

## PROCEEDINGS

# American Academy of Forensic Sciences

## 75th Anniversary Conference



# Science Works

February 13-18, 2023  
Orlando, Florida  
Rosen Shingle Creek Resort



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### ***PROCEEDINGS***

of the American Academy of Forensic Sciences 75<sup>th</sup> Anniversary 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.

Please note that some of the abstracts included in the *Proceedings* deal with topics, results, and/or conclusions that are controversial. The publication of abstracts does not imply that the AAFS, its sections, or the individual section program chairs/committee members have verified or agree with the studies, results, and/or conclusions of each abstract. During the process of planning a scientific program, it is impossible to “peer-review” each abstract and presentation to the degree that is accomplished during manuscript review. Abstracts and presentations are accepted, in part, so they can be critiqued and reviewed by other scientists. Thus, a forum is created to discuss controversial issues. The views expressed in this publication are not those of the AAFS. The data and opinions appearing in the published material were prepared by and are the responsibility of the contributor(s), not of the AAFS nor its respective employees, employers, officers, and agents. The AAFS does not supply copies of meeting papers. Please write directly to individual authors to obtain copies of specific papers. Presentation of some abstracts may have been scheduled or canceled after the publication of this document.

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# ***PROCEEDINGS***

**of the American Academy of Forensic Sciences**

**February 2023  
Volume XXIX**

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## S1 Education, Training, and Experiences: The Ingredients to Make Science Work!

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**Learning Overview:** The Young Forensic Scientist Forum (YFSF) provides students and young professionals with less than five years of experience an opportunity to network, collaborate and learn from fellow peers and experts in the field. This session will prepare attendees for a successful career in their discipline of choice and allow them to look from the outside-in as they evaluate themselves and their future impact on forensic science. Our mission is to provide education, mentorship, and resources to students and burgeoning forensic scientists to promote interest and longevity in the field and encourage participation and advancement in the AAFS.

**Impact Statement:** After attending this session, attendees will better understand how forensic science has evolved over the years and how every forensic science discipline impacts and enhances the field regardless of one's experience and background. In addition, attendees will learn the importance of education, extensive training, and innovative scientific research. Finally, attendees will learn about the "science" behind job interviewing and become adept in resume building in the forensic sciences.

This year the American Academy of Forensic Sciences (AAFS) is celebrating 75 years of global engagement. The overall theme of the conference is Science Works. In the past few years, science and the scientific method have been under attack, denigrated, and dismissed. AAFS President Laura Fulginiti's plan is to offer scientific content demonstrating that forensic science provides trustworthy, objective data that can be relied upon by the judicial system, Congress, and the public. Our Special Session theme, *Education, Training, and Experiences: The Ingredients to Make Science Work!*, was chosen to invigorate young forensic scientists and demonstrate how education and training play a vital role in making forensic science work. This full-day session will show young forensic scientists that their contribution matters and that the decisions they make today can have a positive impact on the scientific community at large.

This year the YFSF is excited to present an outstanding group of speakers. These distinguished presenters have been carefully selected to provide exceptional mentorship to attendees through topics such as how to stay true to science, present results in a scientifically correct and neutral manner, manage high-profile cases, and interact with prosecutors and defense attorneys. The session will also include thoughtful demonstrations on assessing one's professional goals, establishing what success looks like for a young forensic scientist, and thinking about one's responsibilities as a mentor and leader as they advance into a career.

The YFSF Special Session provides an inclusive atmosphere for young forensic scientists to have the opportunity for professional growth/development and scientific engagement with esteemed colleagues. Through these presentations, attendees will hear from experienced practitioners as they discuss their extensive education, training, and experiences that have led to success in their field of study. These presentations include case-driven research, innovative methodology, scientific studies, and personal stories. Additionally, each presenter will include thoughtful discussions on their struggles and setbacks that have ultimately made "science work." Through this session, the YFSF aims to integrate young professionals into the field as they begin their journey into forensic science. The YFSF offers two additional opportunities for young professionals and students: the Bring Your Own Posters (BYOP) Session and the Bring Your Own Slides (BYOS) Session. We are excited to announce that this year we have collaborated with The Center for Forensic Science Research and Education (CFSRE) to honor and award two outstanding scientists for their contributions to forensic science. Finally, the session will end with a resume review workshop, so do not forget to update your resume and bring several printed copies with you to the session!

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### YFSF; Forensics; Special Session



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### S2     **HHRRC Symposium: The Contemporary Global Scope of Forensic Human Rights Investigations and a New AAFS Initiative**

*Dawnie W. Steadman, PhD\*, University of Tennessee, Knoxville, TX; Luis Fondebrider, BA\*, International Committee of the Red Cross, Buenos Aires, ARGENTINA*

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**Learning Overview:** After attending this presentation, attendees will have a greater understanding of the role of and need for forensic science in human rights investigations as well as learn of new initiatives to involve AAFS members in such projects.

**Impact Statement:** This presentation will impact the forensic science community by: (1) highlighting some of the current forensic science human rights investigate efforts, and (2) providing direct access to some of the organizations and individuals currently involved in human rights investigations globally.

The purpose of this half-day international symposium is to provide an overview of contemporary forensic science applications to human rights investigations by some of the individuals and organizations responsible for the forensic responses, including the Equipo Argentino de Antropología Forense, International Criminal Court, and the International Committee for the Red Cross. There will also be a discussion of the challenges of forensic work in these international contexts, beyond the scientific challenges. Current international protocols and standards in forensic human rights investigations will also be presented. In addition, the HHRRC will introduce a new initiative to create a database of AAFS members who wish to be involved in humanitarian and human rights investigations that will serve as a subject matter expert resource for international organizations seeking forensic experts. The second portion of the symposium will consist of a panel of the presenters who will answer questions from the attendees.

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**Global; Human Rights; Investigations**

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## S3 Implementation of Standards: OSAC Registry Checklist

*Sarah Kerrigan, PhD, Sam Houston State University, Huntsville, TX; Allison Getz, BS\*, NIST, Gaithersburg, MD; Laurel Farrell, BA\*, ANSI National Accreditation Board, Firestone, CO; Erika Ziemak, MSFS\*, Houston Forensic Science Center, Houston, TX; Lori Nix, BS\*, Georgia Bureau of Investigation, Decatur, GA; Jason Bundy\*, Florida Department of Law Enforcement, Tallahassee, FL; Chris Krug, MS\*, Johnson County Sheriff's Office, Olathe, KS*

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**Learning Overview:** After attending this presentation, attendees will have gained insight regarding tools, training, and resources to support the implementation of standards on the Organization of Scientific Area Committees (OSAC) Registry. Specifically, attendees will understand how to access and use checklists to document objective evidence of conformance and how to use these checklists within their own organizations for gap analysis or for internal auditing purposes.

**Impact Statement:** Forensic science service providers will benefit from additional resources that are available to facilitate OSAC Registry implementation efforts (i.e., checklists) as part of the National Institute of Standards and Technology-American Academy of Forensic Sciences (NIST-AAFS) Collaborative Agreement.

**Program Description:** In this interdisciplinary symposium, checklists to document objective evidence of conformance, progress toward implementation, and auditing use will be explained. Insights regarding checklist development, beta-testing, delivery, and versioning will be presented. Collaborations with quality management personnel will be explored, and the practical use of checklists within a forensic service provider will be discussed.

During this interdisciplinary symposium, the benefits of checklist use will be presented. Free access to this resource will be provided, and the practical use of these flexible tools will be explained from the perspective of the developers, providers, and other stakeholders. This special session will include instructions regarding the use of checklists for standards on the OSAC Registry, integration in the quality management system, and practical advice regarding their use. This session will also include an interactive session and panel discussion to provide feedback, ask questions, and gain insight from FSPs that are using the checklists.

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### Standards; Implementation; Checklists

## S4 Interdisciplinary Symposium: The Formation of the American Academy of Forensic Sciences

*Paul Messner, MD, Dry Ridge, KY; Jessica Volz, DNP, Adventist HealthCare Shady Grove Medical Center, Brunswick, MD; G. David Annas, MD\*, Forensic Psychiatry Consulting, LLC, Syracuse, NY; Barry A.J. Fisher, MS, MBA\*, Indio, CA; Corina Freitas, MD\*, Freitas and Associates, Alexandria, VA; George W. Hime, MS\*, Miami-Dade Medical Examiner Department, Miami, FL; Carl R. McClary, MS\*, Bureau of Alcohol, Tobacco, and Firearms, Atlanta, GA; Kenneth E. Melson, JD\*, The George Washington University Law School, Washington, DC; Christopher Milroy, MD\*, Eastern Ontario Regional Forensic Pathology Unit, Ottawa, Ontario, CANADA*

**Learning Overview:** The Symposium will provide a historic context for the formation of the American Academy of Forensic Sciences, describe the impetus for its formation, and examine the effects of its members on forensic science.

**Impact Statement:** Attendees will learn about the state of forensic science before, during, and shortly after the formation of the American Academy of Forensic Sciences through the perspective of the six founding Sections: Criminalistics, Jurisprudence, Pathology/Biology, Psychiatry & Behavioral Science, Questioned Documents, and Immunology/Toxicology

The 2023 Interdisciplinary Symposium will examine the post-WW II state of the forensic sciences leading to the American Academy of Forensic Sciences' formation in 1948 and that defined its early years. In 1948, there were six founding AAFS Sections: Criminalistics; Jurisprudence; Pathology/Biology; Psychiatry & Behavioral Science; Questioned Documents; and Immunology/Toxicology. Speakers from each of these Sections will discuss their Section's forensic concepts, research, and applications before, during, and shortly after the Academy's founding (roughly 1945 through 1965). How the Academy's formation and its members helped advance the forensic sciences during those early years will also be addressed.

We will examine how during this era life, law, and forensic science looked so much different than today. This retrospective view of the Academy's founding will help us understand the AAFS' continued efforts today to improve the trustworthiness of forensic science through objective data that can be relied upon by the judicial system, Congress, and the public.

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### AAFS Founding; AAFS Sections; Post WW Forensics

## **CB1 Judicial Misconduct or Judicial Discretion: A Historical Perspective and Case Analyses**

*Patricia Smith, MSL, ACP\*, Harris County District Attorney's Office, Houston, TX; Betsy Adelizzi, PhD, Missouri Southern State University, Joplin, MO*

**Learning Objective:** The goal of this presentation is to create an open and honest dialogue about the criminal justice system and those taxed with the responsibility of ensuring a fair and just system through inclusion, diversity, and with public safety in mind. Attendees will learn about local, state, federal, and international cases and will be afforded an opportunity to be the “trier of fact” and play a role in sanctioning the sanctioners.

**Impact Statement:** This presentation will impact the forensic science community by reminding all attendees of their role in making positive and effective changes to the criminal justice system by focusing on fairness to all parties and accountability for all people involved, through collaborative research and practice with multiple disciplines.

The judiciary is a system of courts and judges interpreting the laws passed by the legislature. The judiciary has evolved over the past thousand years and continues to change in an attempt to meet the needs of society. The makeup of the judiciary originated with a Lord or his stewards and continued to evolve to the elected and appointed judges today. “The very first judges . . . were court officials who had particular experience in advising the King . . .”.<sup>1</sup> The earliest days of the judiciary, prior to the 12<sup>th</sup> century, was barbaric at best and based on a type of divine justice.

Divine justice could involve the accused being forced to sink or swim as a way of proving their innocence. One’s guilt or innocence was not based on law, judicial discretion, or witnesses, but on chance, strength of the accused trying to force himself to sink to show innocence or remain on top, floating, to show his guilt.<sup>1</sup> Another example is a “medicine man” holding two hairs from an accused in his tent and the accused’s guilt or innocence was based on if those hairs remained in place the next day.<sup>2</sup>

The judiciary is arguably a system built around those people with legal expertise, ethical behavior, and fairness. As the judiciary’s evolution continued, the need for codes of conduct and constitutional clarity increased. There are times when the judiciary, or more specifically, certain judges argue judicial independence as a justification for unpopular or sometimes inappropriate conduct. According to Cynthia Gray, “A federal judge argued that the principles of judicial independence incorporated in the United States Constitution barred any sanction for “anything to do with anything that happened when the judge . . . was acting and deciding cases or in any phase of the decisional function,” including “anything that the judge does verbally or physically in the course of adjudication.”<sup>2</sup>

We will review the judiciaries of Canada, California, Texas, the United Kingdom, and Asia. Also, we will discuss specific Harris County cases and engage the audience through polling.

### **Reference:**

- <sup>1</sup> History of the Judiciary. *Courts and Tribunals Judiciary*, London, July 26, 2021. <https://www.judiciary.uk/about-the-judiciary/history-of-the-judiciary/>.
- <sup>2</sup> Gray, Cynthia. The Line Between Legal Error and Judicial Misconduct: Balancing Judicial Independence and Accountability. *Hofstra Law Review*: Vol. 32: iss. 4, Article 11. <http://scholarlycommons.law.hofstra.edu/hlr/vol32/iss4/11>

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## **77Judicial; Judicial Misconduct; Discretion**

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## CB2 Not Always What It Looks Like: Death Due to Tricyclic Antidepressant Overdose

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

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**Learning Overview:** The goal of this presentation is to aid attendees in distinguishing forensic countermeasures used by suicidal persons who do not want to be ruled a suicide.

**Impact Statement:** This presentation will impact the forensic science community by exploring the use of a psychological autopsy when determining manner of death in a possible suicide.

This presentation describes a case study involving possible forensic countermeasures in a suicidal death involving grief, alcoholism, gabapentinoids, and nortriptyline and the process of accurately determining cause and manner of death. Toxicity of tricyclic antidepressants and their role in suicide is discussed, including cardiotoxicity and seizures. Aspects of the case, including investigation and differential diagnosis, are outlined.

As part of the death investigation, a psychological autopsy was performed, with the decedent's prescribing physician and only remaining relative (and heir) interviewed. Of note, the decedent had spent several years in her medical career in a urology clinic and was likely aware of drug toxicity. Her physician stated she specifically requested nortriptyline as an antidepressant. He was unaware of literature available on the internet promoting its use in the process of suicide. The decedent's heir was shocked to hear the death was ruled suicide. She was not aware of any suicidal ideation, but had been requested to relocate to live with the decedent, which she was unable to do at the time. Taking into consideration the decedent's history of alcohol abuse, empty bottles of alcohol at the scene, her knowledge of toxicology, and the location of the three empty bottles of medication found on the floor with their caps on, attempted forensic countermeasures (to preclude suicide) on the part of the decedent cannot be ruled out.

Performed by certified professionals, a psychological autopsy is a best practice postmortem data collection procedure performed in addition to any other official death examination. A psychological autopsy helps to reconstruct the proximate and distal contributing factors of an individual's death by suicide and document the most likely manner of death where that manner of death is equivocal and left undetermined by a medical examiner or coroner. Suicidology is an important methodology in the role of any death investigator.

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### Tricyclic Antidepressant; Suicide; Psychological Autopsy



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## CB3 Postmortem Assessment of Anaphylactic Shock Due to Hymenoptera Stings (Yellow Jacket *Vespula*)

*Elena Forzese, MD\*, Messina University, Messina, ITALY; Claudina Pitrone, MD\*, Messina University, Messina, ITALY; Daniela Sapienza, MD, Messina University, Messina, ITALY; Alessio Asmundo, MD, Messina University, Messina, ITALY; Elvira Ventura Spagnolo, MD, Messina University, Messina, ITALY; Gennaro Baldino, MD, Messina University, Messina, ITALY; Chiara Stassi, MD, Messina University, Messina, ITALY; Cristina Mondello, PhD, Messina University, Messina, ITALY*

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**Learning Overview:** After attending this presentation, attendees will understand that Hymenoptera stings (bees, wasps) are among the most common causes of anaphylaxis, defined as a severe, rapid onset of a systemic allergic reaction which, if not promptly treated, can be fatal.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the role of ancillary postmortem investigations in the diagnosis of anaphylactic shock caused by bee stings when no macroscopically evident signs are detected during either on-spot examination and autopsy.

To this regard, we here present the case of a 45-year-old man who, according to the circumstantial data collected, was hit by a swarm of bees while opening a shed at work, dying shortly thereafter. No signs of bee stings were macroscopically evident during the survey, nor was any sign of anaphylactic shock (angioedema, pharyngeal/laryngeal oedema, petechial hemorrhages) observed during autopsy. The only macroscopic findings consisted of mild pulmonary edema with mild swelling of the upper lip; heart and coronary arteries were macroscopically undamaged.

Considering that the absence of postmortem findings does not necessarily exclude the diagnosis of anaphylaxis (since macroscopic signs can resolve during the postmortem period), to clarify the possible cause of death, histological and biochemical investigations were performed, especially in light of the available circumstantial data. Biochemical assays included serum sampling for Radioallergosorbent testing (RAST) for bee venom specific IgE, which showed positive for the venom of the yellow jacket (type of *Vespula*); other biochemical tests highlighted an increase in tryptase and troponin; samples of heart, larynx, trachea, lungs, and spleen were also collected for immunohistochemical tests in order to evaluate the mast cells infiltrate using anti-tryptase antibodies.

Therefore, based on anamnestic (absence of any other pathology worthy of note), circumstantial (exposure to bee sting), histological, and biochemical data, the diagnosis of death was stated as due to anaphylactic shock although there was an absence of macroscopic signs at autopsy.

The case showed the importance of second-level investigations for the postmortem diagnosis of anaphylactic shock, highlighting the usefulness of biochemical and immunohistochemical analysis. In complex cases, a multidisciplinary approach, such a criteria exclusion cause of death, represents an important method of investigation. Moreover, in such cases, the circumstantial data can provide important data to perform specific postmortem investigations aimed at finding the substance responsible for the anaphylaxis.

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### Bee Sting; Anaphylaxis; Forensic Sciences

## LS1 The Lindbergh Kidnapping Suspect No. 1—The Man Who Got Away

*Lise A. Pearlman, JD (Ret.)\*, Oakland, CA*

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**Learning Overview:** Attendees will gain insight into how key forensic evidence was suppressed and physical evidence altered to convict an innocent man of “the crime of the century.”

**Impact Statement:** The anticipated impact on the forensic science community is greater awareness of ways in which members' forensic analysis and testimony can be misused (e.g., suppression of pretrial notes, overstating conclusiveness of findings, denial of a fair opportunity to the other side to test physical evidence) to cause a miscarriage of justice. This presentation will also provide an example of how forensic scientists can play an important role in revealing the truth and exonerating the wrongly convicted..

This luncheon talk by retired judge and award-winning author Lise Pearlman will discuss a proposed shocking solution to the 90-year-old mystery of the 1932 kidnap/murder of Charles Lindbergh, Jr. based on the best-selling 2020 true crime book, *The Lindbergh Kidnapping Suspect No. 1 — The Man Who Got Away*. The talk will describe the framing of Bruno Richard Hauptmann for the murder of Charles Lindbergh, Jr. and the revelation of Suspect No. 1 whom the New Jersey State Police let get away with the crime. The talk will focus on wood evidence, fingerprints, and handwriting analysis, and address disparities between the "evidence" presented at trial and related contradictory evidence that was concealed by the prosecution and consequently not presented to the jury. Lindbergh “Kidnap”

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**Ladder Evidence; Handwriting Analysis; Fingerprints**

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## LS2 Forensic Sciences and Popular Poetry: A Lighter Way of Teaching People How Science Works

*Jose A. Medeiros, PhD\*, Brazilian Federal Police, Cabedelo, PB, BRAZIL*

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**Learning Overview:** Upon completion of this luncheon seminar, the participant should be able to know how the dissemination of forensic sciences among people can be efficient and lighter through popular poetry, based on this Brazilian testimony.

**Impact Statement:** Showing the international forensic community how the dissemination of forensic sciences through popular poetry is effective among the general public and has encouraged Brazilian forensic scientists to identify themselves with their labor as well as with a literature genre (including woodcutting illustrations) that has become essentially Brazilian and that today is an example of national intangible cultural heritage.

This work is about an initiative that has been developed since 2012: to bring together Forensic Science and popular poetry in a simple and accessible format and share it with the lay public. The path used was “Literatura de Cordel”, a form of literature originally European, but which found fertile ground in the Northeast of Brazil, especially from the 19th century onwards. Like a Limerick, the cordel booklet presents a story usually in a humorous tone and in colloquial language. Today there are already 14 stories about CSI, Forensic Engineering, Genetics, Jurisprudence, Environmental, Toxicology, Children’s History, etc. and more than 18 thousand copies distributed free of charge to people from all Brazilian regions at forensic events, criminalistics units, schools and institutions.

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**Literatura de Cordel; Popular Poetry; Woodcutting Art**

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## LS3 Junk Science and the American Criminal Justice System: An Inside Perspective

*Jose A. Medeiros, PhD\*, Brazilian Federal Police, Cabedelo, PB, BRAZIL*

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**Learning Overview:** After attending this presentation, attendees will have gained insight from an insider's perspective into the heart of a broken American criminal justice system where junk science is just one piece in maintaining the status quo, including insight from the author's recently released, award-winning book, *Junk Science and the American Criminal Justice System*.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the perspective of the premier junk science wrongful conviction investigative and litigation organization—the Innocence Project—that ushered forensics through a time that saw statements such as “one-in-a-million chance” as acceptable science into in a new era of reliability, validation, and, most of all, the accountability we expect to see today.

**Program Description:** In 2012, the Innocence Project began searching for prisoners convicted by junk science, and three men, each convicted of capital murder, became the author's clients. In his book, *Junk Science and the American Criminal Justice System*, the author chronicles his fight to overturn their wrongful convictions and end the use of the bad science that destroyed these men's lives. The quest took many turns and the story weaves together courtroom battles over faulty forensics from Mississippi to Texas to New York City and beyond. The book describes a journey into the heart of a broken, racist system of justice and the role forensic science plays in maintaining that broken system.

In addition to some of the interesting case studies demonstrated in the book, the author's career documents the slow but steady evolution of the field of forensics as a whole and the difficult challenges and barriers that the Innocence Project, lawyers, judges, other stakeholders, and forensic scientists themselves have faced in evolving and moving forward from duplicitous times.

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**Innocence; Courts; Wrongful Convictions**

## W1 Investigating Publishing: How to Disseminate Your Work

*Lerah Sutton, PhD, University of Florida, Gainesville, FL; Gina Londino-Smolar, MS, IUPUI, Indianapolis, IN; Adrienne Brundage, PhD\*, Texas A&M University, College Station, TX; Kelly Elkins, PhD\*, Towson University, Towson, MD; Jason Byrd, PhD\*, University of Florida, Gainesville, FL; Sharon Plotkin, EdD\*, Miami-Dade College, Miami, FL*

**Learning Overview:** After attending this workshop, attendees will be able to review and prepare their own work for a scientific publication; determine the best publication venue for specific educational work; develop case work experiences for publication; and create self-promotion through various means on social media.

**Impact Statement:** This workshop will impact the forensic science community by increasing awareness of the importance of dissemination through educational publications. People within the forensic science community will be able to learn how to best share their work through textbooks, journals, open resources, and social media.

It is important for practitioners and educators to share the work done both in the field and in the classroom. Knowing the best ways to publish various types of research and practice can be a challenging task. This workshop will share best practices in scientific publishing. This will include an introduction to scientific publishing, building a strong foundation for your publication, identifying where to publish, working with the peer-review process, transitioning casework into publications, and promoting your work via social media. There will also be a hands-on session to allow participants to bring questions and examples of work for publication.

Within the forensic sciences, there are many different avenues within which one could seek to publish work. These may include traditional academic avenues for graduate students to publish their thesis or dissertation, various components of textbook publication to include invited chapters/volumes and/or whole texts, and traditional peer-reviewed journal articles. This session will provide an introduction to each of the most common types of publications that a potential author may encounter within the forensic science community such that the participant understands the nuanced differences between each and which type of work would be appropriate to publish in each venue.

Traditional scientific publishing follows well-established guidelines with known expectations. While this method works well it does exclude a whole swath of useful information. Educators, practitioners, even advanced hobbyists are untapped sources of information with few places to disseminate that material. The publication world has room for all manner of publication types, and the ability to make this information known is easier than you may think. This session will review potential publications to determine which type of communication is best suited for your unique information.

Forensic scientists are busy professionals. However, scientific publication is important for courtroom acceptance of new methodologies and approaches to casework and for academics building portfolios for tenure and promotion. There are many publication venues to choose from including university theses and dissertations, peer-reviewed journals, invited book chapters in edited volumes, and textbooks. This session will be focused on illuminating various journals and other modes of publication including peer-reviewed and non-peer-reviewed, open-access and subscription, and invited and contributed submissions.

Many forensic scientists are employed by colleges and universities and are required to meet the employer related obligations of academia for tenure and promotion. In addition to research and instructional duties in the faculty member's field of expertise, there are also employer requirements for publishing and service. This includes serving on university thesis and dissertation committees, publication in peer-reviewed journals, contributing book chapters, creating edited volumes, and authoring textbooks. This session will focus on academic professional development and the value of serving as a peer reviewer, subject matter editor, and editor for professional journals and other published works.

Crime scene investigations college students face the challenge of gaining employment upon graduation. In order to be successful in the interview process, portfolios as a demonstrative aid of student achievements and college coursework is a viable means for showcasing to potential employers that these graduates have the necessary skills to be successful in the crime scene field. Research has shown that portfolios are effective as a demonstrative aid for skill and knowledge acquisition however little research exists in crime scene investigations regarding portfolio use during the interview. This session will address the strengths and weaknesses of portfolios and how to effectively utilize them in the interview for a successful outcome.

As an author, it is important to use communication through various means in order to share your work with others. Ways to do this can be through social media, open-access journals, and in your own classroom. Participants will explore ways to promote their own publications and the importance of their professional profile. The discussion will also include different types of copyrights and the best option to reach specific readers and for individual publications.

During the workshop, participants will be given time to work in small groups with their peers on how to best publish their work. This will include division by participants' specific interest which will be moderated by presenters. This will allow for individuals to work on specific needs related to their own publications as well as get support from others and the presenters.

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### Scientific Publishing; Forensic Science Education; Information Dissemination



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## W2 Implementing Magnetic Flux Measurements for Forensic Document Examinations

*Carrie Polston, PhD\*, Universite de Lausanne, Lausanne, Vuad, SWITZERLAND; Zain Bhaloo, MSc\*, Canada Border Services Agency, Ottawa, Ontario, CANADA*

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**Learning Overview:** After attending this workshop, attendees will be able to recall and discuss the scientific theory that underlies the instrumentation involved with magnetic flux measurements. They will be able to explain what magnetic properties toner has and how they can be measured. Attendees will be able to prepare a laboratory setup with a Magneto-Optical Imaging Device (MOID) and identify critical variables in the laboratory design, which could impact instrument function. They will be able to follow the methodology provided and operate the instrument to collect magnetic flux measurements from different toner samples. Attendees will be able to evaluate the results they obtain to determine if there has been any type of error or if there is bias. Attendees will be able to interpret the significance of the results.

**Impact Statement:** This workshop will impact the forensic science community by providing Questioned Document examiners with the knowledge, skills, and abilities necessary to deploy a new methodology for examination of toner-printed documents in their laboratories. This methodology has been found to meet the needs of the community as stated, being rapid, non-destructive, and cost-effective.

In this workshop, attendees will learn about the foundational theory behind MOIDs and magnetic flux measurements of toners, as well as the variables that must be considered when developing a method for forensic laboratory use and will then get hands-on experience with instrumentation. During the hands-on portion of the workshop, attendees will learn how to set up the workspace and instrument, how to operate the software associated with the MOID, and how to conduct magnetic flux measurements of different toner samples (including text samples, point samples, and text insertion samples). The attendees will then be able to independently conduct measurements on provided samples to demonstrate their proficiency with the instrumentation and methodology they have learned. Finally, as a group, attendees and instructors will evaluate the results of the independent measurements. At this stage, attendees will learn how to evaluate and interpret the significance of the results they obtain from the instrument and how to assess if the data has been affected by any of the variables discussed during the theoretical session.

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### Magnetism; Toner; Questioned Documents

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**W3 Forensic Postmortem Radiology: Crossing the Border Between Radiology and Pathology**

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**Learning Overview:** After attending this workshop, attendees will: (1) learn the basics in how to get started in the field of forensic radiology, (2) understand the current state of the art and levels of evidence in forensic radiology, (3) learn about applications to assist forensic pathologists and practitioners in medical death investigation, and 4) be made aware of potential resources available to interested practitioners.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with expertise from around the world on the use of, best practices and the evidence base of forensic postmortem radiology.

This workshop will give an overview of the current state of forensic imaging in the world, the logistics on how to start a forensic imaging program, and how future technologies may impact the field of forensics.

It has been nearly 13 years since the publication of the National Academy of Sciences report calling for the strengthening of the forensic sciences. In this seminal report, they stated the critical role that imaging plays in the documentation of findings sufficient for courts, for providing the opportunity for review by outside experts, as well as for allowing for reevaluation of evidence as medical knowledge advances. Since then, forensic radiology and imaging has grown significantly as a field around the world.

The “Virtual Autopsy” or “Virtopsy” utilizes multislice computed tomography (MSCT) or post-mortem CT (PMCT) and Magnetic Resonance Imaging (MRI) combined with three-dimensional (3D) imaging technology to create vivid images of the interior of the human body. CT scanning and MRI has been shown to present better visual picture of some injuries, reduce the number of autopsies conducted to rule out occult injury and document the extent of injury in accidents in greater detail. The advantages of the Virtopsy are that it is not invasive or destructive to tissues and can provide clear pictures of skeletal and soft tissue injury. It also provides some information when there is a religious objection to autopsy. Additionally, Virtopsy has the potential to detect internal bleeding, bullet paths, bone and bullet fragmentation, fracture patterns, brain contusion, gas embolism, as well as occult fractures which are technically difficult to demonstrate during the traditional autopsy.

In the United States, only a few Medical Examiners/Coroner’s offices (approximately 20) have access to this advanced medical imaging at this time and very few have the budget to purchase the expensive equipment or to build a suitable facility with staff to maintain it. The current public health crisis of opioid deaths is stretching an already strained ME’s system with regards to autopsy caps set by the NAME standards. Post-mortem medical imaging can play a critical role in easing these demands for full autopsy on offices through triage screening from PMCT.

Within the last years, the National Institute of Justice (NIJ) has fully supported forensic imaging as a supplement to the traditional autopsy and as a triage and long-term evidence documentation tool. NIJ efforts have attempted to bring field standards from around the world and establish major research areas of need in order to help facilitate the field’s growth in the US since it lags behind other countries in the field. Additionally, the post AAFS 2018 attendee survey indicated in several places the desire of members for more information on assisting forensic pathology workloads with the use of CT scans/documentation of trauma via imaging, even going as far as suggesting modifying of National Association of Medical Examiners guidelines to accomplish this. With the field well established in practice outside the US, lessons learned from experts will help those interested in incorporating these technologies into their facility’s everyday practice. Exposure to emerging technologies in the field will also help practitioners be exposed to potential applications that will alleviate their workflow in an already overtaxed death investigation system.

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**Forensic Pathology; Forensic Radiology; Postmortem Imaging**

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**W4 Just Communications: Providing Clear and Accurate Reports and Testimony on Forensic DNA Results**

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**Learning Overview:** DNA experts are asked in court to translate hours of chemistry and complex statistical analysis into yes or no answers. We learn to do this from previous experts who teach us the terms of art, metaphorical examples, and boundaries of permissible expert opinion. Like all human communication this is an evolving art that adjusts to changes in science, computation, law and culture. After attending this presentation, DNA analysts, lawyers, judges, and other criminal justice stakeholders will be better able to evaluate and communicate laboratory procedures and findings. They will be encouraged to adopt agile practices for clear and accurate testimony on the witness stand, and will be familiar with tips and tricks for guarding against common pitfalls. Participants will also learn from examples that have led to wrongful convictions, including bias in DNA mixture interpretation.

**Impact Statement:** This presentation impacts the scientific community by discussing the critical consequences that can result from false or misleading testimony that is anecdotal rather than based on empirical data and ways labs and individual analysts may mitigate some of these consequences through technical training, testimony oversight and quality management.

It is essential that forensic scientists are able to clearly and accurately communicate their work, whether it is casework results or new research. This includes talking to other scientists, investigators, attorneys, judges or jury members, all of which present their own challenges. Based on well-established principles of communication theory and training methodology, this presentation will introduce some of the particular problems faced by forensic scientists in various stages of the justice process. Practical skills and exercises will be introduced to meet the specific communication goals of the forensic human ID scientist. Audience-centered design, creating connections, and accessible language will all be discussed as achievable learning outcomes for forensic scientists in this session. Practitioners and researchers will learn to better communicate their data and its contextual meaning in a way that is accurate, clear, complete and effective.

Forensic labs make significant investments in training DNA analysts on concepts of DNA analysis and profile interpretation. In contrast, very little time is invested in teaching DNA analysts how to communicate those results to laypeople in a clear and unbiased way. In contrast to DNA profile development and the technical checks and balances imposed by the FBI Quality Assurance Standards and ISO 17025 on the DNA testing and reporting process, there is very little oversight or technical assessment of forensic testimony. Forensic DNA testimony is often the result of many processes, procedures and decisions that will only be described in words to the end user, a judge or jury.

The evidentiary value of a DNA profile obtained from the surface of an object is now being commonly questioned in courts. Premature discussions of providing testimony regarding the activities that gave rise to an evidence DNA profile are alarming considering most accredited U.S. forensic DNA laboratories have not adopted the necessary framework to appropriately and accurately evaluate these high-level propositions. While there is a growing awareness surrounding DNA transfer, persistence, prevalence and recovery research, there are many challenges that need to be addressed before attempting evaluations of DNA findings given activity level propositions, such as lack of (1) sufficient research, (2) education and training, (3) availability of data, and (4) standards and guidance.

This presentation describes the hierarchy of propositions, the limitations of forensic DNA analysis to provide certain information in the absence of ground truth and how analysts can best respond to questions that are likely to be posed but are beyond the scope of these limitations. The presenters will discuss language choice and the impact of certain words and phrases in reports and testimony, and methods for resisting pressures from criminal justice stakeholders to stretch testimony beyond the limits of what science can support. Finally, presenters will discuss best practice for teaching science in the courtroom to ensure results are being understood and not misunderstood.

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**DNA Transfer; DNA Prevalence; Forensic DNA Testimony and Reporting**

## W5 Diversity, Equity, and Inclusion in the World of Forensic Practice: Lessons and Actions

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**Learning Overview:** Upon completing this workshop, attendees should be able to acknowledge the need to address historic and ongoing issues of diversity, equity, and inclusion as they impact the forensics community at all levels. With this understanding of the need for action, participants will be armed with a potential path to light the spark of productive change within their own organization in order to move toward parity for all.

**Impact Statement:** This workshop will impact the forensic science community by addressing many issues encountered that are impacted by failure to recognize diversity, equity, and inclusion, thus allowing attendees to see the scope of the problem from a historical and present-day view. This recognition of the breadth and depth of the problem—along with a primer on working within organizations to make changes for the better—will lead the community toward unity and fairness.

This workshop will focus on social justice in the forms of Diversity, Equity, and Inclusion (DEI). *E pluribus unum*. From many one. From its founding, the United States has been seen as a celebration of strength through diversity, although that lesson that can be too easily forgotten, lost, or worse. DEI can be viewed as a means to that lofty ideal—that all people are created equal. Efforts toward social justice attempt to level the playing field for all members of society at local, regional, national, and international levels. Targeted sessions on general forensic practice, medicolegal forensics, representing the hated, and humanitarian efforts will illustrate the need for DEI to strive for parity. The story of the tragic “Pulse shooting” illustrates the depths of depravity, but also tells of the success that can be achieved by embracing unity through diversity. Finally, a primer to ignite organizational efforts will provide a roadmap to a path forward for all.

*E pluribus unum*. From many one. From its founding, the United States has been seen as a celebration of strength through difference. Diversity, equity, and inclusion (DEI) are a means to the ideal – that *all* people are created equal. Social justice attempts to level the playing field for all, at all levels.

How does DEI work in day-to-day forensic practice? How does the *status quo* affect those involved – staff, stakeholders, and others? Failure to recognize or acknowledge existing issues and unwillingness to improve create conflicts. “The way things have always worked” is fundamentally flawed, since it is predicated on a false assumption that things always *have* worked. That might be true for some but certainly not for all.

*Bias* can have many meanings to different people – one definition being “a tendency to believe that some people, ideas, etc., are better than others that usually results in treating some people unfairly.”<sup>1</sup> Bias in the classroom, where future scientists are educated is concerning. Selection criteria for programs, supportive services, and curricula should address DEI issues. Classrooms discussions, including microaggressions, are important considerations. Addressing social justice provides the tools to understand and hopefully address negative bias.

The interface of medicine and the law is both intellectually and professionally challenging. The absence of a singular international definition or practice of Legal & Forensic Medicine underscores that systems and services can and do vary widely. The medical field exemplifies the need to address DEI – intense competition for limited training slots has seen historical discrimination against many and been met with resistance at multiple levels. Patient populations have also been discriminated against with scarce resources inequitably distributed. An overview of the present state of inequity in medical education, practice, and access crystallizes the need for change to ensure movement towards parity.

Socrates modeled the social contract theory by accepting his death at the hands of the authorities, rather than seek escape. Seeking justice is at the foundation of forensic practice. The bias against defending those society dislikes – including those accused of some of the most heinous crimes imaginable – is all too real. Similarly, experience on reintroducing the convicted who have paid their debt in full can prove enlightening and clearly indicates that society has a long way to go before these individuals receive their rightful welcome back into community.

Humanitarian action is founded on a quest for justice for all through application of the fundamental ethical principles of beneficence, non-maleficence, and autonomy. Such efforts can have lasting, global impact. The noblest of humanitarian efforts can too easily get lost in the politics of “othering” and thereby lose the very unity initially sought. Examples of successful efforts help reinforce the need and the purpose required to advance such works.

Tragedy, especially when inexplicable and on a mass scale, is part and parcel of forensic casework. When an event hits close to home, the impact can be severe. The “Pulse Shooting” at a nightclub in June 2016 claimed 49 dead and 53 wounded – the most deadly incident against the LGBTQ+ community in US history. Rather than retreat in the aftermath, the traumatized community came together like never before, forming the One Orlando Alliance to support and strengthen the LGBTQ+ community. This remarkable story of resilience and determination is an exemplar of positive outcomes under trying circumstances.

Fortunately, behaviors can be molded without having to suffer calamities or continue injustices, if history’s lessons are learned. Social justice is a popular subject presently, as many want to “be the change” they desire in the world. Change requires first recognizing the need and then taking action. Motivation to embrace DEI on an organizational level may benefit from a paradigm shift from within. Focusing on DEI as a powerful ally can help

improve institutions, leading to increased productivity, innovation, enhanced employee satisfaction, and greater client satisfaction. A primer on igniting superior organizational performance through such a mind-set endeavors to provide a successful pathway to model organizational success.

*This workshop presented on behalf of the Forensic Science Foundation Education Committee will provide participants with a primer on igniting superior organizational performance through such a mind-set which will provide a successful pathway to model organizational success.*

**Reference:**

<sup>1.</sup> <https://www.britannica.com/dictionary/bias>

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**Diversity; Equity and Inclusion; Social Justice**



## W6 How Science Works to Identify Unknown Decedents Decades After Death

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**Learning Overview:** After attending this workshop, attendees will better understand how science works in a multidisciplinary approach to identify unknown decedents decades after death via multiple lines of evidence and objective data.

**Impact Statement:** The presentations in this workshop will impact the forensic science community by illustrating a trustworthy process that uses anthropology alongside historical research, archaeology, material evidence analysis, odontology, and biological/chemical analyses—underpinned by a comprehensive quality assurance program and case management system—to make identifications.

This workshop is designed to highlight the case resolution process at one laboratory where the identification of decedents decades after death is the norm. This process relies on, and requires, a multidisciplinary approach. Often, Identification Practitioners focus on their own specialty, either because a multidisciplinary approach is not necessary, or because they lack ready access to other disciplines. However, cold cases require multiple lines of evidence for resolution. Historical research provides a critical background on which to build an investigation while forensic archaeology ensures the evidence and contextual data that can be used for identification are collected in a controlled and documented manner. Depending on the items and remains available for examination, material evidence and forensic odontology are useful to exclude individuals from a short list of potential names. Short lists are further reduced via anthropological analysis of collected remains. In some cases, isotope testing can completely exclude a short list, and in other instances, it may only exclude a few. Finally, DNA analysis can narrow, or in some cases, reduce the short list to only one or two individuals. The more lines of evidence that point to the same individual, the more likely an identification can be made.

On one side, attendees will learn how different scientific disciplines are applied to the identification of unknown decedents. This will include discussions of both field science and laboratory analysis; coverage includes archaeology, material evidence analysis, odontology, anthropology, DNA analysis, chest radiographic comparison, and isotope testing. Supporting these methods is a robust surety, or quality assurance, program that ensures all scientific work is above reproach. Finally, a case management system helps to manage multiple types of analyses in a manner conducive to results integration and traceability of casework. The importance and value of each line of evidence, as well as their support structures, will be covered in detail by the presenters.

On the other side, attendees will learn how one specific identification relied on multiple lines of evidence; each presenter will highlight the ways science worked in the case progression. A special emphasis will be included at the end of the workshop to bring all the methods/evidence together to make a complete case for an identification. This integrated portion of the workshop is designed to help the Identification Practitioner understand why a multidisciplinary approach is the norm for identifying decedents decades after death.

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### Forensic Identification; Cold Case Resolution; Anthropology

## W7 The Seven Habits for Highly Effective Standards Development

*Agnes Winokur, MS\*, Drug Enforcement Agency, Miami, FL; Marc A. LeBeau, PhD\*, Federal Bureau of Investigation, Quantico, VA; Teresa L. Ambrosius, BA\*, AAFS Standards Board, Colorado Springs, CO; Allison Getz, MS\*, National Institute of Standards and Technology, Monrovia, MD; Karen Reczek, MLS\*, National Institute of Standards and Technology, Rockville, MD; Kevin P. Kulbacki, MSFS\* KDX Forensic Consulting, LLC, Chicago, IL*

**Learning Overview:** After attending this presentation, attendees will (1) gain a better understanding of the role and importance of consensus-based standards in strengthening forensic science in the United States and internationally; (2) gain a greater awareness of their own individual role in the development of consensus-based standards, and (3) learn how to provide input and get involved.

**Impact Statement:** The 2009 National Academy of Sciences (NAS) Report emphasized the need for standards in forensic science. Since this Report was issued, more than 140 forensic science standards have been published, and more than 100 of those have been placed on the Organization of Scientific Area Committees (OSAC) Registry. Still, many more discipline-specific standards are needed to continue to strengthen the field. Standards development is a long and sometimes contentious process. To be successful, those involved must be able to lead themselves and influence, engage, and collaborate with others. This workshop will provide attendees with information on how they can become more effective in the standards development process.

The 2009 NAS Report, *Strengthening Forensic Science in the United States: A Path Forward*, summarized the challenges facing the forensic science community and emphasized the need for standardization. Administered by the National Institute of Standards and Technology (NIST), the Organization of Scientific Area Committees (OSAC) for Forensic Science was created in 2014 to address the need for discipline-specific forensic science standards. OSAC fills this gap by drafting proposed standards and sending them to standards developing organizations (SDOs), which further develop and publish them (e.g., ASB, ASTM International, ADA, NFPA, and AES).

Standards need to be fit-for-purpose to meet a customer's needs as well as support broad adoption and use. The primary driver for the use of forensic science standards is public welfare and safety. The primary users are the forensic science community and the legal and criminal justice systems. Forensic results can sometimes be misinterpreted or misapplied. Standards that are specific, well-written, and to which personnel are trained, can aid in decreasing errors. Good quality standards have a positive impact on the quality of results produced.

The standards development process starts with stakeholders identifying a concept for a standard. The initiating stakeholders (such as OSAC) may develop a draft "seed" document that attempts to describe the specific need for the standard. After the concept has been accepted, experts within a subgroup or working group will begin to draft the standard. The draft is then circulated for comment, voting, and editing to allow stakeholders the opportunity to provide additional or clarifying language. After the comments have been addressed and the final draft is complete, the standard may move to a higher-level working group for additional voting and editing. Finally, the standard receives final approval and is published. Once published, standards must be continuously reviewed and maintained. Different SDOs have different procedures in place. Understanding each SDO's standard types, processes and structure is important to success.

Anyone who may be impacted by a standard or who has relevant technical knowledge to inform its development can get involved in standards development. According to [OMB Circular A-119](#), a voluntary consensus standards development process is based on the following five principles: openness, balance, due process, an appeals process, and consensus.

In his book, *The 7 Habits of Highly Effective People*, author Stephen R. Covey presents an approach to being effective in attaining goals by aligning oneself to what he calls "true north" principles based on fairness, integrity, honesty, and dignity. Covey believes the way we see the world is entirely based on our own perceptions. To change a given situation, we must change ourselves, and to change ourselves, we must be able to change our perceptions. Covey believes that no matter how competent a person is, they will not have lasting success unless they can effectively lead themselves, influence, engage and collaborate with others and continuously improve and renew their capabilities.

Developing high-quality, scientifically sound standards is a long and sometimes contentious process. It involves bringing together various stakeholders with different backgrounds, experiences, and perspectives to reach consensus on a given topic. To be successful, stakeholders must be able to lead themselves, influence, engage and collaborate with others. Using Covey's *7 Habits of Highly Effective People* as a guide, this workshop will discuss the principles of standards development and provide attendees with information on how they can become more effective in the standards development process.

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### Standards Development; Forensic Science; Consensus-Based Standards

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## W8 Postmortem Interval Estimation Using a Novel Data Set and Methods

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**Learning Overview:** Attendees of this workshop will learn how to use geoFOR application to record decomposition characteristics and estimate the Postmortem Interval (PMI) using the available machine-learning models.

**Impact Statement:** This workshop will impact the forensic science community's practice by demonstrating a procedure for recording scene and decomposition information and machine learning models to estimate PMI.

This workshop will introduce a data repository and associated machine learning models to provide a novel method of estimating the PMI in the geoFOR application. The lack of a large reference dataset for developing reliable and accurate models has been a major barrier for improving methods for determining the time since death.

Since 2019, data from forensic cases and observations from anthropological research facilities have been collected in a reference dataset that contains over 1,500 cases from locations across the United States. This reference dataset is representative of forensic casework; it is geographically diverse and encompasses the various scenarios in which human remains are discovered in forensic casework. Along with decomposition characteristics, the application records location, which allows environmental data from existing databases from the National Oceanic and Atmospheric Administration (NOAA), such as temperature, humidity, and precipitation, to be tied to the location of death to model variables important in the decomposition process. The availability of this reference dataset allows for machine learning models to be employed to provide a robust estimate of PMI with an associated prediction interval.

This workshop will first demonstrate the application, reference data set, and the data entry process. Secondly, Geographic Information Systems (GIS) methods and spatial coding will be introduced to demonstrate how environmental datasets are automatically populated and used in the statistical models and how ArcGIS® is used to store and manage data for collaborators. Third, machine learning models that are used in the predictions of PMI from both observed decomposition variables and integrated environmental variables will be explained. Next, Bayesian generative models will be discussed. These methods are useful for improving research design for future studies of decomposition. For example, using expert knowledge and a priori knowledge, this information can be integrated to provide data-informed stages of decomposition or how decomposition variables are associated with body size or age. Finally, this workshop will provide an overview of how this project aims to follow an Open Science model from the National Institutes of Health with the goal of sharing scientific data to accelerate research discovery, enhance research rigor and reproducibility, provide accessibility to high-value datasets, and promote data reuse for future research studies. Participants will gain experience using the geoFOR application to input data, manage their data in ArcGIS®, and make predictions of PMI.

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### Postmortem Interval (PMI); Forensic Taphonomy; Decomposition

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## W9 Inside the Black Box: Forensic Psychiatry for Lawyers

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

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**Learning Objective:** After attending this workshop, attendees will: (1) have an expanded understanding of mental illness; (2) learn how to effectively utilize an expert psychiatric witness in criminal and civil cases; (3) understand the difference between appropriate and inappropriate consult questions for the expert; (4) learn about unethical expert witnesses (how to spot them, why to avoid them, and effectively cross them); and (5) understand the ethical obligations of an expert witness and anticipate when these can conflict with an attorney's ethical duties.

**Impact Statement:** This workshop will have a positive impact on the forensic science community in terms of helping attorneys work more effectively and ethically with experts in the mental health field. The skill and knowledge acquired will also help inform members of the forensic mental health field to avoid some of the pitfalls that can occur in the midst of complex legal cases. Some of the ethical issues examined will be generalizable to other expert witnesses.

What is inside the black box? Whether criminal or civil, psychiatric experts are frequently called upon to shed light on behaviors, their origins, and their past and future consequences. Many lay people, as well as legal professionals, believe that psychiatric experts have a magical ability to connect behavior with a diseased mind in any circumstance. Misconceptions about this ability result in pitfalls of expert engagement. Knowing how to ethically partner with an expert will facilitate clarity and justice in the legal process. How do psychiatric experts make these connections? Are they even possible? Would you like to take a peek inside the black box? Forensic experts have been used in the legal arena since time immemorial. Criminal behavior and mental illness, likewise, have been misunderstood for the same amount of time. Forensic psychiatry and psychology are specialties specifically created to elucidate the knowledge gap and dispel common mental illness myths.

This workshop will cover the history of forensic psychiatry, the use and types of experts, and the clinical and ethical guidelines followed. We will discuss the crucial differences between a treating clinician and an expert, the use and misuse of psychological testing, and the application of case law and statutes to appropriately answer psychiatric-legal questions. Attendees will gain an expanded understanding of mental illness. We will cover the use and pitfalls of the Diagnostic and Statistical Manual of Mental Disorders (DSM 5-TR). They will also, learn how to effectively utilize an expert psychiatric witness and understand the difference between appropriate and inappropriate consult questions for the expert. Workshop participants will be shown how to spot unethical expert witnesses, and learn how and why to avoid them, as well as how to effectively cross them. Lastly, attendees will gain an understanding of the ethical obligations of an expert witness and anticipate when these can conflict with an attorney's ethical duties.

The workshop is designed to help inform attorneys how to work more effectively with mental health experts, as well as confidently navigate the ethical waters when doing so. This workshop will have a positive impact in terms of helping attorneys work more effectively and ethically with experts in the mental health field. The skill and knowledge acquired will also help inform members of the forensic mental health field to avoid some of the pitfalls that can occur in the midst of complex legal cases. Some of the ethical issues examined will be generalizable to other expert witnesses.

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### Expert Witness; Forensic Psychiatry; Ethics

## W10 Reefer Madness: The History of Tetrahydrocannabinol (THC) Psychosis and Modern Adverse Events

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**Learning Overview:** After attending this workshop, attendees will have a better understanding of the state of cannabis legalization and regulations, the history of cannabis psychosis and adverse events, gaps in the scientific knowledge and promulgated mythology, modern incidents of adverse effects and potential mechanisms, and cases in which evidence and circumstances indicate THC-related psychosis. Content will enable attendees to influence, educate, and collaborate with stakeholders in their communities regarding threats to public health and public safety.

**Impact Statement:** This workshop will impact the forensic science community by increasing attendees' understanding of the complexities of the impacts of cannabis and THC analogs in our communities and the need for a multidisciplinary collaboration to know the incidents of adverse events and importance of appropriately and thoroughly describing the etiology of adverse events.

In many jurisdictions across the United States alternative cannabimimetic products have erupted and proliferated. Due to knowledge gaps, confusion, and lack of enforcement, new products that are claimed to be legal and safe derivatives of hemp have been propagated. The pervasiveness of products containing synthetic (i.e., unnatural) tetrahydrocannabinol (THC) isomers is well known in communities, as they are commonly sold in vape shops, cannabidiol (CBD) stores, gas stations, and convenience stores. These synthetic THC isomers and derivatives include, but are not limited to,  $\Delta^8$ -THC,  $\Delta^{10}$ -THC,  $\Delta^{6a-10a}$ -THC, THC-acetate (THC-O), CBD-di-acetate (CBD-di-O), tetrahydrocannabiphorol (THC-P), exo-THC, hexahydrocannabinol (HHC), tetrahydrocannabutol (THC-B), and halogenated THC. Unknown internet sources have misinterpreted the scientific literature, perpetuating falsehoods and misinformation. Consumers have a false sense of security in the quality and efficacy of these products or are confused by the market. A current and significant challenge facing the United States is that the population of persons taking traditional THC products as well as these THC analogues is exponentially more than those who would consume the "older" synthetic cannabinoids. The incidence of reported adverse effects is on the rise, as demonstrated by the increase in calls to poison control centers. However, attribution of the adverse event to a defined drug rarely happens because the product consumed by the person is not analyzed for chemical content and toxicology testing is often limited. Significant analytical challenges exist since many compounds are isobars, metabolic profiles are not elucidated, certified reference materials do not exist for all compounds, and the cannabis community proliferates at a rapid rate. Conversely, symptomology and general health of persons is also rarely recorded in the event that products are tested from the self-reporting individual. This workshop will describe the state of cannabis legalization and regulations, the history of cannabis psychosis and adverse events, gaps in the scientific knowledge and promulgated mythology, modern incidents of adverse effects and potential mechanisms. Multiple cases will be presented in which evidence and circumstances indicate unexpected THC-related psychosis and illustrate the community challenges to supporting the victims or navigating the legal system. The complexities of the impacts of cannabis and THC analogs in our communities and the need for a multi-disciplinary collaboration to know the incidents of adverse events and importance of appropriately and thoroughly describing the etiology of adverse events will be discussed. Content will enable attendees to influence, educate, and collaborate with stakeholders in their communities to support public health and public safety demands.

### THC-Related Psychosis; Adverse Effects; THC Analogs



## **W11 Acetabular Age Estimation: Theory, Method, and Application**

*Allysha P. Winburn, PhD\*, University of West Florida, Pensacola, FL; Jonathan Bethard, PhD\*, University of South Florida, Tampa, FL; Marta San-Millan, PhD\*, University Girona, Girona, SPAIN*

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**CANCELED**

### **W12 An Introduction to Automatic and Objective Firearm Evidence Identification and Report of Error Rate**

*Robert M. Thompson, MFS\*, National Institute of Standards and Technology, Gaithersburg, MD; Kelly Sauervwein, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Junfeng Song, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**CANCELED**

### **W13 Intraoral Photography for the Forensic Scientist**

*David A. Williams, DDS\*, Trojan Horse Consulting, Randallstown, MD; Jessica Volz, DNP\*, Adventist Health Care, Brunswick, MD; Erin E. Williams, BA\*, Erin Williams Photography, Ridgewood, NY*

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**CANCELED**

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**W14 Transfer and Persistence of Physical Evidence: Deciphering Implications**

*Brooke W. Kammrath, PhD\*, University of New Haven, West Haven, CT; Elaine M. Pagliaro, JD\*, Henry C. Lee Institute of Forensic Science, West Haven, CT; Henry C. Lee, PhD\*, Henry C. Lee Institute of Forensic Science, West Haven, CT; Claire L. Glynn, PhD\*, University of New Haven, West Haven, CT; Pauline E. Leary, PhD\*, KD Analytical, Louisville, KY; David San Pietro, PhD\*, University of New Haven, West Haven, CT; Peter R. De Forest, DCrim\*, CUNY, Greenburgh, NY*

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**Learning Overview:** After attending this workshop, attendees will have a heightened understanding of: (1) age-at-death assessments as well as age assessment of the living from the anthropological and odontological point-of-view; (2) application of alternative techniques, based on the biochemical fundamentals of aging, to improving age estimates; and (3) age assessment implications in humanitarian contexts.

**Impact Statement:** This workshop will impact the forensic science community through the presentation of interdisciplinary approaches toward improving age assessments, encouraging collaboration and innovation among different forensic specialties. This workshop will further highlight the importance of age estimation in various contexts, including humanitarian applications.

The transfer of physical evidence is foundational to forensic science and criminalistics. The Exchange Principle, attributed Edmond Locard (1877-1966), has become a fundamental philosophy. Today, the welcome advances in technology and detection sensitivities have come at an unrecognized cost. This progress demands an increased awareness of issues related to transfer and persistence when interpreting analytical results and assessing their significance. Our ability to detect traces goes beyond our understanding of their presence. Deciphering the implications of the transfer and persistence of traces encompasses two different aspects in this workshop. Although their meanings are distinct, they are interconnected. The first meaning applies to the work of forensic scientists or criminalists in developing an understanding of the physical evidence record produced during the event of interest. This record is comprised of the physical "traces" of the event. The second meaning relates the communication of this understanding of the event to an attorney in reports and in pre-trial meetings so that it can be presented to and understood by the court.

The concept of material transfer, both contact and contactless, is an area of great interest in forensic science. Contrary to what may be the dominant perception in the media and literature, the transfer of evidence via contact is not limited to the idea of "touch" DNA. An understanding of the transfer and persistence of various types of traces, including DNA (or human cells), hairs, fibers, gunshot residue (GSR), paint, illicit drugs, ignitable liquids and more, is essential for a comprehensive interpretation. This workshop will include a review of the numerous studies that have been conducted over the years which examine the nature of the mechanism of the transfer of these diverse traces, what it means in terms of results interpretations, and whether there is any sort of predictive value associated with it.

The persistence of diverse traces remains an important factor for consideration by criminalists, police investigators and attorneys. Numerous investigations have been impacted by the persistence of various traces deposited years prior.<sup>1</sup> There is also a plethora of published research which raise important questions about the interpretation of traces recovered from a person or location given the uncertainties regarding their persistence.

This workshop is intended for forensic scientists, investigators, attorneys, and those interested in expanding their interdisciplinary knowledge of the transfer and persistence of traces. By examining various cases and research studies, as well as conducting hands-on experimentation, attendees will develop a sense of the appropriate questions to be asked, as well as understanding their interpretive and holistic reconstructive significance.

**Reference:**

<sup>1</sup>. Clues point to inside job in Yale killing, NBC News. NBC News. September 14, 2009..

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**Transfer and Persistence; Traces; DNA Transfer**

## **W15 SNP Genotyping for Extended Kinship**

*Erin M. Gordon, MFS\*, Signature Science, LLC; Charla Marshall, PhD\*, AFMES-AFDIL and SNA International, LLC; David A. Russell, MSB, Signature Science, LLC; Jacqueline T. Thomas, MSFS\*, Signature Science, LLC; Daniele Podini, PhD\*, The George Washington University, Washington, DC; Andreas Tillmar, PhD\*, National Board of Forensic Medicine, SWEDEN; Katherine Gettings, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Michelle Peck, MFS\*, Signature Science, LLC; Kimberly Andreaggi, PhD\*, Signature Science, LLC; Rebecca S. Just, PhD\*, National Bioforensic Analysis Center; Stephen Turner, PhD\*, Signature Science, LLC; Jodi Irwin, PhD\*, FBI Laboratory; Matthew Gamette, MS\*, Idaho State Police; Tynan Peterson, MA\*, ThroughLine Consultants; Ray A. Wickenheiser, DPS, MBA\*, New York State Police Crime System; Melanie T. Armstrong, BA\*, ThroughLine Consultants*

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**CANCELED**

## W16 Practical Cardiovascular Pathology for Medical Examiners: Basic Concepts and Advanced Principles

*Lorenzo Gitto, MD\*, Cook County Medical Examiner's Office, Chicago, IL; Ponni Arunkumar, MD\*, Cook County Medical Examiner's Office, Chicago, IL; Sarah Thomas, MD\*, Office of the District 21 Medical Examiner, Fort Myers, FL; Gregory Webster, MD\*, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL; David Waters, MD\*, Cook County Medical Examiner's Office, Chicago, IL*

**Learning Overview:** After attending this workshop, attendees will have an enhanced knowledge base of cardiovascular pathology, as seen in a busy forensic autopsy practice. An approach to dissection techniques and microscopic sampling will also be emphasized. Molecular testing, and an overview of congenital heart disease, will additionally be discussed. Attendees will build skills to identify challenges in cardiac assessments at autopsy and develop techniques to address both common and unusual pathologies. Attendees will be exposed to various specimens through didactic presentations, case-based discussions, and real-time commentary on previously recorded videos.

**Impact Statement:** This workshop will impact the forensic science community by emphasizing the multifaceted nature of cardiovascular pathology in the setting of forensic autopsy. Didactic lectures, as well as practical instruction by use of detailed anatomical images, will be given to advance autopsy performance and competency, with an additional focus on improving awareness of potentially heritable conditions that may require notification of decedent relatives. This workshop's target audience includes forensic pathologists/medical examiners, pathology fellows and residents, physician assistants, and medical students.

This workshop will discuss several topics related to basic and advanced knowledge pertaining to cardiac pathology at forensic autopsy. Topics will range from basic heart anatomy to special dissection techniques and microscopic examination of uncommon entities. Recommendations on how to handle heart specimens in challenging cases will also be provided. Didactic presentations, case-based discussions, videos of heart pathologies detailing traditional and special dissection techniques, and interesting heart histology cases will be shown and commented on by forensic and cardiac pathologists on-site. Interaction with attendees will also be encouraged during the workshop session.

Cardiovascular disease is the most common cause of death in the United States. While most of these deaths do not fall under the medical examiner's jurisdiction for various reasons, cardiovascular disease is often responsible for initially unexplained sudden deaths, often in young individuals or even adults of various age groups without a documented medical history. Furthermore, cardiovascular disease may coexist in forensic autopsies performed for seemingly obvious non-natural deaths, in which case, it is important to understand what influence a particular cardiac pathology may or may not play in such situations.

Cardiovascular disease is a nonspecific term, to include entities that involve both the myocardium (heart muscle) and its adjoining vessels. Entities include hypertensive and atherosclerotic forms of ischemic heart disease (most common); however the term also includes a broad spectrum of other cardiovascular pathology such as the various cardiomyopathies, channelopathies, vasculopathies, and congenital heart disease. Determination of cause and manner of death often relies on the forensic pathologist's solid understanding of these various entities in the context of intervening factors, and furthermore, is dependent upon their ability to identify subtle pathology grossly. In the event of a so-called negative autopsy, especially in the setting of a suspected sudden cardiac death, it is important to understand what next steps to take with regard to microscopic examination and/or molecular testing. These steps will be discussed.

Different heart pathologies may require different approaches, in terms of a detailed external examination of the organ, description of specific pertinent positive or negative findings, dedicated dissection techniques, accurate sampling for microscopic examination, and specimen collection for potential genetic analyses. The dissection of the heart is particularly critical, and as such, multiple techniques will be discussed including the typical direction of flow technique and short axis techniques, as well as long-axis and four-chamber methods. Determining the most appropriate dissection method is based on what pathology needs highlighting, or to search for a particular finding.

Following gross dissection of the heart at autopsy, heart sampling for microscopic or histologic examination may be performed and is often essential for a definitive diagnosis. Traditional and special sampling techniques, such as evaluation of the cardiac conduction system, relies upon appropriate preservation of cardiac tissue if future analysis is warranted.

An entire spectrum of congenital heart disease may be encountered at autopsy, and only minor subtle abnormalities may characterize them. In such cases, medical records are often helpful if a particular defect was previously diagnosed. However, there are times when no diagnosis was previously known or described in the medical record, or the records are not readily available at the time of autopsy. Therefore, forensic pathologists should be trained to recognize both significant and subtle congenital cardiac abnormalities and have some awareness of the many cardiac surgical techniques used to manage these patients. A brief overview of congenital heart disease will be discussed in this context.

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### Cardiovascular Pathology; Forensic Pathology; Dissection

## W17 A First Look at the Draft Recommendations in the Report of the National Institute of Standards and Technology/National Institute of Justice (NIST/NIJ) Expert Working Group on Human Factors in Forensic DNA Interpretation

*Lynn Garcia, JD\*, Texas Forensic Science Commission, Austin, TX; Tiffany A. Roy, JD\*, ForensicAid, West Palm Beach, FL; Nikola Osborne, PhD\*, Human Factors Training and Consultancy, Auckland, AUSTRALIA; Angela Spessard, MSFS\*, Maryland State Police, Pikesville, MD; Mikalaa M. Martin, BS\*, RTI International, Greenville, SC; Melissa Taylor, MD\*, National Institute of Standards and Technology, Gaithersburg, MD; Jarrah R. Kennedy, BS\*, Kansas City Police Crime Laboratory, Kansas City, MO; Hope Zagaria, MS\*, RTI International, Washington, DC*

**Learning Overview:** After attending this workshop, participants will have a deeper understanding of human factors. They will be familiar with the findings and recommendations presented in the report of the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation. Participants will understand the purpose and implications of these recommendations for their own practice (i.e., individual-, team-, and organization-level) and how the recommendations serve to improve the practice of DNA interpretation. Participants will be invited to critique the recommendations and discuss how relevant and feasible they are to their practice and the wider forensic DNA community.

**Impact Statement:** The study of human factors in forensic science is essential to inform our understanding of the interaction between humans and the systems they use. Understanding human factor issues can identify and mitigate the potential for error and bias. Furthermore, we can develop ways to shape positive laboratory culture, improve staff morale, and increase work productivity. This workshop presents three years' worth of effort to understand human factors as they apply to forensic DNA interpretation. This workshop will impact the forensic science community by guiding DNA analysts, laboratory leadership, researchers, educators, and legal practitioners on how to recognize and address human factors influences within DNA analysis and interpretation and, as a result, aid in mitigating the effects of these human factors in justice outcomes.

In this workshop, we will walk participants through the NIST/NIJ Expert Working Group on Human Factors in Forensic DNA Interpretation process to develop the report, highlight key findings, discuss some of the more controversial topics, and present a “first look” at the draft recommendations.

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

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

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

- Education, Training, and Professional Credentialing
- Quality Assurance/Quality Control
- Cognitive Bias and Error Reduction
- Reporting and Testimony
- Management
- Work Environment
- Expressing Evidence Strength (e.g., likelihood ratios)
- Research
- Emerging Technology

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

### Human Factors; DNA; Bias

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## W18 Identification, Analysis, and Interpretation of Blunt Force Skeletal Trauma

*Angela L. Harden, PhD\*, The Ohio State University, Columbus, OH; John H Bolte, IV, PhD\*, The Ohio State University, Columbus, OH; Amanda M. Agnew, PhD\*, The Ohio State University, Columbus, OH; Christopher M. Goden, MA\*, The Ohio State University, Columbus, OH; Kyra Stull, PhD\*, University of Nevada-Reno, Reno, NV*

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**Learning Overview:** Upon completion of this workshop, attendees should be able to: (1) provide an overview of current methods across disciplines for identification and analysis of blunt force skeletal trauma; (2) identify and analyze blunt force skeletal trauma utilizing X-rays, Computed Tomography (CT) scans, photographs, and 3D virtual models; and (3) collaborate with experts across disciplines for a multidisciplinary approach to skeletal trauma interpretation

**Impact Statement:** This workshop will impact the forensic science community by providing attendees the unique and informative opportunity to identify, analyze, and interpret blunt force skeletal trauma in cases with known loading conditions and event data, including instrumentation output, high-speed video, and post-test imaging.

This workshop presents an overview of current methods in blunt force skeletal trauma analysis with a hands-on opportunity for attendees to gain a unique experience and to put these methods to practice. Attendees will gain hands-on experience in identifying, analyzing, and interpreting known skeletal trauma, which is a rare occurrence in forensic sciences. They will also have the distinct opportunity to see exactly how the trauma occurred. All cases included in this workshop are experimental cases of blunt force trauma; therefore, attendees will have access to data collected throughout the entire research design. Specifically, beyond the bone itself, they will be able to evaluate fracture timing, high-speed videos to visualize the response during the event, post-test photographs and radiographs of documented injuries, and 3D models to assist in visualizing skeletal trauma and re-creating the traumatic event.

Foundational concepts will be discussed first in a single element situation, then will transition to the complex whole body situation. This workshop is designed for a broad, multidisciplinary audience and the intention is for the participants to work with colleagues in varying fields to expand their experiences and evaluations. Forensic science is inherently multidisciplinary, but trauma analysis in particular requires a variety of experts. Therefore, this opportunity facilitates communication among colleagues and subsequently substantiates the importance of an interdisciplinary approach to trauma analysis. This novel workshop provides attendees with a hands-on experience in identifying and analyzing skeletal trauma from known loading mechanisms to facilitate an increased understanding of the relationship between trauma mechanism and fracture characteristics, ultimately improving interpretations in real-world cases.

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### Experimental Case Studies; Multidisciplinary Research; Biomechanics

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## W19 Forensic Photography: Photography Basics to Imaging Beyond the Visible Spectrum With Alternate Light Sources (ALS) and Infrared (IR)

*Bryan W. Brendley, PhD\* Methodist University, Fayetteville, NC; David Pauly, MFS\*, Methodist University, Fayetteville, NC; Steve Downs, DBA\*, Methodist University, Fayetteville, NC*

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**Learning Overview:** After attending this presentation, attendees will understand how to properly utilize a Digital Single-Lens Reflex (DSLR) camera in the manual mode. Attendees will also learn how to visualize and image evidence requiring the use of ALS, Ultraviolet (UV), and IR energy. Common types of evidence requiring the use of ALS for visualization and imaging include semen, saliva, urine, gunshot and explosive residue, fluorescent fingerprint powders, hairs/fibers, inks utilized in fraudulent document cases, and other trace evidence. Attendees will learn how to utilize their cell phone cameras to scan evidence for traces of blood and Gunshot Residue (GSR). Attendees will also be exposed to utilizing ALS, including IR energy, to scan for subcutaneous bruising, obliterated tattoos, and obliterated writing with multiple inks.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that whatever the level of understanding of photography may be in the visible spectrum, the crime scene professionals' understanding of photography in the UV and IR spectrums, or spectra outside the visible, remains an elusive concept. With this lack of understanding comes missed opportunities to locate, visualize, and properly capture photographically, evidence that is likely crucial in verifying that a reported crime occurred or to refute a false allegation.

Crime scene and forensic photography within the visible spectrum of light remains the most utilized and thorough means of documenting crime scenes, autopsy findings, physical injury, and forensic evidence. Despite being the most utilized method of documentation, the lack of proper understanding, training, experience and equipment results in photography being underutilized, or at least not utilized to its fullest potential.

Whatever the level of understanding of photography may be in the visible spectrum, the crime scene professionals' understanding of photography in the ultra-violet and infrared spectrums, or spectra outside the visible, remains an elusive concept. With this lack of understanding comes missed opportunities to locate, visualize, and properly capture photographically, evidence that is likely crucial in verifying that a reported crime occurred, or to refute a false allegation.

After attending this presentation, attendees will understand how to properly utilize a DSLR camera in the manual mode. Attendees will also learn how to visualize, and image evidence requiring the use of alternate light sources (ALS), UV, and IR energy. Common types of evidence requiring the use of ALS for visualization and imaging include, semen, saliva, urine, gunshot and explosive residue, fluorescent fingerprint powders, hairs/fibers, inks utilized in fraudulent document cases, and other trace evidence. Attendees will also learn how to utilize their cell phone cameras to scan evidence for traces of blood and GSR. Attendees will also be exposed to utilizing ALS, including IR energy, to scan for subcutaneous bruising, obliterated tattoos, and obliterated writing with multiple inks.

The workshop will begin with instruction of basic photography principles, including the exposure triangle, an introduction – “Tour” – of the Nikon D7000, topic lectures by faculty members, followed by hands-on practical exercises.

Sufficient DSLR cameras, lenses, tripods, alternate light source kits, specific nanometer “barrier” filters, and props will be provided for every two to three attendees.

Forensic science professionals in all disciplines that are charged with imaging crime scenes, or forensic evidence, will benefit from this workshop.

Jurors want, and even expect, to see high-quality images of crime scenes, injuries, and other physical evidence. All too often images exposed in an attempt to capture the various forensic evidence found at crime scenes and other forensic settings are lacking in quality, or do not meet the basic legal standards required. In response to these recognized deficiencies, this workshop has been designed, so that attendees will learn the basic legal requirements for introducing properly formatted images into the courtroom, including when to utilize RAW uncompressed – lossless – settings. Informal surveys of forensic science and law enforcement professionals have shown repeatedly that most law enforcement and crime scene photographers do not understand the nuances in compression levels, such as, when to utilize JPEG vs. RAW, in general crime scene work, vs. when capturing critical comparison, or evidence quality images.

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### Photography; Alternate Light Source; Infrared Light

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**W20 A Gentle Introduction to the Likelihood Ratio (LR): Basic Ideas, Implementation, and Limitations**

*Alicia L. Carriquiry, PhD\*, CSAFE–Iowa State University, Ames, IA; Michael J. Salyards, PhD\*, CSAFE–Iowa State University, Ames, IA; Danica M. Ommen, PhD\*, CSAFE–Iowa State University, Ames, IA*

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**Learning Overview:** Participants in this workshop will understand the basic idea behind the ILR and how to correctly interpret results from an LR analysis. They will also understand some of the challenges that must still be resolved before the LR can be used in a wide range of forensic disciplines.

**Impact Statement:** Forensic scientists are increasingly expected to provide a quantitative, data-based assessment of the strength of the evidence in favor of a proposition. The LR approach has emerged as a plausible approach to do so. Yet, correctly arriving at and interpreting those assessments requires some understanding of the statistical foundations of the LR approach. This presentation will impact the forensic science community by presenting foundations and best practices in an accessible, easy to follow format, aiming to increase the statistical and quantitative literacy of forensic practitioners and provide them with the background they need to more confidently work with LRs.

This workshop will focus on the LR approach to evaluating evidence. The LR is a one-number summary that quantifies the weight of the evidence in favor of the prosecution's or the defense's propositions. While the basic idea behind the LR is simple and intuitive, the challenges arise when trying to implement the approach on different types of evidence. Presenters will discuss the statistical foundations of the LR, but will spend significant time on examples, applications in different forensic disciplines, best practices, and limitations

In recent years, forensic researchers have introduced the idea of *propositions* to formalize the question of interest in a criminal case. Briefly, propositions are put forth by the prosecution and the defense, and the task of the fact finders is to decide whether the evidence supports one proposition over the other. Propositions can refer to *the source* of the crime scene evidence, or to *the activity* that led to the evidence, or even to higher level questions. Regardless of the type of propositions that have been formulated, it is generally accepted that the likelihood ratio (LR) or its close relative, the Bayes Factor (BF) are plausible approaches to estimate the *weight of the evidence* in favor of the prosecution or the defense's hypotheses.

The LR has a long history in statistics and has been used for decades in many different application areas, including medicine, engineering, finance and many others. Its use in forensic disciplines is relatively new, and many wrinkles that are unique to forensic applications still need to be ironed out before the LR can be reliably applied widely in real cases. An exception is the case of forensic DNA analysis, where the LR has been successfully applied since the early 1990s.

In this workshop, presenters will begin by motivating the use of the LR using some simple examples. They will then introduce the statistical foundations of the LR (or BF), and revisit the initial examples to illustrate ideas such as Bayes Rule, a statistical model for discrete observations, a model for continuous observations, the notion of a conditional probability and several other important statistical ideas that are sometimes misunderstood. Of critical importance is the distinction between hypothesis about the *evidence* and hypothesis about the *propositions*; these are often confused in practice, so presenters will emphasize how to correctly think about them and how to correctly interpret the results of a LR analysis. It is expected that this discussion will occupy about a third of the time allocated to the workshop.

Authors will dedicate another third of the workshop to discussing best practices, presenting examples from various forensic areas, and emphasizing the correct interpretation of results in each case. Limitations of the LR approach will also be discussed. Among those, presenters will address practical challenges including the choice of statistical models for the evidence under the competing hypothesis of prosecution and defense, the quantification of the *uncertainty* associated with any LR estimate, and the problem of selecting a *reference population* for computing the denominator of the LR.

Finally, the rest of the workshop will be dedicated to what is known as the *Score-Based LR*, an empirical approximation to the LR that can often be implemented in practice when the LR itself is intractable. This is the case, for example, when the evidence is in the form of an image, as occurs in *pattern comparison disciplines* such as latent prints, footwear and firearm examination. While useful, the SLR has additional limitations that will be discussed. The authors will also (albeit briefly) touch upon the European approach to what might be termed a *qualitative LR (QLR)* and will offer some thoughts about its interpretation

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**Likelihood Ratio; Weight of Evidence; Quantitative Evaluation of Evidence**

## W21 Implementing Forensic Investigative Genetic Genealogy (FIGG): How to Put Figg Into Practice (Without Messing It Up for Everyone Else)

*Ray A. Wickenheiser, DPS\*, New York State Police Crime Lab System, Albany, NY; Kameron Wong, BS\* Verogen, San Diego, CA; Steven Smugeresky, BA\*, Montgomery County Police Department, Gaithersburg, MD; Mandi S. Van Buren, MPharm\*, Kern Regional Crime Laboratory; Connie Bormans, PhD\*, Gene by Gene, Houston, TX; Swathi A. Kumar, PhD\*, GEDmatch, San Diego, CA*

**Learning Overview:** After attending this workshop, attendees will better understand: (1) the standards and policies being created for and by the forensic community; (2) a comparative study of the different technologies available and data-driven guidance on when to use each; (3) database hygiene and best practices for using FIGG databases in alignment with the Interim Department Of Justice (DOJ) policy and evolving terms of services; (4) how to prepare and what to consider when bringing FIGG in-house; and (5) case studies and practical guidance for learning investigative techniques from the perspective of law enforcement.

**Impact Statement:** This workshop will impact the forensic science community by providing the most up-to-date information on the policies, procedures, and best practices associated with putting FIGG into practice in an operational forensic environment. Attendees will receive summaries of all standards and policies presented. Example checklists for implementation, data evaluations, and comparisons will be made available. By sharing experiences and knowledge, the community can build a framework for a system that works together on a wider, coordinated scale.

This workshop will provide scientific, experiential, and collaborative guidance on the critical aspects of a FIGG workflow, namely, emerging standards and policy, DNA technologies and limitations, genealogical databases and terms of use, investigative genealogical skill-building, and laboratory implementation planning. Practical experience and implementation options will be focused by a discussion of how real world cases are solved, which will guide operational and policy choices. Experts from forensic laboratories, law enforcement, and industry will present their consensus, data, and guidance to provide a toolkit for the forensic community to apply to their own investigations.

Forensic investigative genetic genealogy (FIGG) has proven to be an effective tool in generating investigative leads and reanimating cold cases. The intersection of traditional genealogical research, DNA technology, and its application to investigations and casework demonstrates the power of interdisciplinary cooperation. As the application of FIGG to casework increases, the need to share knowledge between disciplines and laboratories to create more standardized investigative and analytical practices becomes more pressing to ensure the continued responsible use and protection of this revolutionary technique. This workshop will provide scientific, experiential, and collaborative guidance on the critical aspects of a forensic investigative genetic genealogy workflow: emerging standards and policy, DNA technologies and their limitations, genealogical databases and terms of use, investigative genealogical skill-building, and laboratory implementation planning. Practical experience and implementation options will be focused by a discussion of how real world cases are solved, which will guide operational and policy choices. By understanding how the different parts of the investigative process play into an actual case, you will more confidently understand how to prepare and plan a technical system that can be both flexible and robust for the requirements of the sample, the investigation, and the information required. Experts from forensic laboratories, law enforcement, and industry will present their consensus, data, and guidance to provide a toolkit for the forensic community to apply to their own investigations.

This workshop seeks to provide the most up-to-date information on the policies, procedures, and best practices associated with putting forensic investigative genetic genealogy (FIGG) into practice in an operational forensic environment. Attendees will receive summaries of all the standards and policies presented. Example checklists for implementation, data evaluations and comparisons will also be made available. By sharing our experiences and knowledge, the community can begin to build a framework for a system that works together on a wider, coordinated scale.

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**Forensic Investigative Genetic Genealogy (FIGG); Forensic Genetic Genealogy (FGG); Investigative Genetic Genealogy (IGG)**

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## W22 An Introduction to Document Security

*Carolyn Bayer-Broring, MFS\*, Immigration & Customs Enforcement, Derwood, MD; Elaine X. Wooton, MFS\*, Homeland Security Investigations, McLean, VA*

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**Learning Overview:** After attending this workshop, attendees will have a better understanding of what constitutes a “secure document,” including the features that comprise those documents, and how those different features and elements function and interact.

**Impact Statement:** This workshop will impact the forensic science community, particularly law enforcement personnel and document examiners, by helping attendees to better understand what they are looking at when handling and examining a variety of documents, and be better able to recognize the variety of production and design features that make documents secure, thereby enhancing that security.

This workshop will provide the attendee with an overview of the fundamentals and methods of document security. The workshop will address the securing of items like identification documents (driver licenses, passports, national IDs, employee IDs); financial documents (checks, currency, money orders, coupons); and commercial products (high-end cosmetics, tobacco products, electronics & software).

Fundamental to all documents are printing and substrate. The workshop will introduce participants to the traditional and digital printing methods used in the production of secure documents, as well as the substrates used for various documents and their interaction with security features, print processes, and other secure elements.

Historically, the default material used for documents was paper. The earliest secure feature was the watermark, and it remains a robust feature for high security documents. Watermarks are created during the paper making process by rearranging paper fibers to make a visible design. Other elements which can be added during the paper making process include: planchettes, fibers, and security threads (strips). Reactive chemicals can also be added, to reveal attempts to modify documents via various methods.

The original substrate used for many “ID cards” and driver licenses was paper, which transitioned to “plastic” cards (originally, typically PVC). In order to prevent alteration, materials like Teslin and polycarbonate were introduced into the marketplace. Eventually (and now ubiquitous), the use of polycarbonate was introduced not only for driver licenses, but also for the bearer photo page in many passports (the remaining pages continue to be paper).

Various traditional printing methods are used for secure documents. Intaglio printing has been used for decades for currency and travel documents, as well as for high value certificates. With its “feel of steel” and slow-drying inks, it is easily recognizable by a layperson with limited forensic knowledge. Other traditional printing methods and techniques include offset lithography (especially front-back registration and split fountain), screen printing, letterpress.

As the quality and flexibility of digital on-demand printing methods improve, they are being adopted not just for the personalization of documents, but for overall production. The inkjet and toner digital presses tend to be high maintenance, but enormously flexible. Rather than being limited to four colors, custom colors and ultraviolet inks are options.

Additional elements covered will include security features such as optically variable devices (holograms and variable laser images) and digital elements (including barcodes and chips).

A critical consideration in understanding security documents is the actual marketplace for genuine and counterfeit documents. Which features are included in a given document are based on the requirements enumerated by a government or business client; the process requires that the customer be familiar with the available features and their current integrity. The decisions about which features to incorporate – and to rely upon for 5 to 10 years (or in perpetuity) - should be informed by the successes of counterfeiters in reproducing those features. The decision-making process is most effective when informed by adversarial testing and up-to-date information about how robust proposed features are.

The various topics covered in the “Introduction to Document Security” workshop will provide a moderate foundation for participants. They will be able to differentiate rudimentary, easily defeated documents from well-designed secure documents.

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### Secure Documents; Substrate; Print Processes

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**W23 Report Writing in a New Rule 702 World—Handwriting Comparison Examination**

*Thomas W. Vastrick, BSc\*, Apopka, FL; Kevin P. Kulbacki, MSFS\*, KDX Forensic Consulting, LLC, Chicago, IL*

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**Learning Overview:** Upon completion of this workshop, participants will be able to understand the potential impact on admissibility of expert testimony under a proposed new Rule 702. Participants will also be introduced to new ideas in report writing and, more importantly, the reasoning behind these changes. The contents of reports will hold added importance under the new Rule 702. Participants will also have begun the process of gaining foundational experience in making these changes to their reports so that the reaction by the forensic community can be proactive rather than reactive. In addition, laboratory management will be aware of the potential to all forensic disciplines and proactively engage all disciplines in the necessary report modifications with sufficient time to develop foundational experience.

**Impact Statement:** Changes in Rules of Evidence pertaining to the admissibility of expert testimony have historically been accompanied by wide-ranging and diverse rulings from courts across the country as the judiciary struggles with the impact of these changes. It should be anticipated that non-uniform rulings will also accompany changes to Rule 702 of the Federal Rules of Evidence and any corresponding changes to state rules of evidence. This workshop will impact the forensic science community by providing an increased understanding of the likely results of the rule changes, which will allow forensic scientists to proactively address the coming challenges by answering questions before they are asked. As a result, proactive responses to the rule changes will decrease rulings of unnecessary exclusions or limitations.

Recently, an advisory committee for the Judicial Conference of the United States' Committee on Rules of Practice and Procedure recommended significant changes to Federal Rules of Evidence Rule 702, pertaining to the admissibility of expert witness testimony. While this specific recommendation is for federal jurisdiction, such changes commonly trickle down to state jurisdictions, and no exception can be expected for this matter. As such, it is important for forensic scientists to be aware of the changes and how they may impact testimony in the near future. It is anticipated that the new rule, if passed as expected, will take effect around December 2023. One of the main changes is the added emphasis for a judge's gatekeeping role for each individual proffer of expert testimony, and it is anticipated that judges will become active in that responsibility under the new rule. So how will that affect forensic science testimony? When the United States Supreme Court changed the basic admissibility requirements for expert testimony based on *Frye* to what is now called the *Daubert* Guidelines, the judiciary responded with less-than-uniform rulings. The results were a marked increase in exclusions in spite of the overall statement that *Daubert* was suppose to be a "lower" bar than *Frye*. While we cannot predict the future under any modified Rule 702, we can certainly look to bolster our report writing in order to proactively minimize any potential negative impacts that could occur. This workshop will explore the potentials and provide recommendations for writing reports that will help stand the test of future Rule 702 scrutiny.

History has taught us that challenges are best addressed head on. The consequences of the aforementioned US Supreme Court ruling that changes admissibility standards away from *Frye* and the "general acceptance" procedure were bolstered regarding handwriting comparisons through a non-peer reviewed article in a law journal. The profession, as a whole, took the position that the changes in admissibility rules and the accusations made in the article would blow over quickly. We were wrong. Courts began to question the foundational bases for reliability as per the new *Daubert* guidelines. Then exclusions started. It took years of research and studies to address the concerns noted by various courts. Attacks on all of forensic science followed demanding reactive responses from the entire forensic sciences disciplines. Hopefully the lesson is learned. It is better to address a potential source of challenge head on and proactively than to scramble with sudden reactive responses.

What can we do? In this workshop, we will address the proactive response through comprehensive report writing that addresses the *Daubert* guideline information. Participants will go through a practical problem from the examination to the completion of a report. We will discuss the information in the report, attachments of pertinent standards and reference materials, and the increased emphasis on details. Other topics for discussion and inclusion in reports will include alternative hypotheses and statistics.

It is not only important to have an increased awareness of what we are about to face but also discuss as a discipline how we can proactively address this challenge. Doing so now allows each of us time to become comfortable with the modifications to our report writing and how they are addressed in court.

Finally, it must be emphasized that those who are in supervisory and executive positions within laboratories, even if not Forensic Document Examination related, will significantly benefit from this information as the principles behind the recommendations are not limited to one discipline but are all encompassing.

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**Proposed Changes to Rule 702; Report Writing; Expert Witness**



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**W24 Public Health Initiatives Informing Forensic Laboratory Practice**

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**Learning Overview:** The goals of this workshop are: (1) the identification of projects in the public health space that focus on substance use; (2) increase understanding of how public health data is influenced by limitations of forensic laboratories; and (3) to foster relationships between public health and forensic laboratories.

**Impact Statement:** This workshop will impact the forensic science community by connecting forensic practitioners with information put forth by public health officials.

After attending this workshop, participants will have an increased awareness of how coordination with public health initiatives can serve the forensic science community by providing information that can assist forensic laboratories in focusing their efforts on implementing changes using data-driven decision making.

Public health is defined as the science of protecting and improving the health of people and their communities. Public health surveillance is the ongoing, systematic collection, analysis, and interpretation of health-related data and is essential to planning, implementation, and evaluation of public health initiatives. Comparatively, forensic laboratories analyze evidence objectively, such as drug exhibits or biological specimens, and these results are typically used in a legal context such as investigation of a suspicious death and/or a drug impaired driving case. Substance use straddles the focus of both the public health and the forensic communities, as substance use may have both health and legal consequences. Forensic laboratories are consistently requested to increase their capacity for more testing completed faster, while also implementing higher standards of practice and improving upon the state of the science in order to meet the ever-increasing demand for information.

Substance use disorders directly impact the public health and legal systems by causing impaired health and well-being, violence, motor vehicle crashes, criminal acts, and sometimes resulting in death. From the public health perspective, people who use drugs are constantly exposed and in some cases are ingesting emerging drugs as part of the ever-evolving synthetic drug market. Further complicating the issue from a public health perspective are the adulterants added to street drugs which create additional harms for people who use drugs. Public health practitioners have access and work directly with individuals who are at the forefront of this dynamic space and may have insights from the drug users on the drugs and adulterants in a specific geographic area and/or specific population. From the forensic perspective, having a collaborative relationship with public health will strengthen the field and assist in early detections of new drugs and/or drug trends.

This workshop will highlight public health projects and work done in the public health laboratories with the goal of informing forensic laboratories so that they can integrate this information into scope updates, platform changes, and appropriately align resources on changes that will have the greatest impact. Many of the projects will touch on various initiatives around toxico-surveillance and how these projects assist forensic laboratories in identifying drug trends, such as emergent novel psychoactive substances or adulterants that should be covered within laboratory testing protocols. The various toxico-surveillance projects focus on different populations (i.e. electronic dance music festival attendees, clinical), therefore providing a variety of perspectives and experiences. Additional topics in the program will focus on the use of forensic laboratory data at the national level to help guide public health policy and practice. The program will conclude with a round table discussion stressing the importance in breaking silos and that collaboration and information sharing is in the best interest of both communities.

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**Public Health; Forensic Laboratories; Toxico-Surveillance**

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## W25 What Did the Lab Bench Say to the Court Bench? And What Did the Court Bench Hear?

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**Learning Overview:** After attending this workshop, attendees will be more aware of how forensic science standards are being implemented in laboratories and the impact of these standards in court.

**Impact Statement:** The use of forensic science standards is increasing exponentially in laboratories and the court system. This workshop will impact the forensic science community by informing attendees about how these standards are being used, and why standards should be adopted in laboratories.

This workshop will present the views and experiences of laboratory practitioners, accreditation experts, attorneys, and judges pertaining to the use of forensic science standards in court.

This workshop considers forensic sciences and forensic standards from two points of view: namely, from forensic laboratory experts, and from legal experts. Attendees will hear how the use of forensic science standards has impacted work from the laboratory to the courtroom. Instead of taking a deep dive into the technical application and implementation of standards in forensic laboratories, this session explores through lively debate, the interface of forensic science laboratories and the legal system.

Forensic laboratories receive, test, examine, evaluate, catalog, identify, store, record, and transfer a huge number of different kinds of materials for a variety of clients, such as law enforcement, prosecution and defense attorneys, as well as the public. Each of these clients relies on the ability of a forensic laboratory to produce accurate results. Lab Accreditation and forensic standards are an element of the labs structure that can add to the trust of a client for a forensic lab, but that does not mean the client necessarily comprehends what accreditation (and similarly personnel certification) means beyond being a vague notion or passive acceptance.

The laboratory expert panel will discuss how performance and quality assurance can be assessed against forensic standards. They will explore both the history and current state of forensic standards as well as the real-world impact of accreditation on the field and how this impacts the delivery of services to their clients.

Legal experts will address the role of forensic standards in the administration of justice. They will talk about forensic standards currently used in the courtroom and provide insight into the profound impact these standards (and the evidence to which they apply) can have on the outcome of cases in the judicial system. They will also comment on the extent to which attorneys and other judicial officials understand the role of accreditation in assessing the value of forensic science output. For example, while attorneys may grasp that having forensic results from an accredited lab may translate into those results being more highly valued, attorneys and courts may not understand the role of accreditation: what it does and, more to the point, what it doesn't do.

Attendees will gain information and insight into how forensic science translates from the scientific environment into the legal environment. They will gain understanding about the what the implementation of accreditation and forensic standards means, and what role it plays in how forensic science is translated into courtroom considerations.

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### Standards; Jurisprudence; Accreditation

## W26 Taming the Wild West of Workflows for Opioids: Emerging and Controversial Drug Threats

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**Learning Overview:** After attending this workshop, attendees will have: a greater awareness of forensic workflows and how they may impact test results; a greater understanding of the challenges in adjusting standardized workflows to address opioids and emerging drugs; and a better appreciation for the importance of accurate substance data and how stakeholders rely on that accuracy.

**Impact Statement:** This workshop will impact the forensic science community by providing information about technological developments and improved workflows for various types of drug testing (toxicology and seized drugs) by discussing the components of a standardized forensic workflow, by showing the effects of deviations or preemptive testing on comprehensiveness, sensitivity and specificity of the test, and by discussing the compliance of test results with emerging standards in forensic science and the acceptance of those results in court and by other pertinent stakeholders.

After attending this presentation, attendees will be able to discuss standardized workflows that are being adopted to better meet regulatory requirements, safety, efficiency, and timeliness in the fields of forensic drug testing, whether in a traditional lab setting, in the autopsy suite, or in the field.

Many types of forensic and clinical investigations involve drug testing. The needs of stakeholders differ but generally they want quick answers, forensic reliability, low cost, and accreditation compliant workflows. Evolving drug markets and prevalent and diverse chemical categories make this even more challenging. In forensic disciplines involving drug testing and clinical drug treatment environments, emerging drugs may remain undetected and invisible by current workflows, and a focus on speed can result in compromises in scope. As backlogs rise for forensic science providers, more focus is being given to exploring ways of triaging casework. However, caution needs to be taken when applying screening technologies that may be inadequate or inappropriate for the detection and identification of opioids and emerging drugs. Stakeholders of drug results include, but are not limited to, the Medical Examiner/Coroner, victims, family, criminal justice system, defendants, epidemiologists, and public health departments.

Without comprehensive drug testing capability, there is a higher probability of clinical and forensic science providers missing critical opioid and emerging drug information when on-site rapid screening is employed. Following a rigorous and standardized testing scheme may be helpful to maintain efficient laboratory operations, but it can also be detrimental in discovering new and emerging substances, that may include potent and lethal opioids and other synthetic drugs not yet scheduled. Any deviations from normal protocol can be advantageous if used appropriately, as well as create controversy if applied incorrectly. Point of use testing, to include a drug screen prior to or during a postmortem examination is gaining popularity and has been implemented in some medical examiner offices, but without any regulatory guidance on the appropriate use of the technology.

This panel convened by the AAFS Opioids and Emerging Drugs Committee has been charged with documenting some of the frustrations and limitations of today's processes, and looking over the horizon to possible new technologies and solutions which may include taking testing out of the lab, and moving it further up the investigative chain, to the autopsy suite or even to the crime scene, to expedite the fact-finding, and provide rapid, actionable data to support not just the prosecution, but also the investigation.

Overall, presenters will identify problems, challenges, and controversies with toxicology and seized drug testing with an assessment of the pro's and con's of these developments, consideration of the maturity of the technology, and the barriers that need to be overcome to allow the results to be presented in court.

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### Forensic Workflows; Emerging Drug Threats; Opioids

## **A1 The Applications of Confocal Laser Scanning Microscopy for Imaging Burned Remains**

*Shelby Garza, MA\*, Texas State University, New Braunfels, TX*

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the practical application of confocal laser scanning microscopy for imaging burned remains.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new method for imaging and analyzing burned remains.

Fluorescence is the property of certain chemical elements, called fluorophores, to emit visible light when excited by incident intense radiation.<sup>1</sup> Previous studies suggest that the presence of an autofluorescence phenomenon in bone samples is likely due to the organic matrix, mainly collagen, of bone tissue.<sup>2</sup> Burning of skeletal remains can alter the structural and chemical properties of bone, and these changes may be dependent on the temperature and duration of heat exposure.<sup>3</sup>

The purpose of this study is to explore the natural autofluorescence of human bone and to evaluate how that autofluorescence may change after the bone has been burned to answer the following questions: (1) are there differences in how burned and unburned bone fluoresce when excited by different radiation intensities, and (2) if differences are observed, are these affected by the severity of burning?

For this study, six donors from the Forensic Anthropology Center at Texas State (FACTS) were placed in various fire-death scenarios at the Forensic Anthropology Research Facility (FARF) at Texas State in San Marcos, TX. Each of the donors or donors' next-of-kin consented to the possibility of being utilized for advanced or traumatic research processes prior to experimentation. Pre-burned and post-burned samples were obtained from the six donors. All pre-burned samples were taken from the left side and all post-burn samples were obtained from the right side for each individual. The pre-burned samples included the left femur, the left fifth metatarsal, and the left sixth rib. The post-burned samples included the right femur, the right fifth metatarsal, and the right sixth rib. Blocks of each bone were embedded in epoxy resin from which cross-sections were obtained, ground, polished to approximately 100µm thick, and mounted to a slide using EUKITT®. Images were collected using the Olympus® FluoView™ 300 Confocal Microscope equipped with five diode lasers: 405, 445, 488, 561, and 640.<sup>4</sup>

The results showed that there is a difference between how unburned and burned bone will fluoresce, with the unburned bone fluorescing with the 405 laser and the burned bone requiring at least a 488 laser to fluoresce. Additionally, the severity of burning affects how the bone will fluoresce, with charred bone fluorescing with the highest laser intensity. Finally, microstructures of the burned bone that can no longer be seen under transmitted light can be viewed with the 561 and 640 lasers.

These results indicate the practical use of confocal laser scanning microscopy to analyze the microstructure of burned bone. Microstructures from burned bone that are not visible under transmitted light can be seen using laser imaging, which is likely caused by the laser reflecting off the elements on the bone surface rather than passing through the bone. While only ribs, femora, and metatarsals were used in this study, early research suggests that collagen is responsible for the autofluorescence of bone; therefore, it is expected that these results would remain consistent if different elements were recovered.<sup>5</sup> Additionally, the difference in fluorescence between burned and unburned bone may be explained by the changes in the physical composition of bone caused by burning (e.g., loss of collagen). Furthermore, these results may provide another method for quantifying the severity of burning from skeletal remains. Collectively, these results can provide evidence for the use of confocal laser scanning microscopy to identify burned human versus non-human remains in a forensic context.

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### **Confocal Microscope; Burned Remains; Forensic Anthropology**

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**A2 A Test of Interrater Reliability Using the Refined Classification System for Thermally Damaged Human Remains by Body Segment**

Chelsey Juarez, PhD\*, California State University, Fresno, Fresno, CA; Elayne Pope, PhD, Pope Consulting, Knoxville, TN; Alison Galloway, PhD, UC Santa Cruz, Volcano, CA

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**Learning Objective:** After attending this presentation, attendees will understand the interrater reliability of the recently published *Refined Classification System for Thermally Damaged Human Remains by Body Segment* method.<sup>1</sup>

**Impact Statement:** A comprehensive classification system for accurately assessing thermally damaged human remains must be easy to use and produce reliable results. In 2022 Pope et al., published a new classification system including detailed illustrations to assist forensic researchers and practitioners to accurately classify levels of thermal damage to burned human remains.<sup>1</sup> In this presentation, we present the first data on the reliability of that system and discuss suggestions to increase ease of use. This presentation will impact the forensic community by providing reliability data on a new classification method for evaluating fatal fire victims.

Eight forensic anthropologists participated in the evaluation and scoring of a series of 20 photographs of burned human remains using the Pope et al. *Refined Classification System for Thermally Damaged Human Remains by Body Segment* method.<sup>1</sup> The goal of the project was to test the ease of use of the method based on examining field photographs and to assess the interrater reliability of the classification method. All eight participants held PhD-level educations and seven were Diplomates of the American Board of Forensic Anthropology (D-ABFA). The photographs were generated over 12 years of field research with the San Luis Obispo Fire Investigation Strike Team's Forensic Fire Death Investigation Course (FFDIC) and represented different levels of burn damage from cases involving minimal heat-related soft tissue damage to full cremation. The study was covered by California State University Fresno Institutional Review Board (IRB) #1177. Participants were asked to use the six-stage scoring system with illustrations to consider and score the changes to: (1) layered soft tissues, (2) exposure of body components (subcutaneous fat, skeletal muscle, internal organs, bone), (3) changes in body position, and (4) skeletal damage from discoloration to calcination- by body segments of head, torso, upper limb, and lower limb. Study participants were able to indicate both a number score (1–6) and a letter score indicating early or late-stage changes for each body segment. In addition to scoring data, each participant included notes within their scoring sheet documenting difficulties or suggesting slight changes that may assist other researchers in the future.

Interrater reliability—the level of agreement between judges—was assessed using Interclass Correlation Coefficient (ICC) in JMP® PRO 16 for Mac® OES® assuming absolute agreement. Number and letter scores were coded where stage numbers (1–6) associated with early phase damage indicated by the letter (a) were simply indicated by the number (e.g., 6a=6). However, scores that included the letter (b) indicating late-stage alteration were given a 0.5 addition, thus, 6b was coded as 6.5. In total, there were 160 scores provided over four body segments for the 20 photographs. The ICC calculated for all 160 scores assuming absolute agreement was 0.907, indicating excellent reliability between study participants using the classification system.

Study participant notes indicated that certain body portions had differing levels of difficulty associated with scoring. In some cases, this was due to the angle of the photographs, but in other instances the thermal modification was difficult to assess even from a clear photograph at the proper angle. An investigation of ICC for each body segment individually revealed variation in reliability ranging from 0.80 for the head to 0.966 for the lower limb. This range was reflected in the comments associated with each area in which many participants noted the difficulty of assessing changes in facial features or movement from photographs. An Analysis of Variance (ANOVA) indicated that the variation between the study participants scores was not statistically significant ( $p=0.06$ ). Although not significant, the largest variance between participants (0.78) was found in scoring the torso.

In conclusion, this preliminary study demonstrates that the recently published Pope et al. classification system for thermal damage to burned human remains produces excellent reliability in results among highly skilled practitioners (PhD education 7/8 D-ABFA).<sup>1</sup> The comments from study participants also indicate the importance of good quality field photographs to properly utilize the classification system from photographs alone.

**Reference:**

- <sup>1</sup>. Pope E, Juarez C, Galloway A. 2022. A regional classification system of thermal damage to human remains. *Journal of Forensic Anthropology*. <https://doi.org/10.5744/fa.2021.0008>.

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**Burned Human Remains; Fire Classification; Interrater Reliability**

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**A3 A Comparison of Patterns of Dismemberment Between Rural and Urban Areas in Northern California**

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**Learning Objective:** After attending this presentation, attendees will recognize the patterns of dismemberment in rural versus urban settings and overall patterns of dismemberment in Northern California as well as the logistical and behavioral factors that influence dismemberment practices.

**Impact Statement:** This presentation will provide the forensic science community with a review of the patterns of dismemberment in forensic cases in Northern California, impacting the forensic community by providing further data and context for dismemberment casework.

In the United States, dismemberments are a small but important portion of unnatural deaths. Regional pattern-based studies and skeletal trauma descriptions of dismemberment have been published.<sup>1,2</sup> This project expands upon previously published information by considering regional dismemberment and comparing rural and urban dismemberment patterns.

Dismemberment is the intentional attempt to separate elements and element portions from the body using force, almost always involving sharp implements. Severe dismemberment consists of more separation and false starts. We predict that urban areas have higher rates of dismemberment and a greater degree of segmentation compared with rural areas. “Urban” is defined as an area where more than 50,000 individuals reside, and rural areas contain less than 2,500.<sup>3</sup> In urban areas, greater segmentation of skeletal elements may expedite body movement and evade detection. Consequently, intentions and motives of dismemberments are discussed as well as their urban or rural classification. This presentation establishes patterns and characteristics of dismemberments in Northern California, providing law enforcement and forensic anthropologists with more data and context for dismemberment cases.

The dismemberments presented are compiled from case archives at the Human Identification Laboratory at California State University, Chico. Sixteen cases dating from 1990 to the present constitute the sample for this study. For each case, several variables are examined, including the county where the decedent was discovered and its population size, the recovery location and context of the remains, the number and anatomical locations of complete bisections of elements and element portions, the presence of false starts, the preservation of the remains on discovery, and inventory of skeletal elements or element portions. Six cases come from urban areas and nine cases from rural areas. Decedents were most often biological males (63.6%). All individuals are 18 years or older, and the average age of identified victims ( $n=7$ ) is 57 years.

The results indicate that severe dismemberments are more common in urban areas than in rural areas, despite a greater proportion of dismemberment cases in rural areas. When both urban and rural data are pooled, complete separation of skeletal elements/element portions occur at both the left and right elbows, left femur shaft, and the cervical spine. There are a total of 54 bisections of skeletal elements in the urban cases, with only 23 complete bisections in the rural cases. So, urban dismemberments show greater severity than rural cases. Regarding criminal intent and motive, the logic and reasoning of the perpetrator cannot be clearly inferred from the pattern of dismemberments or deposition locations.

This work provides a baseline of the similarities and differences between Northern California rural and urban dismemberments. To bolster regional pattern-based studies of dismemberment patterns, future work will include data from other regions of the United States.

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**Forensic Anthropology; Dismemberment; Skeletal Trauma**

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## **A4 Establishing a Skeletal Atlas of Elder Abuse: An Expanded Study of Fracture Patterns in Documented Cases**

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**Learning Objective:** After attending this presentation, attendees will be aware of skeletal fracture patterns associated with the abuse/neglect of elderly individuals as distinguished from those commonly found in accidental ground-level falls.

**Impact Statement:** This presentation will impact the forensic science community by 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.

Increasing rates of elder abuse in the United States have produced an urgent need for improved diagnostic criteria.<sup>1</sup> Physical abuse of elders represents the most severe manifestation of this trend, yet it is difficult to prove.<sup>2</sup> Skeletal expressions of elder abuse offer key indications of inflicted injuries, but often are masked by assignment 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.<sup>3</sup> Our pilot project identified a significant difference in the skeletal manifestation of injury between those individuals involved in accidental falls and those for whom abuse/neglect is suspected.<sup>4</sup> To validate and further explore the skeletal patterns of abuse/neglect vs. accidental falls, cases from the Maricopa County Office of the Medical Examiner dating from June 30, 2017–December 31, 2019, were reviewed. Here, we present the results of this expanded analysis.

Investigative summaries of 235 individuals over the age of 60 years who had sustained skeletal trauma were examined. From these, 33 individuals were included as representative of cases of abuse/neglect based on the following criteria: decedent must (1) have been admitted for a full exam, (2) be associated with an Adult Protective Services and/or law enforcement investigation, (3) have radiographs/samples available for review. Given these strict criteria, this sample is known to be an underestimation of cases of abuse/neglect. To further establish a comparative baseline of those fractures most likely to occur in accidental falls, 150 cases of witnessed, ground-level falls from the same pool were reviewed. Finally, 21 cases involving falls from a sitting position were examined to elucidate potential differences in fracture patterning among non-ambulating individuals.

Results confirm a difference in the skeletal manifestation of injury between those individuals involved in ground-level accidental falls and those for whom abuse/neglect is suspected, as well as provide new information on these patterns. Consistent with previous results, individuals involved in a fall most frequently displayed fractures in the hip ( $n=60$ , 40%) and vertebrae ( $n=21$ , 14%). This new data set revealed a larger proportion of fractures to the arms and cranio-facial skeleton than previously observed in ground-level accidental falls ( $n=26$ , 17% and  $n=11$ , 12%). These fractures typically were in addition to femoral or vertebral fractures in a single individual. In contrast, for those individuals identified as possible victims of abuse/neglect, fractures occurred most frequently in the posterolateral shafts of ribs ( $n=8$ ; 24%) and the arm ( $n=8$ ; 24%), followed by fractures in the facial skeleton ( $n=7$ ; 21%). For these individuals, fractures in the arm and cranio-facial skeleton did not coincide with fractures of the hip or vertebrae.

To better understand potential differences in fracture patterning among non-ambulating individuals in both the categories of accidental fall and potential abuse/neglect, 21 cases involving injuries sustained while in a sitting position, whether wheelchair or bed, were examined. Where there was an allegation of abuse/neglect in such incidents ( $n=5$ ), fractures to the humerus (60%), ribs (20%), and vertebrae (20%) occurred. Where the fractures were the result of a witnessed fall from a seated position ( $n=16$ ), 50% of cases involved fractures to the craniofacial region. These observations contribute to the establishment of a skeletal atlas of elder abuse by providing nuanced patterns of fractures in common circumstances in which elderly individuals may be injured.

Fractures remain the most common musculoskeletal condition requiring hospitalization among individuals aged 65 and older in the United States, and rigorous diagnostic criteria must be developed to differentiate accidental injury from abuse.<sup>5</sup> 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; Fracture Patterns; Skeletal Atlas**



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**A5 Trauma Interpretation: A Case Study in Bone Healing**

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**Learning Objective:** After attending this presentation, attendees will be aware of a situation in which bone remodeling and response to antemortem trauma appears inconsistent with skeletal observations, a condition that may be explained by extreme opioid use.<sup>1,2</sup>

**Impact Statement:** This presentation will impact the forensic science community by raising awareness, especially among forensic anthropologists, of antemortem trauma that presents as perimortem skeletal trauma.

Bone's response to trauma has been well documented in the medical and forensic literature.<sup>3,4</sup> The diagnostic interpretation of skeletal trauma is an important aspect of the forensic anthropology case report and includes estimations of when the trauma occurred and the context of the trauma. The case study highlighted in this presentation concerns unique skeletal trauma for which the exact etiology and timing are documented. This case study features a human donor who was donated to the Northern Michigan University Center for Forensic Anthropology. The individual suffered severe traumatic injury nearly 20 years prior to his death, which involved the pelvis, right scapula, and vertebral column. Recovery from this traumatic event led to an opioid dependence/addiction that spanned nearly two decades. The donor's death was indirectly related to the traumatic injury and opioid use. Multiple regions of the donor's body exhibit skeletal responses to trauma. The locations of all visible trauma on the skeleton are consistent with medical records associated with the decades-old traumatic injury; however, the condition of the bone and apparent absence of remodeling more closely resemble perimortem trauma when viewed macroscopically. It is this disconnect between the known occurrence and timeline of trauma and the presentation of the trauma to which the authors seek to bring attention.

This case highlights the unique situation of when trauma does not align with standard rates of bone healing. It will bring awareness to the forensic anthropological community that we must take great care in assessing trauma. It is the hope of the authors to inspire future research into the holistic interpretation of skeletal trauma in regard to other body systems and health influences.

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**Bone Healing; Skeletal Trauma; Opioid Use**



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**A6 Investigation of Gunshot Homicides in the New Mexico Decedent Image Database**

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**Learning Objective:** Attendees will learn about injury patterns in gunshot homicides and the utility of radiographic scout images in identifying trauma within the New Mexico Decedent Imaging Database (NMDID). The goals of this research were: (1) to explore common injury locations associated with gunshot homicides, and (2) to investigate the utility of scout images in screening skeletal trauma cases in a CT database.

**Impact Statement:** This presentation will impact the forensic science community by providing data on anatomical locations frequently affected by fatal gunshot trauma and insight into the NMDID as a resource for trauma research.

As gunshot-related homicides rise, research is needed to support accurate interpretations of gunshot injuries.<sup>1,2</sup> Since trauma studies are often predicated on the assumption that certain bones or body regions are frequently injured, data on common injury locations can help inform research planning. The NMDID comprises Computed Tomography (CT) data from medical examiner cases and therefore presents a potentially valuable resource for investigating trauma.<sup>3</sup> Research is, however, impeded by an inability to search cases by presence, location, or type of injury. Users can only search by cause and manner of death and must evaluate each case individually to verify trauma presence. Radiographic scout images, which appear in the preview for most NMDID cases, offer a potentially useful tool for narrowing database searches.

A NMDID query of homicides with primary cause of death listed as “gunshot wound of” generated 639 results. Scout images were examined for the presence and anatomical location(s) of fractures and bullets in each case. Chi-squared tests were performed to explore relationships between fracture and bullet presence, fracture presence and location, and bullet presence and location.

Fractures were detected in 272/639 cases (42.6%), bullets in 471/639 cases (73.7%), both fractures and bullets in 239/639 cases (37.4%), and neither fractures nor bullets in 89/639 cases (13.9%). Scout images were absent or unreadable (e.g., incomplete, overexposed, underexposed, etc.) in 46/639 cases (7.2%).

Of the 504 cases in which fractures and/or bullets were detected via scout image, the most common locations affected were the thorax (262 cases, 52.0%) and cranium (214 cases, 42.5%). Gunshots to multiple locations were identified in 104 cases (20.6%). Among these, the most common combination was the cranium and thorax ( $n=40$ ).

Statistically significant relationships were found between fracture and bullet presence ( $p < 0.001$ ) and fracture presence and location ( $p < 0.001$ ). The relationship between bullet presence and location was not significant ( $p = .993$ ).

The results indicate the cranium and thorax are most commonly affected in fatal gunshot trauma. These findings are consistent with Hulse et al. and support prioritization of research on these regions.<sup>4</sup> The cranium and thorax should also be closely examined for trauma in cases involving advanced decomposition or skeletonization.

Additionally, the results offer insight into using the NMDID in trauma research and the utility of scout images as search aids. Fractures were detected in approximately half of cases with readable scout images, indicating they can help users identify skeletal trauma before requesting and downloading CT data. Several issues inhibited their use, however, including lack of availability, image quality, and superimposition of body parts. Another limitation is the ability of observers to detect fractures using scout images. Research shows that observers detect fractures using X-rays with high specificity but low sensitivity, suggesting the current results represent an undercount.<sup>5</sup>

The significant association between fracture presence and location necessitates further exploration to clarify if bone is less often fractured or simply more difficult to detect in certain anatomical locations. The relationship between fracture and bullet presence also requires clarification. Fractures were more frequently identified when bullets were present than absent. This may indicate that projectiles increase fracture detection by drawing focus to the injury location or that projectiles are likely to remain within the body after contacting bone. The next phase of research will investigate CT data to verify the presence of fractures, compare fracture detection on CT and scout images, and explore the quality of 3D renderings for trauma research.

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**Gunshot Wounds; Trauma; New Mexico Decedent Image Database**

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**A7 A Proposal for Cut Marks Classification and Analysis: Serrated vs. Non-Serrated Knives**

*Giada Steiger, MSc\*, Liverpool John Moores University, Liverpool, ENGLAND; Matteo Borrini, PhD\*, Liverpool John Moores University, Liverpool, ENGLAND*

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**Learning Objective:** After attending this presentation, attendees will have a clearer understanding of the most relevant traits to describe and classify cut marks on human skeletal remains.

**Impact Statement:** This presentation will impact the forensic science community by providing a series of traits for the classification of cut marks on human bones. In addition, a logistic regression model has been provided to correctly classify serrated, non-serrated, single- and double-edge blades.

In forensic anthropology, there is a great need to improve the accuracy and reliability of tool mark identification to improve the available data for criminal investigations. Several studies on sharp force trauma have been conducted on skeletal remains. Nevertheless, each author investigates the trauma using different equipment (e.g., high- or low-resolution microscope) and methods (e.g., producing the cut mark by stabbing or slicing the material). Therefore, despite extensive research in the field, there is still a lack of uniformity and standardization of characteristics and methodologies to analyze and describe sharp force trauma marks on skeletal remains. The authors present a proposal for the classification of cut marks on human bones, providing the methodology for their examination and an accurate description of each identified diagnostic trait. The present research focused on cut marks from serrated and non-serrated knives, as well as from single- and double-edged knives.

To develop a classification, the study has been conducted in two phases. In the first stage, seven different knives were used to stab pig ribs (*Sus scrofa*) by an external researcher to guarantee consistency in the knife's handling force applied. A total of 280 cut marks were analyzed under a stereomicroscope (38x magnification) with a tangential light source. The techniques for the morphological examination have been designed to simplify and easily apply the method to guarantee its potential usage in the field. Of the most common characteristics proposed in the literature, 11 traits have been identified as significantly associated with the type of knife used: (1) shape of the cut mark: general morphology, (2) shape of the cut mark's profile (entrance): general morphology, (3) shape of the cut mark's profile (exit): general morphology, (4) rising of the walls (entrance): location, (5) rising of the walls (exit): location, (6) feathering: presence or absence, (7) shards: presence or absence, \*8) mounding (entrance): location, \*9) mounding (center): location, (10) mounding (exit): location, and (11) mounding: general morphology.

The second phase of the research focused on training a logistic regression model based on the identified traits; the model was developed to predict the affiliation group for both serration and blade type. A blind analysis was conducted on 70 new cut marks to validate the model, with 10 cuts made from each knife used in the previous phase. By an initial morphological observation, 5 knives were correctly classified out of the 7 for both serration and blade type. Then, the trained model was applied and predicted the group affiliation for each of the 70 cut marks with an accuracy of 71.6% for serrated and non-serrated knives and 76.1% for single- and double-edged knives. These findings demonstrate how the 11 traits chosen to create the model are appropriate for predicting the serration and type of blade in the 7 knives. Therefore, these traits should be considered in developing future cut marks description and classification standards. In addition, as the study was conducted in controlled conditions, further research should investigate a larger sample to optimize the method for future use in real-case scenarios.

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**Forensic Anthropology; Trauma Analysis; Sharp Force Trauma**

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**A8 Analyzing Chopping/Hacking Cut Mark Characteristics on Bone: Implications for Forensic Analysis**

*Kelly McGehee, MA\*, University of Central Florida, Department of Anthropology, Orlando, FL; John Schultz, PhD, University of Central Florida, Department of Anthropology, Orlando, FL; Sarah Freidline, PhD, University of Central Florida, Department of Anthropology, Orlando, FL*

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**Learning Objective:** After attending this presentation, attendees will not only recognize the different macroscopic cut mark characteristics that occur to bone as a result of chopping/hacking tools but also understand which characteristics are most commonly associated with specific chopping/hacking tools.

**Impact Statement:** This presentation will impact the forensic science community by advancing the field of forensic science as this experimental design aims to develop a standardized protocol for conducting chopping/hacking research by incorporating both the methodological design and data analysis from previous studies.

Sharp Force Trauma (SFT) is a mechanism of traumatic injury involving tools with a slanted/beveled edge that impact the skin and/or bone, producing a penetrating cut mark. While experimental research has been conducted utilizing smaller sharp force tools, minimal research focuses on damage inflicted by a larger class of chopping/hacking tools. Furthermore, the terminology inconsistencies in the literature describing experimental cut marks to bone has resulted in difficulties when comparing results between previous studies. Therefore, the purpose of this research was to macroscopically analyze cut mark characteristics resulting from chopping/hacking trauma inflicted on pig bones (*Sus scrofa domesticus*) to determine if differentiation of chopping/hacking tool class can be made based on the characteristics that occur.

Trauma was manually inflicted to 20 partially fleshed pig hindlimbs utilizing four chopping/hacking tools (axe, hatchet, machete, and cleaver) as well as a carving knife for comparison. The limbs were placed in a standardized position with the downward chopping/hacking force focusing on the femur and the tibia. Once the bones were cleaned of soft tissue remnants, the length, width, and depth of each kerf was measured. Each kerf was then macroscopically evaluated for 16 sharp force cut mark characteristics: presence/absence of five macroscopic chopping/hacking characteristics (chattering, flaking, fracturing, feathering, and peeling) on both kerf edges; presence/absence of kerf bisection; regularity/irregularity of kerf edges, walls, and floor. Data analysis was conducted utilizing the statistical software RStudio (version 4.0.3) to assess statistical significance. Additionally, inter-observer error was undertaken using ten observers who were provided with a visual key and asked to evaluate ten chopping/hacking characteristics (presence/absence of chattering, flaking, fracturing, feathering, and peeling on both Edge 1 and Edge 2) following previous studies. The inter-observer error was conducted utilizing a Fleiss kappa analysis to assess the level of agreement between observers when evaluating cut mark characteristics.

The results of the Kruskal-Wallis analysis followed by Dunn's post hoc test demonstrate statistically significant differences between the tools for both kerf length ( $p=0.014$ ) and kerf width ( $p<0.001$ ); however, a considerable degree of overlap in both the means and the ranges is noted for kerf measurements inflicted by each of the five tools. Utilizing a chi-square analysis, 3 of the 16 cut mark characteristics demonstrate statistically significant differences in relation to the tool utilized: Wall 2 regularity ( $p=0.002$ ), kerf bisection ( $p=0.011$ ), and Edge 1 chattering ( $p=0.010$ ); however, Cramer's V correlations indicate weak to moderate effect sizes for the three characteristics (Cramer's  $V=0.337$ ,  $0.234$ , and  $0.246$  respectively). Evaluation of inter-observer error indicates substantial agreement between observers regarding the ten chopping/hacking characteristics for each tool type ( $Kappa=0.778$ ). Additionally, when combining all chopping/hacking characteristics regardless of tool type, substantial agreement was also indicated between observers ( $Kappa=0.751$ ).

The current research is strongly in agreement with previous studies that have concluded it is difficult to determine tool class based solely on the macroscopic chopping/hacking characteristics left on bone; however, the results of the current experiment indicate that it may be possible to use a combination of cut mark characteristics to potentially suggest the tool class utilized to inflict trauma. At a minimum, if multiple cut marks are present that all share similar characteristics such as a wide kerf width or kerf bisection, these patterns could suggest that a larger, heavier chopping/hacking tool (such as an axe or hatchet) was most likely utilized to inflict the trauma; however, additional research incorporating both macroscopic and microscopic analysis of chopping/hacking cut mark characteristics is a necessity to develop criteria for further tool class differentiation.

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**Sharp Force Trauma; Chopping/Hacking Trauma; Cut Mark Characteristics**

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**A9 Assessing Minimum Kerf Width Measurement Error**

*Alexis VanBaarle, BA\*, Des Moines University, Des Moines, IA; Heather Garvin, PhD, Des Moines University, Des Moines, IA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of intra- and inter-observer error in minimum kerf width measurements across multiple measuring modalities.

**Impact Statement:** This presentation will impact the forensic science community by providing intra- and inter-observer and intermodality error rates for minimum kerf width that can be integrated into best practices.

Forensic cases of dismemberment require forensic anthropologists to assess variables in cut bone surfaces to provide information (i.e., saw class characteristics) about the potential tool used for investigative and legal purposes. One such variable, Minimum Kerf Width (MKW) is defined as the minimum distance across an incomplete cut and has been suggested to be correlated with the width of the saw blade. Much of the research on MKW utilizes different measurement modalities including micro-Computed Tomography (micro-CT) residuals, calipers, and microscope software, but few have assessed the error between these measurement modalities as well as within and across observers. The goal of this research is to assess inter-observer, intra-observer, and intermodality error between three MKW measurement methods.

The research sample includes 351 incomplete cuts created during a previous research project using 27 saws with a variety of saw class characteristics in 19 anatomically gifted macerated human humeri and femora. Three MKW measurement modalities were chosen based on published procedures and practitioner accessibility: (1) MKW was measured within the kerf walls using the inner jaws of the calipers, (2) MKW was measured from the cortical surface using the outer jaws of the calipers, and (3) a stereoscopic microscope was used to view and capture an image of the kerf, from which MKW was measured in the associated software program. For each measurement modality, Observer 1 performed three rounds of measurements and a second observer collected one round of measurements. Error was assessed through relative and absolute differences, relative Technical Error of Measurement (rTEM), Friedman tests, and non-parametric paired *t*-tests.

Intra-observer error within modality was assessed using Observer 1's second and third trials. The internal caliper wall method had the lowest rTEM (3.72%), followed by stereoscopic measurements (rTEM=5.53%). The external caliper method performed the worst (rTEM=8.53%). Observer 1's Trial 2 and Observer 2's MKW measurements were used to assess interobserver error. The stereoscopic measurements had the lowest rTEM (5.42%). The internal caliper method had a slightly higher rTEM (6.15%). The external caliper measurements presented the highest error (rTEM=21.32%). A Friedman test indicated significant differences ( $p < 0.05$ ) between the mean measurement ranks across modalities of Observer 1's trial two measurements, with post hoc non-parametric paired *t*-tests indicating significant differences between internal caliper and stereoscope measurements and between internal caliper and external caliper measurements, but not between external caliper and stereoscope measurements. Internal caliper and stereoscope measurements had the lowest rTEM and average percent difference (rTEM=7.10%, 7.87%), followed by external caliper and stereoscope measurements (rTEM=8.96%, 10.06%) and internal and external caliper measurements (rTEM=9.06, 10.14%).

All rTEM values are higher than previously suggested thresholds for osteometric intra-observer and inter-observer error (1.5% and 2.0%, respectively). Internal caliper and stereoscopic measurements have the lowest inter- and intra-observer error while external caliper measurements have the highest and therefore should be avoided. Higher relative errors are likely related to the small measurement sizes. MKW definition interpretation and orientation of the specimen for the stereoscopic image are likely sources of error. Definitions and measurement techniques should be described in more detail and standardized. Caution is warranted if applying MKW to blade set width equations if they were derived from a different modality.

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**Dismemberment; Sharp Force Trauma; Saw**

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**A10 The Relationship Between Skull Trauma and Demographics in Contemporary New Mexicans**

*Elise Adams, BA\*, Mississippi State University, Starkville, MS; Jesse Goliath, PhD, Mississippi State University, Starkville, MS*

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**Learning Objective:** After attending this presentation, attendees will have developed a better understanding of the interaction between demographic factors and skull trauma and who most often experience these types of injuries in a contemporary population.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the value demographic factors have for interpreting skull trauma in relation to accidental trauma versus interpersonal and structural violence.

During forensic skeletal analyses, the skull is used to score a variety of characteristics related to a biological profile, including age, sex, and evidence of trauma. Contemporary skeletal collections, like the New Mexico Decedent Image Database (NMDID), contain documented demographics and medical histories, including trauma, from hundreds of individuals.<sup>1</sup> These resources can provide another source of potential evidence for identification.

Skeletal remains with evidence of trauma have been known to assist with uncovering the identities of targeted people(s), the specific trauma they suffered, and the lasting biological and cultural impacts.<sup>2,3</sup> Trauma is often associated with violence (structural or interpersonal) and can be determined by assessing the injury mechanisms and other factors surrounding trauma to the skeleton (i.e., accidents or interpersonal violence). The objective of this study was to assess skull trauma from a documented skeletal database and examine how demographics can provide essential information on those who experience these types of injuries.

Data from 40 individuals associated with fatal skull trauma were collected from the NMDID and categorized by whether the trauma was accidental or interpersonal violence. The sample consisted of 10 females and 30 males between the ages of 15–85 years old, which were further divided into individuals older and younger than 40 years old (29 and 11 individuals, respectively). Statistical analyses were performed through Fisher's Exact tests using RStudio Statistical Analysis software (v.4.1.2) to examine whether traumatic events were related to accidents or violence and to compare sex and age demographics. The results of these analyses showed significant differences ( $p < 0.001$ ) with higher rates of accidental skull trauma compared to violent skull trauma, but there was no observed significance with age or sex. However, there was a higher number of accidental incidents among individuals over 40 years old and the data showed a disproportionately high number of males with head injuries compared to females.

While results presented do not indicate significantly higher rates of physical violence, the higher number of accidents among individuals over 40 years old, and an increased number of accidents and violent events among males, indicates that there may be evidence of structural violence. Interpretations of structural violence could include a lack of proper care for older individuals who are at an increased fall risk and the possibility of gender-based violence among males. Further analysis into other societal factors and injury mechanisms could allow for a better understanding of how different forms of trauma relate to evidence of violence and its overall impact on individuals within a population.

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**Biological Profile; Trauma; Structural Violence**

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**A11 Postmortem Interval Estimation and the Recognition of Bone Trauma Through the Analysis of Physicochemical Changes Produced by Bone Diagenesis Over Forensic Timescales**

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**Learning Objective:** After attending this presentation, attendees will understand the potential impact of time and trauma to influence physicochemical alterations occurring to bone left in an exposed deposition environment over short forensic timescales.

**Impact Statement:** This presentation will impact the forensic science community by enhancing existing knowledge of diagenetic effects on the physicochemical composition of skeletal remains over time, with and without the presence of blunt force or sharp force trauma.

The diagenetic phenomenon has been much researched in the hope of understanding the factors that drive this process and establishing the potential for Postmortem Interval (PMI) estimation methods of skeletal remains. Research has shown that the presence of trauma may influence soft tissue decomposition, yet to date no studies have been conducted to investigate how the presence of trauma could affect diagenetic alterations to bone. If bone fractures have the potential to influence diagenetic alterations, this could have implications for PMI research. Due to the occurrence of perimortem trauma, and taphonomic damage occurring to bone postmortem, it is important to understand how bone fractures can influence bone diagenesis.

Presented here are a series of short timescale studies that investigated not only the effects of time and trauma on bone diagenesis, but also the effects of the differing United Kingdom seasonal climate. It was hypothesized that physicochemical changes would occur to the skeletal tissue over time, and that the samples inflicted with trauma would show more extensive changes. Partially defleshed ribs were either left intact or inflicted with trauma immediately (perimortem) or 60 days after deposition (postmortem) and left outside for up to 180 days post-trauma. Collections took place at 90-day intervals. Samples were cleaned and prepared for physicochemical analysis using Scanning Electron Microscopy with Energy-Dispersive X-ray Spectroscopy (SEM/EDS) and Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) spectroscopy.

Results showed statistically significant ( $p < 0.05$ ) changes occurring to the elemental composition of the bones over time with losses in sodium and potassium seen within 30 and 90 days PMI, respectively. Significant increases in crystallinity were also found to occur to the structural composition of the bones over time ( $p < 0.05$ ). These experiments were conducted twice at different times of the year to establish whether the seasonal United Kingdom climate could influence results. This showed that the variable United Kingdom seasons can affect physicochemical changes occurring to bone over time with initial results indicating the exposure to the wet/dry cycles of winter can slow the moisture loss and dehydration of bone. The presence of extensive fractures across a large surface area of the bone caused by blunt force trauma were found to significantly affect the elemental composition of bone. While the structural composition of the bones was not significantly affected by the presence of trauma, evidence suggests this could be due to the short PMIs investigated here and a longer-term study would have elicited significant differences between the trauma and non-trauma conditions.

This study showed that both elemental and structural changes occur to bone in the post-depositional period. Trauma also influences the elemental composition of bone, within 30 days for perimortem trauma and within 90 days for postmortem trauma; however, similar influence on structural composition was not observed until approximately six-months PMI, although this was non-significant. This suggests elemental changes must occur before the mineral lattice is significantly altered. Considering the occurrence of trauma to bone, these results could have implications for forensic anthropology and bioarchaeology as they show that damage occurring to bone can influence the results of bone diagenesis research.

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**Trauma; Postmortem Interval; Physicochemical Changes**



## A12 Forensic Anthropological Studies Published From India During 2011–2022: A Literature Review and Meta-Analysis

*Jagmahender Sehrawat, PhD\*, Panjab University, Department of Anthropology, Chandigarh, Chandigarh, INDIA*

**Learning Objective:** After attending this presentation, attendees will be informed about the trends in forensic anthropological research work in India during the past decade.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees they should focus their studies on more challenging but crucial components of biological profile commonly required in forensic death investigation in India.

**Introduction:** Each type of evidentiary material has its own significance in investigation of matters related to the law. Different scientific methods and techniques have been used to analyze biological materials to estimate their relative contribution and accuracy levels in forensic identifications. A systematic review of articles published during past ten years (2011–2022) in the Scopus® indexed

journals was conducted to examine the current focus of forensic anthropological and odontological research in India.

**Methodology:** A total of 226 research/review articles identified from searches in Scopus® using the keywords of “forensic science” and “India” were further categorized into different sub-disciplines. Only 101 articles were determined to be forensic anthropological or odontological in nature and context; the other publications were excluded from the final sample size. The papers included in the study were categorized according to their year of publication, journal impact factor, article type, and field of inquiry (estimation of age, sex, stature, race, biometric, and molecular identification).

**Results:** Most of the articles represented validated methodologies applied to the Indian population groups. The population-specific anthropological standards were found modified to match Indian contexts for achieving fair accuracy levels for discrimination and differentiation. Of the 101 research articles, 31% analyzed sex determination, 17% age estimation, 16% stature estimation, 11% ethnicity and populational variation, and the remaining were based on biometrical identifications. In addition, only two DNA and stable isotope-based studies were reported from the selected articles.

**Summary and conclusions:** The ulnar and femoral measurements, 2D:4D ratio of index and ring finger and fingerprint ridge density were mentioned as the best forensic anthropological parameters to correctly classify the sex of 90 to 93% of Indian subjects; the dimensions of foramen magnum, and hand and foot indices were found to be the poorest sex estimators. The dental age estimation formulas proposed by earlier researchers were found most accurate in age estimations; the pulp tooth ratio and head dimensions were found least accurate for predicting age. Morphological variation in the ear, acquired body marks, hand-foot correlation, gait pattern, and facial soft tissue thickness are significantly correlated with personal identification traits. Determination of sex and reconstruction of stature were reported as the easiest components of the biological profile to be established from human skeletal remains of unknown origin. More accurate techniques like DNA and stable isotopes were rarely employed for forensic death investigations in India during the past decade. Except for Rakhigarhi, Roopkund, and Ajnala skeletal remains, no other skeletal collection has been reported from India for their forensic anthropological analyses.

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**Forensic Anthropology; Research in India; Systematic Review**



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**A13 The Reliability and Utility of Surgical Materials in Scientific Identification Via Comparative Medical Radiography**

*Rose Leach, MA\*, Kent State University, Kent, OH; Jered Cornelison, PhD, WMU Pathology and the Medical Examiner, Kalamazoo, MI; Christine Pink, PhD, WMU Stryker School of Medicine, Kalamazoo, MI; Carolyn Isaac, PhD, Michigan State University, East Lansing, MI*

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**Learning Objective:** After attending this presentation, attendees will have learned about comparative radiography methods and the use of surgical interventions in comparative medical radiography to confirm identifications in a medicolegal context. Attendees will also learn about the frequency of the presence and position of surgical implant materials, individualizing morphologies that are associated with implants, and be able to use this knowledge to enhance their own identification methodologies and best practices.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into current medicolegal identification practices within the scope of forensic anthropology, allowing professionals to better understand the types and frequency of surgical interventions encountered within comparative medical radiography and how they can be appropriately used in the identification process.

Within the medicolegal system, forensic anthropologists assist with scientific identification of decedents in cases involving decomposition, thermal injuries, commingling, disfiguring trauma, or multiple decedents with similar demographic profiles involved in an incident. Methods available for scientific identification include Comparative Medical Radiography (CMR), fingerprint comparison, comparative dental radiography, DNA, and pacemaker serial numbers. From January 2015 to July 2022 at the Western Michigan University Homer Stryker M.D. School of Medicine (WMed) Department of Pathology, 52.4% of the scientific identifications were accomplished using CMR.

CMR includes not only examination of skeletal elements/morphology in antemortem and postmortem images, but also the presence, position, and/or morphology of surgical materials such as joint replacements, plates, screws, clips, staples, radioactive seeding, and poly-methyl bone cement. While some devices such as pacemakers are individualizing as they have a unique serial code, these codes are often not available for other foreign objects such as orthopedic devices.<sup>1</sup> However, the presence, position, and/or morphology of surgical interventions and the osseous response to these interventions are useful in CMR for facilitating scientific identification.<sup>2</sup> Minimal literature exists on the frequency of these objects in United States populations, and these have focused on skeletal collections, not within medicolegal contexts.<sup>1,3,4</sup> This study aims to contextualize the use of surgical materials in CMR and the frequency of appearance in cases from 2015–2022 at the WMed Department of Pathology.

We reviewed all the CMR identification cases ( $n=515$ ) at the WMed Department of Pathology in decedents identified from January 2015 to July 2022 for any surgical materials referenced in the identification reports, documenting the presence, position, and whether the morphology of the surgical material is individualizing. Of the 515 reviewed CMR identification cases, 114 cases used foreign objects as part of the scientific identification. These cases comprise 22.1% of all CMR identification cases. Within the 114 cases, 189 surgical materials were documented. The highest frequencies of surgical implants include screws (33.9% of total CMR cases with surgical materials), followed by plates (12.7%), joint replacements (12.7%), other hardware (11.1%), and sternotomy wires (10.1%). In addition, 30.7% of all documented surgical materials included individualizing morphologies (e.g., reactive bone formation and surgical putty), which provided stronger evidence toward a scientific identification.

A discussion of the importance and frequency of surgical materials in CMR along with the significance of the presence, position, and individuation of different surgical materials will highlight its role in making scientific identifications within current medicolegal context.

**References:**

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3. Clarkson J, Schaefer M. Surgical intervention. In: Thompson T, Black S, editors. *Forensic human identification: an introduction*. Boca Raton, FL: CRC Press, 2007;127–46.
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**Comparative Medical Radiography; Scientific Identification; Surgical Implants**

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**A14 A Comparison of the Gross and Radiographic Appearance of Classic Metaphyseal Lesions**

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**Learning Objective:** The goal of this presentation is to provide practitioners with an opportunity to compare the radiographic appearance of Classic Metaphyseal Lesions (CMLs) with the gross appearance of the same injury on dry bone.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the more subtle and nuanced radiographic indicators of pediatric skeletal trauma, particularly for infants and toddlers.

CMLs were first described by John Caffey in 1946 as “metaphyseal fragmentation” of long bones that appeared to coincide with chronic subdural hemorrhages in some children. A more current and detailed description of a CML is a fracture that extends from the margin of the subperiosteal bone collar into the metaphysis. A complete CML occurs when the fracture extends from one side of the metaphysis to the other, producing a very thin disk-shaped fragment of bone, which is the mineralized region of the growth plate. An incomplete CML occurs when the fracture only partially transects the metaphysis, separating a fragment of the metaphysis/mineralized region of the growth plate. CMLs are considered highly specific for abusive injury in infants due to the injury mechanism (i.e., pulling or twisting of an extremity or shearing forces sustained by an extremity during shaking). A CML may be difficult to identify grossly at autopsy as this type of injury is deep within the epiphyseal cartilage and may result in little-to-no hemorrhage and/or new bone formation. CMLs can also be difficult to identify radiographically due to: (1) their small size; (2) limited degree of mineralization; and (3) the necessity of obtaining an optimal radiographic view, which is dependent on the specific location of the CML.

Since 2006, the Forensic Anthropology Division (FAD) at the Harris County Institute of Forensic Sciences (HCIFS) has performed 723 pediatric examinations of various types, including seven cases received from other medical examiner/coroner offices. Of these cases, the FAD has performed 253 full Pediatric Skeletal Examinations (PSE), which involve the gross examination of the cranium, clavicles, long bones, and pleural surfaces of the ribs. Additionally, 30 partial PSEs were performed, which involves the gross examination of specific long bones that were targeted based on concerns following radiographic reviews or autopsy findings. Among the cases in which a PSE or a partial PSE was performed, CMLs were identified in 74 cases, constituting 26% of cases in which a full or partial PSE was performed.

Given this volume of pediatric cases analyzed over the past 16 years, the FAD has refined its radiographic techniques, without dosage limitations, to the extent that the postmortem radiographs provide equal, if not better, visibility of CMLs than gross visualization via the PSE. This presentation is a retrospective summary of the pediatric cases performed by the FAD, specifically emphasizing the cases in which CML(s) were identified and analyzed. This presentation will demonstrate the relative effectiveness of high-quality, radiographic imaging and gross visualization. The subtleties of CMLs, particularly when viewed radiographically, frequently still necessitate the resection of the long bone(s) for processing and anthropological assessment; however, many practitioners do not have access to processed bone specimens for comparison with the radiographic appearance of CMLs. This presentation provides a side-by-side comparison of these important pediatric fractures for reference and training purposes across the forensic disciplines.

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**Forensic Anthropology; Child Abuse; Classic Metaphyseal Lesion**

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**A15 The Application of Craniofacial Superimposition in a Case of Death in Migratory Transit on the Coast of Granada**

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**Learning Objective:** Attendees will learn about Alternative Identification (ID) techniques such as craniofacial superimposition, which can be of great use in identification scenarios where primary identification methods such as DNA, fingerprints, or dental comparison are not applicable. This is the case of people who died in scenarios of migratory transit in the Mediterranean, in which most of the time the only Antemortem (AM) material available are photographs provided by relatives.

**Impact Statement:** This presentation will impact the forensic science community by providing tools to the scientific community to apply new identification methodologies by comparing images when the only AM material available are photographs.

The crisis of migrant deaths in the Mediterranean is a shocking reality because of the shipwrecks that leave thousands of bodies on the coasts of Europe every year. In the specific case of Spain, the Alborán route connects the Rif coast with Eastern Andalusia. People move aboard motorboats with the possibility of reaching various destinations, including the coast of Motril in Granada. In 2021, there were 14 deaths and 81 missing persons in migratory transit through this route. Although DNA, fingerprints, and dental comparisons are the primary identification methods, these are not always applicable in scenarios where the deceased are migrants from countries with which there are no collaboration agreements that enable/facilitate access to dental records or previous fingerprints, or even taking DNA samples from relatives. Among the scarce AM material available in these cases, it is very likely that recent digital photographs are found, which opens the door to the application of image comparison identification methods.

The purpose of this work is to describe the methodology used through the analysis of craniofacial superimposition employing the software Skeleton-ID in the case of a male in an advanced state of skeletonization found on the coast of Motril (Granada, Spain) in 2020. For this, a 3D model of the skull was obtained along with three photographs of the possible candidate provided by the Institute of Legal Medicine of Granada. Subsequently, a total of 11 pairs of homologous craniometric and cephalometric landmarks were located in the 3D model of the skull and the three facial photographs. Images were superimposed, and the differences and similarities between the anatomical structures of the skull and face were evaluated. Finally, a very high degree of correspondence was found for 10 of the 11 pairs of landmarks used and between the cranial and facial proportions, especially in the area of the orbits.

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**Human Identification; Craniofacial Identification; Craniofacial Superimposition**

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**A16 A Validation of the FSS Method for Forensic Frontal Sinus Identification Using a United States Sample**

*Patricia Avent, BA\*, Des Moines University, Marshall, IL; Lauren Butaric, PhD, Des Moines University, Des Moines, IA*

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**Learning Objective:** After attending this presentation, attendees will better understand the reliability of the FSS method and its applicability in forensic frontal sinus identification.

**Impact Statement:** This presentation will impact the forensic community by illustrating that practitioners can confidently use the qualitative portion of the FSS method when excluding frontal sinuses for forensic identification.

In response to the *Daubert* standards, objective, code-based methods for frontal sinus comparison in positive identifications have been developed, which focus on standardizing various morphological traits and/or measurements. One such technique is the FSS method developed by Tatlisumak et al., which is named for the morphological analysis of the Frontal sinus's Septa, and Scallops.<sup>1</sup> Utilizing a Turkish sample ( $n=100$ ), Computed Tomography (CT) scans were given scores based on the frontal sinus Presence/Absence (P/A), P/A of an intersinus septa, and number of intrasinus septa and scallops (arcades). Scores from these morphological traits were treated as a numerical string code that were compared to discriminate incorrect identifications. For example, a scan with a present Right/Left (R/L) sinus, complete intersinus septum, no complete or incomplete intrasinus septa R/L, five right scallops, and three left scallops would have the code 112000053. The goal of this preliminary study is to further validate Tatlisumak et al.'s claim of a 93% discrimination rate with a sample partially representative to the United States forensic population.

This study utilizes CT scans of 50 individuals (25M/25F) of European descent from the New Mexico Decedent Image Database.<sup>2</sup> Using 3DSlicer, scans were oriented in Frankfurt horizontal and frontal sinus morphology was observed to assess the qualitative traits in the FSS method described above. Scores were taken for both the left and right sinus—unless an intersinus septum was absent, then the sinus was scored as unified. In cases of unilateral absence, only the side present was scored, and the absent side was filled with a “—”. Codes from the NMDID and those originally published in Tatlisumak's study were then compared for duplicated codes, which would result in a potential mismatch of individuals.

When the 50 codes from the current sample were compared, there were eight duplicates (16% of sample), resulting in an exclusion rate of 84%. Specifically, there were four instances where two individuals possessed exact codes. In one set, two individuals both coded as “112020143,” another two coded as “112000042,” and a third pair as “112000113.” The final pair was two individuals that presented with bilateral sinus absence, coding as “00—.” In all four sets, duplicates were confidently excluded from each other via visual assessment. This duplication rate is lower compared to Tatlisumak's original study, which found 45 duplicates (45%). In their study, the code “112000023” was duplicated seven times, at most resulting in a 93% exclusion rate for that code. No codes from the current study matched those from Tatlisumak's original study, equaling a 98% exclusion rate for the combined datasets. Overall, the results of this study support that qualitative frontal sinus variables from the FSS Method can be confidently used to exclude individuals during frontal sinus comparison. Future research with larger, more diverse samples would provide more insight into potential discrepancies in scoring (such as lack of clear definitions) and the nature of repeated codes.

**References:**

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**Forensic Anthropology; Positive Identification; CT Scans**

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**A17 Cobb Angle Estimation in a Deceased Population**

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**Learning Objective:** After attending this presentation, attendees will better understand how to estimate a Cobb angle from deceased individuals and have an appreciation for methodological challenges inherent in posthumously documenting curvature of the spine.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the Cobb angle can be estimated from Computed Tomography (CT) scans of deceased individuals, which has the potential to aid in identification of previously unidentified individuals. Assessing scoliosis using CT scans provides a more complete picture of spine curvature disorders and their consequences.

Scoliosis, or abnormal lateral curvature of the spine, is generally diagnosed using a series of radiographs (X-rays) and specific body positioning. Clinically, diagnosis can be achieved using a standard anterior-posterior radiograph of the torso; a line is drawn parallel to the superior endplate of the upper vertebra and inferior endplate of the lower vertebra surrounding the curvature. Where the lines intersect is the Cobb angle and the diagnosis is scoliosis if the Cobb angle exceeds ten degrees. In the present pilot study, we attempt to identify and quantify the degree of scoliosis in a sample of CT scans of deceased individuals. Medical CT scans of the torsos of deceased individuals with and without a prior diagnosis of scoliosis from the New Mexico Decedent Identification Database (NMDID) were imported into Dragonfly software ( $n=30$ ). Multiple 2D images were taken in the anterior-posterior direction to capture the entire vertebral column. The 2D images were overlaid in GIMP or Photoshop®, maintaining scale. The Cobb angle was calculated from a composite image of each individual and angles greater than ten degrees were identified as likely scoliotic. Results of the Cobb angle estimation were then compared to the decedent's diagnostic history of scoliosis. Using this method, the correct classification rate was 72%.

During this study, several limitations and challenges were identified. The first is that while each of the scoliotic individuals had a prior diagnosis of scoliosis, the authors had no further information such as etiology, timing of diagnosis (i.e., pubescent versus elderly), potential surgical or orthotic interventions, or comorbidities such as hyperkyphosis or osteoporosis. Further, the authors had no ability to reposition the body to optimize the decedent for measurement; thus, several torsos were slightly askew, introducing more potential error. Despite these challenges, the authors were able to identify scoliosis relatively well, especially in individuals with a double or triple curvature. In future studies, we will examine the effects of scoliosis on the shape of the pelvis.

The results of this study demonstrate that the Cobb angle can be estimated from CT scans of deceased individuals, which has the potential to aid in identification of previously unidentified individuals. Assessing scoliosis using CT scans provides a more complete picture of spine curvature disorders and their consequences.

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**Scoliosis; Vertebral Curvature; Computed Tomography**

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**A18 Inter- and Intra- 3D Modeling Reliability From Computed Tomographic Imaging of the Lumbar Vertebrae**

*Summer Decker, PhD\*, University of South Florida, Tampa, FL; Daniel Martin, MD, University of South Florida Morsani College of Medicine, Tampa, FL; Louis Leon, MD, University of Central FL/HCA Healthcare GME, Lutz, FL; Jonathan Ford, PhD, Department of Radiology\*, Tampa, FL*

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**Learning Objective:** After attending this presentation, attendees will have a deeper understanding of the reliability of 3D modeling from Computed Tomographic (CT) scans of the lumbar vertebra and how that extends to the analysis of medical image data for forensic applications.

**Impact Statement:** This presentation will impact the forensic science community by providing the inter- and intra-modeler results of an ongoing study utilizing part-to-part comparison of the lumbar vertebra for personal identification (NIJ 2019-DU-BX-0031). As the utilization of medical image data in a forensic setting continues to increase, the virtual 3D models and the analyses that derive from those models must be quantitative and reliable. Previous studies have shown that several factors, like scanner settings and image quality, are critical in the development of validated 3D virtual anatomical models.<sup>1</sup> Other factors such as anatomy of interest and 3D modeler reliability also play a role in the accuracy of any virtual forensic analysis.

The use of medical imaging for forensic purposes in pathology, odontology, anthropology, and personal identification is commonly used in everyday practice. Two-dimensional radiographs have been used for forensic applications since the discovery of the technology and continue to be used today. Additional medical image technologies, such as LODOX<sup>®</sup> scanners, Postmortem Computed Tomography (PMCT), and Postmortem Magnetic Resonance Imaging (PMR) are becoming more commonplace in the forensic setting. With its increase in use, PMCT has been leveraged in the virtual forensic analysis of osseous materials. This ranges from the establishment of a biological profile to injury analyses to personal identification. As these methodologies often utilize CT-derived 3D models of bony anatomy, it is critical that these virtual anatomical structures are reliable and reproducible from practitioner to practitioner. The purpose of this study was to analyze the inter- and intra-modeler reliability by comparing 3D modeled L1-L5 vertebrae.

As part of an ongoing personal identification project sponsored by the National Institute of Justice (NIJ 2019-DU-BX-0031), 25 de-identified clinical thoraco-abdominal CT scans were selected at random from the overall study database. Each scan was imported into the Mimics<sup>®</sup> Innovation Suite v. 24 (Materialise). The L1-L5 vertebra were then isolated and modeled via hand-segmentation and thresholding. Two users modeled each set of 25 scans, for a total of 125 lumbar vertebra each. After a period of six months, one user re-modeled the exact 25 for intra-modeler comparison. A part-to-part comparison was conducted comparing modelers' vertebrae and a match ratio was measured. A threshold of  $\pm 1$  mm was set for the part comparison. The slice-thickness of each scan was also recorded to determine any potential influence in outcome as its influence on resultant models has been documented.

Inter-modeler match ratios were excellent, with average match ratios of: L1 0.973  $\pm$  0.048, L2 0.961  $\pm$  0.042, L3 0.957  $\pm$  0.042, L4 0.973  $\pm$  0.048, and L5 0.935  $\pm$  0.053. Intra-modeler match ratios were also excellent, with average match ratios of: L1 0.977  $\pm$  0.017, L2 0.977  $\pm$  0.016, L3 0.965  $\pm$  0.034, L4 0.967  $\pm$  0.034, and L5 0.954  $\pm$  0.037. An Intra-Class Correlation Coefficient (ICC) of 0.75 was calculated comparing the part-to-part inter and intra matches, indicating good agreement. Additionally, a Pearson's Correlation was calculated comparing slice-thickness (which ranged 1mm–3mm, mean 1.51  $\pm$  0.51mm) and lumbar level (L1-L5) was calculated for both the inter- and intra-match ratios. The inter-modeler comparison expressed a weak correlation to slice thickness ( $r=-0.298$ ) and lumbar level ( $r=-0.251$ ). The intra-modeler comparison only expressed a weak correlation to slice thickness ( $r=-0.280$ ).

Overall, there was excellent inter- (0.968  $\pm$  0.029) and intra- (0.953  $\pm$  0.046) modeling match. Additionally, for both inter and intra, thicker images slices were associated with higher rates of modeler disagreement. For inter-modeler comparison, there was also a slight increase in modeler disagreement with the more inferior lumbar vertebra. This disagreement may be on account of the increased facet complexity the more inferior one progresses down the lumbar spine.

With the increase usage of PMCT in the forensic sciences, there is an equal increase in the availability and opportunity to utilize 3D tools. Accuracy, reliability, and reproducibility are critical for any 3D-modeled geometry that is created with the intent for forensic analysis. As the creation of 3D geometries from medical image data is often a mix of manual and semi-automated, one of the key elements for a reliable analysis is the practitioner's skill with the modeling software and anatomical understanding. With properly trained hands, 3D models and their subsequent analysis can be reliably and accurately performed.

**Reference:**

- <sup>1</sup>. Ford JM, Decker SJ. (2016). Computed tomography slice thickness and its effects on three-dimensional reconstruction of anatomical structures. *Journal of Forensic Radiology and Imaging*, 4, 43-46.

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**PMCT; 3D Modeling; Personal Identification**



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**A19 Investigating Forensic Anthropologists' Training and Understanding of Evolutionary Theory**

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**Learning Objective:** After attending this presentation, attendees will better understand forensic anthropology students' and practitioners' working knowledge of foundational evolutionary theory concepts via their responses to a series of survey questions.

**Impact Statement:** This presentation will impact the forensic science community by providing new insights into forensic anthropologists' education in and implementation of this theoretical body of knowledge, highlighting both strengths and deficits, and suggesting areas in which the field can bolster practitioner knowledge underlying forensic anthropological practice.

Central to the training of forensic anthropologists is a deep understanding of evolutionary theory. Both "Evolutionary Biology" (undergraduate level) and "Evolutionary Theory" (graduate level) were highlighted as courses that should be required to practice forensic anthropology in the 2021 American Board of Forensic Anthropology (ABFA) workshop on Forensic Anthropology Education Program Accreditation. While infrequently made explicit in forensic anthropological publications, the relevance of evolutionary theory to forensic anthropologists' practice is implicit and embedded in our methods. This research investigates the training of forensic anthropologists. Specifically, it asks the question of how concepts in evolutionary theory that are relevant to forensic anthropology are taught, understood, and perceived by forensic anthropologists.

A digital survey was created by the authors, reviewed and approved by the Institutional Review Board (IRB) of Truman State University, and circulated online through listservs, social media platforms, and the authors' personal and professional networks to biological and forensic anthropologists. A total of 676 valid responses were collected over a six-week period. Among these, 524 respondents were forensic anthropologists and/or bioarchaeologists that regularly engage in the field of forensic anthropology, while 152 respondents were biological anthropologists that do not engage in the field of forensic anthropology.

Overall, the majority of biological anthropologists, regardless of the major field of study, agreed that evolutionary theory is highly relevant to biological anthropology (89.0% of all respondents) and forensic anthropology (89.3% of forensic anthropologists), and most respondents (92.5%) had taken university-level coursework relevant to evolutionary theory. When asked about the relevance of evolutionary theory to biological profile estimation, more forensic anthropologist respondents deemed evolutionary theory irrelevant to population affinity estimation (20.1% of forensic anthropologist respondents) compared with other aspects of the biological profile (ranging from 13.2% to 19.8% respondents).

When given a "quiz" originally designed for a 300-level undergraduate course about concepts in evolutionary theory that are relevant to forensic anthropology, forensic anthropologists in general scored significantly lower than biological anthropologists that do not engage in forensic anthropology ( $p < 0.001$ ; Analysis of Variance [ANOVA]); however, the difference in scores is not significantly different from 0 between board-certified forensic anthropologists and biological anthropologists with no forensic aspirations ( $p = 0.716$ ; Tukey's Honest Significant Difference [HSD] test).

The results showed that while the majority of practicing forensic anthropologists or forensic anthropology students have relevant experience with university-level courses involving evolutionary theory, their working knowledge (specifically on topics related to the practice of forensic anthropology) is insufficient compared to their biological anthropology counterparts. By demonstrating potential shortcomings of the collective knowledge base in forensic anthropology, the results of this research can serve as a basis for professional organizations and certifying bodies to develop guidelines for education program accreditation and certification. Specifically, these results suggest that extensive literature review during dissertation writing and/or studying for the ABFA Certification Examination allowed ABFA-certified forensic anthropologists to become more familiar with evolutionary theory than non-ABFA colleagues. Incorporating and codifying the study of evolutionary theory into academic and practical training programs may enable more forensic anthropologists to become proficient in understanding how this body of theory informs their practice.

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**Anthropology Education; Knowledge of Evolution; Survey**



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**A20 The Regression Analyses of Nasal Measurements for Forensic Facial Reconstruction: From Juvenile to Adult**

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**Learning Objective:** After attending this presentation, attendees will have obtained information on the relationship between the piriform aperture and soft tissue nasal measurements. This relationship will be exhibited with regression formulae that were designed to estimate the soft tissue nose from the piriform aperture.

**Impact Statement:** This presentation will impact the forensic community by providing a type of guideline to enhance the accuracy of facial reconstruction.

The nose is the most protruded element and is located at the center of the face; therefore, the role of the estimated nose in facial reconstruction and its following recognition is regarded as crucial. Regression analysis was performed to validate the relationship between the soft tissue nose and the piriform aperture in Korean adults and juveniles. Obtained prediction equations for nasal shape referred to the sex and age group to enhance estimation power.

Three-Dimensional (3D) skull and face models were rendered from computed tomography images of 331 males and 334 females, and the 3D models were assessed using Mimics® (ver. 14.0, Materialise). Juveniles, who were under 20 years, were divided into three age groups according to the development of the dentition. Adults were classified into three age groups of two decades each. Nine landmarks and nine measurements were used to measure the soft tissue nose. Seven landmarks and five measurements were selected to measure the piriform aperture. Four measurements were defined to explain the direct relationship between the nasal aperture and nasal shape.

First, descriptive statistics were performed to observe the general shape of the data. Then correlation of soft tissue nose measurements with piriform aperture measurements was analyzed. The relationships of measurements that showed Pearson correlation value above 0.7 were selected for linear regression analysis. Last, we performed linear regression of measurements considering the sex and age group as variables.

Finally, linear regression was performed divided into juveniles, adults, and the total population. The resultant equations estimated nasal bridge length, nasal height, nasal protrusion, and nasal width. Juveniles generally showed larger R-square values than adults with the same equation factors. Also taking into account sex and age group, regression analysis showed enhancement of explanation ability. In juveniles, the age group showed a larger effect on R-square, while sex showed a greater effect in adults. In total population, defining a sample is whether a juvenile or adult showed increment of explanation power. This study would be helpful to approximate nasal shape more accurately in facial reconstruction.

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**Facial Reconstruction; Three-Dimensional Measurement; Regression Analysis**

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**A21 The Significance of Facial Soft Tissue Thickness in Craniofacial Reconstruction: A Systematic Review and Meta-Analysis**

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*Bhavna Ahlawat, MSc, Panjab University, Chandigarh, Chandigarh, INDIA*

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**Learning Objective:** After attending this presentation, attendees will have a conclusive outlook of research trends in Facial Soft Tissue Thickness (FSTT) estimations and its contributions in refining craniofacial reconstruction technology for forensic applications.

**Impact Statement:** This presentation will sensitize the forensic science community about the significance of facial soft tissue thickness in developing automatic facial recognition systems as well as in identification pursuits of unknown skulls retrieved in forensic scenarios.

Forensic anthropologists are traditionally interested in facial approximations and reconstruction of physiognomies of individuals and creation of life-like features onto the unknown skulls retrieved from forensic or bioarchaeological contexts.<sup>1</sup> Craniofacial Reconstruction (CFR) is a complex, challenging task of rebuilding the facial morphology of the deceased; performed manually or using computer-based analogs and algorithms generated from face analytics, soft tissue thicknesses, and craniometrics. The continued advancements in imaging modalities have led to the development of precise and flexible programs for accurate CFRs.<sup>2,3</sup> The reproducibility, reliability, and accuracy levels of tissue thickness estimations can be enhanced and scrutinized through the application of standardized modern radio-diagnostic techniques, use of larger sample size, reporting age-, sex-, Body Mass Index (BMI) - and region-specific variations. Various radio-imaging modalities like radiographs, ultrasound, Computed Tomography (CT), or Magnetic Resonance Imaging (MRI) scanning have been widely used for estimation of FSTT values onto human faces and to analyze the influence of sex, age, ethnicity, body-mass index and body composition, corpulence, subcutaneous fat, hydration status, and weight of an individual on such estimates.<sup>1,4,5</sup>

The present study scrutinizes the role of FSTT in craniofacial reconstruction by revisiting the studies published in the recent past decade (2010–2022). The searches for the published articles mentioning FSTT and related topics spanning over these years were performed using the search-engines like PubMed®, Sciondirect®, Web of Science®, and Scopus®. A total of 325 research articles were identified using different keywords (forensic facial identification, facial soft tissue thickness, skeletal types, effect of age, sex, ethnicity on FSTT, radiological modalities of CT and MRI, etc.), out of which 84 studies were found relevant for systematic review. The inclusion criteria were to consider the studies related to forensic facial reconstruction only and those published during the past decade; the studies not satisfying the present study inclusion criterion were ignored for further analyses. Out of 84 relevant articles, 30 articles presented databases for sex-, age-, and ethnicity-dependent variations in soft tissue thickness measurements. Finally, 17 studies reporting sexual dimorphic variations in FSTT values estimated in supine or upright postured individuals (aged 18–90 years and above) were considered for meta-analysis. The comparative analysis of FSTT data in different age groups revealed that there is a gradual increase in FSTT values in successive age groups in both the sexes. Sex and BMI of an individual has a profound effect on the facial soft tissue thicknesses. FSTT of the upper part of the face is usually thinner than the lower part, and the lower part of the face shows greater variation when compared to the upper part; ergo, special care needs to be applied while reconstructing the lower portion of the face. The study design, instrumentation, and imaging modality used, protocols applied, number of subjects considered, age, sex, and ethnicity-dependent variations in FSTT values, and recent advancements and applications of tissue depth estimations have been systematically reviewed in-depth in the present study.

Present systematic review and meta-analysis gives a conclusive outlook toward research trends in FSTT estimations, its contributions in refining craniofacial reconstruction technology, and identifying where we lack and where we can improve. The detailed results will be presented in the textual, graphical, tabular, and diagrammatic forms in this presentation.

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**Forensic Anthropology; Craniofacial Reconstruction and Facial Soft-Tissue Thickness; Systematic Review and Meta-Analysis**

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**A22 Sixteen Years of Forensic Anthropology at the Harris County Institute of Forensic Sciences (HCIFS): Reflections and Looking Forward**

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**Learning Objective:** The goal of this presentation is to provide a summary of the forensic anthropological contributions to the HCIFS between 2006 and 2022 by demonstrating the breadth of forensic anthropological services offered in a Medical Examiner's (ME) office.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the contributions of forensic anthropologists to medicolegal casework, particularly beyond the estimation of the biological profile.

The Forensic Anthropology Division (FAD) at HCIFS was established in 2006 when the first in-house forensic anthropologist was hired. Since then, the FAD has employed seven American Board of Forensic Anthropology (ABFA) Diplomates and has trained seven postdoctoral grant-funded Fellows. In 2015, the FAD was the first forensic anthropological laboratory in an ME office to be accredited by ANSI National Accreditation Board (ANAB) (**International Organization for Standardization [ISO] 17020**).

Since the FAD's establishment, the seven members have received approximately 5,000 case requests. FAD members conduct "traditional" anthropological analyses such as estimating components of the biological profile, assessing whether remains are human or non-human, and determining whether remains are of medicolegal significance. Responding to death scenes is also a common request of forensic anthropologists, depending on the jurisdiction and type of incident, and FAD members have responded to nearly 400 scenes.

The HCIFS forensic pathologists, in coordination with the forensic anthropologists, increasingly request analyses beyond those "traditionally" considered within the purview of anthropology. Skeletal and cartilaginous trauma analyses are the most frequent request, accounting for approximately 2,500 cases over the past 16 years. Blunt force trauma interpretation is the most common trauma request, with approximately 1,800 analyses performed. Pediatric skeletal examinations, a technique pioneered at HCIFS, or cases involving pediatric decedents have accounted for more than 500 analyses. Finally, FAD personnel have assisted with more than 500 identification cases utilizing medical and/or dental imaging comparisons and/or contextual information.

The HCIFS forensic anthropologists have also published extensively and have served as principal and/or co-principal investigators on numerous grants. Between 2008 and 2022, members of the FAD have published one book, 39 articles in peer-reviewed journals, and 14 book chapters. The forensic anthropologists have been awarded 45 grants, totaling more than six million dollars, from eight granting agencies between 2007 and 2022. FAD members are active within the forensic science community and have been committee members, board members, and/or consultants for numerous organizations including the American Academy of Forensic Sciences (AAFS), the AAFS Standards Board (ASB), the American Board of Forensic Anthropology (ABFA), the Forensic Science Standards Board (FSSB), the *Journal of Forensic Sciences (JFS)*, the Organization of Scientific Area Committees for Forensic Science (OSAC), Scientific Working Groups (SWGs), and the Society of Forensic Anthropologists (SOFA), to name a few. The breadth of our casework routinely inspires these contributions, and we believe they are imperative for the effective practice of forensic anthropology and continued relevance in the Medical Examiner or Coroner (ME/C) context.

The most recent example is the development of the HCIFS's new Postdoctoral Forensic Anthropology Fellowship. This annual, training-based Fellowship will provide the necessary skills, techniques, and procedures required to work in an ME/C office. Considering this new training model, we argue that the next contribution is for forensic anthropologists to standardize the discipline's skillset to be of more value to ME/Cs, even if they are not based within an ME/C office. We encourage students and early career professionals to look forward and diversify their skillsets to best serve the medicolegal system.

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**Casework; Medical Examiner's Office; Fellowship**

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**A23 Forensic Anthropology Casework at the St. Louis County Office of the Medical Examiner**

*Lindsay Trammell, PhD\*, Saint Louis County Office of the Medical Examiner, St. Louis, MO*

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**Learning Objective:** After attending this presentation, attendees will better understand the composition of forensic anthropological consultations at the St. Louis County Office of the Medical Examiner from 2013 through 2021.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the types of forensic anthropological casework within an urban medicolegal system in the Midwestern United States and by providing comparable data for similar jurisdictions.

The field of forensic anthropology has garnered added attention in the past decade as evidenced by more prolific coursework offered among undergraduate and graduate programs as well as concurrent increased recognition of the function of the forensic anthropologist in the medical examiner and coroner office. With this growth, however, the role of the anthropologist in the medicolegal system is still not completely understood, nor is the full scope of their work. This is partly due to the lack of published data on casework composition and detail.

Waxenbaum and Grauer provided insight into forensic anthropological casework at the Cook County, IL, Medical Examiner's Office (MEO).<sup>1</sup> This study incorporates similar variables as it presents data on forensic anthropology consultations within the St. Louis, MO, region as well as nearby counties in Missouri and Illinois where St. Louis County MEO forensic pathologists also provide services.

The St. Louis County MEO employs one full-time forensic anthropologist, who from September 2013 until January 2022 worked concurrently as a medicolegal death investigator. The anthropologist provided consultations at the request of the forensic pathologist or a coroner in a neighboring jurisdiction.

With the goal of eventually directly comparing and collaborating with Waxenbaum and Grauer, the following variables were collected from 2013 to 2021: month of discovery, decedent depositional environment, condition of remains upon discovery, type of anthropology consultation, time from discovery to consultation request, time from consultation request to analysis, and time from analysis to report completion.

From 2013 through 2021, 315 forensic anthropological cases were completed, averaging 35 per year with the lowest in 2013 ( $n=19$ ) and the highest in 2019 ( $n=61$ ). Most consultations were discovered in April ( $n=35$ ), with the fewest being in January ( $n=19$ ); the majority were outdoor, surface recoveries ( $n=173$ ), with the second most common context being indoors ( $n=80$ ).

At the time of original discovery, the state of decomposition of the remains was recorded with the following results: fresh ( $n=33$ ); decomposing ( $n=81$ ); mummified ( $n=10$ ); fully skeletonized ( $n=158$ ); and thermally altered ( $n=44$ ). Consultation requests often included more than one type of forensic anthropological examination and were distributed broadly into these categories: biological profile ( $n=78$ ); osseous trauma analysis ( $n=80$ ); radiographic identification ( $n=111$ ); determination of medicolegal significance ( $n=15$ ); and human versus non-human assessment ( $n=106$ ).

Additional forensic anthropological requests included exhumation assistance, DNA sample procurement, cold case review, and fetal pathological assessment. Excluding human versus non-human cases, the average time from discovery to anthropological consult request was 4.28 days, from consult request to anthropologist involvement was 2.18 days, and from analysis onset to report submission was 7.55 days.

The role of the forensic anthropologist within the medicolegal community is expanding and more open discussion of their roles among practitioners and researchers in the field will continue to contribute to the advancement of the forensic anthropological community and the forensic science community as a whole.

**Reference:**

- <sup>1</sup>. Waxenbaum EB, Grauer AL. 2022. Forensic Anthropology Casework at the Cook County Illinois Medical Examiner's Office, 2012-2020. *Proceedings of the American Academy of Forensic Sciences, 74<sup>th</sup> Annual Scientific Conference*. Seattle, WA 2022.

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**Forensic Anthropology; Casework; Medicolegal Community**

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**A24 Research Needs vs. Publication Trends in Forensic Anthropology**

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**Learning Objective:** After attending this presentation, attendees will be informed about a decade of publication trends in forensic anthropology and how published literature compares with forensic anthropologists' current caseloads and opinions on research needs.

**Impact Statement:** This presentation will impact the forensic science community by examining the extent to which current research meets the needs of forensic anthropology casework.

This research compares data generated through an extensive literature review of forensic anthropology publications with survey data from practitioners who regularly engage in forensic anthropology casework.

Survey data were collected via an anonymous online survey hosted by the University of New Hampshire (UNH) and reviewed and exempted by the Institutional Review Boards of UNH and Texas Tech University. The survey targeted practitioners who regularly conduct forensic anthropology casework, defined for this research as approximately 12 case reports per year or 1 per month. Respondents were asked to: (1) characterize the number, type, and frequency of cases on which they typically consult and testify, and (2) provide opinions on urgent research needs in forensic anthropology. As of abstract submission, 22 participants have responded to the survey, with solicitation to continue through Fall 2022. Preliminary results are presented here.

Publications dated January 2012–July 2022 were reviewed to evaluate the current state of forensic anthropology literature. A title-abstract-keyword search for “forensic anthropology” was conducted within relevant journals, including *Forensic Science International* (n=589), *Journal of Forensic Sciences* (n=500), *International Journal of Legal Medicine* (n=270), *American Journal of Physical Anthropology* (n=88), and *Journal of Forensic and Legal Medicine* (n=146). All American Academy of Forensic Sciences (AAFS) Anthropology section abstracts (n=1,547) and Forensic Anthropology publications (n=144) within the target time frame were included in the review. In total, 3,284 abstracts were evaluated, excluding reprints.

To date, survey respondents reported consulting most frequently on cases involving human versus non-human determination, biological profile, trauma analysis, and personal identification (specifically, radiographic comparison). These results are consistent with previous findings that medicolegal significance, trauma analysis, biological profile, radiographic comparison, and Postmortem Interval (PMI) estimation comprise the most common types of forensic anthropology casework.<sup>1</sup> Respondents reported testifying most often in trauma analysis cases. Survey respondents were generally positive about research presented at AAFS, but indicated that studies are often too simplistic or, while theoretically novel, not practically useful. Respondents were less enthusiastic about published manuscripts, citing a disconnect between academic and applied forensic anthropology.

Several respondents expressed interest in methods that do not require specialized equipment and research to support court testimony. Common practitioner-stated research needs included increased studies on trauma (especially fracture healing and research to support pediatric trauma analysis); updated biological profile methods developed on diversified populations; expansion of validation studies; and estimation of extended PMI.

The literature review revealed that 40.0% of abstracts and manuscripts focused on aspects of the biological profile. Abstracts and manuscripts focusing on age and sex estimation were most frequent across all publications reviewed. Approximately 12.4% of abstracts focused on taphonomy (inclusive of PMI), 10.8% on trauma analysis, 4.3% on personal identification, and 2.0% on medicolegal significance (inclusive of osseous versus non-osseous, human versus non-human, and historic/non-forensic versus modern/forensic).

The results highlight gaps between current literature and practitioner-reported caseloads and research needs. Practitioners report frequently consulting and testifying on trauma cases and list trauma research as an urgent need; meanwhile, trauma studies constitute less than 11% of published manuscripts and abstracts. Similarly, while practitioners frequently consult on personal identification cases, less than 5% of abstracts focus on this topic. Biological profile methods dominate the literature across all examined publications, though survey results suggest practitioners are not entirely satisfied with the diversity of reference samples, practical applicability of methods, and/or amount of research validating existing methods.

These findings are particularly useful for Master's and PhD students interested in developing research projects focused on the most relevant needs of the field. While academic research is often constrained by resource access and/or time limits for degrees, students and advisors can use these data to better direct the scope of their research questions.

**Reference:**

1. Passalacqua NV, Pilloud MA, Philbin C. Caseloads in Forensic Anthropology. A40. *Proceedings of the American Academy of Forensic Sciences*, 73<sup>rd</sup> Virtual Annual Scientific Meeting. 2021.

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**Forensic Anthropology Casework; Research Needs; Publication Trends**

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**A25 A Postdoctoral Forensic Anthropology Fellowship in the Medical Examiner Setting: A Vision for the Future**

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**Learning Objective:** The goal of this presentation is to introduce and discuss competencies that we argue should be standardized for forensic anthropologists working in a Medical Examiner's or Coroner's (ME/C) Office, and the efficacy of a Postdoctoral Fellowship Program as the mechanism for training in these competencies.

**Impact Statement:** The presentation will impact the forensic community by presenting postdoctoral fellowships as a solution to the circular problem of experience and certification in forensic anthropology.

The field of Forensic Anthropology has a well-known circularity problem: that certification by the American Board of Forensic Anthropology (ABFA) requires casework experience, but non-certified anthropologists should not conduct unsupervised casework. Efforts to resolve this problem have resulted in recent substantial changes to the certification process and significant effort on the part of ABFA leadership. The underlying problem is that, unlike other forensic science disciplines, there is no current mechanism to provide experience before certification nor to ensure that a practicing Diplomate has the necessary experience beyond passing an exam. The Harris County Institute of Forensic Sciences (HCIFS) demonstrates its commitment to the advancement of the field of forensic anthropology with the recent development of its permanent Postdoctoral Forensic Anthropology Fellowship. This Fellowship is modeled on those undertaken by medical professionals, such as forensic pathologists, and is intended to address the field's circularity problem by providing intensive training based on the core competencies of the discipline. However, this program cannot be successful without continued progress by the forensic anthropology leadership. We argue that two advancements are specifically necessary for a Fellowship of this kind to adequately fill the gap as intended: (1) the development of the forensic anthropological tool kit based on settled core competencies, and (2) reconciliation of this tool kit with the needs of the forensic pathologist.

The year 2022 marks the inauguration of HCIFS's permanent, county-funded Postdoctoral Forensic Anthropology Fellowship, the first in an ME/C Office in the country. During the development of this position, the HCIFS Anthropology Division has identified a set of competencies that are of primary benefit to our forensic pathologists, and by extension, a likely benefit to MEs beyond our jurisdiction. Based on more than 16 years of continuous casework on more than 5,000 cases, we argue that the following competencies are necessary for employment and relevancy in an ME/C Office: (1) skeletal and cartilaginous trauma analysis and interpretation; (2) recognition and description of biomechanical forces and their relation to patterns of skeletal injury; (3) proficiency in examination and interpretation of pediatric skeletal trauma, pathologies, and anomalies; (4) proficiency in producing and interpreting relevant varieties of medical imaging; (5) gross and/or radiographic recognition and description of bone pathologies; (6) adhering to medicolegal-level evidence handling and management; (7) participating in court testimony and demonstrating an understanding of the judicial ramifications of casework; and (8) adhering to rigorous standard operating procedures, peer-review, and quality management.

As forensic anthropology evolves, we also argue that the following skills should be incorporated into the list of core competencies to expand the tool kit: proficiency in recognition and interpretation of bone histology, particularly fracture healing rates, advanced medical imaging interpretation, and anthropological genetics. Additionally, the structure and function of the medical examiner system requires that forensic anthropology must continue to develop and be responsive to standards and best practices and ensure that these core competencies are standardized in their application. The indicators of success for the HCIFS Postdoctoral Forensic Anthropology Fellowship will be: (1) the Fellowship model will be accepted by the discipline as a novel mechanism to address the circularity problem; (2) that other ME/C Offices will follow suit; and (3) that the program will continue to benefit from advances in the universality of the services provided by the forensic anthropologist and the reliability (based on standard training) of those services.

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**Forensic Anthropology; Postdoctoral Fellowship; Competencies**

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**A26 A Technical Note on Recovery and Processing of Burned Human Remains**

Hayden McKee-Zech, MSc\*, University of Tennessee-Knoxville, Knoxville, TN; Sara Fatula, MA, University of Tennessee-Knoxville, Knoxville, TN; Giovanna Vidoli, PhD, Forensic Anthropology Center, University of Tennessee-Knoxville, Knoxville, TN; Joanne Devlin, PhD, University of Tennessee-Knoxville, Knoxville, TN

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**Learning Objective:** The goal of this presentation is to describe best practices in recovering and processing burned human remains.

**Impact Statement:** Burned remains are difficult to recover and process due to their fragile nature. This presentation will impact the forensic science community by informing attendees that processing of these remains is essential for viewing areas necessary to conduct anthropological analyses or long-term curation for research collections or unsolved forensic cases.

Due to thermal exposure and the loss of water and organic materials, burned bone experiences morphological changes such as shrinking, cracking, and deformation. These changes make the bone fragile and render recovery and processing difficult. Due to their fragility, common methods of maceration or the removal of soft tissue to better visualize the bone for conducting anthropological analyses, including physical maceration and hot water maceration, may not be appropriate for burned remains. Triaca et al. proposed hot water maceration at a temperature of 80°C for 24 hours as the best practice for processing burned bone.<sup>1</sup> However, this study was conducted on burned pig's feet that were exposed to thermal heat for a total of 30 minutes, resulting in a mixture of calcined and unburnt bones lacking most overlying soft tissue. When this processing method was applied to human remains burned at a Glassman-Crow Scale (GCS) of 3, the remains exhibited further fragmentation and damage, suggesting the need for best practices for processing burned human remains when anthropological analysis and long-term curation is needed.<sup>2</sup> The following is a technical note describing best practices for the recovery, processing, and curation of burned human remains.

Twelve human individuals were burned for 2–3 hours at approximately 800°C on an open-air pyre in Knoxville, TN, until a GCS 3 was reached. Burned skeletal remains were packaged for transport by placing loose elements in labeled aluminum foil pouches or in brown paper bags. Laboratory processing of burned remains included a combination of dry brushing, dry picking, physical maceration, hot water maceration, and steaming.

The best practice for transportation of remains utilized tin foil, as it proved to be more secure and supportive than brown paper bags, thus minimizing damage. Best practices for processing the burned bone depended on the degree of burning. For unburned bone, standard processing and maceration with tap water and toothbrushes was optimal. If tissue remains adherent, hot water maceration at 60°C in 45-minute intervals is recommended. If the cortical bone was blackened, best practices were to avoid direct water maceration, instead performing dry picking and gently applying a damp toothbrush, if necessary. If the remains exhibited a combination of blackened and unburned bone, hot-water maceration was applied to the unburned end while the blackened end was maintained above the water line using washcloths for stabilization. If the trabecular bone was blackened, it was imperative that the bone did not receive direct water-based maceration. If tissue was present, a dry toothbrush was lightly applied; if tissue was still adhered, the elements were steamed in 45-minute intervals using a mesh colander suspended above approximately one inch of 60°C water. Following steaming, soft tissue was removed with forceps, dental picks, or toothbrushes. If the bones were calcined, a light brushing to remove any adherent burned soft tissue proved sufficient. Fragile elements were always supported using washcloths, regardless of burn status. The remains will need to be assessed again following 1.5 years of curation to determine the longevity of this method.

**References:**

- <sup>1</sup>. Triaca A, Mahon TJ, Myburgh JA, 2022. Comparison of different maceration techniques on burnt remains. *Journal of Forensic Science*, 67, pp. 676-682.
- <sup>2</sup>. Glassman DM, Crow RM, 1996. Standardization model for describing the extent of burn injury to human remains. *Journal of Forensic Science*, 41(1), pp.152-154.

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**Burned Remains; Maceration; Technical Note**



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**A27 The Role of Anthropologists in the Identification Process Across Medicolegal Jurisdictions**

*Ginesse Listi, PhD\*, Louisiana State University, Geography & Anthropology, Baton Rouge, LA; Teresa Wilson, PhD, Louisiana State University, Baton Rouge, LA; Emily Michon, MA, Louisiana State University, FACES Laboratory, Baton Rouge, LA; Sophia Reck, MA, Louisiana State University, Baton Rouge, LA; Krista Bennett, MA, Louisiana State University, Baton Rouge, LA*

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**Learning Objective:** After attending this presentation, attendees will be knowledgeable about the various responsibilities of forensic anthropologists working in different medicolegal jurisdictions, as well as about the use of biocultural data in the identification process.

**Impact Statement:** This presentation will impact the forensic community by documenting the various responsibilities of forensic anthropologists working in different medicolegal jurisdictions, as well as how biocultural data are used in the identification process.

The role of anthropologists in the identification process varies across medicolegal jurisdictions; however, formal data that document the variation in methods and experiences are not available. The current study addresses this deficiency by documenting: (1) the types of cases on which anthropologists in various jurisdictions and agencies consult; (2) the role of anthropologists in the identification process; (3) the interaction between forensic anthropologists and various case stakeholders; and (4) the use of biocultural data for identification purposes. A survey designed to elicit this information was created, approved by Louisiana State University (LSU) Institutional Review Board (IRB) (#22-0458), and disseminated to the forensic anthropology community through the Anthroforensics and Society of Forensic Anthropologists (SOFA) listservs. To fully complete the survey, participants had to be practicing forensic anthropologists holding a minimum of a bachelor's degree. The survey was active from May 12 through July 8, 2022, and included 17 questions, some with multiple subquestions, that collected information on education, employment, medicolegal jurisdiction, casework experience, participation in the identification process, and the use of biocultural data.

Sixty-nine individuals completed the survey, with a response rate ranging from 50 to 69 for each question; the mode response rate was 51. The majority of participants have PhDs (67%), hold American Board of Forensic Anthropology (ABFA) Diplomate status (38%) or have their work peer-reviewed by a Diplomate (35%), and have conducted casework from 1 to 40 years (mean and mode of 17.3 and 20 years, respectively). Most participants are employed in universities (45%) or Medical Examiner/Coroner (ME/C) offices (24%), perform casework in ME (54%) or Mixed ME/C (14%) systems, and work alone (22%) or with fewer than four colleagues (50%).

Highlights of survey results concerning the role of anthropologists in the identification process, their interactions with other stakeholders, and the use of biocultural data include the following: most respondents (93%) consult on long-term deceased individuals, while fewer (65%) consult on the recently deceased. Aside from skeletal analysis, respondents consistently provide supporting roles in the identification process, including taking DNA bone samples, completing dental charts, obtaining antemortem medical records, and taking dental or other radiographs and photographs for comparative purposes. Respondents less frequently establish positive identification; when they do, the method used most frequently is radiograph comparison (59%). To perform these identifications, most respondents received on-the-job training or mentoring (38%). Case stakeholders with which the most respondents interact include forensic pathologists (98%), DNA analysts (78%), and state's attorneys/prosecutors (88%); the least frequent interactions are with social media and non-law enforcement community groups (both 45%), and with odontologists and fingerprint analysts (both 41%).

The majority of respondents indicated that biocultural data are used in the identification process, either by the anthropologists themselves or by other divisions within their agency, and either concurrent with (53%) or after (44%) skeletal analysis of the biological profile. Specifically, medical implants and skeletal pathology are the most used (both 100%), followed by dental restorations (96%), general (76%) and specific (73%) recovery context, tattoos (69%), personal artifacts (67%) and clothing (61%), and synthetic/natural hair (45%). Most respondents (74%) believe the use of these biocultural data is not contrary to the goal of maintaining objectivity in the identification process.

In conclusion, survey results demonstrate the varied responsibilities of forensic anthropologists regarding the identification process, their interactions with other forensic specialists, and the types of biocultural data they more frequently use. Moreover, while place of employment and medicolegal jurisdiction influence procedure and responsibilities, participants in this survey generally believe the use of biocultural data does not compromise objectivity and, in fact, facilitates identification.

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**Forensic Anthropology; Identification; Biocultural Data**

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**A28 Applying a Four-Field Approach to Forensic Anthropology Casework, Capacity-Building, and Consultation**

*Jaymelee Kim, PhD\*, University of Findlay, Findlay, OH; Lucia Elgerud, PhD, University of Tennessee Knoxville, Knoxville, TN; Megan Moore, PhD, Eastern Michigan University, South Lyon, MI*

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**Learning Objective:** After attending this presentation, attendees will understand how ethnographic, archaeological, linguistic, and biological anthropological training and skills can be combined to successfully engage in contemporary forensic anthropology problems.

**Impact Statement:** Anthropologists are unusually positioned to gain knowledge in executing diverse forms of research (e.g., ethnographic, biological), which is particularly relevant to forensic capacity-building, policymaking, and engagement with sociocultural or sociopolitical issues relevant to medicolegal fields. Rather than debating the relevance of this additional training for forensic anthropologists, this presentation will impact the forensic science community by demonstrating how four-field anthropologists, or those who partner with them, engage in rich and innovative forensic practices.

In recent years, there has been growing interest among forensic specialists in underscoring anthropological training, not just in biological sciences, but also social sciences. Forensic anthropology's increased humanitarian and human rights work, the increased prioritization of evidence-based practice, and ethical reflections on the field's contributions to social inequity have prompted these dialogs. For example, forensic anthropology's continued use of "race" when attempting to identify unknown individuals has undergone continued critique in the field. This is particularly salient when psychological and social science studies have shown implicit bias among medicolegal professionals in solving crimes in which Black, Indigenous, and People of Color (BIPOC) are victimized. At the same time, these efforts to get forensic anthropology to recognize how its efforts may be used in oppressive manners are sometimes met with resistance; United States sociopolitical trends that rely on anti-science, misinformation, and absence of advocacy have caused some forensic scientists to cling to narrow and exclusionary definitions of data. This presentation acknowledges the potential and ability of forensic anthropology to incorporate larger data sets that continue to promote evidence-based conclusions, if the scientists have appropriate education and training across anthropological subfields or collaborate across subfields.

Some forensic anthropologists erroneously cite gathering witness statements or reviewing documents as evidence of qualitative research training without understanding ethnographic methods. At the same time, others reject social science data. Here, the authors argue for inclusion of robust natural and social science methods and data in forensic analyses and demonstrate the relevance of this approach through illustrating how they apply a four-field approach to their casework, forensic capacity-building, and consulting that is rooted in education, experience, and training. Examples of integration of sociocultural data will be drawn from the application of structural violence to casework in cities such as Detroit, MI; forensic anthropology, archaeology, and DNA capacity-building in east African countries; and forensic consultation that is culturally informed domestically and abroad. This is particularly relevant to potential integrations of forensic anthropological findings in public health and other policy-informing fields. With this, the authors argue that those with training across subfields can contribute to a robust forensic anthropological approach that considers multifarious forms of data, ultimately improving and enriching forensic anthropology's contributions to medicolegal processes, policymaking, and the advancement of evidence-based practices.

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**Four-Field Anthropology; Forensic Capacity-Building; Forensic Humanitarian Anthropology**

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**A29 The Evolution of a Holistic Anthropological Approach to Personal Identification at the Pima County Office of the Medical Examiner**

*Jennifer Vollner, PhD\*, Pima County Office of the Medical Examiner, Tucson, AZ; Bruce Anderson, PhD, Pima County Office of the Medical Examiner, Tucson, AZ; Caitlin Vogelsberg, PhD, Pima County Office of the Medical Examiner, Tucson, AZ; Robin Reineke, PhD, University of Arizona, Tucson, AZ; Mirza Monterroso, MA, Colibri Center for Human Rights, Tucson, AZ*

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**Learning Objective:** After attending this presentation, attendees will understand the development and value of a holistic anthropological approach to the identification process of Undocumented Border Crossers (UBCs) at the Pima County Office of the Medical Examiner (PCOME).

**Impact Statement:** This presentation will impact the forensic science community by providing examples and suggestions for expanding the purview of forensic anthropology into a more holistic endeavor, which can potentially increase identification rates.

This presentation proposes that a holistic anthropological approach has been a factor in the relatively high identification rate at the PCOME over the past two decades. Dr. Walter Birkby, the first anthropologist associated with the PCOME, set the groundwork that has shaped the identification process used today with continual augmentation as research, technologies, and techniques have advanced. In the early 2000s, as fatal recoveries of UBCs increased in the Sonoran Desert, Drs. Birkby and then Anderson began to incorporate a “cultural profile” based upon data from identified UBCs. This profile included the geographic location of recoveries, personal effects, and skeletal health indicators in addition to the traditional skeletally derived biological profile when examining decedents.<sup>1</sup> Beatrice and Soler expanded on this original work developing a “biocultural profile” that more accurately described the holistic approach taken in appreciating biological, sociocultural, and structural patterns among UBC decedents.<sup>2</sup>

Since the beginning of the humanitarian crisis on the southern Arizona border, this holistic approach to identification has continued to expand and adapt to recent scientific methods. The observed importance of geographic location has been the impetus for the use of mapping techniques to record recovery location and assist in potential inter-skeletal and identification hypotheses. Personal effects found on or near unidentified decedents help to determine whether an individual is likely to be a UBC and, occasionally, what nationality that individual may be. Languages of documents or written notes have indicated specific regions where indigenous dialects are spoken. More recently, the possession of facemasks has assisted in evaluating the year of death for some individuals. These pieces of information are sometimes key to narrowing down the overwhelming list of potential missing persons.

The collection of missing person data by anthropologists has also been crucial for comparisons between missing persons reports and unidentified decedent profiles. The PCOME works with two non-governmental organizations, the Colibrí Center for Human Rights and Equipo Argentino de Antropología, both of which employ anthropologists. Their training in various subfields, including biological, sociocultural, and archaeological anthropology, assists both in building relationships with families and in the collection of strong antemortem data. For instance, training in osteology allows for improved descriptions of potential skeletal manifestations of previous injuries or diseases in missing persons reports. This level of detail is helpful when building identification hypotheses among unidentified decedents at the PCOME.

The PCOME has an approximate identification rate of 65% ( $n=2,298$  of 3,609) for all UBCs since the year 2000 (when tracking began). This relatively high identification rate can be correlated to the use of holistic anthropology within the PCOME as well as within several of our partner agencies. We propose that the inclusion of similar strategies may aid other agencies in increasing identification successes.

**References:**

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- <sup>2.</sup> Beatrice J, Soler A. 2016. Skeletal Indicators of Stress: A Component of the Biocultural Profile of Undocumented Migrants in Southern Arizona. *Journal of Forensic Sciences*, 61(5): 1164-1172.

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**Holistic; Identification; Undocumented Border Crossers**

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**A30 The Anthropology of Forensic Identification: Four-Field Methods Used by the Louisiana State University (LSU) Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how the application of holistic anthropological methods assists with identification.

**Impact Statement:** This presentation will impact the forensic community by providing an overview of the methods and benefits of applying a four-field approach to forensic anthropology casework at the LSU FACES Laboratory.

The forensic sciences are represented by a wide range of disciplines that focus on various evidentiary, scene, and medical analyses. Anthropologists study humans in the context in which they live and operate; consequently, forensic anthropologists often contextualize the body in social space for individual remains to be reassociated with an identity. Forensic anthropologists also describe and classify skeletal trauma, but even this assessment incorporates more than biological phenomena. For example, research shows that a person's culture and lived experiences affect the type, location, and frequency of skeletal trauma.<sup>1</sup> Thus, forensic anthropology is commonly associated with biological anthropology, but also relies heavily on theory and methodology from other anthropological subfields. Of forensic science specialists, anthropologists are those who primarily consider the intersection of cultural, socioeconomic, geographical, and biological information during the identification process.<sup>2</sup> This range of data is crucial for work concerning border crossers and humanitarian efforts, but is also pertinent and applicable to domestic casework.<sup>3,4</sup> To this end, forensic anthropologists at the LSU FACES Laboratory have been successful in applying a four-field anthropological approach to cases requiring identification.

The goal of the current presentation is to demonstrate the advantage of the four-field anthropological praxis at the LSU FACES Laboratory by highlighting four cases resolved through consideration of various biocultural identifiers. In addition to biological estimations for age, sex, ancestry, and stature, biocultural data including dental modifications, pathology, antemortem trauma, medical implants, tattoos, hair style and type, personal items, clothing, and recovery location were used to create an anthropological profile for the decedents and contributed to the identification process. The case studies will not only demonstrate the importance of using a broad spectrum of data but will underscore the relevance of anthropological pedagogy.

Four-field anthropological training promotes the reflexive examination of the practitioner's positionality and biases, while also recognizing the agency of the deceased. Louisiana's history was shaped by Indigenous, African, French, and Spanish cultures among many others. Familiarity with local histories, cultures, and spaces helps anthropologists address factors that affected an individual in death. As forensic scientists are ultimately public servants, the anthropologists at the LSU FACES Laboratory have found that a four-field anthropological approach enhances analyses and allows for better service to communities in Louisiana.

**References:**

1. Brickley M, Smith M. Culturally Determined Patterns of Violence: Biological Anthropological Investigations at a Historic Urban Cemetery. *Am Anthropol* 2006;108(1):163-177.
2. Michon EF, Reck SI, Listi GA, Wilson TV, Bennett KE. *Facilitating Identification: Using Biocultural Data to Create an Anthropological Profile* [Manuscript in preparation]. Department of Geography and Anthropology, Louisiana State University.
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**Anthropology; Identification; Four-Field Methodology**

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**A31 Identification Investigations: A Collaborative Approach to the Resolution of Long-Term Unidentified Persons Cases at the New York City Office of Chief Medical Examiner**

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**Learning Objective:** After attending this presentation, attendees will consider how forensic anthropologists can contribute to identification investigations through the examination of unknown remains, as well as the comprehensive interpretation of associated contextual information.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview of the investigative model utilized for the resolution of “true unknowns” at the New York City Office of Chief Medical Examiner (NYC OCME). Various case studies demonstrate not only the real-world successes of a holistic approach to complex identification investigations in NYC, but also the adaptability of this framework for practitioners in other jurisdictions.

There are approximately 14,000 unidentified remains cases currently uploaded to the National Missing and Unidentified Persons System.<sup>1</sup> Some of these individuals were recovered decades ago, and the vast majority can be considered “true unknowns” without any tentative information regarding identity. Such cases represent some of the most complex and challenging identification investigations, requiring practitioners to examine biological and contextual evidence and, most importantly, apply a well-informed interpretation of the findings to hypothesize possible socioeconomic, cultural, religious, and social identities. As practitioners broadly trained in critical theory, pattern analysis, and hypothesis testing, forensic anthropologists working within or consulting for medicolegal agencies can significantly contribute to these complex identification investigations.

Holistic approaches to forensic investigations have previously been discussed in the context of ongoing identification efforts along the United States-Mexico border, the identification of United States Service members, and, more recently, in discussions regarding the visibility of transgender individuals.<sup>2-8</sup> The benefits of a holistic approach to identification investigations is less frequently discussed, however, within daily operations of medicolegal agencies and the resolution of long-term unknown persons cases.<sup>9</sup> This presentation demonstrates how forensic anthropologists can and should provide more comprehensive analyses that move beyond the examination of human remains, including the investigation of multiple lines of contextual data.

At the NYC OCME, Forensic Anthropologists (FAs) utilize a mitigated bias approach to analyze both biological and contextual evidence, working collaboratively with Identification Investigators (IDIs) to resolve complex unidentified remains cases. While the investigative model is fluid, the analysis generally starts with a comprehensive examination of the body by the medical examiner and FA. When possible, this includes estimation of the biological profile; review of identifying features; soft tissue, osseous, and dental features suggestive of socioeconomic status, religious, cultural, or social identity; and estimation of the postmortem interval. Simultaneously, an FA and IDI will review the contextual information, including scene location, scene type, and context of how the remains were found; contextual analysis of the personal effects; and the cause and manner of death. Additional forensic testing may also include genetic and isotopic region of origin predictions. These data are then collectively considered to provide a hypothesis of identity that is utilized to direct missing person comparisons and inform referral of identifying information to outside agencies. This may include referral to local and/or federal law enforcement, homeless services, consular offices, religious organizations, local businesses (e.g., tattoo parlors), and non-profit organizations.

This approach to long-term unidentified cases has resulted in the resolution of over 20 cases in the past two years at the NYC OCME, each of which presented its own investigative complexities. Although the application of this model will differ based on the needs of each jurisdiction, this presentation provides a framework for practitioners to meaningfully advance complex identification investigations.

**References:**

1. NamUs. (2022). National Missing and Unidentified Persons System: NamUs. <https://www.namus.gov/>. Washington, DC: National Institute of Justice.
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**Identification Investigation; Holistic Approaches; True Unknowns**

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**A32 Beyond the Science: The Value of Anthropological Forensics Discussion**

*Ginesse Listi, PhD\*, Louisiana State University, Geography & Anthropology, Baton Rouge, LA; Emily Michon, MA, Louisiana State University, FACES Laboratory, Baton Rouge, LA; Sophia Reck, MA, Louisiana State University, Baton Rouge, LA; Krista Bennett, MA, Louisiana State University, Baton Rouge, LA*

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**Learning Objective:** After attending this presentation, attendees will have learned from representatives in different medicolegal jurisdictions and professional settings how the objectives of forensic science are met through the balanced practice of anthropological forensics.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how the objectives of forensic science are met through the balanced practice of anthropological forensics.

The origin of forensic anthropology is attributed to academic physical anthropologists and anatomists who assisted law enforcement by applying skeletal knowledge to questions of identification. From this narrowly focused beginning, forensic anthropology has emerged as a robust contributor to the medicolegal process. Forensic anthropologists today are employed with coroner and medical examiner offices as well as in academia and, in addition to identification, provide assistance with trauma analysis, field recovery, Postmortem Interval (PMI) estimation, mass fatality incidents, and humanitarian crises. Consistent with other forensic disciplines, recent scholarship in forensic anthropology has emphasized scientific rigor and focused on standardization of methods, training, and qualifications. Given the gravity of the forensic anthropologists' responsibilities in medicolegal and humanitarian contexts, such grounding in scientific method and theory is undeniably essential.

However, the variety of circumstances from which forensic anthropologists recover and analyze skeletal remains, and the breadth of their responsibilities, are unlike other forensic practitioners. Moreover, the education forensic anthropologists receive goes beyond the biological sciences to encompass theories and subject matter from the humanities and social sciences. This comprehensive training enables forensic anthropologists to consider and use not only biological but also linguistic, sociocultural, and archaeological methods and data in their analyses. This unique anthropological perspective allows the deceased to be understood as "agents" in the medicolegal process who affect and are affected by their broader community and social networks.

This symposium highlights forensic anthropologists working in a variety of medicolegal jurisdictions and different professional settings who have applied a holistic perspective to their casework. This presentation demonstrates that the practice of forensic anthropology exists on a continuum from the biological to the social sciences, and that anthropologists use multiple types of data, if not equally, then at least regularly, regardless of where they are employed. As such, this presentation demonstrates how the objectives of forensic science are met through the balanced practice of anthropological forensics.

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**Anthropological Forensics; Holistic Anthropology; Biocultural Data**



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**A33 To Scavenge or Not to Scavenge: Rethinking Patterns of Scavenging Behaviors in the Western United States**

*Cheyenne Collins, MA\*, California State University Chico, Chico, CA*

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**Learning Objective:** After attending this presentation, attendees will understand how scavenging patterns within the Willamette Valley of Oregon compares to previous research conducted across the Western United States and how species behavior can vary depending on seasonality, resource availability, and environmental differences.

**Impact Statement:** This presentation will impact the forensic science community by increasing the data available on animal scavenging behavior and its role in the recovery of human remains from the Western United States.

Non-human animal scavengers have been found to play an important role in the decomposition, dispersal, and destruction of human remains in an outdoor environment.<sup>1</sup> Forensic taphonomic researchers have noted a gap in the literature regarding how animal behavior influences decomposition and postmortem modification of human remains.<sup>2</sup> Understanding the intricacies and patterns of scavenging of various species in regard to human remains can allow professionals in the medicolegal field to locate and recover dispersed skeletal elements, understand the difference between scavenger damage and trauma, as well as better inform estimates of the Postmortem Interval (PMI).<sup>3</sup> Scavenging activities can vary depending on the region and climate due to differences in species presence, topography, seasonality, niche specificity, and available food resources. Currently, there are few studies focusing on animal scavenging and species behavior within the Western United States. Adding novel research to the paucity of literature will provide forensic investigators more insight to accurately estimate PMI and construct a search structure that takes into account scavenging activity in medicolegal investigations.

In the current study, game camera data was analyzed from taphonomic projects at the Multidisciplinary Oregon Taphonomy Investigative Studies (MORTIS) facility in Eugene, OR. Approximately one million game camera images were analyzed, representative of all four seasons of the year. Porcine proxies were utilized as human analogs and placed in two separate outdoor environments (woodland and open) at the facility over the span of two years. The remains were distributed across an 80-acre forested parcel of land as well as a separate four-acre disturbed meadow environment.

Observed scavengers included: turkey vultures (*Cathartes aura*), black bear (*Ursus americanus*), Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), gray fox (*Urocyon cinereoargenteus*), striped skunk (*Mephitis mephitis*), spotted skunk (*Spilogale gracilis*), unspecified small rodent species, and various small bird species.

This study found notable differences between animal behaviors previously reported across the Western United States and those exhibited within this project. This included various species actively choosing not to scavenge carrion despite it being an available and high-quality resource to utilize. Furthermore, species that are known to disperse remains either did not during the duration of the study or did so further into the postmortem period than previously reported. The results of this study may be used by medicolegal professionals when constructing search protocols for human remains in outdoor environmental contexts, as animal species who have previously been recorded to scavenge may not always choose to do so.

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**Taphonomy; Animal Scavenging; Search Structure**



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**A34 Scavenging in a Wetland Environment: Documentation of Vulture Scavenging of Fully and Partially Submerged Human Remains**

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**Learning Objective:** After attending this presentation, attendees will better understand the scavenging practices and behaviors of vultures during the decomposition process in subtropical, wetland environments.

**Impact Statement:** This presentation will impact the forensic science community by providing photographic and video documentation of vulture scavenging and manipulation of human remains that are submerged in lentic water. This presentation will add knowledge to an area of minimal research pertaining to vulture scavenging in wet environments with waterlogged carrion.

The forensic literature frequently examines vulture scavenging of human remains, including its effects on the human decomposition process, scavenging rates, behaviors, dispersal patterns, and postmortem damage to bone; however, there is little documentation on vulture scavenging of human remains that are completely submerged in water. This study documents multiple cases of vulture scavenging of human remains that are completely immersed in fresh water and discusses the behavior of vultures feeding on partially and fully submerged carrion.

The Florida Institute of Forensic Anthropology and Applied Science (IFAAS) at the University of South Florida (USF) accepts human donations for taphonomic research. Donations are placed at the USF Facility for Outdoor Research and Training (USF-FORT), a 3.5-acre lot that contains both an open field and wooded section. Due to Central Florida's humid subtropical climate and a month's-long rainy season, the wooded lot becomes a wetland swamp area from summer to winter, resulting in water 20 inches deep or more. From July to October 2019 and in June 2020, ten donors were placed in the swamp to collect baseline data concerning rates of freshwater decomposition in Central Florida. The first donor was left completely uncaged to allow for natural scavenger and fluvial activity to take place. In contrast, two subsequent donors were "fenced in" with mesh netting to contain elements within a smaller area, while still allowing for avian scavenging. To prevent scavenging on the remaining seven donors, cages and/or tarps with wire netting were used to cover the donors. Motion-activated game cameras were placed throughout the area. All donors utilized in this study decomposed in standing water at least temporarily, although there was variation in depth of water and length of time in the water due to natural fluctuations of the water level in the swamp.

Results show that when human remains were completely uncaged and unfenced, fluvial transport and scavenger activity skeletonized remains within 5 days and dispersed elements up to 27 meters from their original placement. The two fenced donors were skeletonized at an average of 12.5 days from placement, with disarticulation and dispersion taking place between 5 to 9 days after skeletonization. Both black and turkey vultures were present. In contrast, caged remains became skeletonized at an average of 181 days post-placement. Vultures scavenged almost all individuals, regardless of their caged status and placement under water. Most caged individuals had limbs completely submerged with the thorax only partially submerged, yet in several instances vultures scavenged the submerged hands and feet. In one instance of an uncaged individual, vultures pulled large soft tissue segments and the lower leg of a fully submerged and disarticulated donor out of the water and onto a dock-like platform to have better access for feeding.

These results demonstrate that in wetland environments, vultures are prominent taphonomic agents. Even in the presence of cages to prevent scavenging, vultures managed to feed on donors despite the depth of water and mesh netting as deterrents. In cases where remains were open to scavenging, vultures contributed to dispersal of skeletal elements similar to scavenging in a dry environment. Although previous research notes that turkey and black vultures prefer forested areas and shrub land for scavenging, discussion of vultures in wetland areas feasting on waterlogged carrion are noticeably absent from the literature.<sup>1</sup> Therefore, vultures should not be overlooked as potential taphonomic agents in waterlogged environments.

**Reference:**

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**Taphonomy; Vulture Scavenging; Decomposition**

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**A35 Estimating Postmortem Submersion Interval: Implications for Varied Water Conditions and Skin Tones**

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**Learning Objective:** After attending this presentation, attendees will have an improved knowledge of the effect of water conditions, including temperature, salinity, hardness, current, and pH, on the process and rate of aquatic decomposition. Attendees will also learn how Decomposition Scoring Systems (DSS) work and their limitations when applied to a variety or range of skin tones or stages of decomposition.

**Impact Statement:** This research should have important implications for the accurate estimation of Postmortem Submersion Interval (PMSI) in forensic cases worldwide. This presentation will impact the forensic science community by improving and validating the current DSS and addressing the gap in knowledge of the effect of different water conditions on the decomposition process and subsequent PMSI estimation. Currently, PMSI estimations are based on experience from casework, anecdotal evidence, or experiments using animal analogs as there is no human taphonomy facility regularly undertaking research on aquatic decomposition. For the first time, data from empirical studies and forensic casework will be brought together to critically evaluate the existing PMSI estimation methods to determine the applicability of such scoring systems for PMSI estimation in Africa and elsewhere.

Drowning is a common cause of death; globally, over 200,000 people drown annually. Aquatic decomposition rate and the accuracy of PMSI estimations are affected by many factors, but these have not been researched thoroughly. The extent of decomposition in bodies recovered from water is measured using visual DSS, such as the Total Aquatic Decomposition Score (TADS), which is adapted from terrestrial DSS for estimating Postmortem Interval (PMI) such as the Megyesi et al. Total Body Score (TBS).<sup>1</sup> The Heaton, van Daalen, and Reh DSS are limited because they have been developed in countries with temperate climates and predominantly using individuals with pale skin. This study aimed to determine: (1) the effect of water hardness, salinity, and current on the decomposition rate in animal analogs (mice and pigs); (2) compare the effectiveness of DSS in animal and human cases for estimating PMSI; and (3) improve the suitability of existing DSS for assessing decomposition changes in individuals with darker skin tones than individuals used in the existing, commonly used DSS.

To address the first aim, a laboratory study was undertaken, where 330 dead mice were divided into groups with varying water conditions (salinity, hardness, movement) and left to decompose for 35 days, with their extent of decomposition measured at 7-day intervals. To compare results with larger analogs, 15 dead pigs were left to decompose in different water conditions for 70 days. The extent of decomposition was measured using adapted Heaton and van Daalen DSS. To compare the accuracy of these DSS in human cases, the extent of decomposition was also assessed in pictures of drowning victims from the Netherlands, the United Kingdom, and South Africa.

The results demonstrated that salinity, hardness, and movement levels significantly affected the decomposition rate ( $p < 0.05$ ); decomposition was faster in fresh water, soft water, and moving water when compared to high-saline water, hard water, and stagnant water, respectively. This study also demonstrated that there are missing decomposition stages in the DSS descriptions and discoloration changes can be misleading when using these DSS on dark skin-toned individuals. Therefore, current speed, salinity, and hardness concentrations should be considered when estimating PMSI of bodies recovered from marine or hardwater environments such as caves. It is also imperative to improve the suitability of these DSS for assessing decomposition in darker skin-toned individuals. This could be done by adapting the descriptions of the discoloration changes noted in these DSS for darker skin tones. This would have important implications for the accurate estimation of PMSI in forensic cases worldwide by improving the accuracy of these DSS when applied to cases with a range of skin tones.

**Reference:**

- <sup>1</sup>. Megyesi MS, Nawrocki SP, Haskell NH. (2005), Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Science*, Vol.50, No.3, pp.19.

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**Drowning; Skin Tone; Decomposition**

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**A36 Assessing Patterns of Moisture Content in Decomposing, Desiccated, and Mummified Tissue in the Southeastern United States**

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**Learning Objective:** After attending this presentation, attendees will understand seasonal patterns of desiccation observed in Western North Carolina between March 2021 and January 2022 and how these data compare to the findings of previous research conducted in central Texas.<sup>1</sup>

**Impact Statement:** This presentation expands on previous research presented at the American Academy of Forensic Sciences (AAFS) 74th Annual Scientific Meeting and will impact the scientific community by providing regional data of desiccation and mummification observed in an area that is classified through Köppen-Geiger as a warm-temperate, fully-humid, warm summer (Cfb) region over a 10-month period.<sup>2,3</sup> This study also provides a direct comparison to the findings of Lennartz and colleagues in central Texas in 2020.<sup>1</sup>

Accurately estimating Postmortem Interval (PMI) is a difficult task, particularly when remains are desiccated or mummified. While desiccation refers to the general moisture loss in tissue, mummification is the point at which decomposition and decay are suspended.<sup>4</sup> Understanding the patterns of desiccation and mummification in different regions can inform PMI estimates and improve methods used to generate them. The goal of this study is to observe natural mummification, with particular attention paid to the sequence and rate at which it occurs in the southeastern United States as compared to those in central Texas.<sup>1</sup> The data from the first trial of this project were presented at the AAFS 74th Annual Scientific Meeting, while the research presented here includes the complete project data and analyses.

This project took place at Western Carolina University's (WCU) Forensic Osteology Research Station (FOREST) between March 2021 and January 2022. Ten donors from WCU's Body Donation Program were enrolled in this study, which was separated into three trials to capture seasonality. Three donors were enrolled in Trial 1 (March–July), three in Trial 2 (June–September), and four in Trial 3 (October–January). Prior to placement, enrolled donors underwent an “intake” procedure where baseline moisture measurements, using the Delmhorst RDM-3™ moisture meter with 22-E electrodes, and initial overall photos were taken. Twenty data points were collected for each donor daily, excluding weekends and inclement weather days, until all points were deemed “inaccessible” due to skeletonization. A HOBO® remote weather station recorded hourly rain, temperature, solar radiation, and relative humidity. The data collected were graphically represented in a scatterplot that utilized Accumulated Degree Days (ADD) as a standardized time scale. A Locally Weighted Scatterplot Smoothing (LOWESS) curve was added to more accurately capture the curve of moisture loss.

The relative moisture contents of the individuals in this study had marginal R<sup>2</sup> values ranging from 0.36391–0.57574, suggesting that 36–58% of the moisture loss was attributed to the fixed (environmental) effects. Multi-level modeling of these fixed effects on each body region demonstrated that temperature has the greatest influence over moisture content with a fixed effects coefficient of -5.15497, humidity and precipitation having a less significant influence on moisture content with coefficients of 0.481891 and 0.11009, respectively. Finally, solar radiation was not found to have a significant effect on moisture content, with a coefficient of 0.0087.

When the data were broken down by seasonality, differences in rates of moisture loss can be seen graphically and the impact of the environmental effects vary between seasons, as shown in the respective R<sup>2</sup> values: Trial 1 had R<sup>2</sup> values ranging from 0.41486–0.62799, Trial 2 from 0.17410–0.59252, and Trial 3 from 0.42199–0.63166. Results from Trial 2 deviated from Trials 1 and 3, as well as the results from central Texas.<sup>1</sup> R<sup>2</sup> values for Trial 2 were lower across every body region, indicating that moisture loss is most likely attributed to the increased scavenging rather than environmental effects. Trials 1 and 3 were most similar to the work of Lennartz and colleagues, in which environmental effects contributed 50–55% of the variation in moisture content, and temperature was shown to have the greatest impact on moisture content.<sup>1</sup> The results from Trials 1, 3, and central Texas suggest a consistency in the pattern of moisture loss and desiccation, regardless of seasonality and geographic region.<sup>1</sup> Mummification was documented in central Texas, but not in Western North Carolina, indicating regional differences in the extent of observed moisture loss.

The results of this study show consistent patterns but continue to demonstrate the need for further research into variation in decomposition and regional development of methods that estimate PMI.

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**Postmortem Interval; Decomposition; Mummification**

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**A37 The Metabolomics-Based Estimation of Postmortem Interval From Bones for Forensic Applications**

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**Learning Objective:** The goal of this presentation is to inform attendees on the application of Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) metabolomics to bone tissue with the purpose of estimating Postmortem Interval (PMI) in forensic science. This aims to provide an efficient extraction protocol as well as standardized assay for achieving an ideal metabolomic coverage.

**Impact Statement:** This presentation will impact the forensic science community by making attendees aware of the great potential that metabolomics has in forensic science, specifically for the estimation of PMI, not only from soft tissues but also from bones.

The implementation of metabolomics in forensic science has considerably increased in the past few decades. This includes the analysis of low molecular weight compounds by means of Liquid/Gas Chromatography coupled with Mass Spectrometry (LC/GC/MS) and provides a comprehensive description of a certain matrix metabolism when the organism is alive. With death and the cessation of the metabolic activity, the metabolome undergoes modifications that seem to correlate consistently with PMI. Despite that several studies on different matrices have demonstrated the potential of metabolomics for short-term PMI estimation (a few hours to days), no comprehensive studies have investigated postmortem metabolome modifications in bone tissues.<sup>1,2</sup> This could potentially allow an extension of the temporal limits of PMI estimation based on low molecular weight compounds.

The present study represents, to our knowledge, the first systematic investigation of the postmortem bone metabolome for PMI estimation. We analyzed non-treated bone fragments taken from the anterior portion of the tibia of 50 individual (male N=27, female N=23, mean age 64.6±15.6 years) from two human taphonomy facilities (Forensic Anthropology Center at Texas State [FACTS] and Southeast Texas Applied Forensic Science Facility [STAFS]) with a PMI ranging between 177 and 3943. Both trabecular and periosteal portions were removed with a lithium-ion drill operated at max. 5,000 RPM to isolate the cortical bone; 50mg of bone powder was processed using consecutive washes of methanol, ethanol, and water. For each step the material was processed with a beads homogenizer, before drying, reconstituting, and injecting the samples in Hydrophilic Interaction Chromatography (HILIC) and reverse phase C18 LC columns in positive ionization mode. LC/MS/MS analysis was performed on a high-resolution quadrupole Time-Of-Flight (qTOF) mass spectrometer and the metabolomic datasets were processed via Progenesis® QI for MS/MS identification and quantification. Preliminary results on a subsample of four individuals carried out on a pre- and post-decomposition sample showed an excellent discrimination between fresh and skeletonized samples, as well as for the four PMIs (up to 872 days). Several metabolites (e.g., hypoxanthine, creatinine, sedanolide) showed consistent trends in their abundances (either positive or negative) with PMI, suggesting they could be valid markers for PMI estimation. By extending the PMI range in this current study, we expect to confirm the previously identified markers and potentially add new ones to develop a model for PMI estimation based on small molecules.

**References:**

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2. Pesko BK et al. Postmortomics: The Potential of Untargeted Metabolomics to Highlight Markers for Time Since Death. *OMICS: A Journal of Integrative Biology* 24, 649–659 (2020).

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**Postmortem Interval; Metabolomics; Bone**

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**A38 The Impact of Fat Mass on Decomposition Rate and Postmortem Interval Estimation**

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**Learning Objective:** In this presentation, attendees will understand how an individual's fat mass, represented by Body Mass Index (BMI), affects the process of decomposition. The accuracy of BMI as an indicator of fat mass and differences in subcutaneous fat deposit locations are examined. Attendees will also learn about the difficulties surrounding the use of decomposition to estimate the Postmortem Interval (PMI) and how the results of this research may impact time since death estimations.

**Impact Statement:** This presentation will impact the forensic science community by advancing knowledge of the effects of obesity on the decomposition process. Currently, there is no consensus in the research literature about the effect of body mass and fat mass on the rate of human decomposition, despite substantial human variation in body fat and the growing prevalence of obesity across the globe. As decomposition rate is often used in forensic contexts to estimate the PMI, it is important to understand how fat mass impacts decomposition rates.

It is unclear how body mass, or fat mass, affects the rate of human decomposition. This study investigates the effect of fat mass on the rate of early to advanced human decomposition to provide more accurate PMI estimations. The decomposition processes of 16 females and 9 males in different BMI categories were monitored. The 25 individuals used in this study were part of the Willed Body Donation Program at Texas State University and were all unautopsied, placed unclothed, supine, and caged at the Forensic Anthropology Research Facility in San Marcos, TX, between 2019 and 2022. The Accumulated Degree Days (ADD) needed to reach skeletonization of the trunk were compared between the BMI categories and among obese and non-obese individuals. Additional subcutaneous fat measurements from nine of the donors were collected to determine if BMI is an accurate indicator of fat mass and if fat deposit locations differ between men and women.

The results indicate that there is not a significant difference in the rate of early to advanced decomposition between individuals in the different BMI categories, but trends toward significance are observed when the categories are collapsed into obese and non-obese individuals. Additionally, the results suggest that there may be a difference in decomposition rate during advanced and later stages of decomposition. BMI was found to not be an accurate predictor of fat mass in obese individuals, and that fat deposit locations do not differ between obese men and women. These findings likely indicate that once an individual is classified as obese, there is not a significant difference in the amount of subcutaneous fat an individual has or the storage location of the fat deposits. In sum, the results of this study suggest the need for further analyses into the effect of fat mass throughout the entire decomposition process.

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**Decomposition; Body Mass Index; Postmortem Interval**

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**A39 A Preliminary Investigation of Fractographic Method Accuracy in Determining Fracture Propagation Direction in Human Tibiae**

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**Learning Objective:** After attending this presentation, attendees will be better informed about the utility of fractographic methods for determining fracture propagation direction in a sample of diverse fracture types and, more broadly, the importance of multidisciplinary research and experimental research design in skeletal trauma analysis.

**Impact Statement:** This presentation will impact the forensic science community by providing a preliminary validation of fractographic methods in determining direction of fracture propagation utilizing strain gage data collected during experimental bending tests of human tibiae.

Forensic fractography of bone demonstrates utility in the identification, analysis, and interpretation of fracture surface characteristics, specifically to determine direction of fracture propagation.<sup>1,2</sup> However, few studies have validated fractographic interpretations from experimental data collected during controlled loading scenarios using human skeletal material. Additionally, limited literature has examined the validity of fractographic methods across different fracture types; therefore, the goal of this study was to validate the determination of fracture propagation direction via fractographic analysis utilizing strain gage data collected during experimental bending of tibiae.

The sample for this study consisted of 21 unembalmed human tibiae (8 male, 69 years  $\pm$  26.7; 13 female, 72.5 years  $\pm$  20.3) loaded in a dynamic bending scenario that resulted in a single fracture per tibia, which is a subsample from a larger on-going research project (NIJ 2019-DU-BX-0040). Prior to testing, two strain gages, one uni-axial and one rectangular rosette, were attached on the medial, lateral, and posterior surfaces of each tibia. All tibiae were impacted in precisely the same controlled, 4-point bending experiment in a lateral to medial direction at 6m/s. Strain data were collected throughout the entire event at 100,000hz and were ultimately utilized as the gold standard to determine cortical surface of fracture initiation. Resulting fractures were classified utilizing the AO/OTA Fracture and Dislocation Classification system and presented as: simple oblique ( $n=3$ ), intact wedge ( $n=6$ ), multifragmentary wedge ( $n=7$ ), multifragmentary intact segmental ( $n=2$ ), and multifragmentary segmental ( $n=3$ ).<sup>3</sup> Each fracture was subsequently analyzed by an unbiased observer following the fractographic methods outlined in Christensen et al.<sup>2</sup> Fracture initiation was determined by the presence of bone mirror, and termination was identified by the presence of arrest ridges and/or cantilever curl, consistent with previous studies.<sup>1,2,4</sup> Together, the identification of these variables resulted in the fractographic determination of fracture propagation direction.

Direction of fracture propagation from fractography was identified in 16 of 21 tibiae (76%) (simple oblique [ $n=2$ ], wedge intact [ $n=6$ ], wedge fragmentary [ $n=6$ ], multifragmentary intact segmental [ $n=1$ ], and multifragmentary segmental [ $n=1$ ]). Five tibiae (24%, simple oblique [ $n=1$ ], wedge fragmentary [ $n=1$ ], multifragmentary intact segmental [ $n=1$ ], and multifragmentary segmental [ $n=2$ ]) did not exhibit the fractographic features (e.g., bone mirror) necessary to determine propagation direction. Fracture propagation direction was determined as either posteromedial initiation with lateral termination ( $n=13$ ) or medial initiation with lateral termination ( $n=3$ ). Fractographic assessment of direction of fracture propagation was found to be accurate in 56% (9/16) of the sample when compared to the strain gage data. The nine tibiae with consistent fracture propagation directions between methods demonstrated the following fracture types: simple oblique ( $n=1$ ), wedge intact ( $n=3$ ), wedge fragmentary ( $n=4$ ), multifragmentary intact segmental ( $n=1$ ). Overall, determination of fracture propagation direction between fractographic and strain gage data were more consistent in simple and wedge fracture types rather than more complex multifragmentary fracture types.

These preliminary findings suggest that current fractography methods are generally more applicable for less complex fracture types but may not be as conclusive when examining multifragmentary fracture patterns. Future work will continue to investigate the accuracy of fractography in determining direction of fracture propagation in a larger sample size with increased variation in fracture types, groups, and number of fractures.

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**Blunt Force Trauma; Trauma Interpretation; Method Validation**



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**A40 An Examination of Strain Mode at Fracture in Experimentally Loaded Human Tibiae**

Angela Harden, PhD\*, Injury Biomechanics Research Center, The Ohio State University, Columbus, OH; Yun-Seok Kang, PhD, Injury Biomechanics Research Center, The Ohio State University, Columbus, OH; John Bolte, PhD, Injury Biomechanics Research Center, The Ohio State University, Columbus, OH; Kyra Stull, PhD, University of Nevada, Reno, Reno, NV; Amanda Agnew, PhD, Injury Biomechanics Research Center, The Ohio State University, Columbus, OH

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**Learning Objective:** After attending this presentation, attendees will understand the importance and value of multidisciplinary experimental skeletal trauma research through an examination of strain modes (e.g., tension or compression) during fracture of human tibiae in a bending loading mechanism.

**Impact Statement:** This presentation will impact the forensic science community by providing large-scale experimental human bone biomechanics data to validate current assumptions regarding strain modes during fracture.

Generalizations regarding the response of human bone prevail across scientific fields without validation from large-scale experimental trauma data. It is consistently stated that fractures initiate in tension, but previous research has shown that human ribs do not always demonstrate initial failure in tension in bending events.<sup>1</sup> In contrast to experimental rib research, research into the strain modes of other long bones in bending has not been investigated. Tibiae are commonly injured in bending scenarios and are therefore a long bone of interest to validate strain mode assumptions. The objective of this study was to determine cortex-specific strain modes at fracture initiation and specifically if the bone failed first in tension or compression, in experimentally loaded human tibiae.

Fifty-four human tibiae were experimentally loaded at 6m/s in a lateral-medial direction in a 4-point bending scenario. Prior to loading, all tibiae were aligned and potted at the 20% and 80% sites using an anatomical coordinate system to ensure consistent orientation across tests. Two strain gages, one uni-axial and one rectangular rosette, were affixed on each tibial surface (i.e., medial, lateral, and posterior) at 45% and 55% of the total length, respectively. For this study, data analysis was limited to the gages measuring along the long axis of each surface (i.e., all uni-axial gages and one of the three gages per rosette). Strain mode (i.e., tension or compression) was documented using microstrain polarity (+ or -) for each gauge at initiation of failure. Failure was determined following the methods of Agnew et al. as a rapid change in microstrain, and the gauge that showed this behavior earliest in time was then used to determine fracture initiation surface.<sup>2</sup>

At the time of fracture initiation, the global behavior of all 54 tibiae was as expected, where tibiae demonstrated compression on the lateral surface and tension on the medial surface. Interestingly, the posterior surface demonstrated variation with strain modes of tension (52%), compression (46%), with 2% ( $n=1$ ) unable to be determined because of broken gages. Likely this variation in strain mode is because of morphological differences in the sample. The strain mode in which fractures initiated was more consistent throughout the sample with fractures most commonly initiating in tension (93%), followed by mixed mode (simultaneously in tension and compression) (13%), and lastly in compression (4%). While these data overall support commonly held generalizations regarding failure, the variation in strain modes on the posterior surface and in fracture initiation should be further explored in greater depth to identify predictors for deviations from the expected. Evaluating the posterior surface of a tibia loaded in bending or assuming that fractures unequivocally initiate in tension could result in misinterpretations of fracture characteristics or the traumatic event. Future work will consist of calculating principal strains and evaluating gross and microscopic morphological variation to allow for further understanding of human tibia behavior during a bending scenario.

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- <sup>1.</sup> Harden A, Kang Y-S, Stammen J, Moorhouse K, Agnew A. Characterization of Strain Mode in Human Ribs Subjected to Anterior-Posterior Loading. *International Research Council on Biomechanics of Injury*; 2020; Virtual.
- <sup>2.</sup> Agnew A, Murach M, Dominguez V, et al. Sources of variability in structural bending response of pediatric and adult human ribs in dynamic frontal impacts. *Stapp Car Crash Journal*. 2018;62:119-192.

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**Skeletal Trauma; Trauma Analysis; Biomechanics**



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**A41 An Evaluation of Blunt Force Trauma Mechanism Interpretations Inferred From Skeletal Fracture Morphology and Patterning**

*Donna Boyd, PhD\*, Radford University, Radford, VA; Victoria Pate, Radford University, Winchester, VA*

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**Learning Objective:** The goal of this study is to evaluate Blunt Force Trauma (BFT) mechanism interpretations derived from skeletal fractures. Fractures derived from a sample of trauma cases with known BFT trauma etiology are used to test a series of hypothesis relating BFT mechanism with skeletal fracture morphology and patterning.

**Impact Statement:** This study will impact the forensic science (including forensic anthropology) community by providing knowledge of the accuracy and error associated with skeletal fracture-based inferences of BFT trauma mechanisms. This will ultimately allow greater standardization of trauma methodology and enable forensic practitioners to provide more objective and precise trauma assessments that meet Daubert requirements for admission of trauma testimony.

Forensic anthropologists are increasingly asked to assess BFT in a medicolegal setting. In this regard, it is important to distinguish between trauma description (reporting of location, dimensions, and other features of skeletal defects) and interpretation. BFT interpretations are explanations focused commonly on identification or confirmation of trauma mechanisms—the external factors (e.g., behavioral and biomechanical) that produce a skeletal defect. Abductive inferences derived from descriptions of skeletal fracture and its patterning (if present) inform medical examiner or coroner attestations of manner and cause of death in the absence of known circumstances of trauma etiology; however, the accuracy of such inferences has not been established. This research seeks to review and validate associations of BFT fracture patterning with the behavioral and biomechanical mechanisms that produced it.

In this study, major BFT mechanisms were identified through a systematic review of over 300 published BFT-themed skeletal trauma references. Mechanisms were categorized into groups of related behavioral and biomechanical etiologies such as falls (from lesser vs. greater heights), beatings (with or without use of external objects), impacts (low vs. higher energy), hangings and strangulations, and torture. For each major trauma mechanism category, a set of skeletal fracture “signatures” was identified, and a series of hypotheses and testable fracture predictions developed. A sample of BFT cases from the Radford University Forensic Science Institute (RUFISI) with known fracture mechanisms was used to test these hypotheses for each major mechanism. The sample of 101 BFT cases consisted of 60 males and 41 females, ranging in age from 1 month to 92 years. Both acute (perimortem) and antemortem fractures were tabulated from these cases in terms of number of fractures observed, type, location, repetition, healing, and degree of refit. These data were then compared to predictions derived from hypotheses for each mechanism.

Results indicate significant specificity (and high predictive value) of fracture number and location with BFT mechanisms involving higher energy events (such as motor vehicle and aircraft impacts). Also highly correlated were non-accidental domestic (including pediatric and elderly) injuries with fracture location and repetition. In addition, hangings and strangulations were significantly correlated with hyoid, mandibular, or associated cartilaginous fractures in the throat. Absence of fracture specificity (and, consequently, weak predictive value) for trauma mechanisms was correlated with complicating variables, including age of decedent, completeness and integrity of sample, and complexity of behavioral events; some fatal events involved several related BFT forces (e.g., a vehicular accident followed by a fall), which complicated their interpretation.

These results are discussed in terms of biomechanical aspects of fracture initiation and propagation and the variability in this process across low energy versus higher energy environments. It is recommended that BFT trauma interpretations of behavioral mechanism, and ultimately, assessments of manner of death from fracture patterning descriptions be made with caution. Construction of trauma datasets with known etiologies, as in this study, allows for testing of hypotheses derived from trauma mechanism inferences and estimation of error associated with their use in Daubert and other court testimony. It also promotes standardized, replicable, and validated trauma methodology leading to a more objective skeletal trauma analysis.

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**Forensic Anthropology; Blunt Force Trauma; Skeletal Fracture**

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**A42 The Application of Forensic Fractography to High-Velocity Projectile Trauma in Long Bones: Relationships to Velocity and Caliber**

*Devin Adcox, BS\*, California State University Chico, Chico, CA; Ashley Kendell, PhD, California State University Chico, Chico, CA; Eric Bartelink, PhD, California State University, Chico, Chico, CA; Hugh Berryman, PhD, The Forensic Institute for Research and Education, Murfreesboro, TN*

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**Learning Objective:** After attending this presentation, attendees will understand how fractographic features can be used to interpret gunshot wounds to long bones and how entrance beveling relates to velocity and caliber.

**Impact Statement:** This presentation will impact the forensic science community by providing a novel method for understanding gunshot trauma to long bones. Attendees will gain a better understanding of the mechanisms involved during high-velocity fractures to long bones and how fractography can be utilized to understand projectile injuries to long bones. With the rising number of gunshot-related deaths in the United States each year, this research should prove invaluable to forensic anthropology and medicolegal communities.

It has been demonstrated that higher velocities will result in decreased bevel angle in plate glass, and fractographic features have long been established for various materials such as steel, ceramic, and glass.<sup>1,2</sup> The same fractographic features found in other materials are predicted to also be present in bone fractured by modern ammunition. In addition, the bevel angle is predicted to decrease with higher velocities.

Fractures to long bones produced by high-velocity impacts are an understudied area in forensic anthropology. This study aims to provide further insight on gunshot fracture patterns in long bones for the medicolegal community. Previous studies investigating high-velocity projectile fractures often focus on the degree of comminution present; however, there have been very few systematic studies investigating these fracture patterns.<sup>3</sup> Fractography offers an ideal method for exploring these fractures because it provides a methodology that is systematic and can be correlated to certain stresses such as tension and compression.<sup>4</sup> This work expands on previous scholarship that investigated the presence of fractographic features in long bones fractured by projectile impacts incurred during the United States Civil War.<sup>4</sup> The current project investigates high-velocity fracture patterns to long bones in a controlled setting. Nine deer limbs, serving as human proxies, were impacted from 15 feet away using two different caliber weapons, a .22 and a .30 caliber with muzzle velocities of 1,070 ft/sec and 2,000 ft/sec, respectively. The limbs were then macerated using an incubator. The fragments from each limb were then reconstructed to orient the fracture surfaces. Then each surface was analyzed macroscopically and microscopically using oblique lighting, and the features were recorded for each surface. These consisted of six of the seven features outlined in Christensen et al., which are as follows: bone mirror, bone hackle, Wallner lines, wake features, arrest ridges, and cantilever curl in addition to mist.<sup>5</sup>

The data indicate that multiple features form a consistent pattern together across each fracture surface, and there appears to be a relationship between the location of bone hackle and velocity. Arrest ridges and cantilever curl often formed on the superior and inferior fracture surfaces, while bone hackle tends to form on the inferior and superior surfaces of lower velocity impacts and on the medial and lateral surfaces of higher velocity impacts. For example, bone hackle was observed 46% on the superior surface and 22% on the inferior surfaces for the .22 caliber, while it was only observed 11% on the superior surface and 20% on the inferior surfaces for the .30 caliber impacts. Wallner lines also appeared to follow this pattern; however, these were infrequently observed. Also, bone mirror and wake features were not observed frequently enough to make any statements regarding overall orientation of these features on the fracture surface.

Bevel formation did not show a pattern between the different velocities. There was considerable overlap between the two calibers, with the angles varying between 60 and 70 degrees. This suggests that bevel angle may not be reliable for differentiating between velocities. This study will be expanded to provide a more robust evaluation of these fractography features and their utility for reconstructing gunshot wounds in long bones.

**References:**

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**Trauma; Projectile Injuries; Fractography**

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**A43 The Microscopic Characteristics of Peri- and Postmortem Fracture Surfaces**

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**Learning Objective:** After attending this presentation, attendees will understand the effect of decreased bone elasticity on the microscopic appearance of fracture surfaces.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of examining microscopic fracture characteristics to elucidate fracture timing.

Differentiating perimortem and postmortem injuries is essential to determining cause and manner of death. Though straightforward when soft tissue is present, these determinations are more challenging with skeletonized remains. Bone retains elasticity well into the postmortem interval, limiting the use of macroscopic analysis to distinguish perimortem injuries from postmortem damage.<sup>1</sup> Similarly, our preliminary research on the fractographic approach described by Christensen et al. found that fractographic features persist into the postmortem interval up to 40,000 Accumulated Degree Hours (ADH) and cannot be used to determine fracture timing.<sup>2</sup> This study uses Scanning Electron Microscopy (SEM) to assess microscopic surface characteristics of experimentally induced fractures in human bone at various postmortem intervals. We hypothesize that microscopic fracture characteristics, including delamination, osteon pullout, and microfractures, may differ as bone elasticity decreases, elucidating perimortem and postmortem events more reliably than macroscopic analyses.

Thirty-seven unembalmed, defleshed human femoral shafts from males and females aged 33 to 81 years were included in this study (periosteum intact). Eight femora were used as the perimortem sample and 29 femora were placed in a ThermoFisher™ Heratherm™ gravity convection oven at 25°C–35°C to simulate postmortem intervals ranging from 1,000–40,000 ADH. A data logger was used to monitor and record temperature and humidity. Bones were weighed before and after removal from the oven to estimate water loss, and a 1cm sample was cut from each shaft for histochemical analysis of collagen. Next, the shafts were fractured with a Columbus McKinnon (CM) Drop Test Frame using three-point bending and drop height of 100–275mm. Fracture tests were recorded with a high-speed camera, and Digital Image Correlation (DIC) was used to calculate stress and strain across the bony geometry, as well as displacement and location of failure.

Fractographic features were documented for each fracture surface, and DIC film was used to verify tension and compression zones. Next, SEM micrographs were collected from the primary tension and compression zones of each fracture, as well as from various fractographic surface features (e.g., hackle, mirror, cantilever curl, arrest ridges). A region of interest was defined within the center of the primary tension and compression zones, and three microscopic fracture characteristics were scored: percentage of delaminated osteons, percent osteon pullout, and number of microfractures. Multiple linear regression analysis was conducted to examine if the microscopic variables inform fracture timing. Additionally, Pearson's correlation tests were run to assess the relationship between each variable and ADH.

The multiple regression model accounts for a significant amount of the variation in ADH,  $F(3, 52) = 150.7$ , adjusted  $R^2 = 0.89$ ,  $p < .001$ . These results suggest fracture surface morphology varies significantly as ADH increases. The effect size is 0.49 (80% power,  $\alpha = 0.05$ ), so a medium effect would be detected with this sample size. Pearson's correlation tests indicated a strong negative correlation between increased ADH and osteon pullout ( $R = -0.91$ ,  $p < 0.05$ ), suggesting this characteristic is associated with perimortem fractures. Delamination and microfractures exhibited strong positive correlations with increasing ADH ( $R = 0.91$  and  $0.85$ , respectively,  $p < 0.05$ ), indicating their higher prevalence in postmortem insults to the bone. Statistically significant relationships also existed between the three characteristics and water loss.

The hypothesis was accepted, suggesting microscopic fracture surface characteristics visible on SEM may predict fracture timing more reliably than macroscopic features. Our results show that postmortem water and collagen loss affect elasticity considerably. Microscopic analysis of fracture surfaces is more sensitive to detecting evidence of decreased elasticity than macroscopic analysis. However, pathological conditions (osteoporosis, cancer) and demographic characteristics (age, sex) may affect elasticity and, consequently, microscopic fracture characteristics. These factors should be documented and factored into forensic analyses and reports.

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**Bone Fractures; Scanning Electron Microscopy; Accumulated Degree Hours**

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**A44 Distinguishing Between Peri- and Postmortem Fractures to Bone in the Sonoran Desert**

Marlen Juarez Lopez, BS\*, Arizona State University, Phoenix, AZ; Kaitlyn Fulp, MA, Arizona State University, Scottsdale, AZ; Katelyn Bolhofner, PhD, Arizona State University, Glendale, AZ

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**Learning Objective:** After attending this presentation, attendees will better understand the characteristics used to distinguish perimortem and postmortem trauma and the timing associated with the transition between these states in a desert environment.

**Impact Statement:** This presentation represents a critical initial step in systematically describing the transformation of fracture characteristics in bone from the perimortem to the postmortem period in the Sonoran Desert. The results demonstrate that these changes can be identified along a continuum, uniquely accelerated by this dry, arid environment in comparison to published timelines for other regions across the United States.

Estimating the timing of injury to the skeletal system, particularly as represented by fractures, is a key contribution of forensic anthropology to the medicolegal system. While variables such as the stage of healing or the type or location of the fracture aid in this endeavor, in many instances distinguishing between peri- and postmortem occurrence is difficult given the ability of bone to remain “wet” for a varied period beyond the individual’s time of death. Investigators often rely on the examination of fracture characteristics, namely color variation, fracture morphology, and microscopic characteristics, to differentiate between peri- and postmortem breakage. Previous research has shown that fractures occurring closer to the time of death exhibit smooth fracture surfaces with visible acute or obtuse angles and “V-” or “C”-shaped outlines. Fractures that occurred after a longer postmortem interval displayed jagged fracture surfaces, right angles, and intermediate to transverse fracture outlines; however, many factors influence the timing and manifestation of these varied characteristics in bone.<sup>1</sup> While the timing of the transition from peri- to postmortem characteristics has been experimentally generated for a number of geographic regions, there is currently no systematic description of this transition for a desert environment. Here, we present experimental data derived from a desert environment for the purpose of better understanding those characteristics associated with peri- vs. postmortem fracture to bone.

The Sonoran Desert, which covers a large portion of the southwestern United States and northern Mexico, is a unique environment with extreme fluctuations in temperature and humidity. These conditions can produce rapid decomposition, unique taphonomic changes, and challenges in estimating the timing and mechanism of fractures observed in exposed remains.<sup>2</sup> To better understand the effects of this environment on fracture characteristics in bone, we utilized a controlled drop apparatus to fracture 26 leg bones from porcine models (*sus scrofa domesticus*) exposed to a natural desert environment, over regular intervals for 12 weeks. Specimens were placed in a fenced environment and temperature and humidity were logged at hourly intervals. A drop apparatus was constructed based on the specifications described in published research to fracture the bones consistently with a dynamic force of 19,876.56 kg/m<sup>2</sup>. Two bones were fractured as a control (week 0), while the remainder were fractured in pairs at weekly intervals. Photographs were taken prior to and post-fracturing, and videos were taken of the fracture process. Fracture angle, outline, and surface texture were recorded.

The results reveal a transition to postmortem characteristics as much as one month sooner in this environment than in other milder regions. This study was conducted in the fall/winter months in the Sonoran Desert, when temperatures ranged from 31°F to 97°F and humidity ranged from 13% to 87%. Bones fractured on weeks 1-3 displayed solely perimortem characteristics: smooth fracture surface texture, acute/obtuse angles, and C-shaped fracture outlines. By week 4, postmortem fracture characteristics were noted, specifically the presence of right angles, but other perimortem characteristics persisted. By week 8, postmortem characteristics were more evident than perimortem, with right angles, transverse outlines, and rough fracture surfaces present. On the final and 12th week, bones fractured displayed predominantly right angles, transverse outlines, and rough fracture surfaces. The timelines produced by studies conducted in more mild climates cannot account for the patterning seen here, thus these models may be inappropriate for use in interpreting peri- and postmortem fracture timing in a desert environment.<sup>1,3,4</sup> This extreme climate alters taphonomic interpretations and conclusions, and further research into the full scope of the implications of this difference in the timing of fracture characteristics is necessary.

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**Sonoran Desert; Trauma; Postmortem**

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**A45 The Thermal Damage Analysis Resulting From a Controlled Burn**

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**Learning Objective:** After attending this presentation, attendees will better understand skeletal thermal patterns resulting from a controlled fire as part of a continuing education course at Western Carolina University (WCU).

**Impact Statement:** This presentation will impact the forensic science community by providing patterns of thermal damage and a greater understanding of the pugilistic pose based on a controlled burn using a willied body donor's remains.

In Spring 2021, forensic anthropology faculty, staff, and students led a continuing education course for public safety officials in conjunction with Certified Fire Investigators from the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF). As part of this course, a willied body donor to the WCU Body Donation Program was clothed and placed inside a constructed flashover cell at WCU's Forensic Osteology Research Station (FOREST). The individual was subjected to a fire of upwards of 1,400 degrees Celsius for seven minutes. Following a two-day cooling period, they were moved from the burn cell to the surface enclosure of FOREST to decompose. A cage was later placed on the donor to limit scavenging from vultures and opossums. The burn process was recorded from multiple angles, permitting a unique opportunity to view the effects of heat flux impact on the human body during the evolution of a compartment fire. This presentation outlines the body's movement into the pugilistic pose and how evidence of the burn process can be observed on the donor's skeletonized remains, helping to inform what thermal patterns can be anticipated on human remains in fires with similar conditions.

On the day of the controlled burn, a small fire was ignited in the southwest corner of the three-walled flashover cell in a trash can containing paper materials to represent a fire started by a cigarette. The fire quickly spread up the walls of the room and migrated to other furnishings until the room reached flashover, or when all remaining combustible materials simultaneously ignite. Soon after flashover, the fire was extinguished by the local fire department so the structure would not become too damaged to safely enter.

The donor started moving through the stages of burning as defined by Pope and colleagues within two-and-one-half minutes after the fire started; the left hand moved up and away from the body, followed by the head shifting toward the fire, then arching at the neck.<sup>1</sup> Within 30 seconds of these movements, the feet angled proximally. The right hand then moved up and away from the body, with all these movements occurring within a one-minute window.

The donor was recovered from FOREST in November 2021. Most of the thermal damage to the skeleton was concentrated on the skull and limbs. The anterior maxillary dentition was burned to the cervicoenamel junction. The cranium had thermal damage to the nasal, frontal, zygomatic, maxillae, and parietal bones; both sides of the mandibular body also had damage. The clavicles had thermal damage to the anterior aspect, with the left being more damaged than the right. Both radii had thermal damage to the inferior aspect of the posterior surface of the bones; the left ulna also had damage to the inferior aspect of the posterior surface. Thermal damage on the left hand extended to the carpals, while only extending to the metacarpals on the right hand; animal scavenging likely led to incomplete recovery of both hands. Both tibiae had thermal damage along the inferior aspect of the anterior surface of the bones. The pedal phalanges were mostly burned away, with thermal damage extended to the tarsals on both feet. All damage was consistent with a normal burn pattern.<sup>2</sup>

The course is offered annually at WCU for law enforcement and fire professionals, with the 2022 donor currently decomposing at the FOREST. This collaboration between WCU and the ATF permits generation of a unique thermal damage collection created from willied body skeletal remains burned under various controlled conditions. This collection has great potential to create a greater understanding of how human bodies burn under various conditions.

**Reference(s):**

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- <sup>2</sup>. Symes SA, Rainwater CW, Chapman EN, Gipson DR, Piper AL. Patterned Thermal Destruction of Human Remains in a Forensic Setting. In: Schmidt C.W., Symes S.A. (Eds.), *The Analysis of Burned Human Remains*. Elsevier Ltd., Burlington, pp. 15-54.

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**Fire; Thermal Damage; Skeletal Analysis**



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**A46 Reference Materials for Stable Isotope Analysis of Bone: Current Availability and Needs**

*Julianne Sarancha, MS\*, Arizona State University, Tempe, AZ; Gwyneth Gordon, PhD, Arizona State University, Tempe, AZ*

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**Learning Objective:** After attending this presentation, attendees will have learned the current availability of Reference Materials (RMs) for research and casework. Attendees will also know how they can implement matrix-matched standards in their own research or casework to improve the reliability of the analyses.

**Impact Statement:** This presentation will impact the forensic science community by discussing reference materials for Quality Control (QC) measures in stable isotope analysis of bone. By discussing current availability and needs in the field, researchers can ensure they are practicing the highest level of quality control currently achievable. This presentation will also open a dialog to consider what reference materials could be developed and implemented to meet the needs of the field.

The reliability of stable isotope analysis heavily depends on the availability and use of RMs and Certified Reference Materials (CRMs) to provide QC measures for analyses. In addition to CRMs and RMs, laboratories also rely on in-house standards. In-house standards are typically easily amassed in large quantities, may be a better matrix-match to common samples in a particular laboratory, and are validated against a CRM or RM. In-house standards provide a cost-effective standard while preserving limited CRMs and RMs. External researchers using centralized analytical facilities commonly cede QC measures to the analytical facility without ensuring QC has been optimized for analysis of a specific material. Without proper QC, stable isotope analysis of bone may provide inaccurate geographic and dietary inferences.

For confidence in the accuracy and precision of data, at a minimum, analyses should utilize normalizing standards, linearity standards, check standards, and matrix-matched standards. Matrix-matched standards can undergo sample preparation in-line with samples, a frequently neglected QC. Isotope analysis of bone in forensic contexts include  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  analysis of carbonate,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of collagen, and  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis of whole bone. Some currently available RMs relevant to isotopic analysis of bone are USGS-88 (marine collagen from wild-caught fish), USGS-89 (porcine collagen), NIST-1400 (Bone Ash), and NIST-1486 (Bone Meal). However, there are critical gaps in available RMs.

For  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of carbonate, there are currently no matrix-matched bone RMs, so geologic analytical standards such as marble are used. USGS-88 and USGS-89 are two collagen supplement RMs often used for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis of bone collagen. While these RMs are useful as analytical standards (i.e., monitoring instrumentation performance), they cannot undergo the sample preparation process since they are powdered and already isolated from the mineral matrix. There are currently no RMs for processing alongside samples to validate protocol precision and accuracy for collagen. For  $^{87}\text{Sr}/^{86}\text{Sr}$  analysis, NIST-1400 and NIST-1486 are CRMs used as in-line standards; however, both CRMs have Ca/Sr that differ from human bone, making them less optimal standards for validating sample preparation methods. Additionally, NIST-1400 has been ashed, and NIST-1486 has been steamed, so organics are degraded in both.

Standards to monitor instrumental performance need improvement. However, there is an urgent and critical lack of matrix-matched standards that can undergo sample preparation. Preparing matrix-matched RMs in-line with samples allows researchers to integrate QC independent of the analytical facility and can also serve to validate a method as fit-for-purpose. To address this challenge, our laboratory sought a potential standard that would provide a substantial amount of solid bone with known geographic origin to be processed and measured to monitor external reproducibility of isotopic analysis. Rib bones from a two-year-old locally raised grass-fed beef steer (“Elton”) were obtained. The rib bones were sectioned into subsamples and characterized as standards that are now used routinely as reference materials for analyses requiring a chunk or powdered bone starting material. A second standard of human cremains (“JWS”) addresses other limitations of the bovine RMs and CRMs. These cremains are powdered ash and are used as a reference material for strontium analyses. Although other labs implement similar sample preparation standards that increase the QC of the analyses, the field lacks suitable in-line standards to validate methods between laboratories.

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**Stable Isotope Analysis of Bone; Quality Control; Reference Material**

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**A47 W.E.I.R.D. Water: Rethinking the Bias in Our Forensic Isotopic Provenancing Methods**

*Taylor Lambrigger, MA\*, California State University, Chico, Gilbert, AZ*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the bias present in existing models relating the oxygen isotopes in human hair to drinking water, and how these biases impact forensic provenancing efforts in global humanitarian crises, like the United States-Mexico border.

**Impact Statement:** The application of stable oxygen isotopes to aid in forensic provenancing efforts for international migrant crises has increased in recent years. However, recent evidence indicates that assumptions relating to oxygen isotope fractionation and drinking water access strongly influence region of origin predictions. This presentation will impact the forensic science community by urging attendees to critically evaluate the methods being used internationally, and to address what may be inherent Western biases in our methods.

Forensic applications of stable isotope analysis have grown increasingly common in recent years. A set of biogeochemical methods, stable isotope ratios in human tissues reflect isotopic composition of the food and water consumed by an individual. Oxygen isotopes in particular represent imbibed water, and because drinking water is geographically patterned, oxygen can elucidate an individual's region of origin. These methodological developments have largely occurred over the past two decades in the United States and areas of Europe, though recent applications of the method span the globe.

The interpretation of region of origin is predicated upon practitioner's understanding of the relationship between the  $\delta^{18}\text{O}$ , or oxygen isotopic value, of hair to  $\delta^{18}\text{O}$  in water. The first fractionation model was proposed by Ehleringer and colleagues in 2008 and was derived from a modern United States population.<sup>1</sup> The model first assumes that the  $\delta^{18}\text{O}$  of food ingested by an individual is constant because of the "continental supermarket diet" present in the United States; however, this concept is highly specific to the United States and other Western industrialized nations. Researchers found that rather than a homogenous continental diet, locally derived diets fit the reality better and, as such, created a local food parameter (l) instead of a constant, allowing this parameter to be tuned to the population being studied.<sup>2</sup> There is, however, a second assumption made in the model: that tap water is isotopically representative of drinking water. Many studies have upheld this assumption, but most were done in the United States and few, if any, were done in Mexico, leaving a gap in the literature.

The development of these methods in the United States and parts of Europe must be addressed. Emerging out of the psychology literature, the acronym WEIRD (Western, Educated, Industrialized, Rich, and Democratic), was introduced as a critique of psychological research focusing their sample populations in these WEIRD societies.<sup>3</sup> Other research into water has developed a similar framework: Modern Water.<sup>4</sup> It is argued that when water is viewed as "modern," it simultaneously becomes associated with uniformity and universality.<sup>4</sup> This study argues that oxygen isotope analyses have developed within this WEIRD, modern water framework: the idea that tap water is universally available and accessed, and thus synonymous with drinking water, is a core assumption in interpretations and applications of the oxygen isotopic method.

This is particularly relevant for forensic practitioners involved in international migrant crises as the literature suggests that the factors that contribute to the structural violence influencing migrants to leave are often also tied to the water insecurity those migrants experience. Previous studies have found that structural vulnerabilities are skeletally embodied, and thus these migrants may also have isotopically embodied the structural vulnerabilities they are fleeing as well.

In recent work done in Oaxaca, Mexico, these assumptions were seen to influence region of origin predictions. Hair samples from locations throughout Oaxaca were put through four iterations of Ehleringer's model, each with different (l) parameters. The predicted drinking water from each model iteration was then compared to measured tap and bottled water from the same locations. Comparing predicted drinking water with measured tap water neared significance much more than those comparisons with measured bottled water. This is expected, as the leading methodology up to this point is to sample tap water; however, previous data has shown that drinking water is not isotopically defined by tap water in Oaxaca, suggesting that the WEIRD water assumptions in existing fractionation models are deficient. Because these models do not tune any parameters related to consumed water source, they fall short in their ability to accurately predict water, and consequently region-of-origin.

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**Stable Isotope Forensics; Global Provenancing; Forensic Anthropology**



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**A48 The Construction of a Bioavailable Strontium Isoscape for South Korea and its Forensic Application**

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**Learning Objective:** After attending this presentation, attendees understand the importance of strontium isotope analysis in forensic provenance and a bioavailable strontium isotope ratio map (isoscape).

**Impact Statement:** This presentation will impact the forensic science community by discussing the process of a strontium isoscape for South Korea and how strontium isotope analysis can apply for forensic provenance.

Strontium isotope analysis has been widely applied for tracing human migrations and provenance in the field of archaeology, ecology, and forensic science. As one of the alkaline earth metals existing in rocks, strontium (Sr) has four naturally occurring stable isotopes:  $^{84}\text{Sr}$ ,  $^{86}\text{Sr}$ ,  $^{87}\text{Sr}$ , and  $^{88}\text{Sr}$ . The radioactive  $\beta$ -decay of rubidium-87 ( $^{87}\text{Rb}$ ) produces radiogenic  $^{87}\text{Sr}$  with a half-life of  $48.8 \times 10^9$  years. Bedrocks of different ages and Rb/Sr ratios have variable Sr isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) that can be transferred to a human body through food intake, including groundwater and local food products. It is expected that  $^{87}\text{Sr}/^{86}\text{Sr}$  of a human body will correlate with environmental samples (groundwater, soil, rocks, plants, etc.) in a specific region. To obtain useful geographical information on specimens, a reference Sr isotope ratio map (isoscape) in the region of interest is required.

This study focused on the construction of a bioavailable Sr isoscape using human tooth (molar) enamel considering environmental contribution. A total of 156 dental samples were collected from individuals with necessary information, including a place of birth and address depending on age classifications. Each dental sample was then prepared for Sr quantification after a series of multiple steps for cleaning, grinding, and acid digestion. The quantification of Sr concentration and measurement of  $^{87}\text{Sr}/^{86}\text{Sr}$  were performed using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS, 8800 Triple Quad, Agilent® Technologies) and Multi-Collector-Inductively Coupled Plasma/Mass Spectrometry (MC-ICP/MS, Sapphire, Nu® Instruments), respectively. Due to a limited number of dental samples, obtained data for  $^{87}\text{Sr}/^{86}\text{Sr}$  of dental samples were interpolated by Inversed Distance Weighting (IDW), kriging, and spline (RBF-ST) methods using a coordinate-based Geographic Information System (GIS). To evaluate the prediction accuracy, the dataset ( $n=156$ ) was randomly divided into the training (80%) and test set (20%). RBF-ST ( $r^2=0.46$ ), which has relatively higher prediction accuracy than IDW ( $r^2=0.36$ ) and kriging ( $r^2=0.40$ ), was selected as the optimal interpolation model in this Sr isoscape. The environmental contribution was also considered by comparing the spatial correlation between tooth  $^{87}\text{Sr}/^{86}\text{Sr}$  and previously obtained environmental samples (soils, groundwater, and hairs). The results showed that the  $^{87}\text{Sr}/^{86}\text{Sr}$  of the tooth (molar) has a higher correlation with that of groundwater ( $r^2=0.76$ ) and a better high correlation for only female tooth ( $r^2=0.88$ ).

As the first bioavailable Sr isoscape for South Korea using dental samples, this Sr isoscape will be a useful tool to estimate the geographical origin of unknown human skeletons such as teeth and femurs in cold cases.

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**Strontium Isotope; Forensic Provenance; Isotope Analysis**

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**A49 The Rapid Association of Commingled Remains**

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**Learning Objective:** Following this presentation, attendees will understand how commingled skeletal remains can be reassociated based on the bones' chemical profiles obtained via Laser-Induced Breakdown Spectroscopy (LIBS).

**Impact Statement:** This presentation will impact the forensic science community by providing a novel tool for sorting mixed skeletal assemblages based on chemical information, supplementing existing physical methods for reassociation.

The commingling of human remains poses an obstacle for death investigations in both modern and archaeological forensic contexts. After recovering a mixed assemblage, anthropologists face the challenge of sorting each skeletal element to its proper individual. Using physical features and osteometric methods, the reassociation process can be a tedious process, especially if bones have undergone fragmentation or taphonomic changes; however, in addition to specific physical traits, bones also have chemical profiles representative of the individual. This information provides useful, discriminatory data for sorting.

This study proposes that the LIBS signatures obtained from bones in commingled assemblages can be used as a preliminary sorting technique. LIBS is an analytical technique well-suited for acquiring chemical information from bones. It requires no sample preparation and provides an emission spectrum within seconds that is representative of the sample surface composition. LIBS is also a quasi-non-destructive method, showing no noticeable indication that material has been removed from the bone during analysis. Further, LIBS technology is available in portable, field-deployable instruments. As much of the casework forensic anthropology begins out in the field, hand-held instrumentation conveniently aids in efficient analysis.

To simulate data collection from a mass grave, the skeletal remains of 12 individuals were obtained from the Forensic Osteology Research Station (FOREST) decomposition facility at West Carolina University. A data set was created by acquiring LIBS spectra from multiple locations on 28 bones for each individual, providing a total of more than 2,000 chemical signatures to classify. Following data reduction and optimization, supervised learning algorithms were used to build discriminant models for the classification of each individual. These models were able to correctly match unclassified bones to their corresponding individuals with greater than 90% accuracy. Further statistical analysis of the spectral data set provided insight on the significance of some trace elements responsible for the variation between each set of skeletal remains.

The results of this study illustrate how the chemical profiles of bones help expedite the sorting process for skeletal assemblages and demonstrate the usefulness of portable LIBS as a potential tool to help forensic anthropologists reassociate commingled remains directly in the field.

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**Commingled Remains; LIBS; Statistical Analysis**

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**A50 An Update on Standards Development Activities Related to Forensic Anthropology**

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**Learning Objective:** After attending this presentation, attendees will have an appreciation of the efforts the Organization of Scientific Area Committees (OSAC) Forensic Anthropology Subcommittee and the Academy Standards Board (ASB) Anthropology Consensus Body have made to draft, develop, and publish standards, as well as better understand the current status of discipline-specific standards related to the field of forensic anthropology.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of forthcoming and existing standards pertinent to forensic anthropology, including ASB publication and OSAC Registry placement. This presentation will also reiterate the importance of active participation in the standards development process by stakeholders during open comment periods.

The OSAC for Forensic Science was established in 2014 to facilitate the development of discipline-specific standards that are of high-quality and technically sound. OSAC provides drafts of proposed standards to Standards Developing Organizations (SDOs), which further develop and publish them. Additionally, OSAC posts these published standards on the OSAC Registry and promotes and encourages their use by the forensic science community.

This presentation will summarize recent standards development activities by the OSAC Forensic Anthropology Subcommittee and the Anthropology Consensus Body of our chosen SDO, the ASB. Updates regarding the status of various drafts, OSAC-proposed standards, and SDO-published standards will be presented, including: SDO-published standards that are currently posted to the OSAC Registry; SDO-published standards that previously stalled in the OSAC Registry Approval process; SDO-published standards that have begun the OSAC Registry Approval Process (1.5 and 2.0 versions); OSAC-proposed standards that have been withdrawn from the OSAC Registry Approval Process; new OSAC-proposed standards at the Task Group and Subcommittee levels; OSAC-proposed (or other) standards that are currently undergoing additional development at the ASB Working Group and Consensus Body levels; and proposed standards that are currently open for public comment or in comment adjudication at the ASB.

Additionally, this presentation will identify topic areas under consideration for future proposed standards and discuss potential collaborative opportunities for standards development relevant to the discipline of forensic anthropology.

Both the OSAC Forensic Anthropology Subcommittee and the ASB Anthropology Consensus Body underwent leadership changes in 2022. As a result, the two entities have renewed their commitments to work transparently and collaboratively on efforts to produce consensus-based standards for the field of forensic anthropology. The breadth of experience and technical knowledge of members of the OSAC Forensic Anthropology Subcommittee and the ASB Anthropology Consensus Body is notable, but the content and clarity of proposed standards are often improved by suggestions obtained during open comment periods. These open comment periods are purposefully built into the standards development process to allow stakeholders and the general public an opportunity to provide feedback. Whether as part of the OSAC, ASB, or commenting public, active participation in the standards development process is of critical importance to the production of high-quality and technically sound standards for the practice of forensic anthropology.

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**Standards; Forensic Anthropology; OSAC**

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**A51 What Makes a “Good” Forensic Anthropologist?**

*Allysha Winburn, PhD\*, University of West Florida, Pensacola, FL; Meredith Marten, PhD, MPH, University of West Florida, Pensacola, FL; Benjamin Burgen, PhD, University of West Florida, Pensacola, FL; Spencer Seymour, MA, University of West Florida, Milton, FL; Taylor Walkup, MA, University of Tennessee, Knoxville, Knoxville, TN*

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**Learning Objective:** After attending this presentation, attendees will appreciate the consensus that exists among forensic anthropologists surrounding what constitutes “good” practice.

**Impact Statement:** This presentation will impact the forensic science community by highlighting areas of agreement among forensic anthropologists from multiple, and sometimes overlapping, work contexts, thus fostering intra-subdisciplinary collaboration.

Forensic anthropology has recently and publicly grappled with fundamental disciplinary issues—including assigning race in decedent identification, the pursuit of objectivity, and the role of bias in medicolegal contexts—that have led to a state of seeming fracture. Using the method of Cultural Consensus Analysis (CCA), this study examined if polarization exists within the subdiscipline, to what degree, and along what lines. Cultural consensus theory derives from cognitive anthropology, which conceptualizes culture as a product of the mind: members of a particular group learn and share cultural ideas and beliefs and organize them conceptually in a coherent way. The degree to which these standards or cultural “truths” are shared among group members can be statistically tested using CCA, a form of factor analysis that ranks how important, relevant, or resonant particular concepts are to members of a group.

Practicing forensic anthropologists were recruited via listservs and social media to participate in two phases of a Qualtrics®-based study. In phase one, respondents were asked, “What makes a “good” forensic anthropologist?” and were provided with text boxes to freelist terms and concepts. In phase two, respondents were presented with the resulting list of 44 terms and concepts and asked to rank them on a scale of one to four, with one indicating lack of importance and four indicating extreme importance to the “good” practice of forensic anthropology. Responses were analyzed using the UCINET software.

In total, 103 respondents completed the survey, 37 of whom were Diplomate of the American Board of Forensic Anthropology (D-ABFA). Many respondents (38, or 36.9%) worked in several professional settings simultaneously, including 26 (25%) working in both medicolegal and academic contexts. Most reported either conducting casework (66, or 64%) or assisting with casework (27, or 26%), with only ten (9.7%) reporting no casework within the last year.

In CCA, a ratio greater than 3:1 between the first- and second-largest eigenvalues indicates a shared cultural model, meaning respondents agree about a shared set of knowledge. In this survey, the eigenratio was 4.506:1, indicating that contrary to widespread concern, consensus exists over the training, experiences, perspectives, and practices important for “good” forensic anthropology. Respondents agreed that “good” forensic anthropologists possess both general scientific acumen and specific expertise in human skeletal and dental anatomy, along with the technical and interpersonal skills necessary to effectively convey analytical results. They are self-reflexive, committed to the process of cognitive bias mitigation, and engaged with a broad body of theory.

The primary area of disagreement surrounded the idea that analysts should remain neutral in the face of issues of social importance. The fault lines of this debate primarily fell along generational lines, with those having earned their degrees earlier believing more strongly in neutrality. This pattern largely maps onto broader (and somewhat routine) disciplinary debates and trends away from positivism, with younger anthropologists more focused on the larger work of decolonizing anthropology and attending to the anti-racist work that figures prominently in anthropology today.

One of the strengths of CCA is that it highlights whether and how contention exists within a field—and when it does not. Despite limited disagreement, a consensus definition of “good” forensic anthropology emerged. Given these results, the idea of subdisciplinary divide may be exaggerated, and continuing debate may be representative of progress, not fracture. Science works, this study affirms, and together, we work toward a shared model of equitable and just scientific practice.

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**Forensic Anthropology; Cognitive Anthropology; Cultural Consensus Analysis**

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**A52 Repatriation Beyond Native American Graves Protection and Repatriation Act (NAGPRA): The Ethics of America's Osteology Collections**

*Lyndsey Hornagold, MS\*, Binghamton University, Johnson City, NY*

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**Learning Objective:** This research will educate attendees on the ethics of non-donor-based osteological collections within universities and museums, as these collections are primarily made up of marginalized groups. Individuals from countries and groups that have been unethically incorporated into osteology collections but receive little coverage will also be discussed. Last, attendees will be given a baseline on how countries outside of the United States have already begun to repatriate individuals within these collections.

**Impact Statement:** The forensic science community, through this presentation, will be forced to think about whether repatriation of individuals collected without consent should go beyond those protected under NAGPRA through a transitional justice perspective. Further, the forensic science community will become more aware of the vast number of ancestral backgrounds that have been incorporated into American osteology collections without consent.

Binghamton University's teaching osteology collection ( $n=26$ ) was used as an example of the ancestral makeup of a non-donor-based collection. Ancestry was estimated by inputting craniometric measurements into AncesTrees. The ancestral makeup of Binghamton's collection is: 19.2% Southwestern European, 15.4% Sub-Saharan African, 15.4% East Asian, 11.5% Australian/Melanesian, 11.5% South American, 11.5% South Asian, 7.7% Southeast Asian, 3.8% Northeast African, and 3.8% Northern Asian/Arctic. Only five (or 19.2%) of the individuals within Binghamton's collection are of European descent, while the other individuals are from historically marginalized, plundered, or colonized countries; however, even those of European descent have been unethically placed in American osteological collections. Impoverished European immigrants that came to the United States in the 19<sup>th</sup> century as well as executed criminals were the main individuals incorporated unethically into osteological and other medical collections.<sup>1</sup>

Historically, marginalized and ancestrally diverse individuals have been non-consensually placed into osteology collections. Many remains acquired by university and museum collections do not have definitive documentation stating the individual's origin. This indicates that there was no consent from the donor or their next of kin for their remains to be donated to science, thus these remains should be repatriated if possible. This ethical debate within anthropology is equivalent to the debate leading up to the passing of NAGPRA. Non-native individuals who have not consented to having their bodies donated to collections are still those who have been impacted by colonization, war, and neglect. Repatriation of these remains would begin to mend the strained relationships between anthropologists and the descendants and countries of these individuals unethically contained within American osteology collections.

However, a complete repatriation of non-donor-based osteology collections would not have to be the end of education or research involving human remains. There are multiple universities within the United States with large, donor-based collections (e.g., the University of Tennessee and the University of New Mexico). These schools could begin to lend these remains to universities or museums that currently do not have a donor donation program. At the very least, these institutions could partner with other universities or museums to foster the creation of more donor-based collections within the United States.

**Reference:**

- <sup>1</sup>. Jones DG, Whitaker, MI. Anatomy's use of unclaimed bodies: Reasons against continued dependence on an ethically dubious practice. *Clin. Anat.* 2012; 25: 246-254. doi: 10.1002/ca.21223.

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**Skeletal Collections; Transitional Justice; Repatriation**

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**A53 Data on Methods Usage in Forensic Anthropology Casework From 1972–2022 Using the Forensic Anthropology Database for Assessing Methods Accuracy (FADAMA).**

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**Learning Objective:** The goals of this study are to understand methods usage across time, assess methods adoption, and discuss potential limitations.

**Impact Statement:** This presentation will impact the forensic science community by providing the most recent data available on methods usage across all components of the biological profile over time. This presentation will examine methods adoption among methods developed pre-2000 and post-2000 and identify possible bottlenecks to adoption.

The use of specific methods for estimating the biological profile in forensic anthropology casework is tied to factors including available elements, methods validation, and practitioner training. This study investigates cases uploaded to FADAMA (<https://www-app.igb.illinois.edu/sofadbf/>) for which data on 101 different methods was available. The case sample is dated from 1972 to 2022 ( $N=410$ ). For sex, age, ancestry, and stature estimation there were  $N=445$ ;  $N=1,076$ ,  $N=356$ , and  $N=168$  instances of methods use recorded by practitioners in the database, respectively. Results indicate a focus on a small group of methods for each category of the biological profile over time and limited adoption of newer methods. Five sex estimation methods represented 85% of the methods used with a median methods date of 2006. Four age estimation methods represented 60% of the methods used, with a median methods date of 1987. Four ancestry estimation methods represent 93% of the methods used with a median methods date of 2005. Two stature estimation methods represent 92% of the methods used with a median methods date of 1996.

Of all methods categories, age estimation demonstrated the greatest variety of methods in the database ( $N=47$ ), the most instances of recorded methods use ( $N=1,076$ ), and the largest variation in methods used. For methods uptake, methods created from 2000 to present were analyzed. There were  $N=16$  methods with a publication year of 2012 or later, and  $N=42$  methods with a publication year between 2000 and 2011. A single factor Analysis of Variance (ANOVA) demonstrated a significant difference between methods usage overtime ( $P=2.19804E-93$ ) with methods published between 2000–2011 representing the majority of usage for both time periods. Taking the dataset as a whole, the use of some older methods (pre-2000) significantly increases over time, while the vast majority of recent methods gain little traction. In addition, the use of non-specific methods (e.g., generalized morphology) was also investigated. For estimation of sex, non-specific methods made up 26.5% of the total methods use over all time periods. Problematically, in the majority of instances, cases indicating the use of “generalized morphology” contained no further indication of what was analyzed or how it was done. These trends suggest the need to investigate how factors like method validation (or lack of), pedagogical traditions, and systematic access to methods training impact the selection of methods used in casework.

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**Methods Usage; Forensic Anthropology; FADAMA**

## **A54 Age-Progressive Acetabular Timing for Age Estimation in African American Males: Preliminary Results**

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**WITHDRAWN**



## A55 Evaluating Aspartic Acid Racemization as a Novel Technique for Forensic Age Determination

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**Learning Objective:** After attending this presentation, attendees will better understand the best sample type that can be selected for the Aspartic Acid Racemization (AAR) age estimation method. A meta data analysis and systematic review of different tissue types ( $N=8$ ) (i.e., dentine, sclera, enamel, femur, cementum, biopsied tooth, alveolar bone, and tooth sample) collected after an autopsy have been done.

**Impact Statement:** This presentation proposes the best-suited source to estimate ages that may be helpful to future researchers by improving the reliability and accuracy of the ages evaluated. The exhibition will provide an in-depth review of the field. It will impact forensic science investigators and researchers by helping them adopt the best parameter for age estimation for missing persons and unidentified bodies.

Estimating the age-at-death of an individual is a challenging task in forensic death investigations, particularly for adults. Different methods developed in forensic anthropology have proven helpful for reliable age estimation of children.<sup>1</sup> However, only a few methods have been suggested for adults with limited accuracy, reliability, and reproducibility. AAR offers an alternative novel method of age estimations in adults, giving age estimates within acceptable forensic thresholds. AAR is the raw conversion of the optically active amino acid from its original Levorotatory (L) form to the Dextrorotatory (D) form over a period of time.<sup>2,3</sup> The accuracy of the AAR technique of age estimation is widely demonstrated and is being scrutinized by various current researchers.<sup>4</sup> However, only a few studies have assessed the comparative accuracy of different tissue samples for extracting aspartic acid and its utility for forensic age estimations. The main aim of the present research is to highlight the current status of research on this topic, the relative importance of different body tissues for the purpose and report accuracy levels of the AAR technique obtained by different researchers around the globe, and its applicability to forensic cases.

Current literature offers a diverse catalog of methods in age determination, targeting different source tissue, population groups, and age groups. A literature search was conducted to identify and shortlist different tissues as sources for age estimation by AAR. The initial search strategy resulted in 128 articles, and data were collected from accessible literature sources and scientific search engines like PubMed®, Scopus®, WoS, ScienceDirect®, and Springerlink®. Data were tabulated and systematically arranged for systematic review and meta-data analysis. Results obtained demonstrated that Pearson product correlation of source samples and age estimation were highly positive and statistically significant for the samples like biopsies tooth ( $N=101$ ) ( $r=0.998$ ,  $p<0.001$ ) error range:-3.2 to +1.7, dentine ( $N=333$ ) ( $r=0.989$ ,  $p<0.001$ ). The error range varied from -10 to +8.8 for tooth recovered after an autopsy, -11 to +13 for dentin and -12 to 0 for enamel ( $N=19$ ). High positive Pearson product correlation observed in sclera ( $N=10$ ) ( $r=0.850$ ,  $p<0.005$ ) with an error range of -11.23 to +32.25, and femur ( $N=39$ ) ( $r=0.854$ ,  $p<0.005$ ) with an error range of -19 to +19 years. Very low positive correlation and no significance was observed in cementum samples ( $N=32$ ) ( $r=0.283$ ) having an error range of -7 to +57 years, and a negative correlation in alveolar bone samples ( $N=47$ ) ( $r=-0.133$ ) with an error range of 28 to +56 years. The results concluded that the overall age estimation from aspartic acid racemization from tooth samples (including dentine, enamel, biopsied tooth, and tooth recovered after autopsy) provides the most reliable results and hence can be used as a source of age determination in the AAR method.

### References:

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### Aspartic Acid Racemization; Age Estimation; Forensic Anthropology

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**A56 The Impact of Pelvic Anomalies on Sacral Joint Surface Aging**

*Krista Bennett, MA\*, Louisiana State University, Baton Rouge, LA*

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**Learning Objective:** After attending this presentation, attendees will better understand how sacralization of terminal lumbar vertebra and unilateral sacral-iliac fusion can impact age-at-death estimations using the auricular surface of the sacrum.

**Impact Statement:** This presentation will impact the forensic community by providing an assessment of the applicability of sacral auricular surface age-at-death estimations from individuals with pelvic anomalies.

Pelvic anomalies (i.e., sacralization and sacral-iliac fusion) occur at varying rates among populations and often affect older individuals as the body attempts to maintain stabilization and functionality.<sup>1-3</sup> Previous research examined the effects of a sacralized terminal lumbar vertebra on age-at-death estimations derived from the auricular surface of the ilium using the Buckberry and Chamberlain revised aging method.<sup>4</sup> Findings suggested that changes in biomechanical load transmission through the pelvic region could result in accelerated age-degenerative changes to the joint surface.<sup>5</sup> Since the auricular surface of the sacrum is covered by a different type and thickness of cartilage, age degenerative features are often delayed and manifest later in life. Therefore, the potential effects of pelvic anomalies on the rate of degeneration of the sacral auricular surface are unknown. The current study seeks to determine whether the Passalacqua sacral aging method should be used when individuals present with either sacralization of the terminal lumbar vertebra or unilateral sacral-iliac fusion.<sup>6</sup>

A total of 150 sacra aged 29 to 96 years from the William Bass Donated Skeletal Collection were randomly selected for examination. Sacral auricular surfaces were scored using the seven features defined by the Passalacqua age estimation method.<sup>6</sup> Each sacrum was also evaluated for the presence and degree of sacralization of the terminal lumbar vertebra and unilateral sacral iliac fusion. For this study, three types of sacralization were identified. First, Type A, where only one side of the transverse process of the terminal lumbar vertebra is fused with the corresponding sacral ala. Second, Type B, where the vertebral body of the terminal lumbar vertebra is fused with the first sacral body segment, but neither transverse process is fused with either ala. Last, in Type C, both transverse processes were fused with corresponding sacral alae; the vertebral bodies of the terminal lumbar may or may not be fused with the first sacral segment. Additionally, unilateral sacral-iliac fusion was defined as a bony fusion between the sacrum and a single os coxae.

Results found that 21 (14%) individuals had one of the three types of sacralization, and nine (6%) individuals developed sacral-iliac fusion. Of the 21 individuals with sacralization, the breakdown of types is as follows: two with Type A, eight with Type B, and ten with Type C sacralization. The accuracy for age-at-death for individuals with sacralization was 62% and 95% for the 68% and 95% confidence intervals, respectively. For individuals with sacral-iliac fusion, accuracy was 56% and 89% for the 68% and 95% confidence intervals, respectively. Individuals who were not assigned accurately to a phase were either too young (i.e., 21 and 33 years) or too old (over 91 years) for either the 68% or 95% confidence intervals.

Individuals that were too young for their assigned phases displayed advanced age-related features such as ring absorption of the first sacrum and microporosity on the auricular surface. These results suggest the Passalacqua aging method can be reliable with sacralization of terminal lumbar or sacral-iliac fusion when using the 95% confidence intervals; however, the method should be used with caution when using the 68% confidence intervals as the accuracy drops significantly.<sup>6</sup> The findings from this study appear consistent with the previous examination of the impact of pelvic anomalies on joint surface aging, whereby changes in biomechanical loading can increase stress and degeneration to the joint surface resulting in older appearing age-related features.

**References:**

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2. Tague RG. (2009). High assimilation of the sacrum in a sample of American skeletons: Prevalence, pelvic size, and obstetrical and evolutionary implications. *American Journal of Physical Anthropology*, 138(4), 429-438.
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**Age-at-Death Estimation; Pelvic Anomalies; Sacrum**

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**A57 The Investigation of the Ectocranial Squamosal Suture to Estimate Age at Death**

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**Learning Objective:** This presentation will discuss the benefits and drawbacks of using cranial suture obliteration as an age estimation method. Attendees will learn about a newly developed technique discussed in the current study, and previously established cranial suture age estimation techniques, such as the one developed by Meindl and Lovejoy.<sup>1</sup> Additionally, attendees will learn about the reliability of usability of the current method presented in comparison with the Meindl and Lovejoy technique.

**Impact Statement:** This presentation will impact the forensic science community by conveying a potential new method that will assist in the age estimation of individuals over the age of 50. Most age estimation methods are more accurate for aging younger individuals and only provide large age ranges for older individuals. Additionally, the method discussed in the current presentation utilizes a small part of the cranium as opposed to the whole element. In forensic contexts where only fragments remain, this method could be useful in providing some insight into age.

A crucial component of forensic death investigations is estimating the biological profile. While there are many methods, there remain challenges for anthropologists in forming a full profile. Estimating the age of an individual, particularly assessing the age of older individuals (50+ years old), remains difficult. The age estimation methods that exist for older individuals are also less precise than methods for juvenile age estimation. Current methods focus on the innominate and the sternal rib margins, which are both susceptible to damage. Remains are often fragmentary or altogether missing, which makes developing the biological profile more challenging. Methods that involve more durable elements, like the skull, are therefore useful to examine as potential indicators because crania are often well preserved. While the examination of cranial sutures as an indicator of age has been addressed by researchers, a single focus upon the squamosal suture for advanced age estimation has not been conducted. Given that the ectocranial squamosal suture is one of the final sutures to close, it may be useful for indicating the age-at-death for older individuals.<sup>2</sup>

This research aims to examine the obliteration patterns of the ectocranial squamosal suture to determine whether there is a correlation between the degree of squamosal suture closure and older age (50+ years old). Additionally, this study compares the age estimation of this novel approach to the previously established method by Meindl and Lovejoy.<sup>1</sup> Measurements of 435 individuals ranging from 25–89 years old from the University of Tennessee, Knoxville (UTK) donated skeletal collection were taken. The squamosal suture was divided into six sections and scored 0–3 based on the extent of obliteration. Composite scores of each individual were calculated using the Meindl and Lovejoy technique.<sup>1</sup> Results from a one-way Analysis of Variance (ANOVA) indicate that four out of the six sections (A1 (F=3.96), P1 (F=3.22), P2 (F=3.53), and P3 (F=5.14)) have a significant relationship between obliteration score and age (all p's < 0.05). Since these four sections demonstrate a relationship between age and suture obliteration, they may be indicative of age and utilized in the estimation of age at death of individuals 50 years of age and older. Kendall's rank-order show equal correlations between all six sections of the squamosal and chronological age (Left: 0.13-0.20; Right: 0.10-0.16). From this, an age estimation method using the composite scores of the six sections of the ectocranial squamosal suture was developed for both left and right sides and appears to be useful for age estimation. We combined this squamosal suture focus with the traditional Meindl and Lovejoy method to establish a two-prong approach to cranial suture age estimation.<sup>1</sup> Results indicate that this novel approach appears to provide more precise age-at-death estimations for older individuals (50+ years old).

**References:**

- <sup>1</sup>. Meindl, Richard S, C Owen Lovejoy (1985). Ectocranial Suture Closure: A Revised Method for the Determination of Skeletal Age at Death Based on the Lateral-Anterior Sutures. *American Journal of Physical Anthropology*, 68(1): 57-66.
- <sup>2</sup>. Lockhart et al. (1959). *Anatomy of the Human Body*. Philadelphia, PA: J.B. Lippincott Company.

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**Age Estimation; Cranial Sutures; Biological Profile**

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**A58 Evaluating the Effects and Timing of Hydrochloric Acid (HCl, 38%) Destruction on Porcine Versus Human Dentition**

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**Learning Objective:** After attending this presentation, attendees will better understand morphological changes occurring from HCl acid immersion in porcine and human dental remains.

**Impact Statement:** This presentation will impact the forensic science community by addressing the ability to recognize morphological changes to tissues and the timing of HCl destruction in the context of body disposal for both porcine and human dentition.

HCl is a known method of destruction used as a means of body disposal. Teeth are more likely than other human substances to remain intact when exposed to an acidic environment; however, few forensic studies explore the timing and effects of acid on dental remains. To date, no studies have tested the effects of HCl on porcine models to determine whether they are comparable proxies to human dental remains for use in experimental design. This lack of information has established a limitation in assessing the effects of an acid environment on remains to establish Postmortem Interval (PMI) or to address other taphonomic factors in an experimental setting. In this study, it was hypothesized that porcine premolars and molars, due to their similar shape, weight, and composition to human molars, are adequate proxies in studies testing the taphonomic effects of HCl on teeth.

Six porcine (*Sus scrofa*) mandibular premolars and molars were used in this study. The teeth were sterilized using an autoclave (121°C, 15lbs psi). Standard odontometric measurements, weight, photographs, and imaging using a Nanoimages SNE-3200M mobile Scanning Electron Microscope (SEM) were taken before and after immersion in a commercially available formulation of hydrochloric acid (HCl, 38%). The teeth were submerged in HCl (38%) in 10 to 15ml of acid. Teeth were submerged for: 6 hours, 12 hours, 24 hours, and 48 hours, and two teeth were submerged until they dissolved completely. These time intervals correspond with previous research using human teeth, allowing for comparisons between porcine and human teeth submerged in HCl. Additionally, a mobile SEM was used to further analyze dental remains that were not identifiable as teeth after at least 48 hours in HCl to assess how their physical makeup compared to mobile SEM images of human teeth.

Data were compared from porcine models to previously published data on the timing and effects of HCl on human dentition. The results of macroscopic analysis showed differences in dissolution timing and overall morphology. The porcine models reduced at a faster rate than human teeth for the first 24 hours; however, after the first 24 hours, the porcine reduction slowed. In both porcine and human teeth, the morphological differences intensified with extended time intervals. After 48 hours, both porcine and human teeth were significantly reduced to the point that neither could be identified as dental remains; however, tissue remained that was able to be analyzed using SEM. When assessing the SEM imagery, both porcine and human teeth showed cracks at low levels of magnification (100X–1,000X). At higher magnification (4,000X–10,000X), both porcine and human teeth showed characteristics that can be described as layering or lamination.

These results were compared to previous studies with human teeth. There were notable similarities in the effects of HCl on human and porcine teeth, which are promising but suggest further studies are needed to support their use as proxies in studies of acid disposal. The SEM imagery results indicated it is possible that the layering or lamination seen at higher magnification are unique to the effects of HCl on dentition, but more SEM studies are needed to validate these results.

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**Forensic Anthropology; Hydrochloric Acid; Body Disposal**

## A59 Testing the Accuracy of Three Dental Aging Methods

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**Learning Objective:** After attending this presentation, attendees will be informed about methodologies used for subadult dental aging applicable to the fields of forensic anthropology and bioarchaeology.

**Impact Statement:** Historically, dental aging methods for subadults underestimates ages for individuals in the pubescent stage. Uncertainty over the reliability of the third molar for teenage aging methods created the need for more accurate methods. This presentation showcases a new methodology created by a collaborative effort of Texas State University and Michigan State University to provide more accurate results in these later subadult years (namely 13–22). The use of transition analysis for subadult dental aging methods will impact the forensic science community by providing a method for practitioners to estimate age from dentition with statistical basis.

The purpose of this research was to test the accuracy of three subadult dental aging methods for a sample of 50 individuals with known age ranges of 3–20 years. The three techniques that were tested were the following: The London Atlas chart technique; the Moorrees, Fanning, and Hunt incremental development stage technique using the Draft Age Estimation Quicksheets™; and the newly developed TA Dental technique that uses transition analysis.<sup>1,8</sup> In dental anthropology and forensic odontology, the use of visual atlases or charts that use dental and root formation stages have historically been used for dental age estimation.<sup>5</sup> The longitudinal study of Moorees, Fanning, and Hunt 1963(a) created a 14-stage graphical representation of incremental stages of dental root and crown formation later tabulated with descriptive statistics by Harris and Buck is most commonly used for dental age estimation in the field today.<sup>4,8</sup> However, past dental age estimation methods do not use statistical understanding of transition analysis, which uses probabilistic information and knowledge of prior distributions for life course analysis.<sup>7</sup> A new analytical tool, TA Dental, created in a collaborative effort by Texas State University and Michigan State University employs transition analysis in R code for subadult dental age estimation in response to this need (Subadult Dental Age Estimation Project website).

This research project compares three dental aging methods for accuracy. To do this a group of five researchers analyzed 50 dental panoramic radiographs and scored root and crown development using the Moorees, Fanning, and Hunt 14-stage graphical depictions.<sup>3</sup> Researchers then implemented these scores into the three methodologies. A Cronbach's Alpha test for inter-observer error showed an alpha score of 0.963. A one-way repeated measures Analysis of Variance (ANOVA) analyzed the scores to their known ages. The results showed that overall TA Dental and the London Atlas were not significantly different, but ultimately the most accurate methodology for dental age estimation is TA Dental. Moorees, Fanning, and Hunt underestimated ages.

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### Dental Aging Methods; Forensic Odontology; Dental Anthropology

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**A60 Evaluating Sacral Metrics for Sex Estimation**

*Alexandra Klaes, PhD, Washburn University, Topeka, KS; Kate Lesciotto, JD, PhD\*, University of North Texas Health Science Center, Fort Worth, TX*

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**Learning Objective:** After attending this presentation, attendees will understand the classification accuracy and observer agreement for sacral metrics.

**Impact Statement:** This presentation will impact the forensic science community by presenting the reliability and validity of the sacral measurements for use in skeletal sex estimation.

The sacrum has widely been considered one of the best skeletal indicators of sex due to sexual dimorphism of the boney pelvis related to parturition. Four measurements of the sacrum are included in the most popular human osteology textbook and/or the computer program FORDISC® 3.1 for combined sex/ancestry estimation: Anterior Height (AH), Anterior Breadth (AB), transverse diameter of the First Sacral Segment (S1), and Maximum Anterior Superior Breadth (MXB).<sup>1</sup> The purpose of this study was to examine the reliability and validity of these metrics as part of larger project attempting to combine metric and morphological features of the sacrum for sex estimation.

Two experienced observers collected the aforementioned four metrics of the sacrum during two trials in a sample of 19 males and 17 females ( $n=36$ ) from the Southeast Texas Applied Forensic Science Facility Skeletal Collection. Reliability was tested by calculating absolute and relative differences and Technical Error of Measurements (TEM and rTEM) between and within observers. Five percent was considered an acceptable rTEM error rate a priori. Intra-observer (Trial 1 vs. Trial 2) rTEM was less than 5% for all measurements and observers except for MXB (7.86% for the first observer). Inter-observer rTEM was also less than 5% for all measurements: AH 4.32%, AB 2.29%, MXB 0.46%, S1 2.49%. These results suggest that overall, the measurements are reliable.

Validity was tested using leave-one-out cross-validated linear discriminant function analysis. Classification accuracy of individual measurements ranged from 42.9% (S1 and MXB) to 77.1% (AB) for the first observer and from 47.2% (MXB) to 70.6% (S1) for the second observer. Individually the traits performed poorly for differentiating the sexes and varied by observer when used in combination: 77.1% for the first observer and 51.6% for the second observer. Using the postcranial reference samples in FORDISC® 3.1 for comparison resulted in slightly higher sex differentiation for both population groups (75%), but with a sex bias: White/of European decent (79.3% females; 73.1% males) and Black/of African descent (67.7% females; 78.7% males).

The measurements are problematic for several reasons and may not adequately capture the subtle shape differences between males and females. For example, AH does not account for: (1) the degree of sacral curvature (e.g., increased curvature reduces height); (2) individuals with more or less than five sacral segments; (3) sacralization or lumbarization; or (4) the inability to take the measurement when the coccyx is fused to the sacrum. The transverse diameter of S1 is complicated by osteoarthritic lipping, which is frequently encountered in this region and requires approximation of the measurement. The MXB is included in the most popular human osteology textbook, but not within FORDISC® 3.1, so it is infrequently used to estimate sex and had the lowest combined classification accuracy (45.1%) in this study. Last, AB can also be impacted by osteophytic lipping like S1, and the measurement can vary based on the angle and orientation of the sacrum during measurement; however, inter-observer agreement was high and intra-observer agreement was the best for this metric.

Although documented to be sexually dimorphic, current sacral metrics for differentiating the sexes are invalid yet reliable. Despite the poor discriminatory power of these metrics, AH is frequently selected in the linear regression equation with the highest stature correlation and can be reliably measured; therefore, this data should continue to be collected.

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<sup>1</sup>. White TD, Black MT, Folkens PA. 2012. *Human Osteology*, 3rd ed. Burlington, MA: Academic Press.

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**Sacrum; Sex Estimation; Technical Error of Measurement**



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**A61 A Content Analysis of Sex Estimation Research in the *Journal of Forensic Sciences* Between 2000 and 2021**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of trends in sex estimation research published in the *Journal of Forensic Sciences (JFS)* over the past two decades.

**Impact Statement:** This presentation will impact the forensic science community by presenting data on the skeletal elements, population affinities, skeletal collections, and statistical techniques utilized in forensic anthropology studies on sex estimation published in *JFS*.

To better understand sex estimation research trends over the past two decades, this study evaluated 111 sex estimation studies published between 2000 and 2021 in *JFS*. The main goals of this content analysis were to: (1) determine how many studies examined metrics versus morphological traits, (2) examine which parts of the skeleton have been most utilized, (3) examine which collections and population affinities have been most frequently sampled, and (4) determine which statistical methods were most frequently implemented.

Thirteen keywords (e.g., “sex estimation,” “sexual dimorphism,” etc.) were used to search the citation database Web of Science™ for sex estimation studies published in the Anthropology/Physical Anthropology section of *JFS* between the years 2000 and 2021. The text analysis software MAXQDA Plus 2022 was then utilized to code the selected articles and perform the content analysis. The materials, methods, and results sections of the articles were coded through a combination of auto-coding and manual coding. Coded information included whether the article focused on metrics or morphological traits, and what skeletal elements, population affinities, skeletal collections, and statistical methods were utilized. The coded data were exported as an Excel® file, and the frequencies for each code were tabulated. Additionally, a Cochran-Armitage test was conducted to test for significant trends in the number of metric- versus morphological-based studies published between 2000 and 2021.

Of the 111 sex estimation articles, 99 (89%) studied the adult skeleton, 10 (9%) studied the subadult skeleton, one (1%) studied the permanent dentition, and one (1%) studied deciduous dentition. While 85 (77%) articles focused on metric methods, 24 (22%) articles focused on analyzing morphological traits, and two (2%) articles combined metrics and morphological traits. The Cochran-Armitage test revealed no significant trends between metric and morphological articles between 2000 and 2021 ( $p\text{-value}=0.4387$ ); however, 29 metric articles were published between 2000 and 2010, compared to 56 between 2011 and 2021.

A number of important trends were noted. The skeletal region most frequently utilized was the cranium ( $n=34$ ), followed by the os coxa ( $n=25$ ), mandible ( $n=15$ ), and humerus ( $n=15$ ). While 80 (72%) studies examined only one skeletal element, 31 (28%) studies examined multiple regions. The most frequently utilized collections were the Robert J. Terry Anatomical Collection ( $n=19$ ), the William M. Bass Donated Skeletal Collection ( $n=19$ ), and the Hamann-Todd Human Osteological Collection ( $n=13$ ). Additionally, 28 (25%) studies utilized computed tomography scans or radiographs to perform their analysis. The most frequently sampled population affinities were “European” ( $n=73$ , e.g., European American, White, South African White, etc.) and “African” ( $n=39$ , e.g., African American, Black, South African Black, etc.). Also, the most popular statistical methods utilized for sex classification were Discriminant Function Analysis (DFA) ( $n=69$ ) and logistic regression ( $n=22$ ). Machine learning methods were only implemented by a handful of studies, including decision trees ( $n=4$ ), support vector machines ( $n=1$ ), and k-nearest neighbor ( $n=1$ ).

Overall, sex estimation research published in *JFS* has prioritized the use of metric methods over morphological methods. Further, only two studies combined metric and morphological data into one statistical framework. The most commonly utilized statistical methods for sex classification continue to be DFA and logistic regression, and only a limited number of studies implemented machine learning. This study also demonstrates that a substantial portion of sex estimation research is being conducted utilizing United States-based collections and limited population affinities. Future sex estimation research should prioritize sampling more diverse populations, exploring machine learning techniques, and developing methods that combine metrics and morphological traits into one statistical analysis.

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**Sex Estimation; Biological Profile; Forensic Anthropology**

## A62 Sex Estimation Using Interlandmark Distances of the Zygomatics

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**Learning Objective:** After attending this presentation, attendees will have information about an approach toward sex estimation using one zygomatic bone and advanced digitizing technology.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that Interlandmark Distances (ILD) of one zygomatic bone provide an option for sex estimation when remains are fragmented, damaged, or commingled.

Advances in technology opened new doors for studies in sex estimation. While many studies using craniometrics for sex estimation have been conducted, few studies examine 3D cranial ILD methods for sex estimation. Recent studies have shown that the zygomatic bones show sexual dimorphism and can be used for sex estimation through landmark data.<sup>1-3</sup> Additionally, the zygomatic bones are elements traditionally more likely to be recovered intact. This research aims to identify if ILD of only one zygomatic bone can be used for sex estimation and to develop a discriminant function for sex estimation.

The crania of 60 individuals from the Texas State Donated Skeletal Collection were selected for this research. Eleven landmarks on each individual's left zygomatic bone were digitized using the 3Skull software following the LNDMARK13F file guide. The 3Skull output of the craniometrics for all individuals was exported into the 3D landmark Coordinate Exporter and ILD Calculator software, which calculated 55 ILD for the left side landmarks. A one-way Analysis Of Variance (ANOVA) was performed to determine that 40 of the 55 ILD measurements were statistically significant for sexual dimorphism. A stepwise discriminant function analysis selected the best combination of measurements for sex estimation, and cross-validation was performed using the leave-one-out method.<sup>4-6</sup>

Discriminant function analyses were used to test if left zygomatic ILD can predict an individual's group membership as either male or female. The stepwise analysis chose radiculare to zygotemporale inferior, frontomale temporale to zygotemporale inferior, and zygion to zygoorbitale as the ILD for the discriminant function. The model correctly classified 81.7% of all individuals into their known groups, with 83.3% (30/36) of males and 79.2% (19/24) of females classified correctly. The leave-one-out cross-validation assessment for validity caused no change in the percentage of correctly classified cases. The results show that zygomatic bones display sexual dimorphism. By calculating ILD from the landmark coordinates, the number of variables for the zygomatic bone was significantly increased. The ILD measurements of only one zygomatic bone provided good classification percentages of individuals into their known groups with the discriminant function analysis. Therefore, ILD can be used for sex estimation based on this model. ILD could provide more variables for sex estimation when limited to fragmented, damaged, or commingled remains. Future studies will explore machine learning and geometric morphometric analysis of coordinate data.

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### Interlandmark Distance; Sex Estimation; Zygomatic Bones

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**A63 Machine Learning and Discriminant Function Analysis in the Formulation of Generic Models for Sex Prediction Using Patella Measurements**

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**Learning Objective:** The main objectives of this study include the formulation of generic models for sex estimation from six measurements of the patella of three of the major population groups from South Africa and to make a comparison between the classification rates from the use of linear Discriminant Function Analysis (DFA) and Machine Learning (ML) algorithms. Three measurements, namely maxh, maxb and maxt, consistently were selected as the best performing variables in both DFA and ML algorithm. The standards generated for sex classification from the use of ML algorithms produced higher average accuracies compared to those generated using DFA.

**Impact Statement:** The results of this study show that the application of novel ML paradigm provides a better classification of sex from the patella measurement compared to the use of the classical DFA. The usage of this method can be further explored in sex classification from measurements of other bones of the body with the potential to provide higher classification rates in sex assignment

Sex prediction from bone measurements that display sexual dimorphism is one of the most important aspects of forensic anthropology. Measurements of bones that do not have distinct morphological traits have been subjected to statistical analysis in the generation of population-specific standards for sex estimation. Recent studies have highlighted some drawbacks in the use of population specific equations, including the need for prior assignment of population groups, which may be difficult if not impossible in cases where complete skeletons are not available or in the absence of bones that display obvious population-specific traits. This therefore makes the process of human identification from skeletal remains demanding, especially in a country like South Africa with diverse population groups. This study aims to formulate generic models for sex prediction using measurements of the patella of South Africans using LDA and ML algorithms.

Six parameters were measured on each of a sample of 260 patellae of South Africans obtained from the Raymond Dart collection of Human Skeletons, University of the Witwatersrand, Johannesburg, South Africa. Stepwise and direct discriminant function analyses were performed for measurements that exhibited significant differences between male and female mean measurements. We also used eight classical ML techniques along with feature-ranking techniques to identify the best feature combinations for sex estimation. A novel stacking ML technique was trained and validated to classify the sex of the subject.

The measurements of the patellae of South Africans are sexually dimorphic and the range of average accuracies obtained for pooled multivariate discriminant function equations is 82–84%, while the stacking ML technique provides 91% accuracy, which compares well with those presented for previous studies in other parts of the world. The generic models proposed in this study from measurements of the patella of South Africans are useful for sex estimation with reasonably high average accuracies.

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**Forensic Anthropology; Sex Prediction; Machine Learning Algorithm**

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**A64 Analyzing Interpretative Approaches in FORDISC® to Improve Sex Estimation Accuracy**

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**Learning Objective:** After attending this presentation, attendees will understand how Posterior Probability (PP) trends can improve FORDISC® craniometric sex estimation.

**Impact Statement:** This presentation will impact the forensic science community by: (1) making recommendations on the most suitable analytical approach to FORDISC® sex estimation taking into account accuracy and sample inclusion, while mitigating sex biases; and (2) providing sex-specific guidelines for interpreting FORDISC® PPs.

Although the FORDISC® software is commonly utilized for osteometric sex and ancestry estimation, the relationship between the obtained PP and accuracy has not been thoroughly investigated.<sup>1</sup> This study focuses on sex estimation using FORDISC® and explores several analytical approaches to interpreting the PP. First, differences in accuracy between two approaches for analyzing the PP output for sex estimation were assessed. Approach 1 estimates sex based on the sex associated with the FORDISC®-assigned reference group. Approach 2 compares the sum of the PP for all the male and female reference samples and assigns the individual to the sex with the greatest summed PP. Leave-one-out cross validation group classifications and associated PPs for each individual in the reference sample ( $n=884$ ) were extracted from FORDISC® using a stepwise forward selection of 18 craniometric variables. Sex estimation accuracy rates for Approaches 1 and 2 were compared and assessed for any sex or population biases. Accuracy rates for Approach 1 (86%) and Approach 2 (87%) were almost identical. With Approach 1, however, of the 125 individuals incorrectly sexed, 65% were erroneously classified as female compared to 35% as male. In contrast, Approach 2 returned 118 incorrectly sexed, of which 55% were classified as female and 45% as male. Given the reduced sex bias, Approach 2 should be the preferred method for sex estimation when using FORDISC®.

Accordingly, Approach 2 was used to explore a Posterior Probability Informed Threshold (PPIT) approach, which takes into account the PP distributions related to accuracy of the sex estimation for the samples of interest to develop PP thresholds with acceptable accuracy levels.<sup>2</sup> Correctly assigned males had a greater median PP and different PP distribution than females ( $p=0.5$ ). Furthermore, accuracy rates above 80% are achieved at lower PPITs for males ( $PPIT \geq 0.75$ ) than females ( $PPIT \geq 0.85$ ). These results highlight how the interpretative approach to FORDISC® sex estimation can affect accuracy, particularly of skulls estimated to be female. FORDISC® users should apply a summed PP approach in conjunction with sex-specific PPIT standards to improve accuracy rates. Establishing a statistically informed approach to incorporating qualifiers for sex estimations promotes standardized forensic case reporting.

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**Sex Estimation; Accuracy; Posterior Probability**

## A65 Determining Sexual Dimorphism From Mastoid Triangle Measurements in a South Indian Population: A Computed Tomography (CT) Scan-Based Study

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**Learning Objective:** After attending this presentation, attendees will be able to comprehend the role of mastoid triangle CT scan measurements for determining the sex in a south Indian population.

**Impact Statement:** This presentation will impact the forensic science community because the importance of CT scan measurements of the mastoid triangle of skull for determining the sex in a south Indian population will be presented.

**Background:** Identification of an individual is one of the objectives of an autopsy. Age, sex, race, and stature can be obtained from dismembered or mutilated bodies, which will help in partial identification of the individual. Bones give important information from which sex can be predicted. The predictive accuracy for determining sex from the skull is 80–85%. The mastoid region of the skull shows resistance to any physical damage owing to its anatomical position and compact structure and, hence, may remain intact in otherwise damaged and fragmented skulls. This explains its utility in sex determination when other parts of the skull may not be fully available. Paiva and Segre developed a method wherein craniometric measurements of a triangle defined by three distinct points on the lateral aspect of the skull in the mastoid region was used for identification of sex using dry skulls.<sup>1</sup> This triangle formed by porion, mastoidale, and asterion, which are the necessary craniometric points, was later described as the mastoid triangle.<sup>2</sup>

**Methods:** The sample size was estimated with an expected Area Under the Curve (AUC) for predicting the mastoid triangle measurements for sex as 0.76. The sample size was 358 with equal distribution from both sexes at a 5% level of significance with 0.05 absolute precision. The sampling technique used was convenient sampling and the data collection period was two months. A record-based retrospective cross-sectional study was done on CT scans of 358 adult individuals as per a pre-defined inclusion and exclusion criteria. The parameters studied were Asterion-Porion Length (APL), Asterion-Mastoidale Length (AML), Porion-Mastoidale Length (PML), Area of Mastoid Triangle (AMT), and Perimeter of Mastoid Triangle (PMT) of both left and right sides.

**Results:** The measured mastoid triangle parameters from both sides were found to have statistically significant differences between the two sexes. The diagnostic accuracy of these parameters in determining the sex was evaluated using the Receiver Operating Characteristic (ROC) curve. AUC with 95% Confidence Interval (CI) was used for each measured parameter. Sensitivity and specificity of the parameters in determining the sex was based on the maximum Youden's index value. The parameter AMT of the left side was found to be most significant in determining sex, with AUC 91.1% at 95% CI, sensitivity 90%, and specificity 76%. APL of the left side was least significant with AUC of 77.3%, sensitivity 76%, and specificity 68%.

**Conclusion:** This retrospective CT-based observational study showed that all the measured mastoid triangle parameters of both the sides were significantly able to determine the sex of the individual in a South Indian population.

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2. Kemkes A, Gobel T. Metric assessment of the mastoid triangle for sex determination: A validation study. *J Forensic Sci*. 2006;51:985–989.

### Sex Determination; Mastoid Triangle; CT Scan Study

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**A66 A Critical Appraisal of Standardization and Integration of Forensic Anthropological Techniques**

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**Learning Objective:** After attending this presentation, attendees will be sensitized to the current limitations of forensic anthropological casework and will demand the utmost care on their part for applying different biological profiling methods and techniques in their research work.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of the current limitations of forensic anthropological casework.

Identification of unknown human remains recovered from forensic contexts is crucially needed for humanitarian purposes, though it remains a perplexing problem for forensic anthropologists. The practice of forensic anthropology is not well regulated or standardized and it currently lacks minimum published and universally applicable standards. Identification of isolated, fragmented, or commingled remains becomes more difficult as such remains may have been discouragingly altered by criminal activity, weather conditions, cultural practices, or extreme temperatures to make their anatomical features unappreciated. An infinite number of such human remains are being reported daily from different corners of the world, but there is no definite set of universal standards that can be applied for identification strategies of these challenged remains. Most of the available standards are population specific, developed from examinations of skeletal remains of known identity or exhumed from the cemeteries or burial grounds depicting credentialed identity of the deceased. Problems arise in cases where even the minute pieces of basic information are not available about the unknown human skeletal remains. Applying a method for the individuals of a population other than for which it is originally developed will expectedly result in anticipated variations or errors. The continued testing of developed methods on other populations with a careful notification of intra- and inter-population variation can lead to universal acceptability of such methods. The lack of precision and the existence of higher intra- and inter-observer variations associated with many of the forensic anthropological methods and techniques is a major concern for their objectivity, reproducibility, and efficacy. The continued testing of anthropological methods for assessing age at death, sex, ethnicity, traumatic and pathological markers of identity, etc. is urgently required.

Another major problem, largely unaddressed, in forensic anthropology is the difficulty of integrating the results of several methods applied by different researchers worldwide. The quantification of complex skeletal and dental features is another cumbersome and difficult task that discourages a quick, cost-effective, and hassle-free assessment of human remains. So, there is a dire need to develop methods that do not rely on such complex traits but on biochemical or nuclear physical markers (such as aspartic acid racemization, stable isotope analysis, radiocarbon dating, and ancient DNA analyses or approaches) that provide high precision in forensic identifications. Determination of geographical origin or ethnicity of remains may be important, especially in terms of investigative efforts by the police. Due to more immigrations and influxes of human capital, the determination of ethnicity and geographical origin has become ever more difficult. Further, documented skeletal collections are not available to most of the global researchers for their examination toward comparative purposes; therefore, building virtual skeletal libraries using high-resolution Computed Tomography (CT) scanning of osteological material of known ethnicity can allow easier access of global skeletal collections to researchers, thus facilitating digital dissemination of skeletal traumas, pathologies, anomalies, defects, etc. and digital rendering of bone structures and features. The molecular, archaeological, taphonomic, and trauma analyses are largely emphasized in forensic anthropological research work these days.<sup>1</sup> Donated cadavers and documented skeletal collections presented to larger interest groups can help remove global barriers in understanding subtle variations in human skeletal remains. In addition to critically examining and continuously assessing the accuracy, precision, and validity of already-developed methods, there is an urgent need to devise and add newer objective methods to the forensic anthropological toolbox to make anthropologists better prepared for future challenging forensic casework. Comparative studies describing the error rates linked with the forensic anthropological methods, updating the existing methodologies of biological profiling, assessing and quantifying patterns of human variation is the urgent need of the hour. There is an urgent need for the improvement and standardization of forensic methods and techniques.<sup>2</sup>

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**Forensic Anthropology; Challenges; Standardization of Methodologies**



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**A67 Differences in Self-Reported Versus Measured Stature**

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**Learning Objective:** After attending this presentation, attendees will develop a better understanding of the differences between reported and measured stature in adults. This has implications for contexts in which stature estimates are employed, including identification of unknown remains.

**Impact Statement:** Self-reported stature tends to be higher than measured stature.<sup>1,2</sup> This presentation will impact the forensic science community by updating this literature with a broader sample by gender and social race than has previously been considered.

Stature estimation is a key component of a biological profile for unidentified remains and is included on missing persons reports. Inaccurate stature reporting or documentation hinders identification of remains. Willey and Falsetti demonstrated that an individual's measured and reported (driver's license) stature differs among a sample of primarily White college-aged men.<sup>1</sup> Using a similar approach among a broader sample of participants, we compared reported and measured stature among adults in upstate New York. Cardoso et al. compared the relationship between cadaver and living stature of a population of male cadavers aged 48-58 years.<sup>2</sup> Our study seeks to replicate previous findings and assess whether they extend to women and underrepresented minorities.

Following Institutional Review Board (IRB) approval, we recruited 52 adults from among Binghamton University's students, faculty, and staff, and compared four estimates of stature: (1) self-reported, (2) reported by a friend or relative, (3) recorded on government identification, and (4) measured with a Seca 217 stadiometer at the time of participation. Data were analyzed with IBM SPSS Statistics 28.

Participants were somewhat diverse by self-reported race/ethnicity using United States Census categories: 7 Asian, 4 Hispanic, 1 Black or African American, 32 White, and 8 biracial and were also diverse by gender: 33 women, 12 men, and 7 non-binary. On average, participants over-estimated stature (self-reported) by 0.36 inches (95% CI: 0.132, 0.589). Family members and close friends similarly overestimated a participant's stature by 0.53 inches (95% CI: 0.197, 0.871). We did not observe this disparity for drivers' licenses (-.06 inches; 95% CI=-0.322, 0.198). Our findings suggest that in-person reports tend to overestimate stature; however, stature estimates taken from driver's licenses or other government identification are generally accurate.

In sum, stature reported by participants and their families and friends tended to overestimate their measured stature. This could confound efforts of identification when comparing biological profiles to missing persons reports, but additional research is needed in this area; such reported statures should be relied upon with caution. Small differences between reported and measured stature are unlikely to make a consequential difference for forensic identification, as forensic stature estimation typically includes a +/- 4-inch range. Differences may be more consequential in a medical setting if patients' estimates are substituted for measurement of stature in, for example, estimation of body mass index. Additional interdisciplinary research with diverse populations is warranted to inform use of reported and measured stature in medical and forensic contexts.

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**Forensic Anthropology; Stature; Identification**

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**A68 An Osteometric Approach to Separating Commingled Pelvic and Foot Joints**

*Helen Brandt-Litavec, MS\*, Binghamton University, Owego, NY*

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**Learning Objective:** The goal of this presentation is for attendees to develop a better understanding of osteometric sorting techniques applied to commingled joint surfaces. Additionally, attendees will understand how to adapt current osteometric sorting models to novel joint areas.

**Impact Statement:** This presentation will impact the forensic community by expanding upon currently available osteometric sorting models to two additional joints in the body.

Commingled human remains can result in a significant loss of information regarding the individuals present and make the biological profile difficult to establish. One common technique for separating commingled remains at joint surfaces is osteometric sorting; however, current models have only been applied to large joints of the body, for instance the hip or knee, while smaller joints, namely the Sacroiliac (SI) or first Tarsometatarsal (1st TMT) joint, are unable to be sorted.<sup>1</sup> Osteometric sorting separates commingled joints with a singular width measurement; however, linear measurements may be limited in their ability to describe the entirety of joint surfaces.<sup>1,2</sup> Therefore, this study's first hypothesis is that commingled individuals can be sorted at the SI and 1st TMT joints using osteometric sorting. The second hypothesis is that the joint surface area values will exclude more potential matches than the width measurements.

The measurements and surface area values were calculated from virtual models created at the William M. Bass Donated Skeletal Collection. Fifty-six individuals' left os coxa, sacra, medial cuneiforms, and first metatarsals were scanned with an EinScan-Pro 2x+ Handheld Surface Scanner. Each articular surface was cropped from the virtual model, and the widest portion of each facet was measured with the distance tool, while the surface area was calculated using the compute area function in Geomagic® Wrap 2017. The measurements were recorded in a Microsoft® Excel® Workbook, where the osteometric sorting models were also calculated.<sup>1</sup> Four reference samples were formed to generate the models: SI width (Model 1), SI surface area (Model 2), 1st TMT width (Model 3), and 1st TMT surface area (Model 4). Each sample was composed of 51 known individuals with varying demographics. Shapiro-Wilk tests were conducted to identify any outliers. These four models were then used to identify commingled individuals in the four test samples. Each test sample consisted of 5 known individuals and 20 artificially commingled pairs. Each model's efficiency was calculated as an indicator of overall success at determining the correct classification rate of true positives and negatives.<sup>3</sup> In all test samples, several potential pairs were excluded through the implementation of the new models. Model 1 was the least efficient (0.28) and only eliminated 10% of the commingled pairs. Model 2 was the most efficient (0.72) and correctly rejected 65% of the commingled pairs. Further, two of the true SI joints were correctly reassociated using Model 2. Model 3 excluded 60% of the commingled pairs; however, it also eliminated one of the true matches (efficiency=0.64). A similar result occurred for Model 4, where only 45% of commingled pairs were rejected as well as one true match (efficiency=0.52).

In sum, these results illustrate that osteometric sorting can help exclude potential matches at these particular joints. The first hypothesis was supported, as the new models did effectively reduce the number of potential pairs. However, the second hypothesis could only be supported for the SI joint, as Model 3 was more efficient than Model 4. It is possible that the overall efficiency is lower than established models due to the small size of the reference samples.<sup>3</sup> If future researchers are interested in osteometric sorting at these joints, then the reference sample sizes should be increased.

*This research is funded by the NIJ Award 2020-R2-CX-0025.*

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**Forensic Anthropology; Commingled Human Remains; Osteometric Sorting**

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**A69 An Automated Deep-Learning Approach to the Segmentation of Vascular Pore Systems and Osteocyte Lacunae in Virtual and Traditional Histologic Datasets**

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**Learning Objective:** The goal of this presentation is to describe the use of a novel method for the automatic segmentation of high-resolution micro-Computed Tomography ( $\mu$ CT) and histologic data. Related objectives include: (1) demonstrating the effectiveness of a trained deep learning model compared to traditional segmentation methods, (2) evaluate its potential to eliminate observer error, (3) reduce data processing times, and (4) assess its application to a host of anthropologically relevant high-resolution data.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that automatic segmentation using deep learning algorithms has the potential to increase the efficiency of virtual and traditional histologic data segmentation and to reduce user inconsistency, error rates, and subjectivity in bone microstructural feature identification used in histologic age-at-death estimation.

The products of bone remodeling (e.g., resorption spaces, Haversian canals, secondary osteons) and features of bone's cellular network (e.g., osteocyte lacunae) are evaluated in histologic age-at-death estimation methods and the assessment of bone quality. Automating the segmentation of these structures in histologic data has the potential to significantly reduce data processing times for large datasets while simultaneously improving results compared to traditionally employed methods.

The described model employed UNet++ Convolutional Neural Network architecture and was trained on synchrotron  $\mu$ CT data of human left sixth rib specimens in ORS Dragonfly (Object Research Systems Inc., Montreal, Canada).<sup>1</sup> Bone tissue specimens were procured via collaboration with an American organ procurement non-profit organization. The donors ranged in age from 21–54 years-at-death (mean age=38 years), and included healthy and pathological bone specimens (e.g., osteoarthritis, osteoporosis) to produce a robust semantic segmentation model.

Multi- Regions Of Interest (ROIs) were used to train the novel UNet++ deep learning model. Dragonfly's ROI thresholding function, morphological operations, and manual brush tools were used to produce multi-ROIs containing two individual segmentation layers (for pores and lacunae) from 12 randomly selected samples. After training, the model was applied to new samples, and segmentation results were compared to those of a traditional segmentation method used for  $\mu$ CT data.

The traditional segmentation method employed is described by Andronowski and colleagues.<sup>2</sup> The data were binarized with a global threshold and artifacts removed with a despeckling feature. Lacunae and pores were selected using 2,743 voxels as the delineating line. 3D and individual object analysis packages recorded morphometric data for the entire sample and individual objects within the sample, respectively.

Independent sample *t*-tests were conducted in SPSS (v. 28.0.0.0) using six test samples segmented by each model. Variables were considered significant with a *p*-value < 0.05. Pore number (*p*-value < 0.001), lacunar volume (*p*-value < 0.001), lacunar surface area (*p*-value=0.040), lacunar diameter (*p*-value < 0.001), and pore surface area (*p*-value=0.014) were significantly different between models. The difference in lacunar number (*p*-value=0.105), pore volume (*p*-value=0.129), and pore diameter (*p*-value=0.198) were not statistically significant. An additional sample was used as a basis for comparing segmentation accuracy between the two automated models. The error rate was calculated by quantifying the mislabeled pixels compared to a user-generated manual segmentation. For lacunae, UNet++ had an error rate of 37.9% while the traditional method had an error rate of 67.7%. For pores, UNet++ had an error rate of 3.9% while the traditional method failed to acquire any pores.

Automatic segmentation using deep learning algorithms has the potential to increase the efficiency of virtual and traditional histologic data segmentation and to reduce user inconsistency, error rates, and subjectivity in bone microstructural feature identification used in histologic age-at-death estimation. Future work will build upon this foundation to further automate the workflow and improve accuracy of segmentation, while more thoroughly evaluating the accuracy of the trained deep learning model in segmenting histological features relevant to age-estimation in forensic anthropology.

**References:**

- <sup>1.</sup> Zhou Z, Siddiquee MMR, Tajbakhsh N, Liang J. UNet++: A Nested U-Net Architecture for Medical Image Segmentation. *Deep Learn Med Image Anal Multimodal Learn Clin Decis Support* (2018). 4th Int. Workshop DLMIA 2018 8th Int. Workshop ML-CDS 2018 Held Conjunction MICCAI 2018 Granada Spain S 11045, 3–11 (2018).
- <sup>2.</sup> Andronowski JM, Davis RA, Holyoke CW. A Sectioning, Coring, and Image Processing Guide for High-Throughput Cortical Bone Sample Procurement and Analysis for Synchrotron Micro-CT. *J. Vis. Exp.* 61081 (2020) doi:10.3791/61081.

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**Image Processing; Deep Learning; Micro-Computed Tomography**

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**A70 When Bone Lights Up: A Novel Way of Labeling Proteins and Cells and Its Potential Uses**

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**Learning Objective:** After attending this presentation, attendees will have learned about an updated and simplified manner of labeling selected bone proteins and cells as well as some potential uses for labeling.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the ability and need to understand all aspects of the bone, and that, of two potential uses for the method, new techniques in areas of postmortem interval calculation and trauma analysis can be devised.

Bone is not a singular material but is rather made up of constituent parts. Among those parts are hydroxyapatite and collagen; however, when we go deeper into bone, we see that it is made up of additional elements, including various proteins and cells. Two of the bone proteins, osteocalcin and osteopontin, are necessary for the binding of the hydroxyapatite to the collagen. Specifically, osteocalcin binds hydroxyapatite to osteopontin, and osteopontin binds osteocalcin to type I collagen. In addition to these bone proteins, various cells play a role in the development and maintenance of bone. Specifically of interest in this presentation are osteoclasts, principally responsible for the resorption of bone, including damaged areas.

While labeling of proteins is nothing new in bone and mineral research, its use in forensic science is still somewhat novel. Further, almost universally, such labeling is conducted using decalcified bone. The method presented here uses undecalcified bone and can be applied to forensic samples with limited pre-treatment before labeling and imaging. The same can be said for the osteoclast imaging, though admittedly the common research protocol does not mention the decalcification process; thus, it is believed that undecalcified bone was used.<sup>1</sup>

The method developed utilized iliac bone biopsies of 12 individuals collected by the Centre Hospitalier de l'Université de Montréal, in Montréal, QC, and divided into three sections per individual, yielding a total of 36 thin-sections. These sections were segregated in such a way that 12 were used for the labeling of osteocalcin, 12 for osteopontin, and 12 for osteoclasts. The osteocalcin and osteopontin samples were then soaked for 30 minutes in a neutral phosphate buffer solution, then labeled for the respective protein. For the osteocalcin, the samples were initially stained in basic fuchsin, then an osteocalcin polyclonal antibody was used in conjunction with goat anti-rabbit IgG AlexaFluor™ 488 secondary antibody, while the osteopontin utilized the osteopontin monoclonal antibody with a goat anti-mouse AlexaFluor™ 555 secondary antibody. Osteoclasts were labeled following the Coxon method, first incubating the samples in a solution of phosphate buffer solution mixed with 0.5% v/v Triton™ X-100, and then staining with basic fuchsin stain and labeling with a solution of 10% Bovine Serum complete with MitoTracker® Red FM Dye in a phosphate buffer solution.<sup>1</sup> Upon a one-hour soak in the solution, the samples were then highlighted using the goat anti-rabbit IgG AlexaFluor™ 488 secondary antibody.

Imaging was conducted using a Carl Zeiss™ LSM800 laser scanning confocal microscope. Two of the four available channels were used including the 488nm and 555nm channels. Imaging was first collected using a 10x objective, and then gradually moving to 40x and 64x to obtain the best images and quantifications. Individual image parameters such as laser power, and gains were all optimized individually to produce the best images. Analysis was conducted using Carl Zeiss™ Zen Blue® Lite 3.4, as well as FIJI/ImageJ. While the uses of this methodology are limitless in our understanding of human bone, concurrent research using these methods are studying its use in the development of a postmortem interval as well as a better understanding of trauma analysis, specifically trauma timing. Preliminary data has shown there is a decline in the number of proteins and cells present in bone fixed at later dates than previous ones, and the labeling has demonstrated a potential congregation of cells and separation of proteins along break areas.

**Reference:**

- <sup>1</sup>. Coxon FP. Fluorescence imaging of osteoclasts using confocal microscopy. In: Helfrich MH, Ralston SH, editors. *Bone Research Protocols*. 2nd ed. New York: Springer; 2012;401-24.

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**Laser Scanning Confocal Microscopy; Bone Histology; Fluorescent Labeling**

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**A71 A Preliminary Investigation of Relationships Between Cortical Porosity and Fracture Type in Human Tibiae**

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**Learning Objective:** After attending this presentation, attendees will understand the importance of investigating microstructural variables in relation to skeletal trauma.

**Impact Statement:** This presentation will impact the forensic science community by presenting innovative and unique methods to develop explanatory mechanisms of fracture pattern variation through an analysis of porosity and cross-sectional areas in human tibiae.

Skeletal trauma analysis is often largely dependent on description, with little interpretation of mechanisms contributing to observed variation in fracture patterns. The Organization of Scientific Area Committees for Forensic Science has identified controlled experimental bone trauma studies as an anthropological research need. Specifically, the role of intrinsic bone features in fracture pattern variability is currently unknown. The goal of this research is to investigate whether porosity and cortical area have relationships with fracture types in human tibiae experimentally loaded in 3-point bending.

Fourteen human tibiae (males  $n=7$  [49–74 years old, mean=64.85], females  $n=7$  [41–79 years old, mean=62.57]) were impacted at 5m/s in a lateral-medial direction at the 50% site. The resulting fracture types were classified as simple ( $n=3$ ), wedge ( $n=8$ ), or multifragmentary ( $n=3$ ). Undecalcified cross-sections from the 66% site were histologically prepared and imaged at 40X magnification on an Olympus® VS120 slide scanner. All histological image analyses were conducted with a custom ImageJ macro, Pore Extractor 2D, which facilitates computer-assisted identification of cortical borders and pore spaces.<sup>1</sup> Cortical area was quantified for each section, then corrected for porosity as bone area. Quantification of pore morphometry included percent porosity, pore density, and pore size and shape descriptors.

Overall, trends in cortical area and pore morphometry demonstrated variation within the sample according to fracture type. Multifragmentary fractures were associated with a lower percent porosity ( $13.4 \pm 0.8\%$ ) than simple ( $25.3 \pm 7.2\%$ ) or wedge ( $21.9 \pm 9.7\%$ ) fractures. Mean pore area was also reduced in tibiae with multifragmentary fractures ( $0.010 \pm 0.003\text{mm}^2$ ) compared to simple ( $0.022 \pm 0.008\text{mm}^2$ ) or wedge ( $0.020 \pm 0.014\text{mm}^2$ ) fractures. Conversely, pore density was increased in tibiae with multifragmentary fractures ( $13.5 \pm 3.7\text{mm}^2$ ) compared to simple ( $11.7 \pm 1.7\text{mm}^2$ ) or wedge ( $12.9 \pm 3.4\text{mm}^2$ ) fractures. Tibiae with multifragmentary fractures also had greater bone area ( $55.7 \pm 1.6\%$ ) than those with simple ( $51.1 \pm 3.9\%$ ) or wedge ( $49.5 \pm 8.9\%$ ) fractures. While pore morphometry did not significantly vary between any predictors (age, sex, fracture type) or their interactions, age-associated increases in percent porosity, pore density, pore size, and pore shape distortion were observed. Principal components analysis identified a separation between multifragmentary and simple fracture types along PC1 (60.9%). This dimension corresponded to mean pore size, percent porosity, and pore density, as opposed to mean pore shape descriptors in PC2 (27.5%).

These findings suggest that elevated porosity, the convergence of discrete pore systems into large voids, and the reduced areal fraction of bone tissue may facilitate less complex fractures, such as simple or wedge types; however, these preliminary findings should be confirmed in a larger sample size. Future work will continue to explore relationships between fracture types and pore morphometry, as well as incorporate cross-sectional areas and associated pore types in a more complex analysis to further understand fracture variation and mechanism.

**Reference:**

1. Cole ME, Stout SD, Agnew AM. 2021. Pore Extractor 2D: An ImageJ plugin for identification, classification, and regional characterization of cortical pores on histological bone images. The 90th Annual Meeting of the American Association of Physical Anthropologists. *Am. J. Phys. Anthropol.* 174(S71): 20-21.

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**Cortical Porosity; Bone Histology; Fracture Analysis**

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**A72 Analyzing the Language Used in Forensic Anthropology Population-Affinity Reporting**

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**Learning Objective:** After attending this presentation, attendees will understand how forensic anthropology practitioners translate the output of a commonly used tool for estimating population affinity into continental, ethnic, racial, and/or regional terms.

**Impact Statement:** This presentation will impact the forensic science community by contributing to the ongoing dialog surrounding how, and whether, to report population affinity.

Forensic anthropologists frequently estimate decedents' biological profiles: their age, sex, stature, and social or biogeographic group membership. Yet, the terms used when reporting the latter category differ, referencing social, ethnic, regional, and continental groups. Reporting terminology has the potential to inform not only identifications but also public perceptions of these groups' biological realities (and the lack thereof). This study investigated whether standardization exists in forensic anthropological reporting of population affinity.

Participants were recruited via email listservs for a Qualtrics® survey. The survey asked participants to report their gender, social race, highest degree, certifications, and work context. Participants were then shown three FORDISC® outputs and told to interpret them as though they were the end state of one of their own metric group-affinity analyses; they were asked to provide a brief statement reporting the results of each output.<sup>1</sup> Study hypotheses held that: (1) reporting terminology would lack standardization; and 2) respondents from systematically marginalized racial groups would be more critical of the practice of affinity estimation.

Of the 48 survey participants, the majority were "female" or "woman" ( $n=32$ ; 67%) with doctoral degrees ( $n=36$ ; 75%); nearly half ( $n=23$ ; 48%) were D-ABFA, and most worked in academia ( $n=12$ ; 25%), medicolegal or government contexts ( $n=9$ ; 19%), or some combination of the three ( $n=15$ ; 31%). Most reported their race as "Anglo," "European/European-American" or "White" ( $n=38$ ; 79%). Eight of the remaining ten respondents identified as "Asian," "Biracial," "Black," "Hispanic," "Indigenous," "Latino," "Mexican," or "N/A I don't abide by social race categories."

The three FORDISC® simulations involved comparisons with the Forensic Databanks's "Black," "Hispanic," and "Japanese" female groups. In all three, most respondents used reporting terminology that mirrored those racial, ethnic, and regional group names. For example, in a simulation involving a two-group comparison with "Japanese" and "Hispanic" females, 16 of the 27 respondents who completed the simulation used one or the other of these terms in their reporting (59%); however, 12 of 27 (44%) translated the FORDISC® group names into terms reflecting continental ancestry (i.e., "Asian" rather than "Japanese" [note: percentages total >100 due to overlap between respondents who used both FORDISC's® regional/ethnic terminology and continental terminology]). Further, when asked which group-membership categories they estimate, 23 of 28 reported ancestry, 14 population affinity, 2 race, and 1 ethnicity. These results support the hypothesis that population-affinity reporting terminology lacks standardization.

Five of the eight respondents who did not identify as "White" (63%) ended their survey after the demographics section, without completing the FORDISC® simulation—over twice the frequency of "White" respondents who did so (26%). Two began the FORDISC® simulation but expressed lack of confidence in their responses due to limitations of the simulation (25%); again, over twice the frequency of "White" respondents who expressed lack of confidence (10.5%). Though hindered by small sample size, these findings provide limited support for the hypothesis that practitioners from systematically marginalized racial groups would be more critical of population-affinity estimation—or at least, less willing to complete a FORDISC®-based survey.

Differing approaches, unstandardized terminology, and practitioner critiques create confusion surrounding what forensic anthropologists are truly estimating when they analyze and report population affinity. The continued reference to "ancestry"—even when continental-based reference groups are not included in the analysis—perpetuates the incorrect notion that human skeletal variation follows continental boundaries. Practitioners who choose to report population affinity should come to a consensus regarding approaches, define the terms they use, and eschew harmful and outdated continental typologies.

**Reference:**

<sup>1</sup>. Jantz RL, SD Ousley (2005) FORDISC 3.1: Computerized Forensic Discriminant Functions. Knoxville, TN: The University of Tennessee.

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**Forensic Anthropology; Population Affinity; Language**



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**A73 Assessing Usage Variability in FORDISC®**

*Sage Pletka, BS\*, University of Indianapolis, Marquette, MI*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the variability in usage of FORDISC®, a computer software program that estimates population affinity using discriminant function analysis. FORDISC® is a flexible software that allows the user to specify many of the parameters to be used in the classification process, which necessitates data and discussion on how it is implemented in casework.

**Impact Statement:** This research will impact the forensic science community by looking into the variability in how the parameters of FORDISC® are selected, which may have implications for the classification outcome and therefore reproducibility. Very little previous research has rigorously examined how FORDISC® is being used in actual forensic casework and if there are large amounts of variation in how the program is run. To answer these questions, we have surveyed self-identifying forensic anthropologists to get concrete data on whether there are large differences in usage between practitioners in the field.

The push in forensic science for standardization has meant for forensic anthropology that biological profile methodology has had to move away from expert opinion and qualitative approaches to statistical-based approaches with the quantification of uncertainty via error rates, validation studies, etc. Population affinity, a parameter of the biological profile estimated by some practitioners, uses multivariate trait/measurement expression distributions of populations to estimate group belonging. One of the most common United States methods to do so is FORDISC®, a computer program which is used to estimate typicality and the posterior probability of an unknown cranium belonging to reference groups based on craniometric measurements.<sup>1</sup>

FORDISC® is a powerful tool that allows the user to specify many parameters of the model such as groups used, number of measurements used, the use of personal datasets, the removal of outliers, and more. It is assumed that the reason for this is to allow for the nature of forensic remains that can be damaged or incomplete, the inclusion of contextual information to be used in the analysis, and to allow the user to stay within the assumptions of the statistical functions as best as possible. While this flexibility gives much power to the user, it also brings the potential for much variability in how the program is used, which has implications for outcomes and, therefore, reproducibility. The use of this statistical model is undoubtedly a step forward beyond classical “trial list” approaches to the estimation of population affinity. However, since there is significant potential for the model itself to be used differentially, it ought to be tested if practitioners do use it differently and how this may affect results.

Data for this study are based on responses to an anonymous survey distributed on the AAFS Anthropology section listserv. The survey asks general demographic questions such as work environment, caseload, education level, and if the participant utilizes FORDISC® in forensic casework. A total of 23 participants took the survey, of which 18 were active forensic anthropologists who use FORDISC® in casework. Results indicate relatively high consistency in parameters such as the narrowing down of populations by sex estimation as opposed to running both sex categories and starting with all possible “ancestral groups” before analysis. Areas that show relatively low consistency in use include whether practitioners use the same process between analyses or not, how variable number and stepwise selection are used, and whether outliers are removed from the analysis. It should be noted that all topics asked about had some variability in use, but some were split much more evenly. This indicates that the allowance of freedom in statistical analysis will lead to differential use of the model, and more work needs to be done to understand how this will affect classification outcomes.

**Reference:**

<sup>1</sup>. Ousley S, Jantz R. (2013). FORDISC® 3. *Rechtsmedizin*, 23(2), 97–99. <https://doi.org/10.1007/s00194-013-0874-9>.

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**Forensic Anthropology; Population Affinity; FORDISC®**

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**A74 Evaluating Expertise in Forensic Anthropology**

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**Learning Objective:** After attending this presentation, attendees will understand how expertise is evaluated within disciplines and recognize the utility of credentials and certification for demonstrating expertise.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the difficulties in identifying disciplinary experts and the need to clearly define qualifications and recognize the value of forensic anthropology expertise in medicolegal death investigations.

Forensic scientists' expertise is evaluated by peers, attorneys, law enforcement, judges, juries, and the public. During testimony, we are considered "expert witnesses," but what defines expertise, and how do we assess it? Collins and Evans developed a model of expertise that defines Specialist Tacit Knowledge as the requisite knowledge to be a practitioner in a discipline.<sup>1</sup> Specialist Tacit Knowledge consists of two forms of expertise: interactional and contributory. Interactional expertise represents the ability to interact meaningfully with contributory experts performing disciplinary work, while lacking the technical knowledge to perform it oneself. Contributory expertise is the highest level of expertise and represents an individual with sufficient knowledge of a subject area to interact with other experts at a complex level and perform complex tasks competently.<sup>2,3</sup> Contributory expertise must be developed through enculturation with other contributory experts.<sup>1</sup>

We examined interactional expertise in forensic anthropology by posing questions ( $n=11$ ) about the discipline and its practice to 27 voluntary participants with varying degrees of experience in forensic anthropology. The authors ranked the respondents' forensic anthropology expertise using a five-point scale ranging from "expert" to "no knowledge" based on their certification, education, and experience in the discipline. All interviews were recorded and transcribed for anonymization. Next, seven voluntary research participants with varying backgrounds in forensic anthropology evaluated the transcripts to determine the level of expertise of the respondents. Evaluators used the five-point scale to rate each of the answers individually and provided an overall rating of perceived expertise in forensic anthropology for each respondent. Evaluators were also asked to identify cues or information they used to reach their assessments. Consistency between evaluators' ratings was calculated using a weighted Cohen's kappa. Polychoric and tetrachoric correlations, and Cohen's kappa were used to examine the relationship between perceived and actual expertise.

Results indicated there was little to no agreement between author-assigned expertise based on credentials and experience, and the expertise level assigned by the evaluators based on respondent answers (polychoric correlation=-0.027; Cohen's kappa=0.32). The correlation increased when expertise rankings were dichotomized into expert and non-expert (tetrachoric correlation=0.45; Cohen's kappa=0.49). Generally, a moderate correlation was found between scores for each answer and the overall rated expertise by the evaluators. The questions most highly correlated with author-rated expertise focused on methods (e.g., FORDISC® and subadult age estimation) and standards development within the discipline. Inter-rater agreement was variable, ranging from concordance smaller than chance, to a weighted kappa of 0.62, and was unrelated to evaluator expertise level. Factors most often reported to assess expertise included the use of discipline-specific terminology and overall length of the answers.

These results indicate that accurately identifying expertise in forensic anthropology may be challenging for both experts and non-experts, at least based on interactional or oral (i.e., non-practical) assessments. The difficulties in distinguishing contributory and interactional expertise highlight the importance of credentials and certification in demonstrating expertise for the purposes of forensic anthropological casework and testimony. Our results suggest that individuals working within the criminal justice and medicolegal death investigation systems (e.g., judges, lawyers, law enforcement) may have difficulty identifying an expert in forensic anthropology without appropriate credentials and/or certification. As we have argued elsewhere, it is necessary to establish disciplinary expertise via certification, with licensure being the next logical step.<sup>4-6</sup> The full and permanent integration of certified forensic anthropology experts into medical examiner's offices may alleviate the burden on stakeholders attempting to identify experts from a pool of practitioners who may or may not have the necessary expertise.

This project was approved by the institutional review boards of all authors' institutions.

**References:**

1. Collins H, Evans R. *Rethinking expertise*. University of Chicago Press. Chicago, IL: University of Chicago Press, 2007.
2. Collins H, Evans R. *Expertise revisited, part I—Interactional expertise*. *Studies in History and Philosophy of Science Part A*. 2015;54:113-23.
3. Collins H, Evans R, Weinel M. *Expertise revisited, part II: Contributory expertise*. *Studies in History and Philosophy of Science Part A*. 2016;56:103-10.
4. Pilloud MA, Passalacqua NV, Philbin CS. Caseloads in forensic anthropology. *American Journal of Biological Anthropology*. 2022;177(3):556-65.
5. Passalacqua NV, Pilloud MA. The need to professionalize forensic anthropology. *European Journal of Anatomy*. 2021;25(S2):35-47.
6. Passalacqua NV, Pilloud MA, Congram D. Forensic anthropology as a discipline. *Biology* 2021;10(8):691-708.

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**Forensic Anthropology; Expertise; Qualifications**

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**A75 The Ceremonial Use of Human Remains: Palo Monte in Maricopa County, Arizona**

*Andrew Seidel, PhD\*, King County Medical Examiner's Office, Seattle, WA; Laura Fulginiti, PhD, Maricopa County Office of the Medical Examiner, Phoenix, AZ*

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**Learning Objective:** After viewing this presentation, attendees will be familiar with the ways in which human skeletal remains have been incorporated into ceremonial objects used within Palo Monte as practiced in Maricopa County, AZ, as well as useful contextual clues for assessing the medicolegal significance of such remains.

**Impact Statement:** This presentation will impact the forensic science community in two primary ways. First, it will detail the skeletal and taphonomic features used to assess the medicolegal significance of the skeletal remains incorporated into ceremonial objects. Second, it will document regional variation in terms of the contents and associations of the items incorporated into objects used within the practice of Palo Monte.

Concerned about the medicolegal significance of human skeletal remains that had been incorporated into ceremonial objects, law enforcement transported the objects in question to the Maricopa County Office of the Medical Examiner for evaluation. These included four containers fashioned from the posterior portions of different cranial vaults as well as five heavy ceramic bowls filled with earth—several of which had evident human skeletal elements visible on their surface. Following radiographic assessment, these ceramic bowls were transferred to Anthropology to facilitate the excavation and documentation of their contents. Based primarily on taphonomic indicators (e.g., the removal of the cranial vault using a sectioning cut such as those used during autopsy or for the preparation of teaching specimens, the numbering of skeletal elements, patina, odor, etc.), the skeletal remains were determined to not be of medicolegal significance.

For comparative purposes, this presentation focuses on the largest and most complex of the bowls. Photographs and written descriptions of its contents and their spatial relationships to one another are provided as well as, where possible, a discussion of the potential symbolism associated with these items. Moreover, the contents of these containers are compared to those documented within published reports originating along the eastern coast of the United States. While similarities between the cases clearly indicate a shared set of practices, the incorporation of novel items, such as Mexican currency and archaeological tools, demonstrate that Palo Monte is a living tradition that is fully capable of taking on characteristics of its practitioners and their local cultural contexts. Forensic anthropologists should be aware of the dynamic nature of these practices so that they may anticipate and document their regional variability. Familiarity with the different ceremonial practices that incorporate human skeletal remains will allow forensic anthropologists to address questions of medicolegal significance more efficiently when they arise.

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**Palo Monte; Forensic/Non-Forensic; Human Remains**

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**A76 In Science We Trust? An Analysis of Demographic Factors Influencing Faith in Forensic Science**

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**Learning Objective:** After attending this presentation, attendees will understand that factors like social race and forensic-science experience can influence an individual's perceptions of forensic science.

**Impact Statement:** This presentation will impact the forensic science community by calling for clear science communication and commitment to continuing theoretical and methodological improvement.

Forensic science is a source of interest to the lay public, with televised, streaming, and podcast content often focusing on its practice. Yet it is unknown to what degree forensic-science conclusions are trusted by non-practitioners versus actual forensic practitioners, nor how social demographic factors affect trust in the forensic sciences.

Using listservs and social media, participants both with and without forensic expertise were recruited for an Institutional Review Board (IRB) -approved Qualtrics® survey. Respondents ( $n=370$ ) were asked to report their age, gender, race, level of forensic-science experience, forensic-science specialization (if relevant), and level of trust in the forensic sciences. The following hypotheses were tested: (1) non-practitioners would express greater trust in the forensic sciences than practitioners; and (2) respondents from majority racial and ethnic groups would express greater trust in the forensic sciences than respondents from systematically marginalized groups, regardless of forensic expertise.

No respondents reported being “completely mistrustful” of the forensic sciences, and few reported having “limited” trust; 98% of 52 non-forensic respondents and 96% of 318 forensic respondents reported either “considerable” or “complete” trust. However, statistically significantly more of the non-forensic respondents reported “complete” trust in forensic science conclusions (27%, vs. 13% of practitioners) relative to “considerable” trust (71%, vs. 83% of practitioners; Chi-squared test;  $p=0.015$ ). When forensic anthropologists were analyzed separately, they had the lowest rates of “complete” trust of all groups (10%), though differences between anthropologists ( $n=185$ ) and other forensic specialists ( $n=133$ ) were not significant (Chi-squared test;  $p=0.219$ ).

Fifteen respondents reported no race category(s). Forty-nine reported group identities that have been historically and systematically marginalized in the United States social context, including (respondent quotes): “African American,” “Asian,” “Biracial,” “Black,” “Hispanic,” “Indian Asian,” “Latinx,” “Mexican,” “Middle Eastern,” “Native American,” “Puerto Rican,” and “Southeast Asian.” Contradicting the second hypothesis, more of these respondents expressed “complete” trust in the forensic sciences than the 302 “Caucasian”/“European”/“White” respondents (20% vs. 14.5%), though the difference was not statistically significant (Chi-squared test;  $p=0.399$ ).

In essence, this survey's lay respondents trusted forensic science significantly more than did the forensic scientists themselves. Given the popularity of the forensic sciences in the media, and their often-unrealistically positive portrayal, this finding is unsurprising. Contrarily, the finding that Black, Indigenous, and People of Color (BIPOC) commonly expressed “complete” trust in forensic science is indeed surprising. Globally, BIPOC experience more racism and socioeconomic discrimination than do White people—systemic inequities that extend to law enforcement and, by extension, medicolegal systems. Yet, lived experiences necessarily differ between individuals, a topic that remains unexplored in this study due to the small sample of BIPOC respondents. A more in-depth, qualitative study should examine how differing experiences of race influence levels of trust in the forensic sciences.

These findings demonstrate the perpetuation of overly high public expectations for forensic science. This has been recognized as a problem for trial by jury and other medicolegal contexts for many years, yet little headway has been made in shaping more realistic expectations. With their holistic training, forensic anthropologists are well poised to communicate with stakeholders clearly about such factors as cognitive bias, method error, probability, the theory-laden nature of the facts they produce, and the unreality of pure scientific objectivity. They might also capitalize on public interest (e.g., host a podcast exploring forensic science myths and misconceptions). Complementing these approaches, practitioners can elevate their science to approximate juror expectations by committing to continued theoretical, technological, and methodological improvement.

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**Forensic Anthropology; Science Communication; Race**

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**A77 An International Validation Study of Artificial Intelligence (AI) -Guided Craniofacial Superimposition in a Contemporary Population Sample**

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**Learning Objective:** After attending this presentation, attendees will better understand the state of the art in AI-guided craniofacial superimposition and the latest developments in the automation of the different steps of the technique. Attendees will be presented with the results of an international validation study of this methodology and learn about the various factors that can influence the accuracy and reliability of the application of the technique.

**Impact Statement:** This presentation will impact the forensic science community by providing an insight into the state of the art in AI-guided craniofacial superimposition. Attendees will gain insight into newly developed tools employed by multiple researchers around the globe over an unprecedented number of comparisons to put its accuracy and reliability to the test. Additionally, this presentation will provide the forensic science community with a replicable validation study for craniofacial superimposition.<sup>1</sup>

Craniofacial superimposition is a forensic technique that aims to identify unknown found human remains through the comparison of an overlay of a skull 2D or 3D image with one or more antemortem facial photographs of a missing person. The expert tries to correlate the morphological relationship of the cranial and facial structures to determine, with a reasonable degree of confidence, whether they belonged to the same person.

Despite its potential, the spread of the technique has been limited because it involves tedious, manual, and repetitive tasks that heavily rely on the knowledge of the expert, and the evaluation of the correspondences can be a subjective and error-prone process. However, for the past decade, researchers from Panacea Cooperative Research and the University of Granada have been researching and developing automatic AI-guided methods in each stage of the process, with the resulting algorithms and tools being added into Skeleton-ID, a comprehensive software tool for human identification.

With the goal of validating the technology, we were granted access to contemporary, real-life cases from the University of South Florida (USF) donated skeletal collection. Using Skeleton-ID and following the Methodologies and Protocols of Forensic Identification by Craniofacial Superimposition (MEPROCS) consortium standards, ten participants were asked to solve a 25 Postmortem (PM) vs 25 Antemortem (AM) scenario. With 47 photographs in the sample, the number of skull-face overlays to perform and evaluate exceeded 1,000 for each participant. To reduce the workload, an automatic ranking that lists candidates from most to least likely to have belonged to the same person was used. To test the accuracy of the ranking, participants were asked to evaluate the first three listed candidates, reducing the comparisons from 1,175 to 141.

In this work, we present the results and insights relayed by the participants and assess the reliability of the proposed AI-guided methodology, while providing the scientific community with a replicable validation study for craniofacial superimposition over an unprecedented number of problems, surpassing 1,000 comparisons.

**Reference:**

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**Craniofacial Identification; Craniofacial Superimposition; Artificial Intelligence**

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**A78 The Life-Long Immobility Effects on Skeletal Morphology: A Case Study Involving Cerebral Palsy**

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**Learning Objective:** After attending this presentation, attendees will have an increased understanding of the effects that a lifelong, non-ambulatory condition can have on the human skeleton.

**Impact Statement:** This presentation highlights the influence of Cerebral Palsy (CP) on skeletal remains in an effort to provide practitioners with a better understanding of how this, and other disease states and pathology, can influence both skeletal health and skeletal analysis. This presentation will impact the forensic science community by sparking discussions on how we as forensic anthropologists can best serve decedents with health disparities in our casework.

A holistic understanding of skeletal development and pathology is imperative within the field of forensic anthropology to better serve our communities and decedents. While forensic anthropologists are well-trained in the assessment of biological profile and trauma interpretation on overall skeletally healthy individuals, few have access to remains of decedents with known pathologies, disorders, or disabilities that may heavily influence the development and condition of skeletal material. This presentation will impact the forensic science community by raising awareness, especially among forensic anthropologists, of how CP may present in the skeleton and how long-term immobility of an individual can affect bone remodeling and bone quality.

In the United States, it is estimated that 1.5–3.0 in every 1,000 individuals has CP.<sup>1</sup> Although the effects of CP are greater in subadults as the disability can affect proper growth and development, this lifelong disability results in permanent effects on skeletal health. This set of neurological disorders often leads to an imbalanced use of the limbs and, as a result, an increase in osteoarthritis and osteoporosis.<sup>2,3</sup> Along with these two disease states, the bone may see a decrease in density and develop an overall abnormal morphology. This conglomerate of skeletal changes must be understood by the forensic anthropology community to serve CP decedents, especially in the context of medicolegal death investigations.

The focus of this study is an adult individual with CP whose donated remains are currently located at the Northern Michigan University (NMU) Forensic Anthropology Research Laboratory. The authors present a list of abnormal skeletal characteristics that were observed and appear to be directly related to the decedent's CP and have the potential to influence the assessment of the biological profile, trauma, and taphonomy. Additionally, this study presents distinctive plastic deformation of the ribs that appears to be related to the donor's prolonged fixed positioning prior to death, as well as some remarkable examples of antemortem trauma affecting the ribs, femur, and humerus.

**References:**

- <sup>1.</sup> Sadowska M, Sarecka-Hujar B, Kopyta I. Cerebral Palsy: Current Opinions on Definition, Epidemiology, Risk Factors, Classification and Treatment Options. *Neuropsychiatr Dis Treat*. 2020 Jun 12;16:1505-1518.
- <sup>2.</sup> Haak P, Lenski M, Hidecker MJC, Li M, Paneth N. 2009. Cerebral palsy and aging. *Developmental Medicine & Child Neurology*, 51, pp.16-23.
- <sup>3.</sup> French ZP, Torres RV, Whitney DG. 2019. Elevated prevalence of osteoarthritis among adults with cerebral palsy. *Journal of Rehabilitation Medicine*, 51(8), pp.575-581.

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**Cerebral Palsy; Immobility; Biological Profile**



**A79    The Mississippi Office of Forensic Laboratories (MOFL) Missing and Unidentified Persons Repository  
Project: A Collaborative Success Story**

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**WITHDRAWN**

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**A80 The Georgian Recovery, Documentation, and Identification Project: Preliminary Results From a Mass Grave Excavation**

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**Learning Objective:** After attending this presentation, attendees will be familiar with the Georgian Recovery, Documentation, and Identification Project (GRDIP) as part of a humanitarian collaborative effort between the Georgian Association of Forensic Anthropology (GAFA), Michigan State University (MSU), and Texas State University (TXST).

**Impact Statement:** This presentation will impact the forensic science community by discussing forensic archaeology in Georgia and the use of advanced mapping techniques to document the excavation of individuals from a mass grave, preserving context in which to interpret physical evidence. This project sheds light on a repressive historical period in Georgia and represents one of the first professional archaeological investigations of a grave from this period. Data and evidence collected during our investigations will be used to clarify the fate of the missing.

In August 2021, the GRDIP collaborative completed excavation of a mass grave containing 28 people in the Adjara Region in Georgia. The grave was one of five located on an old Soviet-era military base, suspected to date to the late 1930s, during Stalin's Repressive period known globally as the Great Terror. During this time, Joseph Stalin and the Soviet Government were responsible for the disappearance of over 1,000 people in Georgia alone.<sup>1,2</sup> In 2017, based on informant information, the Batumi Eparchy trenched the area looking for mass graves.<sup>3</sup> Four mass graves were located and excavated; however, the individuals were removed without systematically recording contextual information. As a result, the authenticity of the graves and the period they relate to has been scrutinized by academics and historians. A fifth grave was found, but not excavated due to protests that the previous excavations did not follow scientific protocols and were not performed by trained experts.

During the August 2021 excavation, various mapping and photogrammetry techniques were implemented to document and record the excavation, minimizing commingling of smaller elements like hands and feet, creating specific mapping points and labels for artifacts, preserving the physical evidence associated with the grave and the contextual evidence of each individual. The positioning of bodies individually and collectively suggests intentionality, which can be visualized with individual and collective photogrammetry models. Furthermore, mapping points collected by the total station for each individual documenting elevation and positioning of the head, shoulders, elbows, hands, hips, knees, and feet on the body can be overlaid with photogrammetry models to understand each individual's position relative to the grave. A Harris Matrix was created to document the stratigraphic and relative position of each individual within the grave.

With careful scientific documentation of this mass grave from the Adjara Region in Georgia, we were able to scientifically record individuals while excavating a mass grave. The process collectively helped to minimize commingling and allows for reconstructing individual placement. We stress the importance of these techniques for future excavations in the region under the supervision of qualified experts like the GAFA with the support of the GRDIP collective to preserve contextual information for further studies and historical documentation.

**References:**

1. Luarsabishvili V. A brief history of the Great Terror in Georgia. *Revista de Historia Actual*, 2017;14:175-183.
2. Avalishvili L. The "Great Terror" of 1937–1938 in Georgia: Between the Two Reports of Lavrentiy Beria. *Caucasus Analytical Digest*, 2010;22:2-6.
3. *Agenda.ge*. 2020. Remains of Victims of Soviet Repression Identified. <https://agenda.ge/en/news/2019/958> (accessed August 15, 2020).

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**Forensic Archaeology; Human Rights; Photogrammetry Modeling**

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**A81 From a Simulated Environment to Real-Life Applications: Human Identification on Maxillary Sinus Morphologies**

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**Learning Objective:** After attending this presentation, attendees will understand the potential of morphological assessments of maxillary sinuses for identification determinations.

**Impact Statement:** This presentation will impact the forensic science community by correlating a potential human identification method on maxillary sinus morphologies in a simulated and real-life environment.

**Introduction:** Analyzing skeletal structures for human identification is a major forensic procedure.<sup>1</sup> Visual comparisons of antemortem and postmortem radiographs are often used as a basis for identification. Maxillary sinuses display a high morphological variability and can be used for identification by examining morphologies on antemortem and postmortem radiographs as found with measurements and volumetric approaches.<sup>2</sup> Furthermore, they offer quick results as the features frequently appear on dental overview images.<sup>3</sup> However, before morphological comparisons of maxillary sinuses can be used to identify remains, the system's applicability must be verified.

This study aims to correlate study results of maxillary sinus morphological comparisons in a simulated environment as well as a real-life environment to further understand the applicability of maxillary sinus morphologies in human identification.

**Materials and methods:** Samples in the study using a simulated environment include a total of 600 maxillary sinuses sampled on Computed Tomography (CT) and X-ray images from nine populations. The sample of the real-life environment used differently aged X-ray images of eight individuals, made available from two populations. All individuals used were over 20 years of age and showed no pathological changes in the maxillofacial area. Analyses of the images were performed separately for the right and left maxillary sinuses. Sinus morphologies got extracted in the software SHAPE™ ver. 1.3, applying Elliptic Fourier Analysis. Ante- and postmortem data of extracted morphologies were analyzed using Euclidean and Mahalanobis distances.

**Results:** Both studies of simulated and real-life environments found extremely low Euclidean distances for matching ante- and postmortem morphology pairs. Those close distances function as preliminary cut-off values between matching and non-matching sinus pairs and could make an application in forensic cases possible. Notably, cut-off values in real-life applications are higher than in the simulated environment. Therefore, those accumulated cut-off values could potentially be used as a threshold providing information about the degree of sinus morphological matching. Correlations of Mahalanobis distances support these results.

With matching morphologies only between each antemortem and postmortem morphology of the same individual, correlations of the two studies support and encourage the future application of maxillary sinus morphologies on radiographic images for human identification.

**References:**

- <sup>1</sup>. Amin MF, Hassan EI. (2012) Sex identification in Egyptian population using Multidetector Computed Tomography of the maxillary sinus. *Journal of Forensic and Legal Medicine*, 19 (2), 65-69.
- <sup>2</sup>. Xavier TA, Dias Terada ASS, da Silva RHA. (2015) Forensic application of the frontal and maxillary sinuses: A literature review. *Journal of Forensic Radiology and Imaging*, 3 (2), 105-110.
- <sup>3</sup>. Altug HA, Ozkan AP. (2011) Diagnostic Imaging in Oral and Maxillofacial Pathology. In: Eröndü, O.F. (ed.) *Medical Imaging*.

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**Forensic Anthropology; Morphological Evaluation; Paranasal Sinuses**

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**A82 Computer-Assisted CT/R Identification (CACTI®)**

*Sharon Derrick, PhD\*, Texas A&M University-Corpus Christi, Corpus Christi, TX; Ruby Mehrubeoglu, PhD, Texas A&M University-Corpus Christi, Corpus Christi, TX; Omer Sevinc, PhD, Texas A&M University-Corpus Christi, Houston, TX*

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**Learning Objective:** Attendees will learn about the progress of the CACTI® Project, a collaboration between computer engineers, computer scientists, a Computed Tomography (CT) nuclear physicist, and forensic anthropologists to develop a new forensic image-matching software program.

**Impact Statement:** This CACTI® presentation will impact the forensic science community by examining the potential usefulness of this software for identification of the completely unknown decomposed or skeletal decedent medical examiner/coroner cases.

The number of people in the United States who die without a name is increasing.<sup>1</sup> Many of these decedents are indigent and homeless. Others have met with violence or a mass fatality natural event. Decomposed or skeletal decedents received by medical examiner/coroner offices without an associated name are typically the most difficult identification cases, especially if there is no family reference sample in Combined DNA Index System (CODIS). Standard radiographic or dental identification methods are useful in these cases, but they require a tentative name to find comparison records. Although radiographic surveys and CT scans are commonly performed as diagnostic tools, especially in emergency departments, the records cannot be accessed without an identifying name and date of birth.<sup>2</sup> Recent advances in Artificial Intelligence (AI) methods and tools are available to support the implementation of deep learning algorithms for a novel identification software model.

In this study, a novel AI-based approach is investigated for the potential to search a large database of antemortem radiology records and generate a statistically based “shortlist” of the best possible matches with the unknown decedent. This presentation describes the results from a comparative analysis of the implementations of three AI networks, including a custom Convolutional Neural Networks (CNN) model, a Resnet-50 transfer learning model, and a Siamese model that incorporates Triplet Networks. More specifically, image processing and CNN methods have been incorporated and the New Mexico Decedent Image Database has been used to train and test the deep learning models.<sup>3</sup> The custom CNN model, the Resnet-50 transfer learning model, and the Siamese models have been tested separately on the dataset and the results compared. The custom model consists of 7 layers, the Resnet-50 has 50 layers, and the Siamese model incorporates 8 layers. The custom model runs with 50 epochs whereas the other networks are executed for 100 epochs.

The findings show that the Siamese model provides the most accurate results compared to the other models with low loss value in predicting the similarity of the image with those held in the database. The Resnet-50 and the Custom CNN models require the whole dataset to be retrained when new data is acquired, creating challenges in expanding databases. To overcome such problems, a novel Siamese network-based architecture that incorporates Triplet Networks was implemented, which not only eliminates new training requirements, but based on the experimental results, also performs better than the other methods. Triplet Network was implemented as a deep learning algorithmic solution to identify the top ten best matches to an unknown input image based on a similarity measure. As the name implies, three input sample images are required as the anchor sample, positive sample, and negative sample. The model easily distinguishes dissimilarities between the target (picked) image and the images in the dataset. The preliminary results of setting up and testing the algorithms to identify best matches to a skull test radiographic image are presented. Results show that the Siamese model gives better accuracy results than the other models with an accuracy of 0.82, where the custom CNN model and Resnet-50 give accuracies of 0.65 and 0.7, respectively.

**References:**

- <sup>1.</sup> <https://namus.nij.ojp.gov/>, *Unidentified Persons Search*, accessed 31/07/2022.
- <sup>2.</sup> <https://idataresearch.com/over-75-million-ct-scans-are-performed-each-year-and-growing-despite-radiation-concerns/>, accessed 31/07/2022.
- <sup>3.</sup> *The New Mexico Decedent Image Database* <https://nmdid.unm.edu/><https://nmdid.unm.edu/><https://nmdid.unm.edu/>, Download Images, accessed 31/07/2022.

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**Decedent Identification; Artificial Intelligence; Radiological Methods**

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**A83 Forensic Identification Using Craniofacial Superimposition: Estimation of Facial Soft Tissue Depth Using Machine Learning Techniques**

*Carlos Sanchez-Muñoz, MSc, University of Granada, 18071, Andalucía, SPAIN; Pablo Mesejo, PhD\*, University of Granada, Panacea Cooperative Research and DaSCI, Granada, Andalucía, SPAIN; Sergio Damas, PhD, University of Granada, Granada, Andalucía, SPAIN*

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**Learning Objective:** The goal of this presentation is to inform attendees of the application of novel machine learning techniques to estimate Facial Soft Tissue Depth (FSTD).

**Impact Statement:** An accurate estimation of FSTD is crucial in craniofacial identification studies. This presentation will impact the forensic science community by informing attendees that our machine learning approach outperforms traditional methods based on the average criterion.

The FSTD is a relevant measure in facial reconstruction and craniofacial superimposition, and it is used to estimate the position of facial landmarks from the corresponding landmarks on the skull. When tackling craniofacial superimposition as a craniofacial landmarks matching problem, the FSTD is traditionally calculated as the average depth per landmark from a certain sample of a particular population. In this presentation, we propose avoiding the mere use of this average, and substitute this strategy by the regression of a series of 3D landmarks on the face from 3D landmarks on the skull in order to estimate the thickness of the soft tissue that separates the skull from the face for each particular individual. The dataset employed includes cranial and facial landmarks of 500 living individuals, along with their sex and age.

The problem tackled in this paper is extremely challenging because of the reduced number of available data (which limits the amount of training examples); the large number of missing values (37% of all feature values); the lack of an initial alignment of the skulls (so the available data are not directly comparable and usable); or the absence of a clear consensus in the scientific community about acquiring and labeling the landmarks, among other issues. In terms of regression analysis, the landmarks in the skull would be the independent variables, or predictors, and since the final objective is the estimation of the soft tissue, the landmarks on the face or the thickness itself would represent the dependent variables. Different regression methods are compared (K-nearest neighbors, multilayer perceptron, regression forest, support vector regression, and partial least squares), and the benefits of using imputation techniques for the missing values and feature selection to choose the set of landmarks to train with, as well as the impact of adding additional landmarks and biological profile information (like sex and body mass index) are studied.

Our results suggest that machine learning models can overcome the average criterion (our baseline) by around 10% error. We also verify that the introduction of biological profile information, such as sex and body mass index, provides a remarkable improvement in terms of performance (5–10%).

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**Facial Soft Tissue Depth; Regression Problems; Machine Learning**

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**A84 The Utility of Digital Bone Loss (DBL) for Biological Profile Reconstruction in Forensic Anthropology**

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**Learning Objective:** After attending this presentation, attendees will understand what DBL is and how it can be used in forensic anthropology. Additionally, a new method for sex and age estimation of the Korean population using DBL will be introduced to the attendees.

**Impact Statement:** This presentation will impact the forensic science community by introducing the utility of a new methodological tool (i.e., DBL) to be used by forensic anthropologists working on Computed Tomography (CT) images.

Recently, 3D imaging technology has attracted interest from many forensic anthropologists as a non-invasive assessment tool.<sup>1</sup> Moreover, rapidly evolving 3D image processing software contributes to the wide use of 3D data (e.g., CT images). Among the common functions incorporated in those software programs is to segment a part of a CT-scanned individual by defining upper and lower thresholds of intensity and selecting the tissues corresponding to the range of intensity (a.k.a. “threshold effect”). Since bones are the hardest tissue in a body, the lower threshold for bone segmentation is generally set high so that other types of tissues can be excluded; however, increasing the lower threshold inevitably results in a loss of part of the bones having the intensity below the lower threshold. In this study, DBL is defined as the partial bone loss by increasing the lower threshold while the threshold effect is performed. Bone density of living people varies by age and sex in the way that it decreases as one gets older, and males tend to have greater densities than females of a similar age group.<sup>2</sup> This study hypothesizes that DBL can be used as a proxy of actual bone density and, when two different lower thresholds are applied to the same bone, greater DBL will be observed in older people (compared to the younger people) and in females (compared to males). The goal of this study is to test this hypothesis and find the most appropriate lower thresholds for sex and age estimation purposes. Additionally, regression equations for sex and age estimation of the Korean population using DBL will be devised. CT scans of the left os coxae and femora from 112 Korean individuals (62 males, 50 females) were used. In 3D Slicer, bones were segmented using four different lower thresholds (450, 500, 550, and 650 for the os coxae; 350, 450, 550, and 650 for the femora), and their 3D models were constructed. After obtaining the number of faces constituting each 3D model in CloudCompare, DBL was calculated as follows:  $DBL(p/q) = [(\#faces \text{ in model P} - \#faces \text{ in model Q}) \times 100] / (\#faces \text{ in model P})$  (where 3D models P and Q are produced using the lower thresholds p and q, respectively)

Female os coxae show a significantly greater DBL than males for all thresholds except for p/q=550/600. Correlation between age and DBL varies depending on the sex and thresholds. In the femora, significant sexual differences were noted only at p/q values of 350/450, 450/650, and 550/650. Age is correlated with DBL only in females for p/q=550/650. Overall, in terms of accuracy, the femur was a better indicator for sex and the os coxa was a better age indicator. As for age estimation, females achieved a greater accuracy than males. Detailed values and equations for sex and age estimation will be provided in this presentation. This presentation introduces a new methodological tool that can be used as a proxy of bone density. Since the concept of DBL is easy to understand and its calculation is quick and simple using open-source software, DBL has great potential to be used among forensic anthropologists working on CT data.

**References:**

1. Jeong Y, Woo EJ, Lee S. 2020. Bibliometric analysis on the trend of the computed tomography (CT)-related studies in the field of forensic science. *Appl. Sci* 10(22): 8133.
2. Alswat KA. 2017. Gender disparities in osteoporosis. *J Clin Med Res* 9(5): 382-387.

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**Digital Bone Loss; Computed Tomography; Korean**



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**A85 Through the Looking Glass: The Application of Aspartic Acid Racemization to Age Estimation in a Spanish Sample**

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**Learning Objective:** After attending this presentation, attendees will consider the possibility of applying aspartic acid racemization in combination with forensic anthropology methodologies to improve age-at-death estimation.

**Impact Statement:** This presentation will impact the forensic science community by providing an extensive study of the applicability of this technique to a Spanish sample and its accuracy for age estimation.

Age estimation represents one of the fundamental parameters in forensic anthropology in creating the biological profile toward the correct identification of an individual. This parameter is particularly important in mass disaster scenarios where skeletons are often incomplete, which makes the correct identification of the victims difficult. Teeth are frequently preserved long after all other tissues have disappeared and are often used to estimate characteristics like age at death.

There are several approaches to estimate the age at death in adult individuals. Forensic anthropology macroscopic techniques are non-invasive methods for this purpose. Although the combination of several of these methods has improved the estimates, still in certain cases the difference between chronological and predictive age may still be around  $\pm 10$  years. New research trends are focused on the inherent process of aging, which produces changes in tissues and organs at different biochemical levels. One of the oldest and most studied approaches in this field is aspartic acid racemization. This is based on the conversion, with the age, of the amino acids in their regular L form into their specular form, D. If these D-amino acids are introduced in metabolically stable proteins, these produce changes in their biological and chemical activities, leading to the degenerative changes of aging. Aspartic acid has the fastest racemization rate and for that reason it is the chosen amino acid when applied to this technique.

The accuracy of aspartic acid racemization in age estimation has been widely demonstrated; however, only a few studies have assessed its accuracy in different populations. The aim of this research was to assess the accuracy of aspartic acid racemization in a Spanish sample and its applicability to forensic cases.

Fifteen healthy erupted third molars from two Spanish populations (ages 19–70 years) were collected from dental clinics. The Smithsonian Institution's ethical committee approved all procedures related to experimentation with human subjects. The teeth were cleaned, and the enamel and cementum were removed. The dentin was isolated, mechanically ground, and divided into aliquots of 200mg each. Then, each aliquot was subjected to chemical derivatization to detect D and L forms of aspartic acid through gas chromatography/mass spectrometry, according to a previously published protocol. D/L ratios were calculated and, after the application of a regression analysis, a formula for age estimation was developed. The results were similar to previous studies, obtaining an  $R=0.91$  between racemization ratios and age and a Mean Absolute Error (MAE) between chronological and predictive age of 5 years. These results were ratified by leave-one-out cross-validation, as well as the application of the formula to five teeth of a known age. Despite these promising results, this technique is not exempt from drawbacks; for example, since racemization is an enzymatic reaction, it accelerates by fire, so it cannot be applied to corpses exposed to fire.

Future studies will be able to expand on these results, using different types of teeth, analyzing other populations, and extending the age range in order to apply this methodology to forensic cases and to combine it with forensic anthropology findings.

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**Age-at-Death; Aspartic Acid Racemization; Spanish Sample**

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**A86 Informative Priors Produce Precision-Accuracy Tradeoff in Dental Developmental Age Estimation**

*Valerie Sgheiza, MA, MS\*, University of Illinois At Urbana-Champaign, Champaign, IL*

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**Learning Objective:** After attending this presentation, attendees will better understand the effects of Bayesian priors and the correlation matrix on estimates of age.

**Impact Statement:** This presentation will impact the forensic science community by providing guidelines for choosing an optimal prior when conducting Bayesian age estimates. This presentation will also serve as the introduction to a publicly available R package for estimating developmental age from the dentition.

Bayesian methods have seen increasing use in dental age estimation; however, dedicated study of priors has been limited. In Bayesian statistics, the prior encompasses the analyst's knowledge of the system before collecting data. In age estimation, the prior consists of the expected age distribution of the population of the unknown individual, such as all forensic cases in a region. The downstream effects of reference sample on age estimates via the model correlation matrix are also not well understood. Ages were estimated from individuals in the New Mexico Decedent Image Database (NMDID) using four different Bayesian priors and nine model correlation matrices with a full factorial design.<sup>1</sup> To avoid effects of overfitting, all model fits were performed on a sample of living dental patients from London, England. The hypotheses tested were that an informative prior would produce more precise and accurate estimates of age and the best performing correlation matrix would be that from the largest reference sample.

The model fitting dataset consisted of dental development scores of left mandibular permanent teeth from 880 London children 3–21 years old.<sup>2</sup> A Bayesian cumulative probit model was fit to the full sample, each half sample split by either sex or ancestry (Bangladeshi or European) and each quarter sample split by sex, for a total of nine model fits. The validation dataset consisted of 381 dental development scores from NMDID publicly available from the Stull lab Subadult Virtual Anthropology Database.<sup>3</sup> Teeth in the lowest stage in the NMDID sample were rescored by the author to match crypt staging in the London sample. Ages were estimated in the NMDID validation sample with a multivariate normal likelihood, one of four priors (uniform, National Missing and Unidentified Persons System [NamUs] missing persons, Centers for Disease Control and Prevention [CDC] total mortality, and CDC homicides) and one of nine correlation matrices (one from each model fit) for a total of 36 rounds of age estimation. The remaining model parameters were from the London model fit to the full 880-individual sample. Performance of each matrix-prior combination was assessed using residual error, 95% High Dynamic Range (HDR) width relative to true age, and 95% HDR success rate at capturing the true age of the individual.

The uniform prior consistently produced the largest age intervals, smallest residuals, and success rates similar to missing persons and total mortality. The homicide prior consistently produced the smallest age intervals, lowest success rates, and largest residuals. The total mortality prior had residuals similar to the homicide prior but intermediate age intervals. The missing person prior had intermediate residuals and interval widths. Residual error had similar results across all nine matrices. Age interval width was larger for matrices from samples containing girls than from those exclusively derived from boys. The opposite trend was seen for success rate. In general, matrix-prior combinations with larger age intervals had lower residual error (correlation = -0.784) and higher success rates (correlation = 0.581). In conclusion, choice of Bayesian prior and correlation matrix represents a tradeoff between precision and accuracy. While the model correlation matrix may not always be under a practitioner's control, when choosing between informative and uninformative priors for age estimation, practitioners should consider whether the accuracy of the point estimate or the precision of the age interval is more important in a particular application.

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**Age Estimation; Dental Development; Bayesian**

## A87 Risser's vs. Schulz's Sonography Staging for Medicolegal Age Estimation in the Living

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**Learning Objective:** After attending this presentation, attendees will be familiar with the two established staging methods (Risser's and Schulz's) of estimating age in the living through ultrasound. Attendees will learn about the conduction of ultrasonography at the iliac crest, which is an important anatomical site to deduce ages in medicolegally significant age groups, otherwise in conflict with the law in heinous crimes.

**Impact Statement:** This presentation will impact the forensic science community by helping attendees infer conclusions about the long-lasting and decisive impact carved out by ultrasonography as a modality of age estimation in medicolegally important age groups of 14–21 years in the living human population, in the past and for years to come.

**Background:** The long-yearned endeavor of researchers all over the world to validate the best modality for age estimation in living populations remains unaccomplished. Radiography is the gold standard technique in this regard. Ultrasonography is prone to an inherent subjectivity regarding opinion about the findings related to age-estimation, or otherwise. Despite this, it is the front-runner modality, which is rigorously assessed by various studies to find its suitability to be the first choice for age estimation in living as part of clinical forensic medicine. This is highly relevant in lieu of the issues of radiation hazards and the high cost associated with sophisticated techniques like tomography and magnetic resonance imaging. These factors inspired the author to conceptualize the testing of ultrasonography for two staging methods of age estimation. It was a step even ahead of the inter-modality testing of ultrasonography in comparison to tomography and magnetic resonance imaging for age-estimation in living.

**Purpose:** This study was intended to assess the validity of ultrasonography for age estimation at a site that is highly relevant in determining decisive ages in medicolegal cases.

**Objective:** This study aimed to compare Risser's and Schulz's staging of age estimation by ultrasonography at the iliac crest for age estimation in living.

**Materials and methods:** The ultra-sonography was conducted at a frequency of 12.5 MHz by using a Philips® HD-I 11 Machine with a linear probe, in a study group of 60 patients (32 male and 28 female). The individual scan of each patient was evaluated with respect to Risser's and Schulz's staging that has been defined for epiphyseal ossification. The data for Risser's and Schulz's staging was evaluated by SPSS 17.0 and EPI-INFO software, respectively.

**Results:** The classification of the ossification by both the staging methods of the iliac crest epiphysis was possible in all 60 cases. By Risser's staging, the minimum age for ossification Stage I was 12.6 years—females, 14.5 years—males; Stage II was 14.6 years—females, 16.5 years—males; Stage III was 16.6 years—females, 18.5 years—males; and Stage IV was 18.6 years—females and 20.5 years—males. The full union was observed at the minimum age of 20.6 years. By the Schulz's staging, the minimum age for ossification for Stage I was 12.1 years—females, 10.8 years—males and Stage II was 13.0 years for both the sexes. The earliest age of the ossification for Stage III was 17.0 years—females and 15.0 years—males, which for Stage IV was 22.0 years for both the sexes.

**Conclusion:** It is clearly seen that the detection of the appearance of the ossification center was possible earlier for the initial stages of the epiphyseal ossification (Stage I and II) by Schulz's staging than Risser's staging. It may prove to be highly important in the proceedings of the cases of heinous crimes perpetrated on females, or otherwise, where estimation of decisive ages within a narrow bracket is of much essence and requirement of the natural justice. The author humbly asserts that it is now high time to revamp the system of age estimation with not just the consideration of ultrasonography as the modality of first choice, but by validation of this technique by Schulz's staging of epiphyseal ossification for the medicolegally important ages of 14–21 years.

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### Age Estimation; Endochondral Ossification; Ultrasonography

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**A88 Radiographic Age Estimation Based on Vertebral Degenerative Changes**

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**Learning Objective:** After attending this presentation, attendees will better understand a new method to estimate age from a radiographic evaluation of specific vertebrae.

**Impact Statement:** This presentation will impact the forensic science community by introducing an aging method that will assist in the determination of the biological profile for unidentified decedents. This method will be especially useful in age estimation of older adults and may eliminate the need for skeletal sampling in medical examiner cases where advanced degenerative changes are radiographically observed in the lower thoracic and/or upper lumbar vertebrae.

Age estimation is an important component of decedent identification. Anthropologists frequently assist in this role through gross examination of skeletal elements, such as clavicles, ribs, and pubic symphyses.<sup>1-3</sup> For fleshed bodies, this requires removal of these elements and maceration prior to analysis. A new method was developed using radiographic images to estimate age from degenerative changes of the lower thoracic (T11 and T12) and upper lumbar (L1–L3) vertebrae. This technique will complement anthropological age estimation methods in young and middle-aged adults and may serve as a stand-alone method for older individuals.

Existing digital radiographs from a sample of 240 medical examiner cases at the New York City Office of Chief Medical Examiner were randomly selected for evaluation. The sample includes 120 females and 120 males between the ages of 18 and 101 years. Individuals were targeted in various age cohorts to keep the sample balanced. All individuals were still fleshed at the time of X-ray, although in some cases there may have been advanced decomposition. Digital radiographs were evaluated through a web-based Picture Archiving and Communications Systems (PACS) system. Frequently there were multiple digital images available for a case.

Only the lower thoracic (T11 and T12) and upper lumbar (L1–L3) were evaluated. These vertebrae are more easily visualized on radiograph than other vertebrae since there is less soft tissue and organ interference in this area of the body. Only cases with at least two scoreable vertebrae were included in the study. In some cases, full body images were present, which allowed for all five vertebrae to be scored. In other cases, only chest radiographs were available, which generally permitted full observation of just T11 and T12. Any individuals with surgical intervention of the vertebrae were excluded. Individuals with pathological conditions unrelated to age (e.g., paraplegia) were also excluded from the study group.

A 3-phased scoring system was determined to sufficiently capture various stages of degenerative changes with minimal inter-observer variation. Phase 1 vertebrae have a youthful appearance without any degenerative changes, Phase 2 vertebrae show some degenerative changes, and Phase 3 vertebrae have advanced degenerative changes. For each of the selected vertebrae, individuals associated with Phase 1 were generally found to be under 50 years of age. Individuals in Phase 3 were generally over 60 years of age. Those vertebrae in Phase 2 showed too much age variation to be informative.

An averaged phase score, which factors in the scores of multiple vertebrae, was found to be the most informative value to use with the method since it provides a more accurate picture of the overall degenerative state. Any averaged score greater than 2 (meaning at least one vertebra had a score of 3), was always associated with an individual over 50 years of age, and approximately 87% of these cases were 60 years or older. Individuals with an average score of less than 2 (meaning at least one vertebra had a score of 1) were generally under 50 years of age (approximately 85% of the cases). Individuals with an average score of 1 (all observable vertebrae scored as Phase 1) were generally under 45 years old (88% of the cases). No significant differences were found between males and females.

These findings will be especially useful in age estimation of older adults and may eliminate the need for skeletal sampling in medical examiner cases where advanced degenerative changes are radiographically observed in the lower thoracic and/or upper lumbar vertebrae.

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**Age Estimation; Degenerative Changes; Vertebrae**

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**A89 The Age Changes in Cranial Base Synchondroses in a Cadaveric Population**

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**Learning Objective:** Attendees will learn from this presentation that there are quantifiable changes in joints of the cranial base that develop and function differently from cranial sutures commonly evaluated for determining age of adult skeletal remains.

**Impact Statement:** Results presented here should encourage osteologists to reconsider the value of cranial morphology for age assessment of forensic skeletal cases. The goal is to contribute to the challenges faced by forensic anthropologists in determining age from skeletal remains of older adults.

This presentation addresses functional histological differences between joint types in the human skull to investigate why one type remains unossified until later decades. This study tests the null hypothesis that there are no quantifiable osseous changes in the cranial base past 40 years of age.

A review of research published in anthropology, medicine, developmental biology, and forensic science directed the study to focus on the Petro-Occipital Fissure (POF) and Petro-Sphenoidal Fissure (PSF), synchondroses anterior and posterior to the petrous portion of the temporal bone. Cadaveric samples were collected from 45 donors (ages 48 to 91 years) approved for research and defleshed using a novel technique for removing embalmed tissues. An educational skull served as a “control” adolescent. An experienced osteologist developed a scoring system and trained research assistants to collect data from the endocranial aspect. Observers were blinded to donor information. Composite scores from both synchondroses were plotted against age at death, and inter-observer variability was calculated.

Synchondrosis ossification and age at death were positively correlated ( $r=0.4587$ ) with statistical significance ( $P<0.05$ ). Inter-observer reliability varied significantly based on the joint evaluated, with scoring of the PSF having the highest percentage of agreement (69%).

Based on these data, the null hypothesis is rejected. There are quantifiable changes in cranial base synchondroses after the age of 40 years, but the correlation between age and joint fusion is not clearly linear. Morphological variability in bone along both the POF and PSF can obscure observation of osseous changes, resulting in higher inter-observer variability. Future research will examine head computed tomographic imaging of patients of known age and medical history. This will increase the sample size significantly, help include a greater range of subject ages, and will provide a clearer view of ossification progression along joint margins.

This research aims to identify morphological changes in the skull base related to advanced aging to help refine age estimates of human skeletal remains from individuals over the age of 40 years. This information is also relevant to osteopathic clinical practice and neurosurgery on mature adult patients, since undergraduate medical students rarely learn about anatomic variability that undoubtedly affects efficacy of manipulative treatment and surgical approaches.

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**Skull Base; Age Determination; Ossification**



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**A90 Introducing OnSEt: The Ontogenetic Subadult Sex Estimation System**

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**Learning Objective:** After attending this presentation, attendees will be familiarized with OnSEt, a new software program that utilizes skeletal maturation indicators associated with puberty and traditional morphological sex traits within an ontogenetic framework to estimate sex in subadults.

**Impact Statement:** This presentation will impact the forensic science community by providing a unique approach to subadult sex estimation that is as accurate as methods used for adults.

Morphological traits of the skull and pelvis are commonly used for estimating sex from the human skeleton. Currently, these traits are recommended for application in individuals estimated to be 18 years of age and older; however, these traits are associated with the onset of puberty and, therefore, should be applicable prior to adulthood. Research has shown that dimorphism in the morphological traits of the skull and pelvis is expressed by 13 years of age.<sup>1,2</sup> However, application of these traits in subadults requires age to be estimated, which could result in compounded errors if the age estimate is incorrect. A better way of deciding if an individual is mature enough for sex to be estimated is to evaluate skeletal indicators associated with puberty, which can provide insight into the status of maturity without relying on age.<sup>3,4</sup>

A new two-step method for estimating subadult sex has been developed using an ontogenetic framework that will be freely available as a graphical user interface (The Ontogenetic Subadult Sex Estimation System [OnSEt]) and is anticipated for release in December 2022. Skeletal maturation indicators associated with puberty are first scored and entered into the OnSEt system, which will generate a random forest model with a Proceed/Caution outcome to assist in deciding whether an individual is mature enough for sex to be estimated.<sup>5</sup> The maturation indicators include: humeral proximal epiphysis, humeral distal epiphysis, humeral medial epicondyle, ulnar proximal epiphysis, radial distal epiphysis, iliac crest, ischio-iliac epiphysis, calcaneal tuberosity, and mandibular canine. Visuals of male and female sex trait scores by each maturation indicator are also provided to further aid in deciding if the individual is mature enough for sex to be estimated. If sex estimation is deemed possible, morphological sex traits of the skull and pelvis that have been modified for use with immature remains are scored and entered into the OnSEt system and a second random forest model with a Male/Female outcome will be generated.<sup>6</sup> The sex traits include: glabella, nuchal crest, mastoid process, mental eminence, supraorbital margin, ventral arc, pubis morphology, subpubic contour, ischiopubic ramus, greater sciatic notch, preauricular sulcus, and obturator foramen. The OnSEt system will produce a report including all maturation indicator and sex trait scores, as well as the outcome and posterior probability of each random forest model, for inclusion in bench notes or case reports.

A sample of 625 subadults (M=386, F=239) aged 8 to 20 years from the Subadult Virtual Anthropology Database was used in the development of OnSEt. Prior to incorporating the maturation indicators, model classification accuracies for the skull, pelvis, and skull/pelvis combined exceeded 80%. After incorporating the maturation indicators, model accuracies ranged from 88–98.5%. The high classification rates demonstrate that traditional morphological sex traits can be accurately applied prior to adulthood when used in an ontogenetic framework and skeletal maturation indicators provide a valid way to capture maturity without needing to rely on age. Development of the new subadult sex estimation method and its distribution through OnSEt provides practitioners with a user-friendly interface to include sex as part of the subadult biological profile, ultimately helping to facilitate identification and improve outcomes in medicolegal death investigations involving children and young adults.

*Funding provided by the FSF (Lucas Grant) and NIJ (2015-DN-BX-K409, 2020-R2-CX-0024).*

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**Sex Estimation; Juvenile; Puberty**

## A91 The Utility of Combining Morphological and Metric Data Into Statistical Frameworks for Classification of Osteological Sex in the Pubis

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**Learning Objective:** The goal of this presentation is to inform attendees of the impact of data type (morphological, metric, or combined) on the classification performance of Logistic Regression (LR) and Discriminant Function Analysis (DFA) for the estimation of osteological sex of the pubis.

**Impact Statement:** This research will impact the forensic science community by highlighting the potential for developing novel statistical methods by combining morphological and metric data for the estimation of osteological sex.

Forensic anthropologists estimate osteological sex as a component of the biological profile to aid in the identification of skeletal remains. The pelvis is argued to be the best indicator of osteological sex due to the obstetric demands placed on the female structure to facilitate childbirth. Within the pelvis, the pubis is frequently regarded as the most preferred region from which to estimate osteological sex. Morphological methods are often favored over metric approaches due to their practicality as well as the difficulty with identifying landmarks on the innominate, though both methods are frequently independently utilized; however, limited research has investigated the utility of combining data types into statistical frameworks for classification to generate a single estimate of sex. Therefore, the purpose of this research was to investigate the impact on classification performance when combining morphological and metric data.

Data sets were collected using established scoring criteria of morphological (ventral arc, subpubic contour, medial aspect of the ischiopubic, and pubic body shape) and metric (pubic body width, pubic symphysis height, pubic symphysis breadth, maximum pubis length, and minimum pubis length) variables of the pubis to estimate osteological sex.<sup>1-7</sup> The sample consists of adult African American and European American females ( $n=217$ ) and males ( $n=241$ ) curated at the William M. Bass Donated Skeletal Collection and the Hamann-Todd Human Osteological Collection. LR and DFA were selected to analyze the data as they are statistical methods frequently employed in forensic anthropology research and casework. While DFA is frequently employed, it is not necessarily always applied appropriately, which may impact the model accuracy. Classification statistics were undertaken on morphological data, metric data, and combined morphological and metric data to assess accuracy rates of each method and data set. The models were validated either through a training and test sample (LR) or leave-one-out cross-validation (DFA).

Of the two types of data, morphological data outperforms metric data for both LR and DFA. Morphological data results in higher overall classification accuracies of 99.29% and 98.23% for LR and DFA, whereas overall accuracy rates for metric data are substantially lower at 84.02% and 88.43%. Additionally, metric data also produces a larger sex bias, with females being less accurately classified than males when relying on metric data alone. When morphological and metric data were combined, accuracy rates either remained unchanged (LR at 99.29%, overall) or minimally decreased (DFA at 97.81%, overall) when compared to models that only incorporate morphological data. Further, LR outperformed DFA for morphological (99.29% vs. 98.23%) and combined (99.29% vs. 97.81%) data sets while DFA outperformed LR for metric (88.43% vs. 84.03%) data.

These preliminary results indicate that combining morphological and metric data into statistical frameworks for classification of osteological sex in the pubis does not increase the classification performance of either LR or DFA compared to only using morphological traits that already provide very high classification accuracies. However, this is likely due to the underperformance of metric variables in comparison to morphological data, especially those of the pubis, which are documented to be highly sexually dimorphic and already have high accuracy rates alone. Metric variables, while more objective, are also harder to define in a manner that captures the variation in shape between the sexes. However, it would be beneficial to continue exploring the utility of more metric variables of the pelvis and their performance when combined with the morphological traits of the pubis as well as the remainder of the pelvis. Additional exploratory research could result in higher classification accuracies of other regions of the pelvis when the pubic regions are damaged or absent in forensic contexts.

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### Sex Estimation; Statistical Methods; Combined Data

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**A92 The Effect of Age on Sex Estimation of the Greater Sciatic Notch Morphology Using Walker in an Australian Population<sup>1</sup>**

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**Learning Objective:** After attending this presentation, attendees will achieve an awareness of the effects of age on the distribution of Greater Sciatic Notch (GSN) scores based on applying the Walker method in Australian adults.<sup>1</sup>

**Impact Statement:** This presentation will impact the forensic science community by demonstrating an average narrowing of the GSN with age. Not considering age in sex estimation practice, especially for older females, may lead to an inaccurate assessment.

Sex estimation is a key aspect of a forensic biological profile. The pelvis, being the most dimorphic part of the skeleton, has been studied in considerable detail relative to morphological and metric variation; however, quantitative data concerning the effect of age on pelvic morphology relative to sex variation is limited. This study aims to assess whether there are age-related differences in the distribution of the Walker morphological scores for the GSN in an Australian population.<sup>1</sup>

Three-dimensional volumetric reconstructions derived from Multidetector Computed Tomography (MDCT) scans of 567 pelves, of 258 females and 309 males, aged 18 to 96 years, were scored using the Walker method.<sup>1</sup> Differences in score distributions and means by sex and age group were tested using chi-square and Analysis of Variance (ANOVA), respectively. The accuracy of sex estimates derived from logistic regression equations were explored and confirmed using leave-one-out cross-validation. In this study, individuals were considered correctly classified as females when scored 1 and 2 and as males when scored 3 to 5, in accordance with Walker's reported scoring system results.<sup>1</sup>

Significant differences were found in score distribution and means among age groups in females, but not in males. There was a tendency toward higher scores (maximum 3) in older females. Males aged 70+ years had on average significantly higher scores (4 and 5) compared with males aged 18–49 years. The overall sex estimation accuracy with, and without, age as a confounding variable was 87.5%. When comparing the age groups 18–49 and 70+ years, the estimation accuracy decreased in females (99% v. 91%), while the opposite was found for males (82% v. 87%).

These findings suggest that age affects the morphology of the GSN and, by association, the scores assigned following Walker's method.<sup>1</sup> Higher mean scores in older females imply that GSN becomes on average narrower with increasing age, which has been previously assumed considering changes of hormonal levels related to bone metabolism during aging. Walker's interpretation of young males having lower scores has not been statistically supported in this study. Rather, older males had on average higher scores than younger males. This may be explained by differences in health and lifestyle, but also potentially by ancestral composition. To conclude, it is recommended that one considers estimated age when assessing sex based on the GSN in unidentified human remains. Further research regarding the effect of demographic and health variables on sex estimation would be beneficial to improve the accuracy of sex estimation.

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<sup>1</sup> Walker PL. Greater Sciatic Notch Morphology: Sex, Age, and Population differences. 2005. *Am J Phys Anthropol* 127(4):385-91.

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**Sex Estimation; Greater Sciatic Notch; Age**

## A93 A Case Study for the Impact of Gender Affirming Hormone Treatment on the Gross Morphology of the Bony Pelvis in Gender Variant Individuals

Raphaella Meloro, MA\*, University of Florida, 01 Department, Gainesville, FL

**Learning Objective:** After attending this presentation, attendees will understand how Gender Affirming Hormone Treatments (GAHTs) may impact skeletal morphology in gender variant individuals.

**Impact Statement:** This presentation will impact the forensic science community by giving insight into potential skeletal impacts of the use of GAHTs, thereby impacting biological profile estimations of unidentified decedents.

Forensic anthropology standards for the estimation of skeletal sex have historically treated assigned skeletal sex as a binary system that is separate from, but typically aligned with, an individual's gender identity.<sup>1-3</sup> Although not yet reflected in standards, recent research has addressed the need for the development of methods inclusive of gender variant populations, recommending changes for the phrasing of sex and gender in osteological reports.<sup>1-3</sup> Currently, few studies have investigated the impact of GAHTs on the gross morphology of the bony pelvis, particularly in relation to the efficacy of anthropological sex estimation methods.<sup>3,4</sup> Forensic anthropology studies on the impact of GAHTs on skeletal morphology have been limited by the lack of accessible scan data for direct research.<sup>3</sup> Studies that have investigated the impact of GAHTs on the morphology of the bony pelvis are typically of a clinical nature that place the focus of their research on bone density and bone microstructure.<sup>5-12</sup> These studies have produced inconclusive results on the effects of GAHTs on the gross morphology of the bony pelvis, suggesting that further research is warranted.

This case study analyses the impact of GAHTs on the gross morphology of the bony pelvis using metric and non-metric methods. Computed Tomography (CT) scan data from two transgender individuals who utilized GAHTs in their transition for an extended period of time (> 3 years) were obtained from the University of Florida Integrated Data Repository. Both individuals had CT scans taken at different time points during their treatment, with a two-year period between their respective scans, and only began treatment after the age of pelvic fusion. Under non-metric analysis, no major changes in shape were detected between the scans; however, a geometric morphometric analysis, including a comparative control sample, discerned slight but present changes in pelvis breadth between the first and second scans of each individual. While both individuals clustered with their sex assigned at birth in the geometric morphometric analyses, the individual who underwent treatment for the longest period of time slightly shifted toward more indeterminate morphology. Although future research is warranted, the results of this analysis suggest that GAHTs may produce slight changes to pelvic shape but that these changes may be difficult to discern when only performing non-metric analyses, highlighting the importance of metric analysis in sex estimation. Furthermore, this research may suggest that an extended period of time or earlier initiation is needed for GAHTs to impact the morphology of the bony pelvis.

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### Forensic Anthropology; Geometric Morphometrics; Skeletal Sex Estimation



## A94 A Novel Method for Estimating Sex in New Zealand and Thai Populations Using Enthesis-Based Osteometrics From the Cranium and Clavicle

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**Learning Objective:** After attending this presentation, attendees will be familiar with a new metric sex estimation method based on sexual dimorphism observed in existing morphological methods and osteometrics (skeletal measurements) developed from soft tissue attachment sites (enthese) associated with the nuchal crest, mastoid process, and rhomboid fossa in European New Zealand and Thai populations.<sup>1-3</sup>

**Impact Statement:** This presentation will impact the forensic science community by providing a new method for estimating sex from unknown skeletal remains from European and Asian populations. When working with unknown human skeletal remains, sex estimation can assist with identification of missing persons. This method has demonstrated >80% correct classification rates in European New Zealand and Thai skeletal samples, meeting the threshold for use in a medicolegal context.<sup>4</sup> Additionally, it complements existing methods following recommendations that multiple traits and approaches should be used to obtain accurate sex estimations.<sup>3,5-8</sup>

The aim of this research was to create a metric sex estimation method from the cranium and clavicle based on existing morphological methods, since morphological scoring can be subjective and require high levels of expertise.<sup>1-3,9-12</sup> The addition of a metric method to an overall sex estimation may improve accuracy by introducing standardized osteometrics that can be applied by less-experienced practitioners.<sup>13</sup>

This presentation is part of a larger project, which analyzed relationships between sexual dimorphism and muscle, ligament, and entheses size through the dissection and maceration of European New Zealand cadavers donated to the University of Otago (ethics reference, H18/113) and Thai cadavers donated to Khon Kaen University (ethics reference, HE621296). With these data, osteometrics were developed to create the metric sex estimation method using univariate and multivariate logistic regression equations. The osteometrics used for this method were chosen separately for each population based on both significant sex differences and positive correlations to entheses associated with the nuchal crest, mastoid process, and rhomboid fossa. The equations were then tested on modern documented skeletal samples from New Zealand (H18/113;  $n=16$ ; seven males, nine females) and Thailand (HE621296;  $n=100$ ; 50 males, 50 females) for accuracy.

Using nine measurements (New Zealand sample: three cranial, one clavicle; Thai sample: two cranial, three clavicle), population-specific univariate equations were developed for estimating sex in these populations. Additionally, multivariate equations using a combination of measurements were created for the New Zealand (8 equations) and Thai (15 equations) samples. The metric method performed well in both skeletal samples, with total correct classifications of 88% (New Zealand) and 81% (Thai), when making an overall sex estimate from both univariate and multivariate equations. The method performed best when considering results from multiple equations together (either univariate, multivariate, or both); however, when using only single equations to estimate sex, multivariate equations performed best.

The overall recommendation for using this method to estimate sex is to calculate results from multiple equations to complete a final sex assessment. Further, it is important to apply the appropriate population-specific equations, which have been developed from individuals broadly representing European and Asian populations, and, therefore, may not be accurate for estimating sex in other populations.<sup>3</sup> This new method presents a way in which sex can be estimated in modern European and Asian populations using cranial and clavicle metrics, which complements existing sex estimation methods and is particularly useful when the pelvis is not present for analysis.<sup>1-3,13-17</sup> Further research into how well this metric method estimates sex in various other European and Asian populations is essential to understanding its full potential for use in forensic anthropology.

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### Sex Estimation; Osteometrics; Enthuses



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**A95 Examining Evolutionary Significance of Population Affinity from Craniofacial Morphological Features**

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**Learning Objective:** After attending this presentation, attendees will understand how evolutionary quantitative genetic methods estimate the combined genetic and climatic influences on craniofacial form and how evolutionary mechanisms control geographic patterning of human variation used in establishing population affinity.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of foundational anthropological and evolutionary theory in shaping forensic methodological approaches for estimating the biological profile. This quantitative genetic modeling aids in moving the discipline toward a more holistic approach of population affinity estimation by controlling for genetic forces of variation to explore influences of novel variables in an empirical way.

Anthropology is shifting from reductionist approaches associated with ancestry estimation and toward a more holistic approach that estimates population affinity beyond simply trying to measure genetic relationships.<sup>1</sup> To achieve this, forensic anthropologists can adopt evolutionary modeling from other ecological and biological disciplines to examine other potential forces responsible for patterns in human population variation while building this relatively new theoretical approach.

This research applied a Bayesian Sparse Factor Analysis of Genetic Covariance Matrices (BSFG) model from quantitative genetics, which controls for genetic population structures to examine climatic influences on craniofacial morphological variation. Here, the influence of temperature, absolute humidity, and the degree of annual fluctuations in temperature and humidity was examined on cranial Macromorphoscopic (MMS) trait expression between a series of nine geographic populations on a global scale. The populations chosen emulated previous work on craniometric findings for comparative purposes.<sup>2,3</sup> Populations that overlapped in geographic space from microsatellite data and the Macromorphoscopic Databank (MAMD) were selected, while climate data locations were selected based on close proximity to MAMD populations.<sup>4,5</sup> Climate data were obtained from online databases available through the National Oceanic and Atmospheric Association and the Climatic Research Unit.

Genetic matrices were constructed from microsatellite data while phenotypic matrices were constructed from dichotomized macromorphoscopic trait data. Mantel tests of these two lines of data were significantly correlated ( $r=0.62$ ,  $p=0.00$ ), supporting previous findings that cranial MMS traits can serve as genetic proxies.<sup>6</sup> Climate coefficients generated from the BSFG were further explored in R. In general, the features associated with the nasal complex become narrower with greater projection from the face in colder environments, particularly those in regions with less climatic fluctuations throughout the year. This pattern follows known thermoregulatory responses in the midface. Detailed analyses of the influence of each climate variable on all cranial MMS traits are presented, as well as the evolutionary significance of those relationships.

MMS traits are often used in forensic investigations of population affinity estimates, yet evolutionary significance of these traits has never been established. This research employs a statistical model that can control for genetic relationships to test other causative forces of human variation, which moves us away from reductionist methods. This study demonstrates that cranial MMS traits are as theoretically robust as craniometric methods.

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**Macromorphoscopic Traits; Quantitative Genetics; Human Variation**

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**A96 The Covariance, Correlation, and Mutual Information of Cranial Macromorphoscopic Traits and Interlandmark Distances**

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**Learning Objective:** After attending this presentation, attendees will have an increased appreciation for the interrelationships of cranial Macromorphoscopic (MMS) traits and Interlandmark Distances (ILDs).

**Impact Statement:** This presentation will impact the forensic science community by providing empirically grounded results that refute the usage of typological suites of MMS traits. Furthermore, comparisons between metric and MMS data types demonstrate the differing information they both capture regarding the cranial phenotype.

Forensic anthropology often utilizes cranial indicators to estimate parameters of the biological profile; however, the relationships between variables or their developmental trajectories are not fully understood. While research on the variation of cranial MMS traits and ILDs within and across populations is relatively abundant and growing as access to more diverse samples increases, little research has specifically focused on quantifying the degree of shared information within and across these variables. This research explores the covariation, correlation, and the amount of Mutual Information (MI) shared between MMS traits and ILDs in a modern sample of subadults and young adults from the United States. The sample is comprised of 461 male and female individuals aged between birth and 20 years from the Subadult Virtual Anthropology Database (SVAD). Twelve MMS traits were scored and 24 ILDs were derived from volumetric models of the cranium reconstructed from computed tomography scans.<sup>1,2</sup> Scoring and landmark placement was conducted without *a priori* knowledge of an individual's age, sex, or reported population affinity. Individuals were categorized into Life History Stages (LHSs) following chronological age: infancy (0–2.99 years; females=17, males=38), childhood (3.00–6.99 years, females=35, males=27), juvenile (7.00–12.99 years; females=32, males=53), adolescent (13.00–17.99 years; females=74, males=129), and adulthood (18.00+; females=84, males=113). Correlation coefficients (polychoric and Spearman's rank order) and covariance were used to assess relationships between MMS traits. MI analyses measured the degree of shared information between MMS traits and ILDs, while polyserial correlations quantified the strength of their relationships.

No pairwise comparison of MMS trait correlations is greater than 0.53, regardless of age. MMS traits assessing the nasal region tended to have the strongest relationships across all LHSs, though the correlations are still quite weak. MI scores range between 0 and 1.95 where 1.95 represents pairwise comparison of a variable against itself. MI scores between MMS traits remain very low (0–0.12). In contrast, MI scores between ILDs are higher (0–1.39), indicating more information is shared between ILDs than between MMS traits. In comparisons conducted across MMS traits and ILDs, MI scores remain relatively low with the most shared information provided by traits and measurements assessing the interorbital (0.06–0.13) and nasal breadth (0.08–0.18) regions. Polyserial correlation analyses further corroborate stronger relationships between MMS traits and ILDs in the interorbital (0.37–0.54) and nasal breadth regions (0.50–0.60).

The lack of strong relationships between most MMS traits suggests they are measures of human variation that are largely independent from each other. These results are consistent with recent population-specific non-metric research and refute the usage of typological trait suites that reinforce inaccurate population typologies. Moreover, except for traits assessing analogous interorbital and nasal breadth regions, most cross-trait analyses also lack strong relationships. These results indicate that most MMS traits provide different information about individual cranial variation than ILDs. The consistency of results across LHSs posit a limited influence of developmental patterns on these relationships throughout ontogeny.

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**Macromorphoscopic; Craniometric; Virtual Anthropology**

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**A97 Biological Distance Assessment Using Matched Data From Two Geographically Proximate Samples in Latin America**

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**Learning Objective:** After attending this presentation, attendees will be familiar with biological distance analysis using matched biological data to differentiate among Latin American samples.

**Impact Statement:** This presentation will impact the forensic sciences community by illustrating how matched biological data from two data types in forensic anthropology, cranial Macromorphoscopic (MMS) and craniometric data, can be used to differentiate between samples from Mexico and Guatemala. This analysis provides insight into the utility of combining these data types in forensic applications within the United States

Forensic analysis of unidentified human remains centers on the creation of the biological profile or estimation of demographic variables from the skeleton. Specifically, population affinity assesses the similarity between skeletal features and data from known reference samples to arrive at a probabilistic statement of group membership. Designations are based on social or biological groups defined by research parameters.<sup>1</sup> Group assignments are often based on population structure, shared population history, and geographic proximity to other groups. In the United States, these labels often correspond to socially constructed ideas of race. Individuals with familial origins from or born in Latin America are often grouped together under the heading “Hispanic” following United States governmental classifications. Under this broad heading, there is little regard for individual population histories, population structure, or evolutionary events impacting Latin American populations on a macro and micro scale. This leads to a generally poor understanding of human variation in Latin American groups.<sup>2</sup>

This study explores human variation in Latin America using samples from the Yucatán State, Mexico, Guatemala City, Guatemala, and comparative data collected in migration contexts from the Mexico- United States border. The Yucatán sample comes from individuals interred in the Xoclán Cemetery in Mérida (cranial MMS [ $n=109$ ]; craniometric [ $n=159$ ]), housed at the Universidad Autonoma de la Yucatán (UADY). This sample comprises modern individuals with Indigenous Maya heritage living near or in the city of Mérida. The Guatemalan sample (cranial MMS [ $n=40$ ]; craniometric [ $n=32$ ]) comprises individuals from forensic casework at the Instituto Nacional de Ciencias Forenses de Guatemala (INACIF). Data from individuals in migrant-related contexts (Identified Guatemalan Migrants, Identified Mexican Migrants) are included. All samples used in this study are considered “Hispanic” within current classification systems.

Each sample is subject to biological distance analysis following the methods most appropriate for each data type. Mahalanobis Distances are calculated from craniometric data, while Smith’s Mean Measure of Divergence is used for cranial MMS data. Distances are then transformed into the same multivariate space using a Procrustes transformation. Two patterns emerge. First is the craniometric and cranial MMS data for each populational sample correlate in Cartesian space. This indicates that cranial MMS data and craniometric data are identifying similar patterns of human variation in the cranium regarding populational groups. Second, not all groups included in this analysis are similar to each other. The two Guatemalan samples (Guatemalan Migrant and INACIF) cluster together, while the two Mexican-derived samples are spatially distant from each other. This suggests that Mexican migrants come from regions other than the Yucatán, which is corroborated by migration statistics.<sup>3</sup> The UADY sample is geographically close to Guatemala, so expectations hypothesize a smaller biological distance. However, the UADY sample is dissimilar to the Guatemalan samples, indicating the impact of micro evolutionary cultural events on cranial variation in the Yucatán, and/or high variability in the Guatemalan sample.

Overall results indicate differences among samples currently grouped under the Hispanic heading using biological distance analysis, supporting the refinement of this broad category, as the data allow. Additionally, this research demonstrates that craniometric and cranial MMS data types are providing similar information regarding cranial variation. Results from this study can be used to inform population affinity research in forensic anthropology.

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**Forensic Anthropology; Craniometric Data; Cranial Macromorphoscopic Data**

## **A98 A Survey of Population Affinity (Ancestry) Estimation at the Defense Prisoners of War/Missing in Action (POW/MIA) Accounting Agency**

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**Learning Objective:** After attending this presentation, attendees will understand how approaches to population affinity, or ancestry estimation, have changed over time at the Defense POW/MIA Accounting Agency (DPAA) and how method selection is predicated by laboratory accreditation requirements and appropriateness for casework in the agency's specific historical context.

**Impact Statement:** This presentation will impact the forensic science community by illustrating the applicability of the discipline's current methodology in this context and the need for identifying appropriate reference samples for comparison.

To address growing concerns regarding the estimation of population affinity in forensic anthropology casework, a critical review of 711 forensic anthropology case reports was undertaken to evaluate the efficacy of methods used by analysts at the DPAA compared to the reported perceived race in servicemember personnel records.<sup>1</sup> Of the 711 case reports, 537 reports included analyses to estimate population affinity with methods and syntheses stemming from 33 articles or book chapters. These methods utilize metrics and morphology of the cranium, mandible, dentition, and long bones as well as cervical vertebrae morphology.

Most reports employed multiple methods for estimating population affinity with one case reporting nine separate analyses, but most reported either two ( $n=219$ ) or three ( $n=127$ ) methods. Morphoscopic traits of the cranium were the primary approach, appearing in 402 case reports: 377 reports followed Hefner, Hefner and Ousley, or a combination of both, and 25 additional reports employed both references as well as Hefner and Hefner and Linde.<sup>1-4</sup> The descriptive cranial morphology approaches of Gill and Rhine were utilized in 162 and 115 reports, respectively.<sup>5,6</sup> Craniometric ( $n=372$ ) and postcranial metric ( $n=120$ ) analyses performed in FORDISC® v3 were the second most-common approach with 12 reports concluding indeterminate, and five estimations were not consistent with reported perceived race.<sup>7</sup> Eighty-eight reports had no cranial analyses present either because there was no cranium present ( $n=54$ ), or the cranium was too fragmentary for analysis ( $n=34$ ). Of those with no cranial analysis, 42% estimated population affinity as indeterminate.

Mandibular metrics and morphology appeared in 118 reports with 115 following Berg and Kenyhercz and 3 reports from 2012 citing Angel and Kelley.<sup>8,9</sup> Dental morphology was employed in 66 reports. Other postcranial metric methods used involve the platymeric index and were used in 78 case reports with 27 estimated as indeterminate.<sup>10-12</sup> The remaining references observed were used to support analytical decisions, including measurement selection and atypical results.

A total of 11 unique population estimations were observed including "probable" categories. Correspondingly, a total of 7 distinct perceived races were reported in the servicemembers' personnel records including White ( $n=502$ ), Black, Japanese, Native American, Chamorro, Filipino, and Puerto Rican (collectively  $n=23$ ). For cases with an estimated population affinity of "European" ( $n=470$ ), 96.8% were consistent with a reported perceived race formally associated with "European" populations (i.e., White or Caucasian). Population affinity estimates including "African" ( $n=10$ ) or "Asian" ( $n=2$ ) were 100% consistent with the reported perceived races. Indeterminate estimations were observed in 55 case reports with all respective servicemembers having either White or Black reported perceived race.

The high consistency between affinity estimates of European and the personnel records is not surprising given the preponderance of casework at the DPAA involves servicemembers of White ethnicities; however, the 3.2% raises some concerns. It is possible that there is an inherent confirmation bias, but it is more likely that the methods permissible to use did not include adequate reference samples reflecting the range of diversity of the servicemembers. Methods included in the laboratory Standard Operating Procedure (SOP) are required to be validated, peer-reviewed methods shown to be applicable to the historical context of the case work, limiting the application of some available methods. Thus, remains that express complex population affinities continue to be a challenge for anthropologists regardless of the associated context.

Reported perceived race refers to the category of race in Individual Deceased Personnel Files for all deceased servicemembers relevant to DPAA investigations. This category was completed based on the perceived race of servicemembers during the enlistment process. For the cases examined, these date to enlistments for World War II, the Korean War, and the Vietnam War.

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## Population Affinity; Forensic Anthropology; Ancestry Estimation

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**A99 Exploring Macromorphoscopic Traits in Relation With Craniofacial Ratios**

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**Learning Objective:** This research will provide attendees with a better understanding of the relationship between two cranial Macromorphoscopic (MMS) traits: Interorbital Breadth (IOB) and Nasal Aperture Width (NAW), and four craniofacial measurements: Interorbital Breadth (DKB), Minimum Frontal Breadth (WFB), Nasal Breadth (NLB), and Bizygomatic Breadth (ZYG) during ontogeny.

**Impact Statement:** This presentation will impact the forensic science community by exploring two MMS traits capturing facial ratios in relation to their craniometrics “counterparts.” Previous research has demonstrated the applicability of scoring MMS traits on immature individuals and the current project will increase confidence in applying these scores on immature individuals.

The forensic anthropological field has posited that there should be a better understanding of MMS traits regularly used to capture human variation. Unlike other MMS traits, NAW and IOB definitions utilize a facial ratio to determine trait scores.<sup>1</sup> This research aims to test the relationship between MMS traits approximating ratios and calculated ratios of craniometric variables defined by interlandmark distances. The results can potentially answer questions regarding analogies between MMS and craniometrics, as well as examine MMS methodology and its applicability to subadult individuals.

A subset of 816 individuals aged between birth and 20 years from the United States subset of the Subadult Virtual Anthropology Database (SVAD) were included in this study. NAW and IOB MMS traits, the DKB, WFB, NLB, and ZYG interlandmark distances were used in the analyses.<sup>1</sup> In comparing NAW and NLB, NAW scores increased as NLB increased. Calculated NAW scores, defined as the NLB/ZYG ratio for 54.6% of individuals were equivalent to those originally assigned through the MMS approach. Of the individuals that received a different NAW score, the majority of individuals (29.6% or 241 individuals) changed scores from a 2 (intermediate NAW) to 1 (narrow NAW). Percent changes in MMS scores were computed to assess any age-related changes based on the ratios. Using the calculated ratio (NLB/ZYG) to predict the NAW score resulted in a change of score for 26% to 55% of individuals across the life history stages, with the greatest percent change in NAW scores observed in adolescents (aged 13 years and above) and adults (55% and 54%, respectively).

In contrast, IOB showed no notable trend with the DKB measurement. IOB scores were also compared to DKB/ZYG ratios and DKB/WFB ratios. The IOB scores were associated with increasing ratios of both DKB/ZYG and DKB/WFB. The IOB scores calculated from the two ratios were then used to assign an IOB score based on the MMS trait definitions. Comparably to the original IOB scores, no individual was assigned an IOB score of 3 (broad IOB, requiring a ratio of at least 1:3) for either of the IOB ratios (DKB/ZYG and DKB/WFB). The individuals that had previously been scored with an IOB of 3 during the MMS analysis either measured to a ratio of 1:5 or 1:4 and were assigned a predicted IOB score of a 1 or 2. Using the DKB/ZYG ratio, 27.5% (212 individuals) received the same IOB score as originally assigned, while the DKB/WFB ratio resulted in 61.9% (477 individuals) with consistent IOB scores. Change in scores for the two IOB ratios from the original IOB ranged from 64% to 74% for DKB/ZYG and 28% to 50% for DKB/WFB without any obvious age-dependent patterns.

Results of this study suggest that IOB based on the MMS trait definition and craniometric analyses should be assessed conjointly for a given individual to describe the variation of cranial features more completely as they do not present redundant information. Furthermore, it supports the use of ratios to inform MMS scores; however, the disparity between MMS scores dependent on the specific craniofacial ratio applied speaks to the complexity in the IOB definitions of “overall facial breadth,” as well as the NAW definition of “overall width of facial skeleton.”<sup>2</sup> A lack in clarity of the exact craniofacial ratio being visualized may lead to lower repeatability of IOB and NAW scores.

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- <sup>2</sup> Hefner Joseph T, Plemons Amber M, Kamnikar Kelly R., Ousley Stephen D, Linde Kandus C. (2018). *Macromorphoscopic Software Version 1.61 User Manual*.

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**Macromorphoscopic; Craniometric; Virtual Anthropology**



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**A100 The Effective Use of Stature in Forensic Identification**

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**Learning Objective:** After attending this presentation, attendees will be aware of a new approach to the evaluation of stature in forensic identification of human remains.

**Impact Statement:** This presentation will impact the forensic science community by offering new tools for practitioners to better utilize stature and bone sizes in the forensic identification process.

The evaluation of stature has a long history in biological anthropology as it is one of the obvious and interesting aspects of human variation. Bioarchaeologists estimate stature from skeletal remains of long-ago deceased individuals to characterize populations. In such cases, there is a bone that can be used, in conjunction with a statistical model, to predict the stature of the individual with a prediction interval. In forensic anthropology, where personal identification is a primary concern, we are often interested in a related, but different question: Given the known stature of a candidate for identification, are these bones of the correct size to be his/her remains? Also, how good or poor is the fit? A direct way to address these questions is to utilize a regression model with stature as the independent variable to predict bone size. This makes intuitive biological sense given stature is a good proxy for overall body size, and bone sizes are determined by the underlying genetic and developmental factors that drive overall body size. To explore the efficacy of this approach, the **Defense POW/MIA Accounting Agency (DPAA)** reference data was used in regression models predicting humerus, radius, femur, and tibia lengths, respectively, based on stature.<sup>1,2</sup> Next, *t*-tests were used to generate p-values for each individual in the reference data to evaluate model performance (using a cutoff of 0.05) in the context of testing the association of bone size to stature. The power of this method as a forensic tool was evaluated by generating random pairings of statures and bone lengths, performing the *t*-test, and observing how many incorrect associations can be rejected. Since skeletons are often segregated from larger assemblages of commingled remains, it can be prudent to simultaneously evaluate all the bone sizes against a candidate's stature using an omnibus test rather than assume a single bone represents the entire skeleton.<sup>3,4</sup>

This procedure was demonstrated by combining the p-values from the *t*-tests of the individual bone elements in the reference data in an omnibus test, then counting the erroneous rejections (using a cutoff of 0.05). The power of the method was evaluated by generating random pairings of sets of bone elements and statures, generating the omnibus results, and observing how many incorrect associations were rejected. Results for the regression model *t*-tests applied to the reference data were: humerus (*N*=172) error 4.1%; radius (*N*=168) error 4.2%; femur (*N*=170) error 3.0%; tibia (*N*=162) error 4.3%. These errors were close to expected for a 0.05 cutoff. The power of the models, or proportion of successful rejections among randomly paired measurements, was: humerus (*N*=994) 44.6%; radius (*N*=1,000) 39.8%; femur (*N*=1,000) 50.3%; tibia (*N*=1,000) 46.7%. The omnibus test applied to the reference data (*N*=144) yielded an error rate of 4.8% using a 0.05 cutoff. The power of the omnibus method was 48.4% (*N*=1,000). It should be noted that the power will be greater or lesser depending on the size disparities actually encountered in case work. The random pairings were next run including only pairings known to be from individuals with >7.5cm disparity in stature to yield 60.2% (*N*=2,514) successful rejections, then including only pairings with >10.5cm stature difference (*N*=2058) to yield 69.5% correct rejections. The power to exclude roughly half of incorrect associations can be significant when working cases with many remains and many potential candidates for identification. Power depends ultimately on size disparity in the case applications.

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**Stature; Regression Models; Omnibus Tests**

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**A101 A Reassessment of Stature for Modern Native Americans Using the New Mexico Decedent Image Database (NMDID) Data**

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**Learning Objective:** After attending this presentation, attendees will be familiar with stature estimation equations for modern Native Americans (NA) derived from current forensic casework.

**Impact Statement:** This presentation will impact the forensic science community by providing updated stature estimation equations for NA calculated from Computed Tomography (CT) scans collected in routine forensic investigations in New Mexico. Using CT scans to develop stature estimation equations is innovative, allowing skeletal data collection without documented skeletal collections. This approach allows improved anthropological methods for groups reluctant to donate human remains, such as NA. This research fills a significant gap and improves methods for an underserved group by providing the first stature estimation equations based on contemporary NA samples.

Stature estimation is core to the biological profile for limiting the pool of missing persons to compare with unidentified human skeletal remains. Stature estimation calculates forensic stature, usually height recorded from a legal document.<sup>1</sup> Forensic stature is related with error to other descriptions of stature, including cadaver length and family-reported height. We use a mathematical method to estimate stature, regressing stature on long bone measurements to produce regression equations. These equations can use single or combinations of long bone measurements. The most accurate estimations derive from long bones that directly contribute to stature (femur, tibia, fibula); however, equations using upper limb bones are sometimes useful. Due to secular change, mathematical equations are population and time specific.<sup>2</sup>

Equations commonly used to estimate stature for NA derive from archaeological material and a low socioeconomic status sample from Mexico; neither may be appropriate for modern NA.<sup>3,4</sup> This study presents new stature equations for NA in New Mexico and the United States southwest.

Maximum lengths of femora, tibiae, and humeri of NA (males=160; females=67) were collected from CT scans stored in the NMDID. The NMDID contains whole-body CT scans and associated metadata from individuals examined at the Office of the Medical Investigator in Albuquerque, NM, 2010–2017.<sup>4</sup> All individuals were described by next of kin as members of the Navajo or Jicarilla Apache Nations, or the Pueblos of Acoma, Laguna, Santo Domingo, and Zuni. Separate equations were calculated for males and females.

Cadaveric length was used to represent stature. Mean stature for males is 175.1cm; the mean for females is 161.3cm. These means are higher than those presented by Auerbach and Ruff for prehistoric southwestern NA males (160.79 +/- 5.33) and females (150.52 +/- 5.17), and for Mexican males (161.50cm) and females (149.80cm) in Genovés' sample.<sup>4,5</sup> Stature equations were determined using R, applied to the sample, and compared with estimates using equations in Genovés.<sup>5</sup> We noted differences in the slopes for males and females, indicating the NMDID sample is taller with longer limbs. This is true for the femur and tibia for males and females and humeral length for females. Consistent with Genovés, humeral length did not perform well for NMDID males.<sup>5</sup> Stature equations calculated from the NMDID will be presented for maximum lengths of the femur, tibia, and humerus, and for bicondylar femoral length.

We demonstrate secular change between prehistoric and contemporary NA in New Mexico. Furthermore, the NMDID data indicates allometric differences in limb proportions when compared to Genovés' sample. The stature equations presented here are more appropriate for calculating stature for modern NA individuals that are a part of forensic casework in New Mexico, and perhaps more broadly across the American southwest. Additional modern datasets are required to assess the viability of new equations for modern NA in casework across other regions of the Americas.

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**Forensic Anthropology; Biological Profile; Computed Tomography**

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**A102 Expanding the Scope of Subadult Stature Estimation**

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**Learning Objective:** From this presentation, attendees will understand how using cross-sectional data and non-linear regression can expand the scope of stature estimation for the subadult biological profile.

**Impact Statement:** This presentation will impact the forensic science community by providing updated and expanded methods for estimating stature in subadult skeletal remains.

Stature estimation of immature skeletal remains is one aspect of the biological profile that has been underrepresented in the forensic anthropology literature.<sup>1</sup> Investigations into subadult stature estimation methods have been hampered by small samples of known skeletal remains and the challenge of capturing the relationship between skeletal measures and stature over ontogeny. Consequently, subadult stature estimation methods have been primarily regression based (i.e., mathematical), trained using longitudinal samples with homogenous demographics, have only used long bone lengths, and have restricted age ranges or age-specific equations for easier modeling (i.e., diaphyseal vs. maximum measurements, non-linearity).

The present study aims to address several of these challenges by: (1) using a large, contemporary, cross-sectional sample of subadult skeletal remains; (2) generating regression models using both lengths and breadths; and (3) utilizing both linear and non-linear regression models to accommodate the nonlinear shape of long bone growth. The resulting models encapsulate the ontogenetic variation between skeletal measurements and stature and provide more options for subadult stature estimation that does not rely on the recovery of complete long bones or estimating age prior to stature estimation. These modifications better align stature estimation within the scope of forensic casework.

Eighteen long bone lengths and breadths were measured on 349 individuals (M=214, F=135), aged birth to 14 years, from the United States subset of the Subadult Virtual Anthropology Database.<sup>2</sup> Data were partitioned into training (80%) and testing (20%) sets. Kendall's tau correlations were first conducted to evaluate the strength of the linear relationship between all long bone measurements and stature. In line with previous stature estimation methods and for comparison, ordinary least-squares regression was used to model the relationship between each measurement and stature. Bivariate relationships between length and breadth measurements and stature were also visually assessed for shape, resulting in the choice of two nonlinear functions: a three-parameter asymptotic exponential model for lengths and a three-parameter asymptotic logistic model for breadths. In addition, one multiple linear regression equation was generated using stepwise regression including both lengths and breadths. In total, 35 linear and non-linear models were generated and compared.

Correlation coefficients between each measurement and stature ranged from 0.78 to 0.91. Testing accuracy for all linear and non-linear models ranged from 0.90 to 1.0, with Standard Error of Estimate (SEE) ranging from 2.64 to 2.93cm and Mean Absolute Deviation (MAD) ranging from 3.30 to 8.67cm. Interestingly, the linear and non-linear breadth models outperformed the length models when compared using testing accuracy and SEE. The SEE falls well within the ranges reported by previous subadult stature estimation methods.<sup>3-6</sup> In contrast, MAD for the current models is higher than was reported by a recent comparison of two subadult stature estimation methods.<sup>7</sup> Deeper investigation into these models show higher MAD for linear breadth models. The comparable SEE ranges and high testing accuracy of breadth models suggest these models are appropriate for use in forensic casework because the 95% prediction interval is more important than the point estimate.

Results of this study demonstrate the utility of cross-sectional data and non-linear regression models to expand the scope of subadult stature estimation for forensic casework. To make all stature estimation equations accessible, a freely available graphic user interface was created for practitioner use.

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**Non-Linear Regression; Juvenile; Graphical User Interface**

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**A103 Investigating the Reliability and Validity of the Portable Osteometric Device**

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**Learning Objective:** Attendees will learn about the novel Portable Osteometric Device (POD) and its reliability and validity. The POD is an osteometric measuring device that uses laser sensors to measure bone length with a time-of-flight technology.

**Impact Statement:** Unlike the current osteometric board design, the POD uses modern technology to take measurements, allowing it to decrease data collection time and transcription errors. Compared to the current conventional osteometric board design, the POD is smaller, making it easier to transport for researchers traveling to conduct fieldwork and conducting data collection in local and abroad field settings. This advancement in data collection and osteological analysis ultimately contributes to larger questions concerning the identification of unknown decedents and improving the accuracy and reliability of osteometric data collection.

Metric analysis of skeletal material is integral in the analysis and identification of human remains, though the osteometric board—an instrument used for osteological analysis—has lagged in advancement and improvement. Traditional osteometric boards are expensive, bulky, and require the user to read measurements manually, leading to intra- and inter-observer error. To address these functional limitations, this project tests the reliability, validity, and intra-observer and inter-observer error of the Portable Osteometric Device Version 1 (PODv1), a novel laser-measuring device. The PODv1 uses laser sensors with time-of-flight technology to measure distance. To test the reliability and validity of the PODv1, 55 volunteers from the Mississippi State University (MSU) community and the American Association of Biological Anthropology (AABA) 2022 conference meeting measured four different skeletal elements using both the PODv1 and the PaleoTech devices over three different rounds. An Institutional Review Board (IRB) approval was obtained before any MSU or AABA volunteer collected osteometric data for this project (IRB-21-457). The measurements of the tibia ( $w=8,530$ ,  $p=0.4789$ ), humerus ( $w=9,386.5$ ,  $p=0.5908$ ), and femur ( $w=8,585.5$ ,  $p=0.4095$ ) taken from both devices showed no statistical significance between them; however, there was a statistically significant difference between the two devices' length measurements of the ulna ( $w=10,437$ ,  $p=0.03758$ ), likely due to unfamiliarity of the device and measurement instructions.

These results support the PODv1 as a reliable and valid measuring device when compared to the PaleoTech. Although both devices are prone to the same issues such as calibration issues, transcription errors, experience level, and user errors, the PODv1 can improve on these issues with more modifications and research, unlike the current osteometric board designs. This advancement in data collection and osteological analysis ultimately contributes to larger questions concerning identification of unknown decedents and improving the accuracy and reliability of osteometric data collection.

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**Lasser Measuring Device; Osteometric Board; Anthropometric**

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**A104 How Complete Are These Remains? An Assessment of Training and Experience**

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**Learning Objective:** After attending this presentation, attendees will have insight into the relationship between skeletal analytical experience and the description of incomplete human skeletal remains, as well as the importance of using standardized quantification methods.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness of the lack of standardization in quantifying incomplete or fragmented remains and provide suggestions to remedy this shortcoming.

Forensic anthropologists commonly overlook a basic yet integral component of skeletal inventories: how to calculate and report the amount of remains present in an incomplete, fragmented, and/or commingled assemblage. Describing the amount of remains present is critically important because it can impact the trajectory of death investigations and provide insight into whether additional recovery efforts are needed, scavenging and other postmortem effects that led to the dispersal or loss of material, the minimum number of individuals represented, commingling, and the suite of analytical methods that can be applied, among other considerations. Despite this, the forensic anthropology community does not have agreed-upon standards to assess completeness, and therefore, it is highly subjective.

Recent Data Collection Procedures (DCP 2.0) advise analysts to specify the extent of the remains present using generalized terminology or descriptions. Generalized descriptions are not used for any meaningful quantification—instead, analysts are directed to rely on detailed skeletal inventories. While this approach has few implications for single individuals, it may not be appropriate for assemblages that are fragmented, commingled, or otherwise complicated. Numerous published methods exist for quantifying the amount of remains present in complex or larger assemblages, many intended for bioarchaeological audiences. However, it remains unclear to what extent forensic anthropologists utilize these methods and whether factors like degree of expertise influence analysts' ability to objectively report skeletal completeness.

This study describes the relationship between experience and assessments of skeletal completeness using the results from an online survey that was sent to individuals both with ( $n=75$ ) and without ( $n=31$ ) human skeletal analytical experience. Participants examined six photographs of incomplete human skeletal remains. For ease of photographic assessment, each image depicted an Minimum Number of Individuals (MNI) of one. No contextual information was provided with the photographs. Individuals were asked to describe the completeness of the skeletal remains using percentages, words, or other means. Forensic anthropologists were encouraged to describe the methods.

Respondents primarily used a mixture of percentages, descriptive terms, and/or inventories to describe the photographs. Only nine respondents (8%) indicated the method used. Kruskal-Wallis and Mann-Whitney U tests were performed to compare the responses of individuals with and without skeletal experience. Responses from only two of the six sets of remains demonstrated significant differences ( $p < 0.05$ ), suggesting that skeletal experience does not facilitate better quantification of remains. Additionally, no significant correlation was observed between years of experience and precision in skeletal quantification. In other words, forensic anthropologists are not getting better at quantifying incomplete remains with years of experience. Furthermore, the presence or absence of diagnostic elements (e.g., the skull or os coxae) significantly shifted perceptions of completeness ( $p < 0.05$ ) in images with similar percentage of skeletal elements present—both for respondents with and without skeletal analysis experience.

These outcomes indicate that experience can impact quantification assessments; however, the lack of agreed-upon standardized methods results in imprecise interpretations of skeletal completeness that cannot be meaningfully understood. Considering that forensic anthropology reports are used by other medicolegal experts, the language we use in reports can influence investigations and potentially bias individuals. Therefore, forensic anthropologists must be more conscientious about how they describe incomplete skeletal remains. This study highlights the need to critically consider how incomplete remains are quantified and to report quantification methods explicitly, particularly in complicated assemblages.

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**Forensic Anthropology; Commingling; Completeness**

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**A105 Sorting Successive Thoracic Vertebrae in Commingled Contexts: A Geometric Morphometrics Approach**

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**Learning Objective:** After attending this presentation, attendees will have insight on the issue of commingled human remains and the application of geometric morphometrics in skeletal reassociation.

**Impact Statement:** This presentation will impact the forensic science community by presenting a new approach in reassociating commingled human vertebrae.

Commingling refers to intermixed skeletal material derived from different contexts and is common both in forensic and bioarchaeological research.<sup>1</sup> Until recently, sorting methods were focused on optical similarities or metric reassociation.<sup>2</sup> Nevertheless, a novel method for reassociation based on geometric morphometrics shape analysis has shown promising results.<sup>3</sup> Given that, an approach that utilizes new methods of sorting designed for 3D models would help minimize the human factor error and reach more reliable and reproducible results.

Two of the typical thoracic vertebrae (T4-T5) from 65 individuals showing no anatomical difference were scanned with a structured-light 3D scanner. These vertebrae were chosen based on their preservation since T4-T5 was the most frequent pair in the sample. The sample derived from three skeletal collections of different geo-chronological contexts. Namely, the skeletal material was obtained from modern collections from the Universities of Athens and Crete and from an archaeological assemblage of the Democritus University, Greece. Fourteen landmarks were placed on the inferior rim of the body and the articular facets of the fourth thoracic vertebrae. Another 14 landmarks were placed on the superior rim of the body and the articular facets of the adjoining fifth thoracic vertebrae. The landmarks' raw coordinates were transformed into Procrustes coordinates for removing the factor of size. The adjoining skeletal elements (T4 and T5) that resembled the most based on their shape were the ones that presented the smallest Procrustes distance across the sample and, therefore, it was expected to belong to the same individual. The results showed that in 66.2% of the cases, the correct T4-T5 match was found between the three first skeletal elements that presented the three smallest Procrustes distances compared with all the other elements. It is recommended that this method would be used in rejecting an approximate 70% of the possible matches based on shape differences and to reevaluate the remaining 30%. Additionally, further investigation based on more of the typical vertebrae with more landmarks and/or semi-landmarks could possibly improve the results.

Future research could further investigate the applicability of landmark- and/or semi-landmark-based methods in sorting commingled skeletal elements.<sup>4</sup> This approach adds valuable information to the existing sorting methods through its ability to retrieve and evaluate shape information for each skeletal element of the sample.

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**Forensic Anthropology; Commingled Remains; Sorting**



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**A106 The Impact of Age on Pair Matching Accuracy**

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**Learning Objective:** Attendees of this presentation will learn how human skeletal element pair matching is often used by forensic anthropologists to segregate commingled individuals, determine the minimum number of individuals represented, and guide genetic testing strategies. Given the increasing number of Commingled Human Remains (CHR) projects at the **Defense POW/MIA Accounting Agency** DPAA and other forensic anthropology laboratories around the world, studies to determine pair matching accuracy and inter-observer error are timely and necessary.

**Impact Statement:** This research impacts forensic anthropologists conducting CHR projects. As individuals with unfused and fusing epiphyses are often encountered during DPAA casework, knowing that pair matches can accurately be made in any age group directly contributes to DPAA's primary objective of identifying United States military personnel unaccounted for from past conflicts. Further, given recent discoveries of commingled subadult assemblages from various contexts, this research indicates that experienced analysts can confidently pair match subadult remains.

A study recently conducted at DPAA on nearly 600 commingled humeri demonstrated that analysts could identify correct pair matching elements with 80–100% accuracy depending on their CHR experience (preliminary results). The study sample, however, was exclusively United States World War II servicemen 17–52 years of age at death. As only late adolescents and adults were represented, all humeri had attained adult size, but the proximal epiphyses were unfused, actively fusing, recently fused, or completely fused. While differential epiphyseal fusion aided analysts in sorting, preliminary results indicate reduced pair matching confidence and increased determinations of “no match” among younger individuals.

Theoretically, subadult skeletal remains of the same age are products of differing genetics, maternal environments, and post-natal biomechanical loading environments. Thus, size and shape variability is expected during growth and development, suggesting subadult humeri should vary enough between individuals to accurately pair match. Notes from the adult humeri pair matching study, however, revealed that although analysts relied predominately on diaphyseal lengths, decisions to assign a pair match and pair match confidence were often influenced by muscle insertion site morphologies. Since muscle insertion sites develop with increasing use—and age—, we hypothesized that subadults would be more difficult to accurately pair match than adults due to underdeveloped individuating morphologies.

To test this hypothesis, 84 subadult individuals were analyzed from the Hamann-Todd Osteological Collection. All atraumatic complete subadult humeri were gathered ( $n=72$  individuals) and separated into three age cohorts: 0–6 years ( $n=47$  specimens), 7–14 years ( $n=27$ ), and 16–19 years ( $n=54$ ). Not all humeri had a pair match; thus, analytical groups were unequal in size. Any markings were covered, and humeri within each age cohort were commingled. The analysts pair matched each assemblage individually. “Match” (confident), “probable match” (fairly confident), “cannot exclude” (not confident), and “no match” data were recorded by each observer, along with documentation of how they reached their conclusions. Following the humeri pair matching study, two additional pair matching tests were completed by each observer: all femora of subadults 2–19 years presented as one commingled group ( $n=99$  femora; 53 individuals); and all long bones from individuals 0–1 year of age organized by element type ( $n=138$  long bones; 17 individuals).

Unexpectedly, subadult age had little bearing on experienced analysts' abilities to identify correct pair matches (100% accuracy for the humeri and femora tests; analysts varied only in match confidence). Rather, diaphyseal lengths, torsion, epiphyseal fusion stages, and muscular insertion sites were used more confidently in this group than among adult remains, likely due to fewer subadult asymmetrical differences. However, the morphological variability of long bones for individuals 0–1 year of age diminished, which resulted in lower overall accuracy (95.9%) and observer confidence in pair matches.

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**Commingled Human Remains; Pair Matching; Subadults**

### **A107 The Reliability of Visual Pair Matching in a Commingled Skeletal Assemblage**

*Abigail K. Kindler, MA, SNA International\*, Hickam, HI; Cortney N. Hulse, PhD, SNA International, Hickam, HI; Carrie LeGarde, MA, Defense POW/MIA Accounting Agency, Hickam, HI*

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**WITHDRAWN**

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**A108 The Construction of a National Hydrogen and Oxygen Isoscape for Colombia as a Baseline for the Identification of the Region of Origin of Unidentified Victims of the Internal Armed Conflict**

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**Learning Objective:** After attending this presentation, attendees will know how the water sampling process was developed and carried out during the mobility restrictions placed by the national government due to the COVID-19 pandemic throughout 2020 and 2021, how virtuality allowed for the formation of a network of volunteers that made possible the sample collection, and how the analysis of the stable isotopes of hydrogen and oxygen ( $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ ) from those water samples were used to construct an initial predictive isoscape as a baseline for the determination of the probable region of origin of unidentified remains.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that the construction of a national hydrogen and oxygen isotope reference map for Colombia based on water samples will guide both the identification of unidentified persons within the country, contribute to global reference databases, such as the University of Utah's database [WaterIsotopes.org](https://waterisotopes.org) and help increase the reference baseline for water isotope concentration in the Latin American region.

The armed conflict in Colombia left countless victims, many of whom are still missing, their remains unidentified in laboratories and cemeteries throughout the country. To assist with the identification of these victims, this project aimed to construct a national reference map of stable isotopes (isoscape) of hydrogen and oxygen from water that can be used to estimate possible geographic regions of origin for these unidentified individuals.

To develop this, samples were taken in 138 population centers, with population density and the impact of the armed conflict in those regions as the primary selection criteria. A total of 245 samples of drinking water were taken in 25 departments (states). Analysis of the samples was carried out at the Stable Isotope Ratio Facility for Environmental Research (SIRFER) laboratory of the University of Utah, where the samples were analyzed for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  isotopes with a Picarro L2130-I isotope and gas concentration analyzer. The values obtained were calibrated for the reference values on the Vienna Standard Mean Ocean Water-Vienna Standard Light Antarctic Precipitation (VSMOW-VSLAP) scale. The values of  $\delta^2\text{H}$  range from -0.9 ‰ in the Amazon region to -80.6 ‰ in the Santander region. The values of  $\delta^{18}\text{O}$  range between -0.1 ‰ in the Amazon region and -11.3 ‰ in the Santander region. Predictive oxygen and hydrogen isoscapes were developed from these values.

Isotope ratios of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  of the water samples, as well as their geographic coordinates in latitude and longitude (WGS 1984), were imported into the ArcMap® GIS platform. The points were then aligned with a base map of Colombia, color-coded according to the measured isotopic ratios, and used as input for interpolation using the "Nearest Neighbor" method. This process resulted in the creation of a continuous surface covering the entirety of the country, with the predicted isotope values at intermediate locations constrained by the measurements that were taken at the sample sites.

This isoscape does not cover the entire national territory. Therefore, it is necessary to continue collecting samples in the eastern and southeastern regions. These regions are composed of departments (states) spanning large areas that represent accessibility challenges such as remote locations or security situations that may adversely affect the safety of the sampling teams.

These results are the first step in generating multi-isotopic models to determine the region of origin and migration patterns in modern populations in the Latin American region. As a next step, these isoscapes should be validated by collecting water samples from several new sites throughout Colombia to determine to what extent the isotopic values at these sites correspond to those predicted by the isoscape. Once this has been evaluated, these new measurements can then be incorporated into the isoscapes, refining their accuracy even further.

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**Stable Isotopes; Provenance; Identification**

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**A109 Isotopic Human Geolocation in North America: A Data Review and Compilation of Oxygen and Strontium Isotopic Databases**

*Kirsten Verostick, PhD\*, University of Utah, Salt Lake City, UT; Alejandro Serna, PhD, Department of Archaeology, University of York, York, ENGLAND; Gabriel Bowen, PhD, Department of Geology and Geophysics, University of Utah, Salt Lake City, UT*

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**Learning Objective:** After attending this presentation, attendees will have an improved understanding of the current state of using oxygen and strontium isotopes for geolocation and provenancing of unidentified persons in North America, along with understanding the areas of strength and weakness in these types of studies.

**Impact Statement:** This presentation will review data compiled from known and assumed origin human tissues throughout North America, highlight how the data have been collected and used in the past, and suggest improvements for future isotopic data collection studies.

Various studies have reported human tissue isotope data related to or reflective of the location of tissue growth. While the goals of these studies vary, the data presented constitutes a collective resource that may document spatial patterns in human tissue isotope ratios and relationships between tissues and environmental proxies (e.g., water, soil). The degree to which this is the case depends on factors including the standardization of metadata, analytical protocols, data reporting, and the geographic coverage of data from any given tissue and isotope system.

We reviewed >40 studies that use, discuss, or include strontium or oxygen isotopic data from human tissues, to include journal articles, reports, book chapters, theses, and dissertations. Human tissues represented include hair, bone, nail, teeth, and urine. A metadata compilation was performed, recording each study's methods and contextual information, and divided the dataset based on the types of geographic and demographic information reported.

Our dataset focuses on 23 studies where the geographic origin of the sampled tissues was known or could be assumed. We systematically evaluated the isotopic data, along with pretreatment protocols, reporting of standards and calibration, and demographic or life history data. The compiled dataset comprised >2,500 isotopic data points, with 56% from the United States, 18% from Mexico, and 26% from Canada; 52% of the data represented hair or nail keratin, 46% bioapatite (primarily tooth enamel), and the remaining samples urine. Most of the isotopic data (85%) are from known origin tissues, whereas for 15% the location of tissue growth had to be assumed. Geographic coverage and methodological standardization differed among studies and tissue types. The broadest coverage in North America available is for keratin; however, a large fraction of these data lack true "known" geographic origin, limiting the utility of the dataset. The second broadest coverage is for tooth enamel isotopic values, and while all these samples are of known origin, the preponderance of this data is from the United States. This data review highlights the strengths and weaknesses of the current tissue data supporting isotope-based human geolocation, allowing us to propose guidelines for future reporting of data and methods, and may help to guide future sampling efforts.

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**Forensic Anthropology; Stable and Heavy Isotopes; Forensic Provenance**

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**A110 The Utility of Birthplace Prediction From Stable Isotope Analysis in Migrant Identification**

*Ivanna Robledo, MA\*, Texas State University, San Marcos, TX; Krysten Cruz, MA, Texas State University, Round Rock, TX; Elisabeth Cuerrier-Richer, MSc, Texas State University, Austin, TX; ChristiAna Dunham, MS, Texas State University, San Marcos, TX; Kelsey Fox, MA, Texas State University, Austin, TX; Michelle Hamilton, PhD, Texas State University, San Marcos, TX; Kate Spradley, PhD, Texas State University, San Marcos, TX*

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**Learning Objective:** After attending this presentation, attendees will understand the applicability of stable isotope analysis on unidentified migrant remains and their use in providing possible identification hypotheses.

**Impact Statement:** This study will impact the forensic community by examining whether birthplace predictions obtained through stable isotope analysis aid in the identification process, and it will allow researchers to better understand the challenges faced when utilizing stable isotope analysis in a forensic context. Additionally, this study will impact the migrant community by demonstrating that further research is being conducted to aid in the identification of unidentified presumed migrant remains to potentially decrease the time from recovery to repatriation.

Recent research suggests that the incorporation of stable isotope analysis for unidentified remains can be useful in aiding identification efforts through the reconstruction of an individual's past. Kramer and Bartelink aimed to demonstrate the application of stable isotope analysis as a tool for provenancing potential birthplaces of deceased migrants that are part of Operation Identification (OpID), an identification initiative that locates and repatriates unidentified migrant remains along the Mexico-Texas border.<sup>1</sup> Estimated birthplace predictions were analyzed through dental samples, which reflect the food and water consumed during an individual's childhood. Kramer and Bartelink assessed the childhood birthplace predictions through strontium and oxygen isotope analysis to test whether the number of missing persons cases in the National Missing and Unidentified Persons System (NamUs) can be narrowed down when incorporating the estimated birthplace predictions obtained through stable isotope analysis.<sup>1</sup> The goal of this study was to test the usefulness of Kramer and Bartelink's birthplace predictions and their applicability to aiding in providing identification hypotheses.<sup>1</sup>

The sample comprised 18 unidentified OpID forensic cases, including 10 males and 8 females. Kramer and Bartelink's birthplace predictions were further tested by applying search parameters in NamUs, namely sex and population affinity, birthplace, age, and date of last contact.<sup>1</sup> Once all parameters were applied, the remaining missing persons reports were reviewed to determine if any identification hypotheses could be made with the OpID cases, based on stature, personal effects, dental records, and antemortem trauma.

While no positive identifications were made, each parameter reduced the number of NamUs profiles for each OpID case by an average reduction of 92.9% for birthplace, 45.7% for age, and 67.1% for date of last contact. Identification hypotheses were found for 13 of the 18 OpID cases. For these 13 cases, 96% of identification hypotheses fell within estimated stature ranges and 16% matched dental and personal effects information in the OpID case files.

The chosen parameters narrowed down the number of identification hypotheses significantly; however, a combination of incomplete and vague information in missing persons reports in NamUs, as well as indeterminate aspects of biological profiles or unclear anthropology reports, were a few factors that contributed to preventing a strong probability of an actual match. As Kramer and Bartelink have also mentioned, there may not have been matches due to the lack of a centralized effort among professionals in Texas, as well as the fact that the individual may not have been entered as a missing person in NamUs at all.<sup>1</sup> Additionally, while stable isotopes have repeatedly been shown to be accurate, incomplete isoscapes could be leading to inaccurate birthplace estimations.

Improving identification hypotheses requires refining and standardizing data collection techniques for NamUs; however, NamUs is not the only missing persons database. Searching additional missing persons databases in the United States and Latin American countries, and collaborating with national and international organizations such as the Colibri Center and the Equipo Argentino de Antropología Forense (EAAF), would increase the potential for migrant identifications. For stable isotope analysis to effectively limit the time interval between recovery of remains and identification, there needs to be standardization of stable isotope data collection techniques, as well as a development of robust isoscapes outside of the United States. These improvements would reify stable isotope birthplace predictions and make identification hypotheses more reliable.

**Reference:**

- <sup>1</sup>. Kramer, RT, Bartelink, EJ. 2022. Childhood and Adulthood Residency Prediction Using Strontium (<sup>87</sup>Sr/<sup>86</sup>Sr) and Oxygen ( $\delta^{18}\text{O}$ ) Isotopes for Unidentified Deceased Migrants Recovered in Southern Texas. *Forensic Anthropology* (University of Florida Press). 5(1): 25-45. DOI: 10.5744/fa.2020.0054

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**Stable Isotopes; Migrant Identification; NamUs**

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**A111 Isoscapes Around the World: Mapping International Forensic Stable Isotopes Research**

*A. Skylar Joseph, MS\*, International Committee of the Red Cross, Geneva, SWITZERLAND; Stephen Fonseca, BS, International Committee of the Red Cross, Pretoria, Gauteng, SOUTH AFRICA*

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**Learning Objective:** After attending this presentation, attendees will better understand the various stable isotope projects currently ongoing in different regions around the world.

**Impact Statement:** This presentation will impact the forensic science community by highlighting a broader selection of recent research ongoing globally to help improve overall awareness and potential collaboration opportunities across regions and disciplines. Through promoting an increase in awareness and collaboration on a global scale, this presentation will ultimately aid in improving and supporting the role of medicolegal systems and forensic institutions in preventing and resolving the issue of missing and deceased migrants around the world.

According to the latest data reported from the International Organization for Migration (IOM), there were 281 million international migrants in 2020, up from 272 million in 2019.<sup>1</sup> Of these migrants, between 3,900 to 5,400 are reported officially missing or deceased each year; however, this number is likely much higher due to frequent underreporting. Missing migrants may not be reported to authorities due to many factors, the most common of which are often related to either fears of repercussions to family or community members who would typically report an individual missing, or due to a lack of knowledge that an individual has gone missing if contact was lost with the person during the migration process itself. Furthermore, many mortuaries around the world may accumulate thousands of unidentified individuals whose location of origin or migration status may be unknown, and therefore would not be officially reported as migrants even if they are suspected of originating outside the city, country, or region. When it is unknown where an unidentified individual even originated from, it can be impossible to start a meaningful search and identification process, which is where the benefits of creating a global isoscape become most apparent.

The International Committee of the Red Cross (ICRC), with its global mandate aimed at ensuring protection and dignity for the dead, has recently been working toward encouraging more global collaboration on the topic of stable isotope analysis for forensic purposes. With global migration consistently increasing, techniques to help understand and estimate an unidentified individual's potential migration history have become pertinent to the overall search and identification process.

Stable isotope analysis, while still a relatively new field of study within the forensic sciences, has shown promising results in numerous regions around the world toward provenancing human remains. While many projects have been conducted or are currently ongoing in many countries around the world, it can often be difficult for researchers from different regions to locate past research or data sets. Furthermore, a general lack of standardization in the field has resulted in many studies being difficult to compare due to the different methodologies used. As a first step toward addressing some of these issues, the present research has sought to map as many recent or ongoing stable isotope studies as possible, with the goal of increasing overall awareness of the current research available and institutions around the world currently engaged in research. The ICRC, with its unique ability to act as a global convener, has also recently started to promote collaboration more actively among researchers in this field with the aim of improving the global collaboration and data sharing of stable isotopic research, with a vision to eventually create a global isoscape that could be used as a tool in unidentified persons cases from around the world.

**Reference:**

<sup>1</sup>. McAuliffe M, Triandafyllidou A. (Eds.), 2021. *World Migration Report 2022*. International Organization for Migration (IOM), Geneva.

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**Stable Isotope; Migration; Unidentified Persons**



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**A112 The Region-of-Origin Predictions of Arroyo Grande Jane Doe: A Cold Case Isotopic Study From Nevada**

*Alina Tichinin, BA\*, California State University, Chico, Chico, CA; Taylor Lambrigger, MA, California State University, Chico, Gilbert, AZ; Elizabeth Hannigan, MA, California State University, Chico, Chico, CA; Eric Bartelink, PhD, California State University, Chico, Chico, CA*

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**Learning Objective:** After viewing this presentation, attendees will understand the role of stable isotopes in predicting region of origin of unidentified human remains cases.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the use of stable isotope methods to advance cold case investigations.

Stable isotope analysis has become an increasingly used method to aid in the identification of human remains, including cold cases, undocumented border crossers, and service members from past wars and conflicts. Carbon and nitrogen isotopes of human tissues reflect diet and can provide information about food consumption patterns of deceased individuals. Oxygen, hydrogen, strontium, and lead isotopes reflect origin and can provide more specific information regarding a decedent's residence patterns at different points in time depending on the tissues sampled (e.g., hair, nails, bones, and teeth). Isotopic reference data analyzed within a geospatial framework can be used to create isotopic landscapes ("isoscapes") to predict possible regions of origin of unidentified human remains. The current study applies these methods to a cold case from Nevada.

In 1980, the body of a female teenager (Arroyo Grande Jane Doe) was discovered along a freeway in Henderson, NV. Despite being found within 24 hours of death with minimal decomposition and the evidence of a tattoo and dental procedures, the decedent was not able to be identified. In subsequent decades, the remains were exhumed four times to obtain a DNA profile, a facial approximation, and to conduct other forensic tests. In 2020, law enforcement requested stable isotope testing to predict region of origin of the decedent and to generate new investigative leads. Samples for isotopic testing were taken from a rib, a first molar tooth, and from hair that was obtained at autopsy and stored in evidence.

The hair sample was prepared at California State University, Chico (CSU, Chico) and submitted to the Stable Isotope Ratio Facility for Environmental Research (SIRFER) Laboratory at the University of Utah for analysis of oxygen and hydrogen isotopes. The bone sample was prepared at CSU, Chico and submitted to the Stable Isotope Facility at University of California, Davis (UC Davis) for analysis of carbon, nitrogen, and oxygen isotopes. Finally, tooth enamel from the first molar was submitted to the Department of Geology and Geochemistry at Vrije University for analysis of carbon and oxygen isotopes. Dietary isotopes values (C and N) of bone collagen and bone and tooth enamel bioapatite were consistent with the United States population. Isotopic prediction maps were next generated using the ASSIGNR package for Oxygen (O) isotope values from the hair, bone, and tooth samples. The tissue prediction maps showed substantial geographic overlap for the hair, bone, and tooth samples, and included states within the Pacific Coast, the Southwest, the Great Plains, the Midwest, and the Northeast. While the state of Nevada could be excluded, California, Arizona, and New Mexico represented the geographically closest prediction areas for consideration.

In 2021, Arroyo Grande Jane Doe was identified through forensic genealogy as a missing 17-year-old female from Artesia, NM. Although the isotope data was not available until after they were identified, this case study provided an opportunity to evaluate the potential investigative value of the isotope data. Odds ratios computed for predictions of origin were 15.348, 7.314, and 17.819 times more likely for New Mexico versus Nevada for the hair, bone, and tooth samples, respectively. These data constrained the possible region of origin substantially for the decedent and provide a useful approach for other cold cases.

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**Forensic Anthropology; Stable Isotope Forensics; Cold Cases**

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**A113 The Off-Season of Dental Cementum Investigations**

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**Learning Objective:** After attending this presentation, attendees will be aware of the importance of critically evaluating dental cementum-based methods for the season of death estimation.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of standardized protocols in dental cementum investigation and by demonstrating the prospects and limitations of dental cementum as a method for estimating the seasonality of death.

Dental cementum refers to the mineralized connective tissue covering the root and shows a layered structure under light microscopy. An annual deposit consists of a translucent band delimited by a dark incremental line. Dental cementum is increasingly considered a reliable age predictor and has been adopted to broaden the age-at-death methods available in forensic anthropology, even if its limitations are just beginning to be acknowledged.

Even if the underlying mechanisms driving cementum apposition remain unclear, the dark incremental line is supposed to grow from late autumn to early spring while the brighter band grows from spring to autumn. Based on this assumption, it has been suggested, for humans and non-humans, that death seasonality could be estimated based on the outermost cementum deposit. Fifteen years ago, a study of human teeth indicated that dental cementum predicted the season of death with 99% accuracy.<sup>1</sup> In the absence of further studies validating this approach, the question regarding whether estimating season of death based on dental cementum represents a realistic and achievable goal can arise. This work, based on a software-assisted procedure, aims to critically evaluate the applicability of dental cementum to estimate the season of death on a human dental reference sample.

The study sample consists of 20 canines extracted from individuals from the 21<sup>st</sup>-Century Identified Skeletal Collection, University of Coimbra, Portugal, with documented age at death, sex, date of death, and dates of inhumation/exhumation. Fifty percent of the individuals died at the end of the summer and 50% at the end of the winter. For each tooth, five non-decalcified 100µm cross-sections were prepared using a standardized protocol. Images of the region of interest, with the acellular cementum layered structure, were captured and imported into ImageJ software/Fiji package. To reduce subjectivity in identifying the bright or dark nature of the outmost deposit, alternating luminance intensities were identified across the dental cementum, and these were then compared with the ones of the outermost deposit.

The protocol enabled the software to categorize 95% of outermost cementum deposits into dark or bright classes, based on the luminance values; however, the accuracy of season of death predictions was very low, reaching only 47.4%. This study shows that the identification of the outermost deposit is highly challenging due to physical properties of dental cementum and the optical properties of the microscope's objective lenses.

Interestingly, the accuracy rate for those who died at the end of the winter was higher (70%), likely due to a tendency for the software to interpret the outermost deposits as dark.

Even with the use of a standardized protocol for preparation and a software-assisted analysis of luminosity, this study reveals inconsistencies in the perception of the last band within and between cross-sections. Even more worrisome, the results demonstrate a low chance of correctly correlating the outermost deposits with a spring/summer or fall/winter season and undermines the possibility of estimating the season of death of human remains, particularly those exposed to taphonomic factors.

**Reference:**

<sup>1</sup>. Wedel VL. Determination of season at death using dental cementum increment analysis. *J. Forensic Sci.* 52, 1334–1337 (2007).

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**Cementochronology; Postmortem Interval; Season of Death**

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**A114 A New Method to Estimate Time Since Death Using Binary Observations and Random Forest Regression**

Michael Kenyhercz, MSc\*, Defense POW/MIA Accounting Agency, Kailua, HI; Giovanna Vidoli, PhD, Forensic Anthropology Center, UTK, Knoxville, TN

**Learning Objective:** After attending this presentation, attendees will understand a new method for estimating time since death as measured through Accumulated Degree Days (ADD) using a suite of 85 binary observations with random forest regression. Additionally, attendees will learn techniques in model tuning and optimization for large datasets.

**Impact Statement:** The present research will impact the forensic community by allowing for more precise and less biased estimates of ADD and by demonstrating alternative ways in which the postmortem interval can be studied and leveraged for practical application.

Estimating the postmortem interval through observed changes in human decomposition has been notoriously difficult within the medicolegal community, especially once rigor and algor mortis have passed. Megyesi et al. introduced the Total Body Score (TBS) wherein qualitative observations of the head and neck, trunk, and limbs were weighted and summed to provide a quasi-continuous score that can then be used to estimate ADD.<sup>1</sup> The final model provided by Megyesi et al. resulted in an r-squared value of 0.84.<sup>1</sup> The aim of the current study is to divide the body into more specific anatomical locations using only a suite of discrete observations (yes/no questions) with random forest regression.

The current research took place over a span of three years and eight seasonal trials at the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville, using a total of 51 human donors from the Forensic Anthropology Center's (FAC) body donation program. Donors were included in the study based on: (1) known time of death; (2) natural cause of death with no external trauma; (3) donor weight between 150–250 pounds; and (4) not autopsied or embalmed. Once received at the FAC, each donor was set in a deep freeze for at least 24 hours to equalize body temperatures. Each cohort of donors was removed from the deep freeze at the same time to allow them to thaw to ambient temperature for 24 hours prior to placement at the ARF. All donors were placed unclothed in the supine position at least three meters apart to ensure that microenvironment, topography, and exposure conditions were comparable among donors without risking a bias in insect diversity.

The body was divided into 16 anatomical regions: head/neck, chest, abdomen, genitals, left and right (L/R) upper arm, L/R lower arm, L/R hand, L/R upper leg, L/R lower leg, and L/R feet. Each body region was scored independently using the same set of binary observations that describe aspects of bloat, skin coloration, skin appearance, purge, and insect activity for a total of 596 binary variables scored per individual. Each donor was scored and photographed each day from placement through 2,000 ADD or until only skeletonized and desiccated tissue remained. The final dataset includes 2,457 observation events, each with 596 binary responses. Temperature data were recorded from the National Oceanic and Atmospheric Administration's National Climatic Data Center, wherein mean temperature in Celsius was calculated for each observation day. Any temperature below 0 Celsius was still recorded as 0 Celsius because the freezing temperatures inhibit decomposition processes.

Random forest regression was used to estimate ADD from the binary variables. Prior to model construction, the data were optimized through a variable selection process, Variable Selection Using Random Forest (VSURF), and model parameter tuning for the optimal number of variables to be tested at each decision node and trees for the forest. The final optimized model utilizes 85 variables, 40 of which are tested at each node, and a forest of 870 trees. For the current study, the dataset was split into a 70% training set, a 15% validation set, and a 15% testing set.

The random forest regression resulted in an overall r-squared of 0.83. The average absolute difference between actual and predicted ADD (residual [R] ADD) is 82.28 ADD. Further, 50% of the predictions are within 57.97 ADD, 95% are within 259.54 ADD, and 99% are within 376.60 ADD. The R ADD is consistent from 0 through 1,000 ADD where the model tends to overestimate ADD that continues until about 1,500 ADD wherein the model overcorrects and begins to underestimate ADD until the 2,000 ADD limit was reached.

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**Forensic Science; Human Decomposition; Data Science**

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**A115 Animal Scavenging of Human Skeletal Remains in Western North Carolina**

Maggie Klemm, BS\*, Department of Anthropology, University of Nebraska, Lincoln, Yorkville, IL

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**Learning Objective:** After attending this presentation, attendees will gain an understanding of animal scavenging patterns and behaviors associated with common scavengers in western North Carolina.

**Impact Statement:** This presentation will impact the forensic science community by providing regional data regarding animal scavenging and behaviors observed in western North Carolina.

Animal scavenging is an important forensic taphonomic process that influences the rate of decomposition and often results in dispersal and damages to bone. Despite their influences in taphonomic processes, extensive research related to animal scavenging and their effects on decomposition has yet to be conducted. Currently, regional data pertaining to animal scavenging and behaviors is not well documented. A more thorough understanding of the scavengers and the types of damages they cause in a particular region will aid professionals in the medicolegal field to analyze the events occurring after death more efficiently.

Skeletal remains of 12 donors from the John A. Williams Human Skeletal Collection were examined for evidence of animal scavenging and subsequent damages. The 112 donors were placed in the Forensic Osteology Research Station (FOREST) in Cullowhee, NC, between the years of 2019 and 2021, leaving them exposed to natural elements and scavenging until they were mostly skeletonized. Donors were covered with plastic tarps until skeletonization was nearly complete before being recovered for processing. Some donors were covered with wire caging to deter scavengers and keep skeletal elements from being scattered in the facility. Vultures were unable to get into this wire caging, but opossums were able to fit underneath and continue scavenging. Motion-sensing game cameras were placed near each donor and the footage was analyzed to document the damages and dispersal of skeletal elements and identify which scavengers caused these changes. The collected skeletal elements from each donor were placed in an anatomical position to be examined. Using an Excel® spreadsheet, notes were taken regarding the presence and absence of each bone, as well as indications of damages on each bone caused by scavengers. Data were examined through determining the percentage of each of the skeletal elements present for the 12 donors. These data were compared to the percentage of each skeletal element having damages resulting from scavengers. Of the 12 donors, 19 of the 24 (79.16%) tibiae had puncture damages resulting from animal scavenging. The specific location and frequency of animal scavenging was analyzed, concluding that for the 12 donors, 13 of the 24 tibiae (54.16%) had damages from animal scavenging located on the proximal end, and 11 of the 24 tibiae (45.83%) had damages located distally. Of the skeletal elements, the hand and foot bones belonging to the donors were among the least frequent to be recovered, largely due to scavenger dispersal and/or consumption.

This survey reveals that in the western North Carolina region, common animal scavenger species include turkey vulture (*Cathartes aura*), black vulture (*Coragyps atratus*), Virginia opossum (*Didelphis virginiana*), black crow (*Corvus brachyrhynchos*), and brown mice (*Mus Musculus*). Of the noted animal scavengers, both species of vultures and opossums had the most significant impacts on the rate of decomposition of the donors in this study and frequently left distinctive damages on the skeletonized remains as a result. Vultures consume soft tissues, accelerating the rate of decomposition, often leaving scratches on the surface of the skeletal remains from their beaks and talons and contribute to the dispersal of remains.<sup>1-3</sup> Opossums were observed to consume hands and feet partially or completely, their teeth producing gnaw marks, punctures, and splintered ends.<sup>4</sup> This survey suggests that when estimating a postmortem interval and evaluating events at a scene, the effects of animal scavengers, such as acceleration to decomposition rate and damages to skeletal elements, must be considered. Additional research in this region and other geographical areas is required to offer more comprehensive knowledge of animal scavengers and their behaviors, which will aid in forensic contexts and scenes that have been modified by scavengers.

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**Decomposition; Taphonomy; Animal Scavenging**

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**A116 Future Avenues in Research Standardization for Terrestrial Human Decomposition**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how recent research has responded to calls to improve standardization and further substantiate terrestrial human decomposition research.

**Impact Statement:** This presentation will impact the forensic science community by discussing recent advancements in terrestrial human decomposition research and will identify key areas that remain to be addressed.

Over the past several decades, and through the establishment of multiple decomposition facilities throughout the world, anthropologists have striven to observe and explain the extensive variability seen in human decomposition. In recent years, in an attempt to meet the *Daubert* criteria and standardize practices within decomposition research, anthropologists have sought to ground observations and patterns of morphological changes in statistical frameworks.<sup>1</sup> Several scholars have called for shifts in both research methodologies and research perspectives, encouraging movement away from traditional attempts to isolate singular drivers of decomposition, and toward more holistic approaches to improve the validity, reliability, and accuracy of decomposition studies.<sup>2,3</sup> Therefore, the aim of this research was to examine the responses of researchers to the aforementioned calls to action to implement considerable changes in our approaches to decomposition research.

Research articles were retrieved from the database Web of Science™ using different combinations of the following search terms: forensic anthropol\*, entomolog\*, forensic entomolog\*, forensic taphonom\*, decomposition, human decomposition, taphonom\*, postmortem interval (all spelling variants), PMI, scaveng\*. Papers published between 2018 and 2022, following suggestions outlined in Wescott, and pertaining specifically to terrestrial decomposition, were retained for this study.<sup>3</sup> As a result, any articles pertaining to aquatic decomposition, purely entomological studies, postmortem cellular degradation, review articles, and technical notes were excluded. From this, a total of 39 articles were identified and evaluated on the basis of eight criteria outlined following the recommendations made in previous research.<sup>2,3</sup> These eight criteria included: use of standardized data collection procedures, inclusion of statistical analyses, examination of an extended PMI (> 1 year duration), an emphasis on carrion ecology and environmental interactions, interdisciplinary research efforts, discussion of animal proxy limitations, incorporation of theory, and inclusion of a robust sample size (n greater than or equal to 10).

The results of this study indicate that more careful consideration of these recommendations is necessary in future decomposition research. During the time period examined, the criteria use of standardized data collection procedures (97% of papers evaluated), use of a clear statistical framework (72%), and an emphasis on carrion ecology (69%) appear to be well-established standards in the field. The criteria utilization of interdisciplinary research efforts (49%) and robust sample sizes (49%) show room for improvement though they face the inherent challenges of accessibility to human donation programs and human variation. The remaining three criteria, extended PMI (28%), the use of theoretically grounded concepts (33%), and acknowledgement of animal proxy limitations (21%), were identified as the areas needing the greatest improvement; however, the criterion acknowledging limitations of animal models were only assessed when applicable (*n*=20 papers).

Given these results, the authors echo previous calls for future research to move toward non-descriptive, systematic, statistically validated studies from a multivariate approach. Additionally, in specific regard to postmortem interval estimation research, increased understanding of interactions between known variables involved in human decomposition research, and increased taphonomic research in general, is necessary before future researchers attempt to develop additional postmortem interval estimation models.

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- <sup>2.</sup> Passalacqua NV, Megyesi MS. 2015. A look into the past, present, and future of decomposition research and the estimation of the postmortem interval. *Proceedings of the American Academy of Forensic Sciences*, 67<sup>th</sup> Annual Scientific Meeting, Orlando, FL. 2015. 20:93.
- <sup>3.</sup> Wescott DJ. 2018. Recent advances in forensic anthropology: Decomposition research. *Forensic Sciences Research* 3:278–293.

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**Terrestrial Decomposition; Forensic Anthropology; Research Standards**

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**A117 Setting a Baseline: An Analysis of Florida's Forensic Institute for Research, Security, and Tactics (FIRST)**

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**Learning Objective:** This presentation will highlight the importance of collecting baseline data on Florida's Forensic Institute for Research, Security, and Tactics (FIRST) Forensic Field and compare those data to an existing decomposition facility in Florida.

**Impact Statement:** The benefit of this research is to establish a baseline for soil and temperature data for the facility to inform future casework involving Florida buried body cases.

This presentation will highlight the importance of collecting baseline data on Florida's FIRST Forensic Field prior to placing human cadavers within or on the soil surface as recommended by Wankmiller.<sup>1</sup> Baseline data include subaerial surveys (e.g., moisture and temperature) and ambient weather data from a weather station on the FIRST Forensic Field. This research is essential for future comparison studies to Postmortem Interval (PMI).

At its foundation, FIRST is about the partnership between scholars and practitioners used to create innovative training in forensics, security, and tactics. Our academic affiliation with Florida Gulf Coast University (FGCU) highlights such partnerships. Forensic casework and research have identified a gap in the knowledge for climate-specific research in Florida that can inform the forensic community about the taphonomic processes associated with the postmortem deposition of human remains. Based in Land O Lakes, FL, the FIRST Forensic Field is centrally located to allow for research into the effects that an inland-wetland environment has on human decomposition patterns relative to the biota found in this environment.

Established in December 2020, the FIRST Forensic Field is approximately 156 miles north of FGCU's Buckingham Forensic Facility (BEFF) in Fort Myers. The purpose of this research is to examine the differences between these two facilities. We are conducting this long-term study in phases. The first phase will examine differences between the two facilities in climate/weather and soil composition. FGCU's Human Identity and Trauma Analysis program's WeatherSTEM was installed in December 2021. Shortly after, WeatherSTEM installed our FIRST weather station in March 2022. The weather stations are solar and cellular powered giving them the ability to collect weather data 24/7. Additionally, the stations have soil sensors collecting moisture and temperature data at approximately 50cm. These data are then stored and accessible on the stations' websites.

The FIRST and FGCU Human Identity and Trauma Analysis (HITA) WeatherSTEMs are relatively new installations. Therefore, they did not have data going back to 2019. As such, we selected other WeatherSTEMs within 15 miles of both facilities.<sup>2</sup> Those stations are:

- Sunlake High School WeatherSTEM (28°12'04.67"N, -82°30'26.54"W) = 7.40 miles/11.91 kilometers
- FGCU's WeatherSTEM at the Food Forest (26°27'42.26"N, -81°46'47.48"W) = 13.45 miles/21.64 kilometers

Using these two stations, we collected quarterly data from 2019, 2020, 2021, and 2022. Average annual data from 2019 to 2021 exhibits an approximate three-degree difference in temperature data and a miniscule difference in humidity. Soil data at an approximate 50cm depth between the FGCU and FIRST facility show an approximate five-degree difference in temperature and a considerable difference in moisture.

The benefit of this research is to establish a baseline but also to inform future casework in Florida buried body cases. Contemporaneous data collection will help researchers understand the differences between soils with decomposition versus soils without decomposition to inform future estimations of PMI.

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**Forensic Anthropology; Taphonomic Research Facility; Human Decomposition**



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**A118 The Skeletal Manifestations of Striped Skunk (*Mephitis mephitis*) Scavenging on Human Remains**

*Alexander Smith, BA\*, Colorado Mesa University, Grand Junction, CO*

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**Learning Objective:** After attending this presentation, attendees will understand how striped skunk (*Mephitis mephitis*) scavenging behaviors can both directly and indirectly cause a range of defects on human skeletal remains.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of documenting minor scavenger-created skeletal defects as indicators of scavenger interference.

While striped skunks are not known to gnaw on bone or target skeletal material, their scavenging behaviors do create defects on soft tissue.<sup>1</sup> Seven human donors at the Forensic Investigation Research Station (FIRS) in Whitewater, CO, were scavenged by striped skunks in a manner that impacted both bone and soft tissue. Skeletal material at the FIRS can remain protected by desiccated tissue for years after death unless removed by other means. So, when present, insects and scavengers like skunks act as major drivers for decomposition and bone weathering. Striped skunks are not as destructive to bone as many larger scavengers, but they are pervasive throughout North America and can be disruptive to remains.<sup>1,2</sup> So, understanding their scavenging patterns is important to understanding the decomposition trajectory as a whole.

The human donors in this study were allowed to decompose naturally in the arid climate of western Colorado. The research area is fenced, excluding large scavengers, but except for one individual (who was briefly caged), no other action was taken to prevent scavenger activity. The scavenging activity occurred mainly during moist decomposition, but remains were left outside and monitored until the end of other decomposition studies, about 15 years depending on the individual. Remaining tissue was then removed through warm water maceration techniques. Skeletal defects and weathering were documented and compared to known scavenging using field photographs taken regularly throughout the decomposition process.

Striped skunk scavenging at the FIRS produced both direct and indirect evidence on bone. Direct evidence included scratch marks, tooth scores, and tooth depressions. These defects were correlated to observed skunk behaviors, including digging, limb stabilization, and tissue consumption. While present, these marks were uncommon. Some donors showed a single pair of claw marks across multiple scavenged limbs, while others presented many marks on a single bone. These differences can likely be attributed to the effort required for the skunk to access the remains. The most direct evidence of scavenging was observed on the body that was protected by a cage for part of the scavenging window. The skunk dug underneath the cage and pulled at the limb to access and consume tissue. The other scavenged donors were more accessible, so this effort was not necessary.

Due to the long-term soft tissue preservation observed at the FIRS, early skeletal exposure caused by scavengers can cause differential weathering patterns. This indirect evidence of scavenging was observed in all scavenged donors where bone was exposed. In these cases, most of the weathering on the skeleton was noted in the scavenged areas. The weathering in the scavenged areas was generally far more advanced than that observed in other areas of the skeleton. The pattern of weathering on the bone was often distinctive relative to weathering observed elsewhere in the same skeletons. Areas exposed due to scavenging frequently presented a speckled pattern where weathered bone and non-weathered bone were interspersed. This pattern appears to primarily result from small tufts of tissue and soil adhering to the bone while there was still grease and moisture present, then desiccating in place.

Scavenger-induced damage to the human skeleton is often only discussed in its most extreme forms. More minor defects, such as those described here, may be overlooked. The presence of these subtle defects indicates scavenging activity, which can impact the decomposition trajectory through tissue removal, disruption of insect activity, and alterations to the scene. While more destructive scavengers can have a more substantial impact on remains, smaller scavengers, such as striped skunks, can still cause significant deviations from the expected course of decomposition in ways meaningful to an investigation.

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**Striped Skunk; Postmortem Scavenging; Taphonomy**

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**A119 Surveying Scavengers and Their Taphonomic Effects in Murfreesboro, Tennessee**

*Miranda Fain, BS\*, Middle Tennessee State University, Murfreesboro, TN; Yangseung Jeong, PhD, Middle Tennessee State University, Murfreesboro, TN*

**Learning Objective:** After attending this presentation, attendees will learn what scavengers are around and effect decomposition of carcasses in Murfreesboro, TN. Additionally, an overview of the new decomposition research facility, Middle Tennessee State University (MTSU) Outdoor Forensic Facility (MOFF), will be provided to the attendees.

**Impact Statement:** This presentation will impact the forensic science community by providing forensic investigators of the Middle Tennessee area with practical knowledge about scavenging activities, which will help reconstruct the context of crimes more accurately when scavenging evidence is noticed on victims.

Dead bodies in an outdoor setting are susceptible to animal scavenging. When trauma or damage is noticed on a victim recovered from a crime scene, forensic investigators must determine if it was due to human activities or animal scavenging.<sup>1</sup> Knowledge about local scavengers and understanding of the species-specific activities is required to make the determination.<sup>2,3</sup> Thus far, no effort has been made to study scavengers present in Murfreesboro, TN, from a forensic perspective. The goal of this study is to survey active scavengers in Murfreesboro and to understand their taphonomic effects on the carcasses.

Experiments took place at MTSU MOFF, an outdoor decomposition research facility where forensic taphonomy research and related trainings are conducted. All MOFF operations are administered by the MTSU Forensic Science Program. Two goat and one deer carcasses were placed at MOFF on October 26, 2021, and January 8, 2022, respectively. Each carcass was surrounded by 3-foot-high metal fences and monitored by two motion-activated cameras for three months. Identification of the scavengers and analyses of their activities were conducted based on the photos captured by the cameras. A total of five species of scavengers were identified: two diurnal (turkey vulture, black vulture) and three nocturnal ones (opossum, bobcat, brown rat). Most dominant scavengers were opossum (on the goats), bobcat (on the deer), and vultures (both on the goats and deer). Scavenging by vultures, opossum, and bobcat was initiated in three, five, and nine days after placement, respectively. In terms of the decomposition states of the carcasses, bobcat scavenging was observed only at the fresh stage, while opossum and vulture scavenging continued all through the stages. Two to three opossums tend to work on the same carcass together and initiate scavenging on the region that vultures already opened. The size of the vulture group varied between 2 to 10+. In addition, several species of by-passers (e.g., red-tailed hawk, red fox, mockingbird, deer, and song sparrow) were observed.

This is the first experimental study documenting scavenging activities and their taphonomic effects in the Middle Tennessee area. The results will provide forensic investigators with practical knowledge about scavenging activities in this area and help them: (1) discern the animal scavenging evidence from human-induced trauma, (2) reconstruct the context at the time of crimes more accurately, and eventually (3) help resolve forensic cases where disturbed remains are recovered.

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3. Connor M, Baigent C, Hansen ES. 2018. Testing the use of pigs as human proxies in decomposition studies. *J Forensic Sci* 63(5): 1350-1355.

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**Scavenging; Murfreesboro; MTSU Outdoor Forensic Facility**

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**A120 The Applicability of the Postmortem Submersion Interval Estimation Formula for Human Remains Found in Subtropical Aquatic Environments**

*Kara DiComo, BA\*, University of South Florida, IFAAS, Loxahatchee, FL; Erin Kimmerle, PHD, University of South Florida, IFAAS, IFAAS, Lutz, FL*

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**Learning Objective:** From this presentation, attendees will acquire a better understanding of the processes behind aquatic decomposition and the importance of region-specific studies for improving location-specific methodologies.

**Impact Statement:** This presentation will impact the forensic science community by improving upon and facilitating the use of standardized methodology in the Postmortem Submersion Interval (PMSI) estimation process for human remains found within aquatic environments in subtropical regions.

Within the past decade, several attempts have been made to standardize a method for estimating PMSI; however, the majority of these studies have focused on data from a temperate climate, which can not be taken as representative of large portions of the globe. Thus, there are large portions of the earth in which the methodology from these studies may not be able to accurately estimate PMSI, which has the potential to leave investigators in these other climatic zones at a disadvantage. This presentation presents a case study into the applicability of two Total Body Scoring Systems (TADS) utilized for estimating PMSI by Heaton et al. and van Daalen et al. for remains found within aquatic environments in subtropical climates.<sup>1,2</sup>

To this aim, data was collected from temporal photographs of cadavers donated to the Institute for Forensic Anthropology and Applied Sciences (IFAAS) that were taken while the donors were placed at the University of South Florida (USF) Facility for Outdoor Research and Training (USF-FORT) located in Tampa, FL. The nine donors eligible for this study had been placed in a naturally occurring, freshwater, ephemeral, aquatic environment and allowed to decompose with limited intervention throughout a period of time between July 7, 2019, to March 15, 2021 (between 31 to 600 days). From this period, a random date, for which photographs were available, was chosen that fell within a period in which the remains were in a state of active decomposition (between 4 and 31 days) to serve as the date on which the donor would be scored with the TADS systems and would provide a known PMSI with which the estimated PMSI could be compared. The results of this analysis indicate that the inclusion of temperature data (and thus Advanced Degree Days [ADD]) is paramount to the estimation of PMSI, as only five out of the nine donors were able to achieve an ADD score within the 95% confidence interval of those calculated by Heaton et al based on their TADS system.<sup>1</sup> There is also a strong likelihood that modifications will need to be made to the TADS systems as estimated PMSI was approximately four times longer than the actual PMSI for the majority of the donors, which can be explained by how regions of the body, specifically the face, tend to decompose at a faster rate in subtropical climates than temperate ones.

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**Decomposition; Subtropics; Water**

## A121 The Effect of Multiple Interments on Soil pH and Decomposition in Two Cemeteries in Cyprus: Implications for Future Cemetery Use

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**Learning Objective:** After attending this presentation, attendees will understand how soil pH in Cypriot cemeteries can affect the decomposition of interred bodies and how multiple interments has the effect of reducing the soil pH and speeding up decomposition rate.

**Impact Statement:** The results of this preliminary study show that the way the burials are managed in a cemetery, and particularly the number of interments placed in one grave, can have long-lasting effects on the soil pH and the decomposition rate of individual bodies. This has important implications for the prediction of the time it takes for a body to become fully skeletonized and, therefore, how often and when a grave can be re-used. This is important for cemetery management and planning, not only in Cyprus but in many areas where burial space is limited.

The management of the dead is an important aspect of all societies. Greek-Cypriot authorities currently operate a cemetery system whereby skeletonized individuals are exhumed after five years and the burial space is re-used, in order to accommodate the local population in relatively small cemetery spaces. Over the past few years, a slower rate of decomposition has been noted anecdotally in two cemeteries in Limassol, Cyprus. The skeletonization rate has increased from three to five years to seven to ten years, and more cases of differential decomposition have been noticed. It was hypothesized that continued grave use, as measured by Post-Burial Interval (PBI) and Number of Interments (NoI), as well as soil type and soil pH, may affect the rate of decomposition in these cemeteries.<sup>1</sup> These variables were compared to the rates of body decay (fleshed/fresh, mummified/desiccated, and skeletonized/dry) seen in 23 graves from two Cypriot cemeteries in order to test this hypothesis.

Soil is a facilitator of decomposition for buried remains. South Cyprus soils are alkaline, with a typical pH of 8.6 or higher in Limassol.<sup>2,3</sup> When soil pH in these cemeteries was measured as a soil slurry, an interesting difference was seen. Soils from graves of multiple interments exhibited slightly lower pH than single interments and the surrounding areas. These grave soils showed a range of neutral and weakly alkaline pH values between 6.93–8.08 ( $n=12$ , mean=7.58).

Individuals from graves where multiple interments had taken place over time were consistently fully skeletonized, lacking the odor of decomposition. In contrast, remains from single interment graves with slightly more alkaline soil pH ( $n=11$ , mean=7.82) were not fully decomposed after five years. A weak negative correlation was found between NoI and soil pH. Statistical significance was found between NoI and extent of decomposition ( $p=.0026$ ) but not between PBI and extent of decomposition ( $p=.277$ ).

The results of this preliminary study suggest that the re-use of graves and prolonged decomposition activity within them can lead to a slight reduction in pH and be associated with increased decomposition rate. Soil characteristics may be more influential than time on the extent of decomposition, and in areas with very alkaline sandy-silt soil, multiple interments in graves may be a way to mitigate against slow or reducing decomposition rates. It has also been observed that placing cemeteries in alkaline soils should be avoided, especially if full skeletonization of remains is required within five years for cemetery management purposes. However, to further understand the impact of soil on rates of decomposition in Cyprus, the carbon-to-nitrogen ratio of soil must be measured. This has important implications for the long-term use and re-use of the cemeteries in this way, so the need for more sustainable burial solutions is becoming increasingly important.

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### Soil pH; Decomposition; Cemetery Management

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**A122 Using Fiber-Optic Reflectance Spectroscopy (FORS) to Identify Humans Decomposition Characteristics in Plants and Soil**

Anielle Duncan, BA\*, University of Tennessee-Knoxville, Knoxville, TN; Sierra M. Bow, PhD, University of Tennessee-Knoxville, Knoxville, TN; Amy Mundorff, PhD, University of Tennessee-Knoxville, Anthropology, Knoxville, TN; Dawnie Steadman, PhD, University of Tennessee-Knoxville, Knoxville, TN

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**Learning Objective:** After attending this presentation, attendees will understand how FORS can be used to identify spectral signatures from human decomposition in plants and soil.

**Impact Statement:** This presentation will impact the forensic science community by exploring how spectroscopic methods can aid in locating missing deceased individuals via examining how human decomposition fluid can be identified in nearby plants and soil.

Where an individual decomposed has the potential to aid law enforcement by providing information including site of deposition and Postmortem Interval (PMI). Hyperspectral drones may be used to detect an individual decomposing on the ground surface through the spectral signature in soil and adjacent plants. This study was designed to determine whether human decomposition could be identified in the Visible Near-Infrared (VNIR) and Short-Wave Infrared (SWIR) spectral regions and if human decomposition impacts the surrounding environment enough to be identified spectrally.

Using FORS to analyze the spectral signature of decomposition in surface soil and plants can inform whether cadaveric materials are present and persist in the surrounding environment. This project demonstrates how FORS can be used to identify spectral signatures unique to human decomposition. In forensic contexts, FORS is advantageous due to its portability, non-destructive data collection, and rapid analysis.

The goal of this study was to determine: (1) whether cadaveric materials provide a spectral signature for human decomposition identifiable in nearby plants and soil, and (2) if the cadaveric materials can be viewed spectrally outside the bounds of a visible Cadaver Decomposition Island (CDI). Conducted at the Anthropology Research Facility (ARF) at the University of Tennessee, Knoxville, this study examined two prevalent plants at ARF, amur (*Lonicera maackii*) and winter honeysuckle (*Lonicera fragrantissima*), and soil surrounding human body donors. Using a Malvern Panalytical® ASD FieldSpec 4 Standard-Res Spectroradiometer, data were collected from April to November 2021.

A total of eight human donors were utilized during three separate trials. Three donors were placed on the ground surface in April, two donors were placed in June, and three were placed in September. The spectral signature of plants ( $n=23$ ) around these eight donors were collected at weekly intervals during active decomposition. Following the cessation of active decomposition, data collection occurred bi-monthly. There were 13 data collection days for trial one, 18 collections for trial two, and 9 collections for trial three.

For soil, spectral data were collected around one donor during the April trial and one donor during the September trial. A 3x3m grid was placed around each donor and sampling occurred at 50cm increments. There were 11 data collections for the April trial and 7 for the September trial.

The results of this initial study are qualitative. SWIR spectral features unique to human decomposition fluid, such as lipids and proteins, were all identified in both honeysuckle plants and in soil within the visible CDI. A spectral feature for lipids was identified at 1,725, 1,762, 2,309, and 2,348 nanometers for lipids and 2,052 and 2,177 nanometers for proteins. These features were identified during the initial release of decomposition fluid. Plants and soil outside of the CDI did not display spectral features indicative of decomposition. Plant results indicate that spectral features associated with cadaveric materials can only be identified in leaves after an initial release of decomposition fluid. Plants metabolize organic compounds in decomposition fluid after uptake, resulting in undetectable spectral changes if release does not reach the plant. For soil, the signature was found to persist in ground soil for several months following initial decomposition through skeletonization.

Results indicate that techniques such as FORS can be used to identify human decomposition markers in plants and soils. The spectral features identified were consistent across all trials. Identifying cadaveric materials via FORS in plants can only be done during the release of fluids following initial decomposition, thereby limiting its applicability. Given that cadaveric materials persisted in soils, FORS may be particularly advantageous as a method to aid agencies in determining the location and extent of decomposition.

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**Human Decomposition; Spectroscopy; Remote Detection**

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**A123 The Effects of Varying Soil pH and Aluminum Foil Wrapping on the Elemental Composition of Bones**

*Raeann Motacek, BA\*, Indiana University of Pennsylvania, Indiana, PA; Lillian Gonzales, BS\*, Indiana University of Pennsylvania, Hamilton, NJ; Andrea Palmiotto, PhD, Indiana University of Pennsylvania, Indiana, PA*

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**Learning Objective:** After attending this presentation, attendees will understand the complications of bone diagenesis as it relates to elemental composition, soil conditions, and storage practices.

**Impact Statement:** This presentation will impact the forensic science community by expanding on existing research regarding how soil conditions and storage practices impact bones in a forensic setting.

Postmortem conditions can impact the quality of bone and subsequently analytical capabilities. As such, it is important to understand taphonomic factors and their general influence on the state of human remains. This study examines the relationship between bone elemental composition, soil conditions, aluminum foil storage, and bone type. From a forensic perspective, it is unknown how elemental composition may be impacted for bones recovered from different types of soil conditions. Foods wrapped in aluminum foil, particularly those marinated in acidic solutions, have demonstrated intake of aluminum and other metals during cooking. Because of this, there are concerns that using aluminum foil to store and transport bones may contaminate the bone. Previous anthropological tests of bone elemental composition after aluminum foil wrapping did not observe significant aluminum leaching.<sup>1</sup> In previous studies, remains were not buried in soil prior to testing, nor were multiple types of aluminum foil tested; however, different soil pH and aluminum foil brands may also influence elemental composition.

For this study, all elements were collected from an open-air decomposition pit in rural western Pennsylvania where hunters and farmers discard carcasses of deer, cow, and other animals. All remains were completely skeletonized (though the exact postmortem intervals are unknown) and stored at room temperature prior to this study. This sample includes bovine metapodials ( $n=10$ ), radii ( $n=7$ ), and vertebrae ( $n=17$ ). Prior to the onset of the study, each element was inspected to ensure no trauma, pathology, or other damage was present that might impact results. Then the bones were submitted for initial X-Ray Fluorescence (XRF) testing and divided into five groups based on soil conditions and foil types.

Control Group 1 consisted of two bones that were neither buried nor stored in foil but stored in an archival box at room temperature. Group 2 consisted of bones only wrapped in foil ( $n=8$ ). Groups 3–5 were stored in different types of soil for four weeks, then wrapped in aluminum foil for four weeks ( $n=24$  bones total). The three types of soil included: acidic, ~5pH; neutral, ~7pH; and alkaline, ~8.5pH. Four types of locally available aluminum foil were used: Reynolds Wrap®, RW® Recycled Product, Great Value®, and Amazon® brands. The bones were submitted for XRF testing in between and after each phase of the study.

Shapiro-Wilk tests were used to determine that the data sets were not normally distributed. Wilcoxon Signed-Rank tests were used to compare the calcium, aluminum, and strontium values before/after burial in soil and before/after wrapping in foil. Results indicate that burial in soil does impact the elemental composition of bovine bones ( $p < 0.05$ ), particularly calcium values in all soil types and aluminum levels in acidic soils. Additionally, remains wrapped in Amazon® foil exhibited significant differences in calcium, while remains in Reynolds Wrap® exhibited significant differences in aluminum values. Furthermore, remains exposed to acidic conditions then wrapped in foil displayed significant differences in all three element values. Mann Whitney U tests were used to determine that metapodials and radii exhibited more changes in elemental composition, regardless of treatment, than vertebrae ( $p < 0.05$ ).

These results suggest that soil conditions, the type of aluminum foil used for storage, and bone type can impact the elemental composition of bones and therefore can impact subsequent analyses. Additional research is required to better understand these factors and their impact on remains of medicolegal significance.

**Reference:**

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**Forensic Anthropology; Taphonomy; XRF**



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**A124 Decomposition in the Desert: An Examination of the Relationship Between Exposure to Ultraviolet Radiation and the Process of Decomposition**

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**Learning Objective:** After attending this presentation, attendees will better understand the relationship between Ultraviolet (UV) exposure as measured by the UV Index (UVI) and the process of decomposition, particularly in reference to estimating a Postmortem Interval (PMI) in a forensic context.

**Impact Statement:** This presentation will present data examining the relationship between the UVI and progression through visual stages of decomposition using the Total Body Score (TBS) for means of PMI estimation in forensic cases.

The Sonoran Desert, which covers a large portion of the southwestern United States and northern Mexico, is a unique environment with extreme fluctuations in temperature and humidity, even in a single season. Estimating a PMI in this region is imperative for medicolegal death investigations, yet it remains a difficult task given the inapplicability of models generated in mild climates to cases in the desert. Previous research suggests that neither temperature nor humidity appear to be driving the progression of decomposition in this environment.<sup>1,2</sup> These studies demonstrated that: (1) decomposition does not follow expected or established timelines based in more mild climates, and (2) the Accumulated Degree Days (ADD) method does not accurately estimate PMI here. To further explore these observations and their implications for death investigation in a desert environment, we examined a novel variable in decomposition research—UV radiation exposure levels.

The UVI is a measure of expected intensity of UV radiation from the sun calculated by the National Weather Service (NWS) based on ozone levels, cloud cover, and elevation.<sup>3</sup> UVI is calculated by the NWS for 58 cities across the United States. While decomposition and insect activity have been compared in areas with differential sun exposure, to our knowledge measured UV radiation has not been explored as a variable that may influence decomposition.<sup>1,4,5</sup> To explore the potential relationship between UV exposure and decomposition, we use the NWS UVI and the TBS. UVI covers a range of 0 to 11+ and is broken into categories Low (<2), Moderate (3–5), High (6–7), Very High (8–10), and Extreme (11+).<sup>3</sup> TBS is based on visual stages of decomposition in three separate body segments to produce a “score” of decomposition.<sup>6</sup> Over the course of 3 years, spring and summer, six porcine models (*Sus scrofa*) were placed in an area of native desert, clothed, and in cages to deter large scavengers. Cameras were attached to each cage and photos were taken at intervals of 20 minutes to 1 hour and temperature and humidity readings were taken at 10-minute intervals. UVI data from the NWS’s Prediction Center archives was used for the periods corresponding to the studies.<sup>7</sup>

Our results reveal that there does appear to be a relationship between UV exposure and the rate of decomposition, particularly when this exposure is higher during the initial stages of decomposition. Simple regression analysis demonstrated a strong correlation between UVI stage and TBS (adjusted  $R^2 > 0.80$ ) in the six porcine models. Further, higher UVI is associated with a rapid initial progression through the visual stages of decomposition compared to more moderate UVI exposure. Porcine models placed when UVI ranged from “Very High” to “Extreme” (10–11) reached maximum TBS (most advanced decomposition observed) between 5 and 8 days. Porcine models placed when UVI ranged from “Moderate” to “Very High” (~7) reached maximum TBS between 20 and 33 days.

These results demonstrate a correlation between UV exposure and the rate of decomposition that is stronger than the relationship observed between temperature or humidity and decomposition in this region. We propose that there is likely a significant relationship between UVI and TBS in other geographic regions as well. In future studies, we will further explore this relationship and the utility of UV radiation as a component of PMI estimation.

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1. Fulp KJ, Gast MM, Harris JA, Bolhofner KL. Decomposition in the Sonoran Desert During the Summer. *Proceedings of the American Academy of Forensic Sciences*, 73<sup>rd</sup> Virtual Annual Scientific Meeting. 2021.
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**UV Index; Decomposition; Extreme Climate**

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**A125 The Effects of Topography and Animal Scavenging on the Dispersal Patterns of Surface-Scattered Human Remains: A Study of a Forensic Sample From Actual Casework**

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**Learning Objective:** Attendees of this presentation will gain a deeper understanding of the effect of taphonomic factors such as topography and animal scavenging on the spatial patterns displayed by surface-scattered human remains.

**Impact Statement:** This retrospective study of an actual forensic sample impacts the forensic community by presenting new insights on the influence of taphonomic factors such as surface slope, Postmortem Interval (PMI), and animal activity on the spatial distribution and completeness of human remains at outdoor forensic scenes, as well as on the data collection methods and analytical techniques useful to assess, detect, and characterize those effects in their forensic interpretations.

An understanding of the common dispersion patterns and the effects of various taphonomic processes on surface-scattered human remains is crucial to the taphonomic interpretation and reconstruction of the death event.<sup>1</sup> The analysis of the distribution patterns of remains and associated evidence in relation to environmental factors and conditions, including local topography, helps to address questions related to the PMI, damage to skeletal elements, and scattering and absence of these elements; however, at present there are still limited actualistic quantitative data from actual case scenes available to address these issues.<sup>1</sup> Many of the current studies on distribution patterns of surface-scattered remains have utilized small samples of non-human proxies with short PMIs. While these studies are useful for identifying candidate taphonomic factors, processes, agents, and effects with potential forensic applications, the lack of actualistic data and studies on human remains from forensic cases, covering the whole range of PMIs typically encountered in forensic casework, limits our ability to test the hypotheses raised by the proxy studies and the applicability of their findings in real forensic contexts.

The most recent retrospective study on a northeastern forensic anthropological sample further assessed that potential influence of PMI on the preservation and spatial distribution patterns of surface scatters of human remains.<sup>2</sup> It was found that contrary to what those previous studies suggested, while spatial scattering and element loss appeared to increase with time at low PMIs, the remains with very long PMIs indeed appeared to be less dispersed and more complete than those from intermediate, much shorter PMIs.<sup>3,4</sup> While that research described common patterns of surface scattering and preservation, it did not examine the effects of other specific taphonomic variables, such as clothing, scene topography, and vegetation density.<sup>1</sup>

The goal of this research was to quantify the effects of taphonomic variables, such as topography and animal scavenging, on the preservation and spatial distribution of surface-scattered human remains over a wide range of PMIs. The study utilized a forensic sample of 41 forensic anthropological cases with a known PMI range between approximately 2.5 months and 22 years. Each case was recovered through forensic archaeological methods and had full forensic osteological and taphonomic analyses. The PMI, presence/absence of each skeletal element and evidence of animal scavenging was recorded for each case. Spatial data was collected by assigning cartesian coordinates for each individual skeletal element, collected from detailed scene maps through image processing software. The overall dispersion of each case was quantified by calculating the scatter area using convex hulls as well as calculating the distance of each skeletal element from the median of all the elements. The spatial distribution of each case was analyzed in relation to the topographic data collected from the scene, the skeletal inventory, and osteological evidence of animal scavenging. The relationship between the dispersion and recovery of skeletal elements and taphonomic variables, including scene topography and animal scavenging, were assessed through regression and Analysis of Covariance (ANCOVA) methods. The overall and relative dimensions of the dispersion clouds (centroid size, mayor axes lengths, perimeter length, and point density) were treated as dependent variables, and topographic measures (median slope) and PMI as independent variables. These results were then analyzed to elucidate the relationship between taphonomic variables, including animal scavenging and topography, on dispersion and element recovery.

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- <sup>1</sup> Dirkmaat D.C., 2012. *A companion to forensic anthropology*. Sussex, UK: Wiley-Blackwell.
- <sup>2</sup> Kortonick KA, Cabo L. 2022. Identifying taphonomic factors affecting the dispersal patterns of surface-scattered human remains: A study of a forensic sample from actual casework. *Proceedings of the American Academy of Forensic Sciences*, 74<sup>th</sup> Annual Scientific Conference. Seattle, WA 2022.
- <sup>3</sup> Haglund WD, Reay DT, Swindler DR. 1988. Tooth mark artifacts and survival of bones in animal scavenged human skeletons. *Journal of Forensic Sciences*. 33(4):985-997.
- <sup>4</sup> Manhein MH, Listi GA, Leitner M. 2006. The application of geographic information systems and spatial analysis to assess dumped and subsequently scattered human remains. *Journal of Forensic Science*. 51(3).

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**Forensic Taphonomy; Animal Scavenging; Postmortem Interval**

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**A126 Carnivore-Related Bone Damage and Dispersal in the Swiss Alps: A Forensic Anthropological Analysis**

*Lara Indra, MSc\*, University of Bern, Bern, SWITZERLAND; Sandra Lösch, PhD, Department of Physical Anthropology, Bern, SWITZERLAND*

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**Learning Objective:** After attending this presentation, attendees will have an overview of postmortem damage on human bone caused by animals in a high-altitude environment.

**Impact Statement:** This presentation will impact the forensic science community by showing casework in which animals affected the anthropological analyses.

Signs of vertebrate scavenging are relatively common in forensic anthropological investigations, especially on remains from outdoor contexts and long Postmortem Intervals (PMIs). Scavenging can alter or obscure identification features as well as modify perimortem trauma. Animals can further influence decomposition by removing soft tissue or consuming insects associated with decay of the body. Additionally, vertebrate activity often causes disarticulation of body parts and subsequent scattering.

About 20% of the Alps lie in Switzerland where they cover about 60% of the country, although only 11% of the Swiss population live in the Alps. Forty-eight summits in Switzerland reach an altitude of at least 4,000m, while glaciers cover roughly 1,140km<sup>2</sup> of the country's surface. Approximately 211km<sup>2</sup> of those glaciers and firns lie in the Canton of Bern in Switzerland; however, the glacier-covered area is decreasing.

From 2010 to 2022, we investigated 14 cases from the Canton of Bern, yielding human bones from above 2,000m of altitude. Eight of these cases were recovered from glaciers.

We observed signs of carnivore scavenging in five cases (36%), belonging to four genetically identified males. In most cases, the skeletal recovery rates were less than 25% and the dispersal rates had a radius of over 300m. Their PMIs ranged from 0.5–3 years. Most long bones exhibited missing epiphyses, hollowed-out shaft ends with irregular and crenulated margins, as well as scores on the outer cortical bone. Pits and punctures were seen mainly on irregular bones such as the pelvis.

The damage observed on the remains and the high dispersal is consistent with carnivore scavenging as described in the literature. Given the environment, canids are the most likely cause. Our study raises awareness of the impact vertebrate scavengers can have on human remains in high altitudes in Europe. This knowledge will help with trauma analyses and can inform search strategies for missing skeletal elements in the future.

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**Animal Scavenging; Bone Lesions; Swiss Alps**

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**A127 The Effects of the Fluvial Environment on Saw Mark Evidence in Bone**

*Tyra Volney, BS\*, John Jay College of Criminal Justice, Brooklyn, NY; Alicia Grosso, PhD\*, Clarkson University, Potsdam, NY*

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**Learning Objective:** Through this presentation attendees will gain knowledge of how saw cut bone as exhibited in dismemberment cases wears over time in a fluvial environment with water and sand.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that saw mark evidence, such as tooth hops and breakaway spurs, are still available and measurable after 96 hours of exposure to a dynamic fluvial environment, although knowledge of how to manipulate oblique lighting is key to detection when bony surfaces exhibit mottled staining.

Saws are a common tool utilized in postmortem dismemberment to alter and/or transport human remains. Forensic anthropologists are well-equipped to evaluate resultant saw mark evidence in bone; however, dismemberment may not occur in isolation from other concealment methods such as burial, disposal in an aquatic environment, or cremation. This study focused on the exposure of saw cut bone to fluvial environments. Cross-sections from pig humeri cut with a 7 Teeth-per-Inch (TPI) rip saw were macerated for microscopic saw mark analysis as part of another investigation.<sup>1,2</sup> Cross-sections were from the shaft, consisting primarily of cortical bone. Both proximal and distal cut surfaces were evaluated for Tooth Hop (TH), exit chipping, and the presence of a breakaway spur/notch. All saw mark evidence was measured by two observers using a stereomicroscope. Observer A was an anthropologist with prior experience using microscopic saw mark analysis; Observer B had no prior experience but received hands-on training before data collection. A one-dimensional profilometer was used to assess bone surface roughness, with three measures collected along each surface prior to fluvial exposure. Bones were randomly assigned to control or experimental groups and, within each, further divided by duration of exposure (24, 48, 72, and 96 hours). Control groups were exposed solely to moving water ( $n = 4$  cross-sections), while experimental groups were exposed to moving water with sediment ( $n = 20$  cross-sections). A 12-meter-long sediment recirculating hydraulic channel containing a mixture of sand and stone with a flow depth of 0.135m contained the experimental groups, while a 3-meter-long hydraulic channel with a flow depth of 0.07m contained the control groups. Both flumes had a water velocity of 0.45m/s. Each group was removed at the conclusion of their designated exposure time and had all microscopic and roughness measurements repeated.

Analysis found that TH count varied before and after fluvial exposure between observers, with bone staining from the flumes enhancing or hindering an observer's findings, depending on experience level. Pre-fluvial testing, 268 TH were recorded by Observer A with a mean of  $3.67 \pm 0.46$ mm; Observer B recorded 247 with the mean of  $3.48 \pm 0.45$ mm. Post-fluvial testing, the number of TH recorded by Observer A increased by 16.79% with a mean of  $3.73 \pm 0.49$ mm; the number of TH recorded by Observer B decreased by 29.15% with a mean of  $3.36 \pm 0.33$ mm. Differences between TH means by observer both pre- and post-fluvial exposure were significantly different when assessed in millimeters using a two-sample Wilcoxon test (Pre:  $W = 41508$ ,  $p < 0.05$ ; Post:  $W = 41883$ ,  $p < 0.05$ ); however, this difference was not reflected in estimated TPI ranges as all included the 7 TPI saw blade. Exit chipping was identified on 95.83% and 93.75% of surfaces according to Observer A and B; post-experiment, these numbers decreased by 18.75% and 2.08%, respectively. Exit chipping lost flakiness post-fluvial exposure (in both flumes), although staining was limited to the experimental groups exposed to sand. When broadly comparing surface roughness (Ra) pre- and post-fluvial exposure, a Kruskal-Wallis test showed significant differences in roughness values, with bone smoother post-fluvial exposure ( $H = 4.80$ ,  $p = 0.03$ ). Overall, the fluvial environment did not overtly obscure or erase saw mark evidence from bone. Observer experience should be considered when estimating TPI from stained bone, with casting preferable if staining is mottled. Reflecting on the effects of water velocity and sediment type in fluvial environments on saw cut bone, investigators may better estimate duration of exposure.

**References:**

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**Saw Mark Analysis; Taphonomy; Fluvial Environment**

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**A128 Postmortem Skeletal Damage Characteristics From Coffin Contexts Mimicking Thermal and Sharp Force Indicators**

*Rebecca Wilson-Taylor, PhD\*, POW/MIA Accounting Agency, Kapolei, HI; Janet Finlayson, PhD, Defense POW/MIA Accounting Agency, Ewa Beach, HI; Katie Rubin, PhD, Defense POW/MIA Accounting Agency, Honolulu, HI*

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**Learning Objective:** After attending this presentation, attendees will be aware of certain taphonomic signatures of the coffin microenvironment that can resemble thermal and sharp force modifications of bone.

**Impact Statement:** This presentation will impact the forensic science community by providing case examples where broad details of the postmortem intervals are known, including long-term, often cyclic, exposure to a wet environment. These examples show how such exposure may affect skeletal remains in ways that imitate other damage etiologies that are well-recognized in forensic anthropological casework and will aid practitioners in distinguishing between pathways.

A critical component of forensic skeletal analysis is the recognition of taphonomic processes that affect bone and distinguish them from other skeletal modifications. The reconstruction of the events surrounding an individual's death relies on these determinations. Using case examples from the Defense POW/MIA Accounting Agency (DPAA), this presentation discusses similarities between the taphonomic effects of water within coffin environments and commonly recognized thermal and sharp force modifications.

As part of the mission to account for missing United States service members of past conflicts, the DPAA exhumes remains interred as unknowns in American memorial cemeteries that are often the site of post-primary interment. The interval between interment and disinterment for analysis by the DPAA varies by associated conflict but typically spans several decades. These remains are often overlaid with preservative powder and are typically shrouded in cloth and buried in metal caskets. The extent to which groundwater contacts the remains is a highly variable and influential microenvironmental factor affecting skeletal preservation.

Various skeletal changes may be facilitated via water in the burial environment.<sup>1</sup> In particular, the coffin burial microenvironment introduces unique damage characteristics due to repeated exposure to alternating wet and dry conditions.<sup>2</sup> In DPAA casework, these exposure cycles commonly produce patterns of skeletal staining, alteration, and destruction analogous to thermally altered bone, including: black, white, and grey/blue coloration; warping; and degraded bone integrity with production of superficial patina fracturing. Although this coloration may be influenced by a variety of agents, loss of organic bone components, microorganisms, and manganese dioxide from soil may be key influences in damp environments.<sup>3</sup> Areas of patina fracturing vary in size and may be circumscribed by borders of altered color. These signatures often present in heterogenous, highly localized patterns throughout the skeleton, with affected regions directly adjacent to unaltered bone. The similarity in damage features from acidic water and thermal exposure likely results from similar alteration of the bone at a chemical level, with local loss of collagen fibrils essential to bone's material, microarchitectural integrity. However, in cases of water-facilitated damage, these signatures lack indications of thermal soft tissue shielding and their focal distribution pattern typically makes little interpretive sense in the context of thermal alteration outside of complex exogenous shielding of the remains. The observed distribution pattern may be associated with burial cloths "wicking" moisture in and out of contact with specific skeletal regions, thus introducing differential presentations even within elements.

Contact with acidic groundwater in the coffin microenvironment may also manifest in skeletal remains in a manner that imitates sharp force modifications, particularly those associated with transection and dismemberment. The pooling of acidic groundwater in the coffin causes dissolution of bone along relatively level (liquid meniscus) planes. Subsequent abrasion of skeletal elements against the coffin confines or sediment, as from oscillations of groundwater flow, creates a localized, flat, "sheared" appearance of bone, widely recognized as coffin wear.<sup>2</sup> Both coffin wear and dismemberment are commonly observed at epiphyseal ends of bone. To distinguish coffin wear from possible sharp force etiology, the analyst should consider context, skeletal condition, associated fracturing, and the plane of sheared bone.

As demonstrated by case examples from the DPAA, the ability of water to facilitate skeletal modifications mimicking disparate etiologies—particularly those commonly associated with intentional, anthropogenic, remains disposal tactics—necessitates careful examination and consideration of case context. Although the cases derive from formal interment contexts, similar alterations may occur in any contained burial context. An analyst's interpretation of these alterations drives their reconstruction of past events in ways that can have real-world implications, especially for forensic anthropological casework beyond the purview of the DPAA and in more traditional medicolegal settings.

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- <sup>3.</sup> Fernández-Jalvo Y, Andrews P. 2016. *Atlas of Taphonomic Identifications: 1001+ Images of Fossil and Recent Mammal Bone Modification*. New York: Springer.

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**Burned Bone; Sharp Force; Forensic Taphonomy**



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**A129 Trends in Forensic Taphonomic Research Over Time Within the American Academy of Forensic Sciences**

*Rhian Dunn, MS, Michigan State University; Paige Lynch, MS, University of New Mexico, Albuquerque, NM; Andrea Zurek-Ost, MS\*, University of North Carolina at Chapel Hill, Durham, NC*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how taphonomy is represented within the field of forensic science research.

**Impact Statement:** This presentation will impact the forensic science community by providing a critical review of taphonomic research at the American Academy of Forensic Sciences (AAFS) conference over the past 20 years.

It has been argued that forensic anthropology has entered a new paradigm, one in which forensic taphonomy plays an integral part through examination of the processes, agents, and transformations that affect an organism from its time of death and deposition to the time of its recovery.<sup>1,2</sup> Thus, taphonomic research has seen a shift in focus from bone surface modification to the ability of forensic taphonomy to contribute to outdoor crime scene reconstructions, including the estimation of the postmortem interval. To assess the extent and foci of current forensic taphonomic research topics, the authors conducted a comprehensive review of taphonomic research at the AAFS annual conference. This was accomplished through three principle aims: (1) understand the extent of forensic taphonomic research over time within the AAFS conference; (2) identify the source of that research (e.g., section within the AAFS conference, geographic origin); and (3) determine overall trends and which methodological approaches to forensic taphonomic research are most utilized.

Abstracts available in the online AAFS Reference Library were examined to identify their participation in forensic taphonomic research based on the inclusion of “taphonomy.” Abstract data (titles, dates, authors, keywords, etc.) underwent text analysis in R using the “tidytext” package to assess word frequency, correlations, and predictors (via term-frequency-inverse document frequency scores). Abstracts were compared by their overall research foci, keywords, country of origin, section within the AAFS conference, and publication date.

Overall, 307 of 16,183 abstracts (1.90%) from 2003 to 2022 were focused on taphonomic research and were primarily published in the Anthropology section, followed by the Pathology/Biology and General sections. Within Anthropology, 224 of 2,518 abstracts (8.90%) were focused on taphonomic research, peaking in appearance between the years of 2012 and 2016 (10.6–17.9%). On a global scale, forensic taphonomic research was most common in the United States (79.2%), followed by the United Kingdom (6.2%) and Canada (4.2%).

Keywords in the abstracts included in this study indicated that “taphonomy” is more likely to be used than “forensic taphonomy” ( $n=189$  and  $n=41$ , respectively) and that there is a concentration of research on “decomposition” (27%) and the “postmortem interval” (17.3%). Text analysis revealed shifts in foci across time, with a decrease in differential trauma research and a steady increase in the use of archaeology and total body scores. Additionally, text analysis found that research in the United States had a higher focus on scavenging and validation of methods for the estimation of the postmortem interval than other geographic areas.

This comprehensive review indicates that research has reflected a paradigm shift in foci, but forensic taphonomy research remains a small percentage of research in anthropology and minimal across all AAFS sections.

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**Forensic Taphonomy; Text Analysis; Research Trends**



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**A130 A Comparison of Anthropological Methods and Next Generation Sequencing for Population Affinity in the East Marshall Street Well**

*Amber Mundy, MS\*, Virginia Commonwealth University, Richmond, VA; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA; Joseph Jones, PhD, William & Mary University, Williamsburg, VA; Erin Gorden, MFS, Signature Science LLC, Barboursville, VA; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA*

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**Learning Objective:** This presentation will provide attendees with information on comparison of anthropological methods used to assess population affinity and results from Next Generation Sequencing (NGS) to assess ancestry, as well as a better understanding of how optimization studies for skeletal DNA are performed.

**Impact Statement:** This presentation will impact the forensic science community by providing a reference study for obtaining genomic DNA from heavily degraded cranium and mandible samples.

Extraction of DNA from skeletal samples that have been exposed to harsh environmental conditions (e.g., submerged human bones) has proven to be extremely challenging with respect to both DNA quality (due to both DNA degradation and Polymerase Chain Reaction [PCR] inhibition) and quantity (less quantifiable human DNA; higher relative proportion of microbial DNA) for forensic laboratories.<sup>1,2</sup>

Over 500 commingled skeletal remains from the East Marshall Street Well in Richmond, VA, were discovered in 1994 during construction of the Kontos Medical Sciences Building on the Virginia Commonwealth University (VCU) medical campus. The skeletal remains were analyzed at the Smithsonian National Museum of Natural History and were revealed to be primarily individuals of African origin, with recovered artifacts suggesting a 19<sup>th</sup>-century context. Some of the “Ancestral Remains” may have been procured through grave robbing by “resurrectionists” and medical students, a practice that disproportionately targeted the bodies of enslaved and poor African Americans.<sup>3</sup> To restore dignity to these individuals, the Family Representative Council, a group of descendant community members chosen to stand in for relatives of the deceased, requested the re-association of these remains so they could be properly and respectfully buried.

Pair matching and articular grouping of bones were accomplished by the Smithsonian anthropologists but, due to the inability to associate non-articulating bones, they were unable to re-associate discrete individuals. DNA analysis of the crania and mandibles found in the well was undertaken to individuate and confirm the estimated biogeographical ancestry. The genetic characteristics will be compared to the metric and morphological estimation of ancestry undertaken by the Smithsonian in the 1990s, as well as the new estimations that were undertaken by VCU at the beginning of the project.

For the first part of the project, measurements were entered in FORDSIC® 3.1, a program that classifies adults by ancestry and sex using a combination of standard measurements of known crania of different population affinity. All but 1 out of the 12 cranium was classified by the program to be of African descent, which was consistent with the initial Smithsonian analysis. The second component of the project investigated optimizing a protocol for the extraction of DNA from the heavily degraded skeletal remains. Different extraction methods, such as the commercially available QIAamp® DNA investigator kit and the Charge Switch® gDNA Plant kit, were tested in comparison to organic extraction methods, including a total demineralization lysis step for the bones. The total demineralization protocol, which utilizes 0.5M Ethylenediaminetetraacetic Acid (EDTA) with 1% Sodium Dodecyl Sulfate (SDS) and 200uL ProK, had a yield two times the amount as the other protocols tested.

In the third stage of the project, the extracted human DNA will be utilized for generation of human DNA profiles using NGS. The ForenSeq® DNA Signature Prep Kit generates DNA sequence profiles from approximately 200 nuclear DNA loci, for individualization, biogeographical ancestry, and various phenotypic characterizations of these skeletal remains, which will assist in determining the most culturally appropriate burial and memorialization of these individuals.

**References:**

1. Booncharoen P, Khacha-ananda S, Kanchai C, Ruengdit S. Factors influencing DNA extraction from human skeletal remains: Bone characteristic and total demineralization process. *Egypt J Forensic Sci* 11, 2 (2021) <https://doi.org/10.1186/s41935-021-00216-8>.
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**Next Generation Sequencing; Skeletal Remains; FORDISC® 3.1**

**B1      Enhancing the Detection and Analysis of Low-Copy Number DNA From Fingerprints on Commonly Found Substrates at Crime Scenes**

*Sulekha Coticone, PhD\*, Florida Gulf Coast University, Fort Myers, FL; Camila Garcia, BS. Florida Gulf Coast University, Fort Myers, FL*

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**WITHDRAWN**

## **B2      Exploring DNA Quality of Nucleic Acid Dye Staining for Portable Fluorescence Microscope**

*Chao-hsing Fan, PhD\*, Tainan City Police Department, Tainan City, Tainan, TAIWAN; Ming-Sie Pan, PhDc, Department of Industrial Management, Kaohsiung City, Kaohsiung, TAIWAN*

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**WITDRAWN**

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**B3 An Investigation of Trace DNA Samples From Objects Stolen in Pickpocket Scenarios**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how the relative proportions of DNA deposited by different individuals who handled the same object changes through time as well as the impact on the final DNA profile.

**Impact Statement:** This presentation will impact the forensic science community by specifically investigating the deposition and persistence of DNA on objects collected in pickpocketing cases. This presentation will provide insight and information on trace DNA left behind on objects commonly stolen in pickpocket scenarios and will aid investigators in gauging the possibility of detecting an individual's DNA profile using substrate-type and history of a forensically relevant trace recovered from an evidentiary item.

Trace DNA, also known as “touch” DNA, is found at every crime scene and can be invisible or latent. This DNA is left behind due to a person coming directly or indirectly into contact with an object.<sup>1</sup> To evaluate trace DNA evidence, relevant factors, including Transfer, Persistence, Prevalence, and Recovery (TPPR) of DNA must be considered.<sup>2</sup> The evidentiary trace samples left behind at the scene of the crime are being submitted to crime laboratories for DNA testing and following STR profiles are submitted to the CODIS database for matching purposes.

This study was designed to investigate how the relative proportions of DNA deposited on objects handled by first and second users varies over time in pickpocketing case scenarios.<sup>3</sup> Three simulations were set up and seven pairs of volunteers (4 males and 9 females) were recruited to handle objects of different substrate-type for varied amounts of time. One male participant is in two different pairs. The list of objects used in each of the simulations included a wallet (leather), a credit card (plastic), a money clip (metal), a handbag (fabric), and a pair of sunglasses (plastic). Each simulation required the first handler (object owner) to handle the object for 20 minutes each day for 8 consecutive days. The second handler used the object previously handled by the object owner for 1, 3, and 20 minute(s) in three distinct simulations. The first and second users were required to thoroughly wash their hands 20 minutes prior to handling the objects. The time between the first user and second user handling the object was less than 24 hours. Reference samples from all volunteers were collected from buccal swabs, extracted using Chelex<sup>®</sup> resin, amplified using the GlobalFiler<sup>®</sup> Amplification kit, and run on the SeqStudio<sup>™</sup> Genetic Analyzer. Trace samples were collected by double swabs, extracted using the QIAshredder<sup>®</sup>/QIAamp<sup>®</sup>, quantified using the Trio Quantification kit on a QuantStudio<sup>®</sup> Real-Time PCR system and amplified using the GlobalFiler<sup>®</sup> Amplification kit. The STR fragments were then separated using the SeqStudio<sup>™</sup> Genetic Analyzer and DNA profiles analyzed with the GeneMapper<sup>®</sup> ID-X Software.

Overall, preliminary results on an initial set of 42 traces collected on credit cards and wallets showed that the major donor in a mixed STR profile was observed to correspond to the first handler. Nonetheless, a gradual increase of the DNA contribution from the second handler, in relation to the first handler, was noted over time in the traces analyzed, which is in line with previous works.<sup>3,4</sup> In addition, no single-source STR profiles were obtained from the tested trace samples, some of which exhibited alleles not from either individual of the pair.

In conclusion, a thorough analysis of the full sample-set is ongoing to elucidate the contribution of DNA deposited by the two volunteers as well as the presence of extra alleles derived from indirect DNA transfer.

**References:**

1. Burrill, J., Daniel, B., & Frascione, N. (2019). A review of trace “Touch DNA” deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics*, 39, 8-18.
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**Trace DNA; STR Typing; Pickpocket Case Scenario**

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**B4 The Optimized Recovery of DNA From Exhaled Breath Devices: From Drug Detection to Human Identification**

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**Learning Objective:** After attending this presentation, attendees will learn about the effectiveness of various methods to retrieve DNA from breath devices to ensure sample integrity and safeguard the chain of custody.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a method that could be used to confirm the identity of the user and ensure sample integrity of breath devices that are used for drug detection.

Recovery of DNA from exhaled breath is extremely difficult. Due to the low levels of starting template, stochastic events occur during the Polymerase Chain Reaction (PCR) that often result in unwanted effects, such as allele peak imbalance, increased stutter, and allele drop in or out, making interpretation more challenging.<sup>1</sup> In this study, we explored whether DNA could be captured from exhaled breath using two different collection devices (Breath Explor<sup>®</sup> and SensAbues<sup>®</sup>). These devices are typically used for drug detection after in-field breath collection, and they are sent to laboratories for further analysis. Therefore, it is important that the chain of custody must be maintained to ensure sample integrity; hence, the suggestion of processing these breath devices for drugs of concern and DNA to confirm identity. Several DNA collection methods were compared to determine if quality STR profiles could be attained from the mouthpiece and/or internal filters of the devices. The mouthpieces of both devices were swabbed with cotton and microFLOQ<sup>®</sup> swabs, and all swabs yielded complete profiles. The filters were subjected to swabbing with a microFLOQ<sup>®</sup> swab and two soaking methods (EZ1 Investigator<sup>®</sup> chemistry and a previously published method by the San Diego Police Department [SDPD]). Poor DNA recovery and incomplete STR profiles were observed from both filter types.

In order to seek a method to improve the capture and preservation of DNA in breath, we inserted wet or dry FTA card punches into the mouthpiece of the Breath Explor<sup>®</sup> device. However, this approach did not result in any improvement, with less than 10% of samples yielding detectable amounts of DNA. Therefore, laboratories may only swab the inside of the mouthpiece to safeguard the chain of custody. To further expand this study, Diamond Dye<sup>®</sup> was used to visualize where the DNA is located in the device to establish if cells or cell-free DNA is being transferred from breath to the device, and if so, then where to focus sample collection.

**Reference:**

- <sup>1</sup>. B. Budowle, A.J. Eisenberg, A. van Daal, Validity of low copy number typing and applications to forensic science, *Croatian Medical Journal*. 50 (2009). <https://doi.org/10.3325/cmj.2009.50.207>.

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**Low-Template DNA; Trace DNA; Exhaled Breath DNA**

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**B5     The Impact of 1,4-Dithiothreitol (DTT) on the Microbial Community Structures Associated With Human Semen Samples**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the microbial DNA associated with human semen samples and how the presence or absence of DTT impacts the bacterial structure associated with these samples.

**Impact Statement:** This study will aid the forensic community in establishing more robust extraction protocols and methods, specifically regarding samples often associated with sexual assault cases such as semen.

Previous studies suggest that body fluids have distinct and distinguishable microbial signatures. Identifying these microbial markers associated with forensically relevant fluids not only enhances current identification methods, but this method can also be easily implemented into comprehensive High-Throughput Sequencing (HTS) panels such as those currently used in the forensic workflow integrating human identity with human phenotypic marker characterization. Body fluid identification is a crucial step in the forensic workflow as this can aid in crime scene reconstruction as well as support and corroborate victim and witness statements. Semen is one of the most common body fluids encountered at a crime scene. However, while human and bacterial DNA are co-extracted without the need for additional steps to the forensic DNA workflow, the extraction of human DNA from semen samples typically requires the addition of DTT. As the developed body fluid identification method was designed for the use with unknown samples, any variations to current extraction methods should be investigated; specifically, determining how DTT impacts the microbial signatures associated with semen.

This study was designed to investigate how the addition of DTT during DNA extraction influences the microbial signatures in semen. Semen samples were collected in duplicate from 19 donors using an IRB-approved collection method ( $n=38$ ). All samples were then extracted using the DNA Investigator® kit, with one set of duplicates being extracted with the addition of 20µl DTT during the lysis step. This was to determine whether the addition of DTT significantly impacted the microbial signatures associated with semen.

Immediately after extraction, all samples were quantified using an in-house developed bacterial DNA quantification method.<sup>1</sup> Using the dual-indexing protocol as described by Kozich et al., the V4 region of 16S rDNA was sequenced on Illumina's® MiSeq® FGx™ platform.<sup>2</sup> Sequences were then analyzed using the open source microbial analysis platform, mothur version 1.47, and statistical analysis was performed using the "car" package in R version 4.2.1.<sup>3,4</sup>

When comparing the bacterial DNA yields between the DTT and non-DTT group, bacterial DNA yields did not significantly differ between treatments. While the relative abundance of some bacterial taxa varied, specifically in the low abundance taxa, the presence of DTT did not significantly impact the bacterial communities, and therefore the bacterial signatures associated with semen samples. This suggests that the bacterial signature-based body fluid identification method is well suited for the implementation into the forensic DNA workflow regardless of whether the analyst uses DTT for extraction or not.

Findings from this study will help minimize errors associated with the accuracy of microbial signature-based method for body fluid identification, while enhancing current extraction protocols.

**References:**

1. Seashols-Williams S, Green R, Wohlfahrt D, Brand A, Tan-Torres AL, Nogales F, et al. An accurate bacterial DNA quantification assay for HTS library preparation of human biological samples. *Electrophoresis* 2018;39(21). <https://doi.org/10.1002/elps.201800127>.
2. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. 2013. <https://doi.org/10.1128/AEM.01043-13>.
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**Body Fluid ID; DTT; Microbiome**



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**B6 Can We Predict DNA Shedding Propensity From Other Individual Characteristics?**

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**Learning Objective:** After attending this presentation, attendees will have learned which biological factors may affect differences in individual DNA shedding propensity.

**Impact Statement:** This presentation will impact the forensic science community by adding information on the variability of individual shedding propensity. Data on individual variation of how much DNA is deposited affects the probability of seeing certain DNA results under different transfer scenarios.

Determining the probative value of DNA traces, especially in the absence of body fluid attribution, requires the consideration of passive transfer scenarios. One factor playing a role is the well-established variation in individual shedding propensity.<sup>1</sup> A more standardized approach for measuring these differences could aid in transfer assessments. In some circumstances (e.g., with an uncooperative or deceased person of interest), it may be necessary to predict the relevant shedding propensity based on other physical characteristics. This study attempted to correlate individual characteristics to the amount of DNA recovered from a person's hands.

We used adhesive D-SQUAME<sup>®</sup> tape disks to collect DNA from the fingers of over 100 volunteers 30 minutes after hand washing and no activity, and after 60 minutes with controlled activity. The volunteers provided information on personal habits like smoking or use of medication, and physical characteristics such as height, weight, tanning, and sweating propensities. The research team used a C+K Multiprobe instrument to measure each volunteer's skin hydration, melanin level, and sebum content. Observers counted each participant's involuntary self-touches during the 60-minute activity period. After processing the finger samples with Qiagen<sup>®</sup> QIAamp<sup>®</sup> DNA Investigator extraction chemistry, LifeTechnologies Quantifiler<sup>®</sup> Trio quantification and GlobalFiler<sup>®</sup> STR typing the self-DNA concentrations were compared to the volunteer characteristics.

As expected, finger surfaces contained more DNA after the 60-minute wait with controlled activity. STR typing was successful for most samples. Both finger sample types allowed for a separation of low and high shedders, with most individuals in the intermediate category. Results showed that time since last shower, self-declared sweating, the number of self-touches, and age are biological factors that possibly correlate to DNA shedding propensity. Other characteristics were less promising. Body mass index, sebum, melanin content, and skin hydration so far did not show a correlation to DNA shedding propensity ( $n=22$ ) but are being revisited in the larger sample set.

**Reference:**

- <sup>1</sup>. Szkuta B, Ballantyne KN, Van Oorschot RAH. Transfer and persistence of DNA on the hands and the influence of activities performed. *Forensic Sci Int Genet.* 2017;28:10–20.

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**Contact Traces; DNA Shedding Propensity; DNA Transfer**

## **B7 The Multiple Reaction Monitoring Tandem Mass Spectrometry Approach: A Tool for Latent Stains of Biological Fluids Identification in Forensic Sciences Laboratory**

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**Learning Objective:** After attending this presentation, attendees will understand the necessity of a Multiple Reaction Monitoring Tandem Mass Spectrometry approach for latent stains study. Body fluid identification can be as important as DNA tests in linking a biological stain to a crime scene event.

**Impact Statement:** This presentation will impact the forensic science community as it can modify the traditional approach to the study of latent stains. In these circumstances, enzymatic and immunological tests are easy to use but can give false-negative results.

Biological samples found at the crime scene play a crucial role in forensic investigation by providing valuable evidence. Body fluids identification can be as important as DNA profiling when linking a biological trace of a person to the crime scene.<sup>1,2</sup> Investigators can highlight the presence of body fluids on many specimens (i.e., clothes, surfaces, items). In general, white light enhances evident stains of human blood, sperm, and saliva, while a poly-light (ranging from 320-490nm) exalts latent biological traces. Chemiluminescence tests are also employed to highlight latent bloodstains. Traditional techniques currently used in the laboratory for forensic fluid identification are microscopy, enzymatic, and immunological tests. These tests are easy to use, but forensic operators have demonstrated that they could give false negative results when applied to latent traces.

Researchers have developed new methods that involve messenger RNA (mRNA), micro-RNA (miRNA), DNA methylation, and peptide studies.<sup>3-5</sup> However, RNA is less stable than peptides, and DNA methylation is still considered a relatively new area of forensic analyses, so we have approached the peptides detection techniques that involve tandem mass spectrometry in Multiple Reaction Monitoring (MRM).<sup>6-8</sup>

This report compares the routine identification techniques to the LC-MRM/MS. Saliva, blood and semen were analyzed to determine the concentration of the proteins: amylase, hemoglobin, and PSA. Hence, 100µl of specimens, obtained by serial dilutions of blood, semen, and whole saliva, were placed on sterile swabs, dried, and analyzed. The outcomes were useful for evaluating the methods' sensitivity, thought to be the lowest analyte concentration measured with acceptable accuracy and precision; 108 biological swabs and 27 blank swabs were analyzed. The study showed the LC-MRM/MS was the reference technique to identify latent biological traces. After performing immunological and LC-MRM/MS analysis, results were correlated to DNA profiles obtained from each sample. We found the maximum dilutions at which it was still possible to get a complete genetic profile and to identify a biological trace.

### **References:**

1. Roewer L. DNA fingerprinting in forensics: past, present, future. *Invest. Genet.* 2013; 4(22): 1-10.
2. Ja Hyun An, Kyoung-Jin Shin, Woo Ick Yang & Hwan Young Lee. Body fluid identification in forensics. *BMB Reports.* 2012; 9(20): 546-53. <http://dx.doi.org/10.5483/BMBRep.2012.45.10.206>.
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### **Biofluid Identification; Forensic Sciences; LC-MRM/MS**

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**B8 The Enhanced Collection and Recovery of Touch DNA Samples From Challenging Surfaces Using a Venturi Vacuum Device (VVD) Across Two Forensic DNA Workflows**

*Joseph Rahm, BS\*, Florida International University, Miami, FL; Charday Ward, BS, Florida International University, Miami, FL; DeEtta Mills, PhD, Florida International University, Miami, FL*

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**Learning Objective:** Through the comparison of cotton swabs and a VVD with polycarbonate filters used to collect real touch DNA samples from brick and steel bar materials, attendees will learn about a novel vacuum collection approach for touch DNA from challenging surfaces and its subsequent effect on DNA profile quality downstream.

**Impact Statement:** This presentation sets to improve the standard approach to collection of touch DNA samples from challenging surfaces at crime scenes.

Metal and brick materials are commonly encountered in forensic investigations where touch DNA evidence remains a challenge to collect with a profiling success rate of 0–26%.<sup>1,2</sup> Often, cotton swabs remain the standard tool when collecting evidence for possible touch DNA from crime scenes.<sup>2,3</sup> However, cotton swabs often rip and tear on challenging surfaces like brick.<sup>2,3</sup> Furthermore, cotton swabs have been shown to not release cells effectively.<sup>2,3</sup> A new approach for collecting cells from challenging surfaces was tested using a novel VVD equipped with a Polycarbonate Filter (PCF) and compared to traditional cotton swabs. This device allows for evidence to be collected from hard-to-reach areas or from rough surfaces such as brick. The PCF also appears to release cells from the collection matrix more efficiently, which improves downstream analyses.

Touch samples were collected with cotton swabs or the VVD/PCF and compared across two DNA workflows, traditional-extraction processes, or direct PCR, and assessed by comparing STR profile quality. QIAGEN® manual extraction and PowerUp Syber qPCR kit were used for the extraction workflow and compared to direct amplification. All STR amplifications for both workflows were done using a Fusion™ 6C kit containing 24 core STR loci, and a half-reaction protocol. The VVD/PCF and cotton swabs were used to collect real touch DNA samples from stainless steel bars and brick cinder block substrates handled separately by ten individuals in replicates of three, forming eight different treatment groups. The number of expected alleles recovered was recorded for each treatment group, converted to a percentage, and analyzed with a one-way ANOVA. Preliminary data for the post-extraction DNA workflow for steel bars showed 59% recovery of expected alleles for cotton swabs and 58% for VVD/PCF, and were not significantly different from each other. However, data for the extraction DNA workflow for brick showed 11% recovery for cotton swabs and 45% recovery of expected alleles from VVD/PCF collections. Data for direct PCR workflows is still ongoing.

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**Touch DNA; Collection; Direct PCR**

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**B9 Touch DNA: An Evaluation of a Novel Collection Method**

*Jamie Fredericks, PhD\*, Eastern Kentucky University, Richmond, KY; Hannah Varner, BSc, Eastern Kentucky University, Richmond, KY*

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**Learning Objective:** Attendees will learn about a simple and effective collection method for analyzing touch DNA samples deposited on various surfaces in a direct STR PCR amplification protocol.

**Impact Statement:** This novel collection method will allow DNA analysis to be streamlined into a more efficient process by utilizing the advantages of direct STR PCR amplification. It has the potential to reduce the time it takes to profile samples and financial impacts on laboratories.

Touch DNA, skin cells that have been left behind when an individual has handled an object, has become an important source of evidence. Obtaining a DNA profile from touch DNA can be challenging. When an individual handles an object, only a small amount of DNA is deposited. Therefore, it is important that all DNA from the source is collected for STR amplification. There are numerous variables, including the shedding potential of the individual, the pressure applied, or surface type of the object being touched, which cannot be controlled by the investigator but can influence the amount of DNA present. In addition, choosing the right collection method, extraction method, or STR amplification kit may also influence the likelihood of successfully amplifying a DNA profile. Direct PCR amplification (direct PCR) has focused on genotyping samples from source, avoiding the time-consuming extraction process attributed to traditional methods and reduces the amount of DNA lost from sample transfer (during the multi-step purification processes of traditional methods).

A novel collection method was investigated to determine whether it could improve analysis of touch DNA through a direct STR PCR protocol. The collection method involved moistening a swab (cotton or nylon swabs were tested) with a minimum volume (50-100 µl) of a direct PCR amplification reagent. Prep n Go™, SwabSolution™, and low TE was used to collect touch DNA samples from various handle surfaces. Surfaces tested included rubber, metal, wood, and plastic handles. Using DNA IQ™ Spin baskets, the volume was removed from the swab and used in an STR PCR amplification kit (PowerPlex® Fusion and GlobalFiler™ were used) as described by the corresponding manufacturer's protocol. When using low TE, 15µl was added to each STR PCR amplification kit.

Our preliminary investigation has shown that Prep n Go™ and SwabSolution™ are able to produce full profiles, using GlobalFiler™ and PowerPlex®, respectively, from handles made from rubber, plastic, and metal, irrespective of the swab used. On the same surfaces partial profiles were obtained when using low TE. Only partial profiles were observed when testing wood surfaces, regardless of the collecting reagent or swab type, suggesting that potential inhibitors (from the wood or varnish) were present. This study demonstrates that our novel collection method could be used on certain surfaces to obtain a full DNA profile in a direct STR PCR amplification.

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**Touch DNA; Collection Method; Handles**

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**B10 Exploring Target Areas for Recovering Informative DNA Profiles From Eyeglasses**

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**Learning Objective:** This presentation aims to inform attendees about targeted sampling of eyeglasses as a way to identify the primary wearer.

**Impact Statement:** Attendees will better understand why targeted sampling of eyeglasses increases the statistical support for inclusion of the primary wearer over an approach that swabs the entire item.

The recovery and analysis of transfer DNA is an essential, routine method used in forensic investigations to suggest suspects' presence at crime scenes, either with a victim or an object. However, the increased sensitivity of modern DNA technology has also enhanced the likelihood of detecting mixtures from swabbed objects, some of which are the result of innocent indirect transfer.

As a commonplace item, eyeglasses can be an object of interest when discovered at crime scenes. The ability to obtain an informative profile from eyeglasses can provide investigative leads. The most common swabbing method involves swabbing the entire item with a single swab to recover the greatest quantity of DNA for subsequent profiling. This approach likely collects non-wearer DNA transferred to the eyeglasses from the environment and the wearer's own hands. This research was designed to identify a specific target region(s) on eyeglasses more likely to produce a single-source DNA profile consistent with the primary wearer. A more targeted approach to evidence sampling could add efficiency to investigations and reduce backlogs by eliminating potentially uninformative DNA profiles.

To test the hypothesis that a single-source profile consistent with the primary wearer will be detected using a targeted swabbing approach, eyeglasses with different primary wearers were utilized. The eyeglasses were not sterilized to imitate eyeglasses recovered from a crime scene. Each pair of eyeglasses was then swabbed on the following surfaces: nose bridge (internal, external), nose pads (left, right), lens (left, right, internal, external), rims (left, right, internal, external), temple (left, right, internal, external), and temple tip (left, right, internal, external). The six surfaces were swabbed using a separate swab, totaling six swabs per pair of eyeglasses. Samples were extracted utilizing the QIAamp® DNA Mini Kit. Samples were processed through DNA analysis workflow utilizing Applied Biosystems® products: quantified with Quantifiler® Trio DNA Quantification Kit on a 7500 Real-Time PCR System, amplified using Globalfiler® Amplification Kit on a ProFlex™ PCR System, and analyzed on a 3500xL Genetic Analyzer. DNA profiles were deconvoluted and likelihood ratios generated using STRmix™ v2.6.1.

When comparing the wearer's profile to the samples collected from their eyeglasses, only 53% of the likelihood ratios provided "very strong support" that the wearer contributed to the DNA recovered from their eyeglasses. "Strong support" was assigned to 7% of the samples, 33% were "uninformative," and 7% were excluded from contributing to the samples collected from their own eyeglasses. These results are interesting as 87% of the profiles appeared to have only one contributor based on observations of the electropherogram. This suggests the project's initial goal of identifying a region that would produce a single-source profile is oversimplified and should instead seek to identify a region that provides the strongest statistical support for the contribution of the wearer to the sample. In this study, the portions of the eyeglasses in direct contact with the temple (temple tip and temple region) and the nose pads produced the strongest statistical support for inclusion of the wearer. The extraneous alleles detected in the other samples complicated the ability to associate the wearer with their own eyeglasses. Therefore, the practice of swabbing the entire item is more likely to detect extraneous alleles and sampling practices should focus on just those areas of the eyeglasses in direct contact with the wearer.

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**Transfer DNA; STRmix™; Eyeglasses**

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**B11 The DNA Transfer to Bedsheets in Differing Cohabitation Environments**

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**Learning Objective:** This presentation aims to inform attendees about the transfer and recovery of DNA on bed sheets in differing habitation situations.

**Impact Statement:** Attendees will be impacted by better understanding the ease of DNA transfer within dwellings and among social circles as observed by direct and indirect DNA transfer to bed sheets.

The collection of biological material from bed sheets could serve as valuable evidence when a sexual or violent assault has occurred on the linens. However, sampling bed sheets differs between forensic laboratories and agencies. Some agencies utilize swabbing techniques and others take cuttings. The location sampled also varies based on the presence or absence of staining and the circumstances of the case. With increasingly sensitive technologies that no longer rely on the generation of DNA profiles from body fluids, a systematic test of different target locations on the bed sheet under different habitation situations may inform sampling strategies.

This project compared DNA profiles collected from linens located in households with different cohabitation patterns (two individuals living in the same household with separate bedrooms and two individuals living in the same household who share one bed) to determine if the living pattern and sleeping arrangements impact DNA transfer. Identical fitted bed sheets were purchased, labeled to identify the sleeper(s), and divided into thirds horizontally creating a top, middle, and bottom section. Following the sheet manufacturer's instructions, sheets were washed separately in a singular load in the personal washing machine of each participant. After the initial wash, sheets were swabbed to investigate the presence of DNA before sleeping. One swab was taken from the entirety of each section. The bed sheets were then placed on each participant's bed and used for one week. At the end of the week, all three sections were swabbed again. Each set of participants' sheets was washed as previously described and replaced on their respective beds. This procedure was repeated for four consecutive weeks. Participants went about their normal activities to accurately investigate DNA transfer in an uncontrolled environment. This research design allows for an investigation of hypotheses concerning the transfer and recovery of DNA in different cohabitation situations.

Samples were extracted utilizing the QIAamp® DNA Mini Kit. Samples were further processed through DNA analysis workflow utilizing Applied Biosystems® products: quantified with Quantifiler® Trio DNA Quantification Kit on a 7500 Real-Time PCR System, amplified using GlobalFiler® Amplification Kit on a ProFlex™ PCR System, and analyzed on a 3500xL Genetic Analyzer. Additionally, DNA profiles were deconvoluted and likelihood ratios generated using STRmix™ v2.6.1. A total of 97.8% of the samples, representing all sections of the individual bedsheets, generated profiles suitable for STRmix™ analysis. This is noteworthy as many previous studies suggested a wet swabbing technique, as used here, was inefficient in collecting enough biological material from bedsheets for subsequent analysis.<sup>1,2</sup> Results demonstrate DNA transfer to bedlinens occurs frequently, since approximately 60% of samples from single occupant beds have multiple contributors and 42% of samples from the shared bed have more than two contributors. Additionally, outside interactions between participants is enough for DNA transfer to occur, since the DNA of individuals were detected on the bedsheets of households they never visited. This research shows that even the “strong support” of an individual's DNA on bedsheets does not confirm physical contact with the bedsheets.

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**Transfer DNA; Bed Sheets; STRmix™**



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**B12 Morphological and Autofluorescence Signatures for the Identification of Vaginal Cells in Mixture Samples Containing Saliva and/or Epidermal Cells**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of vaginal cell characteristics and how differentiation of this cell type based on size, morphology, and autofluorescence profiles can be a crucial step in the forensic science workflow, particularly for sexual assault cases.

**Impact Statement:** This work has the potential to improve the methods for identifying, differentiating, and confirming vaginal cells and fluid in forensically relevant samples, as well as serve as a predictive tool for the quantity and quality of DNA from specific contributors in a biological mixture.

In forensic casework there are several bodily fluid identification methods to characterize unknown stains recovered as evidence. However, there has not been a reliable method for detecting the presence vaginal cells and/or differentiating it from other forensically relevant tissue types that may be recovered from a crime scene. This is particularly a challenge in sexual assault cases where samples may be a mixture of vaginal cells with epidermal cells and/or saliva cells. To address this, we tested a new approach for identifying vaginal cells within mixture samples based on Imaging Flow Cytometry (IFC) profiling, which can occur before DNA profiling. During IFC, the morphology and autofluorescence profile is characterized in a high throughput and non-destructive fashion, which provides unique signatures for cell populations based on their intrinsic biochemical differences and source tissue.

To develop a robust signature for vaginal cells, we first collected reference vaginal tissue from 50 different individuals and compared it to IFC signatures obtained from reference samples representing saliva and epidermal tissue sources. Following collection, each sample was stored dried for time periods ranging between one day and six months. Results showed that vaginal cells could be reliably differentiated from epidermal and saliva cells with a classification accuracy of ~92% (by donor cell population). Analysis of multivariate discrimination functions for each tissue group indicated that differentiation was primarily based on size/morphology of vaginal cells as well as the intensity of autofluorescence at wavelengths between 500nm–600nm and optical contrast. The length of time the sample was dried did not appear to negatively impact differentiation signatures.

Next, we tested whether vaginal cell signatures could be used to identify contributor cell populations in mock casework samples consisting of mixtures of: (1) vaginal and penile epidermal cells and (2) vaginal and saliva cells. A total of 12 samples of each mixture type were collected and stored dry for approximately two months prior to IFC analysis. To enhance the probative value for DNA casework, IFC signatures were used to create a predictive framework for classifying unknown/blinded biological samples where the confidence/likelihood that a particular cell population originated from a specific tissue type was described with a dissimilarity index value and a posterior probability. Results suggest that vaginal cells could be successfully detected in approximately 80% of mock casework samples involving either penile epidermal cells or saliva cells. Further, when vaginal cells were present, it was associated with relatively high confidence,  $>0.90$  posterior probability  $< 3$  Mahalanobis distance. Samples where vaginal cells were present but not detected were generally associated with post-deposition activities such as surface washing. This approach offers a new method for the presumptive identification of vaginal cells and, importantly, may be one of the first methods to provide a probabilistic framework for interpreting serological analyses.

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**DNA Mixtures; Sexual Assault; Imaging Flow Cytometry**

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**B13 The Application of an Improved Processing Method of Touch DNA Samples**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the importance of improving methods to process forensic samples that present low quantity and low quality DNA.

**Impact Statement:** This presentation will impact the forensic science community by presenting the application of a modified protocol that can be used to improve the processing of touch DNA samples.

In many cases, DNA is obtained from biological material that is transferred from a donor to an object during physical contact. This type of sample is known as “touch DNA” and often presents low quantity and low quality DNA. Despite this characteristic of touch DNA samples, they can play an essential role in forensic casework and are considered an important tool for investigators. Due to the usefulness of this type of evidence, successful sample collection and processing of touch DNA samples is crucial for obtaining an efficacious downstream analysis.<sup>1</sup> Touch DNA can present low copy number when the contact between object and donor is not long enough to leave behind enough donor’s cells or when the deposition of the cells is compromised by the item’s surface. Also, touch DNA can be a vulnerable sample by being easily exposed to environmental conditions and therefore suffering degradation.<sup>2</sup> Samples such as fired cartridge cases (FCCs) are examples of touch DNA samples but because they are so difficult to process and obtain suitable DNA profiles, they are typically not processed for DNA analysis.<sup>2</sup> However, new techniques to improve the analysis of fired cartridge cases were demonstrated very recently by the United States Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF), National Laboratory Center.<sup>3</sup> Despite showing an elevated success rate in obtaining useful information, this new protocol of collection and analysis seems to not have been applied to other types of touch DNA samples.

The goal of this study was to demonstrate the application of the improved collection and processing technique developed by ATF to increase the success rate of analysis of different types of forensically relevant touch DNA samples. To achieve this goal, different mock forensic samples were created, and DNA samples were collected and processed using the ATF modified protocol. Some samples were also first exposed to different environmental conditions before DNA collection. The results obtained in this study are important to show that a higher success rate in generating informative DNA profiles can be achieved with the use of modified and improved protocols of collection and processing of touch DNA samples.

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**Damaged DNA; Low Copy Number; Sample Processing**

## B14 Trace DNA: Recovery Efficiency From Patrimonial Crimes in the Brazilian Federal Police

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**Learning Objective:** The present study aims to evaluate the percentage of recovering interpretable genetics profiles from the different types of traces collected in crime scenes of patrimonial crimes so this information can be used by crime scene investigators to select the traces that have the greatest probability of providing a useful result.

**Impact Statement:** This presentation will impact the forensic science community by helping to increase the efficiency of genetic analysis of crime scenes related to patrimonial crimes.

The use of forensic genetics to help solve crimes has increased considerably in the past years, with a consequent increase in the analysis of biological evidence. However, resources are limited, making it necessary to optimize the work in order to allow results to be obtained in a timely manner and to avoid the occurrence of backlogs. This work evaluated the potential for recovering interpretable genetic profile from the different types of traces collected at property crime scenes; 958 reports issued by the Federal Police's forensic genetics laboratory in 2020 were analyzed, resulting from 2,872 samples. A percentage of recovery of an interpretable genetic profile was observed in 32.42% of the analyzed samples, with blood being the evidence that presented the highest recovery rate, followed by traces resulting from oral mucosa and finally by material resulting in trace DNA. Overall, human biological tissue from teeth/dental prosthesis and razors showed a higher percentage of useful results, both with 100% ( $n=4$  and  $n=1$ , respectively), followed by blood with 96.30% ( $n=260$ ), and the remains of foods consumed, with 84.62% ( $n=11$ ). Considering the number of samples and the percentage of success, blood was considered the best result. Among the traces with the highest percentages of non-useful results are backpacks and bags, with 93.18% ( $n=41$ ), followed by socks, with 90.91% ( $n=10$ ) and surface swabs, with 90.00% ( $n=477$ ). The research produced a schematic view of the percentage of useful and non-useful profiles for each type of trace.

The descending order of recommendation for collection (Position/Material/Number of samples successfully obtained):

1. Blood 260 (96,30%)
2. Human biological tissue from teeth/dental prosthesis 4 (100,00%)
3. Razor 1 (100,00%)
4. Partially consumed food scraps 11 (84,62)
5. Toothbrush 20 (71,43%)
6. Cigarette butt 77 (69,37%)
7. Underwear/panties 4 (57,14%)
8. Cups/cans/bottles/cutlery/straw 124 (51,45%)
9. Towel 3 (42,86%)
10. Balaclava/ninja headgear 19 (39,58%)
11. Face mask 13 (38,24%)
12. Cap/hat/cap/hood 45 (37,82%)
13. Hair 8 (36,36%)
14. Glove 49 (35,25%)
15. Adhesive tape/plaster 18 (33,96%)
16. Shirt/coat/vest 46 (31,29%)
17. Pants/shorts/shorts 4 (25,00%)
18. Feces/urine 5 (29,41%)
19. Shift swab/steering wheel/handbrake 36 (21,18%)
20. Glasses 6 (17,14%)
21. Flip-flops/sandals/sneakers/shoes 8 (16,33%)
22. Objects and tools 111 (15,02%)
23. Helmet 2 (11,76%)
24. Surface swab 53 (10,00%)
25. Socks 1 (9,09%)
26. Backpack/bags 3 (6,82%)

The success of recovering an interpretable genetic profile is directly related to the concentration of genetic material present in the sample. The higher the concentration of DNA, the greater the effectiveness of recovering an interpretable genetic profile. This way, traces that are most likely to have high concentrations of DNA should be sought. The present work showed that blood is the trace that presents the best recovery, and preference should always be given to this trace when found. However, in crime scenes against property, blood is not always present. In the absence of this trace, preference should be given to the collection of traces that had contact with the oral mucosa or saliva, such as partially consumed food, glasses, cans, bottles, toothbrushes, and cigarette butts, and only then move on to the collection of clothing in general, such as shirts, gloves, and caps. Understanding the success rate of recovering interpretable genetic profiles from the different types of traces present in crime scenes is essential to direct prioritization in the collection and processing of DNA evidence.<sup>1-35</sup>

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### Forensic Genetics; Trace DNA; Forensic DNA

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**B15 Improving the Analysis of Forensic Samples Using a Microwave Treatment and Rapid, Direct Polymerase Chain Reaction (PCR)**

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**Learning Objective:** After attending this presentation, attendees will understand how a microwave treatment can be used on forensic samples to quickly “extract” their DNA.

**Impact Statement:** This presentation will impact the forensic science community by introducing attendees to a unique application of a microwave oven for forensic DNA analysis and by showing the ability of the microwave treatment to recover alleles of low-template DNA samples.

In forensic DNA analysis, generating a complete DNA profile from low template samples still represents a challenge. There is an ever-increasing demand to speed up the process and increase the sensitivity of the results. Despite the progress and the introduction of rapid instruments, sensitivity and speed remain an issue when dealing with complex, low template samples. In this study, a novel extraction protocol for forensic samples using a microwave oven and a homemade PCR multiplex of 9 mini-STRs was developed and optimized for the following body fluids: saliva, blood, and semen.

This project represents a novel application of the microwave treatment for use in forensic DNA analysis. The hypothesis is that microwaves can be used to rupture the cell membranes, making the genetic content available without the use of chemicals or toxic reagents. The method includes a microwave treatment to increase the yield of the samples, followed by a direct-rapid PCR protocol, therefore skipping the conventional extraction step. The homemade direct and rapid PCR protocol, which includes 9 mini-STR loci in a multiplex, was developed and optimized for the three body fluids, amplifying them in less than 15 minutes. The experimental design occurred in three phases: (1) developing a method for calibration of the microwave ovens, (2) optimizing a rapid PCR protocol, and (3) testing the protocol on forensic samples and in a round-robin inter-laboratory study.

The results obtained demonstrated the ability of the microwave treatment to increase the yield of all the samples analyzed. For each body fluid, microwave settings were optimized. Blood and saliva samples were microwaved for 20sec at 300W while semen was microwaved for 40sec at 300W. An increase in average peak heights and recovery of alleles was observed for samples subjected to the microwave treatment. In 1% diluted blood samples, the average allele peak height increase was >300%, while the 1% diluted saliva samples had an overall increase of 55% and 0.5% diluted semen samples increased 96% in peak heights. When analyzing mixtures of blood and saliva, an increase of 10% in allele recovery was observed, including increased peak heights of the minor contributor ranging from 106% to 130% increase, after microwave treatment. Inter-laboratory studies resulted in similar increases in allele recovery when a microwave treatment was applied.

Overall, the use of a microwave coupled with rapid direct PCR represents a valuable addition to an analyst’s toolkit, increasing the DNA yield as well as the speed of the experiments. Moreover, it is less expensive and less toxic than other pretreatments. Combining microwave pretreatment with direct PCR may also impact medical and environmental analysis in which rapid and fieldable sample extraction requires high sensitivity and rapid analyses.

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**Forensic DNA Analysis; Microwaves; Direct and Fast PCR**



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**B16 Finding the DNA: How Much Was Recovered and Where Did the Rest Go?**

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**Learning Objective:** This presentation reviews data from a controlled study designed to evaluate DNA recovery from bloodstains using the DNA IQ™ extraction chemistry. Attendees will learn: (1) how DNA recovery can be easily estimated, and (2) which steps in the DNA IQ™ protocol contribute to DNA loss.

**Impact Statement:** This presentation will impact those in the forensic science community who use DNA IQ™ reagents or other silica-based DNA extraction methods. Laboratories using DNA IQ™ reagents should be aware that a significant amount of the sample DNA may be lost when following the DNA IQ™ System Small Sample Casework Protocol.

Evidentiary samples often contain small amounts of DNA; therefore, maximizing the amount of DNA recovered is essential to increase the probability of generating a complete STR profile. While silica-based DNA extraction methods remove PCR inhibitors, they have multi-step procedures that can lead to partial DNA loss.

Evaluating the efficiency of an extraction method can be difficult, as the original amount of input DNA may be unknown. Knowledge of the amount of DNA present in the starting sample provides the quantitative information needed to calculate DNA recovery. The use of a direct lysis DNA “extraction” method lyses all the cells in the sample and can be done in a single tube. Therefore, qPCR values from a set of identical starting samples, extracted using a direct lysis method (in these experiments, forensicGEM™ was used) can be used to calculate the expected total DNA that was present in the samples pre-extraction.

The Maxwell® FSC instrument is a robotic DNA extraction instrument designed to remove PCR inhibitors from forensic samples. The Maxwell® FSC and the Maxwell® FSC DNA IQ™ Casework Kit were used to extract DNA from bloodstains made using fixed volumes of progressively larger dilutions. Upon comparing the amount of DNA recovered using the Maxwell® FSC instrument to the amount of DNA obtained using the direct lysis method, which in theory would recover 100% of the DNA, the percent yield for serially diluted bloodstain samples was low, ranging from 8–14% DNA recovery.

To provide additional data on DNA recovery, the Maxwell® FSC study was replicated using the DNA IQ™ System Small Sample Casework Kit, which uses the same chemistry and reagents as the Maxwell® FSC DNA IQ™ Casework Kit. The manufacturer’s instructions for manual extractions were followed for all subsequent samples. Repeating the experiment by hand showed similar results, with DNA recovery yields ranging from 1–22%, depending on the level of the blood dilution.

The purpose of this study was to determine which steps in the DNA IQ™ process contribute to low DNA recovery by comparing the amount of DNA obtained from samples extracted with DNA IQ™ and the amount of DNA obtained using direct lysis with forensicGEM™. Steps from the DNA IQ™ System Small Sample Casework Protocol were identified where, potentially, the DNA loss could occur. These included: (1) DNA left on the swatch, (2) DNA left in the supernatant after incubation with the IQ resin (referred to as “waste”), and (3) DNA left on the IQ resin after elution. The laboratory-made stain extraction buffer was also checked to ensure that there was no issue related to the use of this buffer.

Study data identified two major sources of DNA loss: the “waste” and the swatch. When quantifying DNA remaining in the “waste,” for almost every individual sample, more DNA remained in the waste than was found in the initial extract using the DNA IQ™ System Small Sample Casework Kit. The high amounts of DNA in the waste indicates that the DNA IQ™ resin does not bind to all the available nucleic acid in the solution. This data and other data characterizing DNA loss at particular steps of the protocol are described in this study.

Additionally, modifications to the protocol are described, which were made in an attempt to increase the amount of DNA bound to the resin that would then subsequently be recovered. Preliminary results indicate that yield can be improved by modifying the procedure to either increase the resin volume, increase the incubation temperature of DNA with the resin, or recapture DNA from the first waste.

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**DNA Recovery; DNA IQ™; Silica-Based DNA Extraction**

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**B17 Using Exact Likelihood Ratio (LR) Distributions for Probabilistic Genotyping Software Validation**

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**Learning Objective:** After attending this presentation, attendees will understand a fast and easy approach to constructing exact LR distributions that help calculate accurate sensitivity and specificity error rates when validating Probabilistic Genotyping (PG) software.

**Impact Statement:** This presentation will impact the forensic science community by showing a method for calculating precise LR distributions for sensitivity and specificity error rates. These distributions consider every possible reference genotype. They are helpful for PG software validation, and for establishing scientific reliability in the courtroom.

Testing DNA PG interpretation software is important in forensic science. Empirical testing ensures a method works as expected. Validation studies test the PG method on representative data sets, reporting LR match statistics. These studies typically include sensitivity and specificity error rates. Sensitivity evaluates the inclusionary strength of true contributors to DNA. Specificity examines the ability of DNA evidence to statistically exclude non-contributors. The log(LR) number is used to measure sensitivity and specificity information. From LR distributions, false exclusion and false inclusion error rates can be immediately calculated.

To use PG software for DNA interpretation, applicable validation standards require sensitivity and specificity studies, as well as error rate determination. Legal admissibility standards encourage PG software validation and error rate calculation—both of which are *Daubert* prongs.

LR distributions for examining system sensitivity and specificity can be developed either by limited sampling or by exact convolution. Both calculation methods produce distributions of log(LR) statistics. The sampling method approximates exact log(LR) distributions by comparing a set of evidence genotypes with a set of randomly sampled reference genotypes. Sampling is incomplete, only testing a thousand (103) or so references, which is a miniscule fraction of possible genotypes. Calculating by sampling is tedious in validation; comparing a thousand (103) evidence genotypes with a thousand references entails a million (106) match statistic calculations.

The exact method accurately calculates log(LR) distributions for evidence genotypes. The requisite convolution can have any preset numerical resolution. The convolution approach is complete, with one distribution accounting for all (e.g., 1,024) possible reference genotypes.<sup>1</sup> The calculation is fast; a hundred genotype distributions can be constructed in one second. Many evidence genotype distributions can be averaged to represent a set of genotypes in one composite distribution. This composite feature is highly useful for validation studies.

We assessed both the sampling and convolution methods on the same DNA laboratory's mixture validation data set. We constructed contributor (posterior evidence probability weighted) and non-contributor (prior population probability weighted) genotype log(LR) distributions. We calculated error rates from these distributions to measure sensitivity and specificity. The data came from single source and DNA mixture samples.

Sampled contributor distributions were limited to the provided matching references, which severely under-sampled reference genotypes, and gave limited false exclusion rates. But the exact distributions spanned the entire range of expected log(LR) match values and provided accurate false exclusion probability for the tested data sets.

Non-contributor distributions were calculated by limited sampling and exact convolution. The distributions from both methods appeared qualitatively similar. But more random reference sampling—and time—was needed to better approximate the true distribution. Building exact convolved distributions was far faster than using sampling.

Using exact convolution, rapid calculation of sensitivity and specificity from the log(LR) distributions on multiple datasets sped up the PG validation, relative to sampling methods. Human operator time was significantly reduced. User interfaces for non-contributor, contributor, and composite distributions simplified PG validation.

Calculating exact composite log(LR) distributions by convolution—and determining associated error rates on genotype subsets—improves on LR sampling methods. Convolution construction is easy, fast, complete, and accurate. The method lets forensic scientists readily determine error rates for PG methods of interpreting complex DNA evidence. Moreover, the exact convolution LR distribution construction approach has applicability to other forensic subdisciplines, providing accurate error rate determination for reliable scientific validation and reporting.

**Reference:**

- <sup>1</sup> Perlin, M.W. Efficient construction of match strength distributions for uncertain multi-locus genotypes. *Heliyon*, 4(10):e00824, 2018.

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**Probabilistic Genotyping; Mixture Validation; Exact Convolution**

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**B18 The Algorithm Performance on a Firearm Examiner Black Study**

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**Learning Objective:** Attendees will learn how firearm evidence comparison algorithms perform compared to firearm examiners. Applications of these algorithms and how they would assist examiners in routine casework will also be discussed.

**Impact Statement:** As comparison algorithms continue to advance, they will begin to be applied in casework and presented in courts. This research will show the usefulness of these algorithms on fired cartridge cases when using a three-dimensional imaging instrument and will display data examiners can reference when discussing algorithm performance.

Firearm evidence comparisons have traditionally been performed through visual examination with use of a comparison microscope. In recent years, three-dimensional imaging technology has become more prevalent in crime labs, allowing for the collection of cartridge case and bullet surface topographies with micron-scale lateral and depth resolution. These high-resolution scans can be used for Virtual Comparison Microscopy (VCM) where comparisons are performed on a computer using the surface scans rather than physically comparing the evidence with a comparison microscope. A benefit to the use of VCM is that comparison algorithms can be applied to collect objective data on the similarity between cartridge cases and bullets. For these algorithms to become widely accepted, their performance must be measured under similar conditions to those that are expected to be encountered in casework, which includes a variety of firearm models representative of those that are recovered in connection with crimes. The purpose of this study was to evaluate performance of the Cadre Forensics breech face comparison algorithm on cartridge case test sets from an examiner error rate study.

In this study, cartridge case test sets from a previous study on examiner error rates were selected.<sup>1</sup> There were 20 test sets, each composed of three test fires from a known firearm and one questioned, or unknown, cartridge case. In this previous study, the examiners were tasked with determining if the questioned cartridge case was fired by the same firearm as the three knowns. In sets where the ground truth was an elimination, meaning the questioned cartridge case was not fired by the same firearm as the knowns, the questioned cartridge case shared the same class characteristics so that eliminations could not be made based on class characteristic differences.

All 20 test sets of cartridge cases that were used in the examiner error rate study were scanned on a Cadre Forensics TopMatch-GS 3D system that measures surface topography. The Cadre Forensics comparison algorithms were applied to the breech face area of the cartridge cases to assess their similarity within each test set. The similarity score values ranged from zero to one, where larger values indicated more similar features than lower scores. Algorithm score data were extracted for analysis using R via RStudio.

Results show that the Cadre Forensics breech face comparison algorithm is indicative of the ground truth for a majority of the test sets. Combining these data with the examiner conclusions, algorithms would be a good quality control solution. After an examiner performs a visual comparison, an algorithm can be applied, and the similarity values analyzed. Our data show that algorithms may help to prevent false positives and false negatives, as well as transition some inconclusive conclusions to identifications or eliminations.

**Reference:**

- <sup>1</sup>. Law, E.F. and Morris, K.B. (2021), Evaluating firearm examiner conclusion variability using cartridge case reproductions, *J Forensic Sci*, 2021;00:1-17. <https://doi.org/10.1111/1556-4029.14758>.

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**Firearms Identification; Algorithm Comparisons; Error Rates**

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**B19 An Assessment of a Novel Method for Physical Fit Examinations Using an Extensive Database of Casework-Like Samples and Interlaboratory Studies**

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**Learning Objective:** After attending this presentation, attendees will have learned about two developed systematic methods for reporting the quality of a physical fit of duct tape and textile edges, the performance rates derived from large experimental datasets of fractured materials, and the results of interlaboratory studies conducted utilizing the developed protocols.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the reliability and validity of physical fits in trace evidence materials. This presentation will also illustrate the development of systematic methods to assess the quality of a fit using quantitative metrics, the influence of various factors on edge morphology, and the performance of the method in both large populations and when utilized by practitioners in interlaboratory studies.

Duct tapes and textiles are commonly encountered at crime scenes and can be relevant evidence in a wide variety of cases. When these materials are fractured, a physical fit examination can demonstrate that the items were once joined together. While standard practices for conducting physical fit evaluations are still developing, recent studies have shown the potential value of physical fit exams in many materials. A critical aspect of enhancing these examinations is the development of systematic methodologies, identification of relevant comparison features, and testing of the method's validity with large populations of samples that simulate items received in casework. In addition, testing the methods through interlaboratory studies allows for refinement of the method and evaluation of factors that can improve the inter-examiner consistency in their decision and reporting procedures. This study developed modern and practical methods for physical fit examinations of duct tapes and textiles. The tape dataset encompassed more than 2,800 comparison pairs, consisting of samples collected from three different tape grades and incorporating two separation methods and stretching that simulates challenging case specimens. The textile dataset included more than 960 comparison pairs, using textiles of different compositions and construction while torn by hand or stabbing the fabric. All comparisons were completed blindly, with the participating analysts unaware of the pairs' true fit/non-fit ground truth before analysis. The systematic methods utilized defined comparison features relevant for each material and estimated an Edge Similarity Score (ESS) to quantify the similarity or dissimilarity between edges.

Interlaboratory studies were designed to validate the ESS methods, following training of the participants on the comparison, documentation, and reporting protocols. Each participant received a set containing seven comparison pairs in the form of physical samples (round robin) for tapes and digital samples for textiles.

Overall, the populational data from the two methods demonstrated high accuracy. The accuracy of the tape and textile sets ranged between 85% to 100%, depending on the set. While the tape sets had no false positives reported, ten false positive misclassifications were reported from the textile dataset (2% false positive rate), demonstrating the importance of assessing the suitability of a material to undergo physical fit examination, estimating the quality of a fit, and recognizing which factors can lead to errors or uncertainty on the fit examination. Several false negatives were reported for both material types, mainly influenced by the tape quality and textile composition and construction. For both materials, ESS scores below 20 provided support for a non-fit decision, while scores above 80 provided support for a fit decision. Depending on the material, recommendations are also provided for a threshold for inconclusive results.

In the interlaboratory studies, variation between examiners was within expected ranges. The auto-populated cells and color-coded templates designed for the study clearly indicated the examiners' decision process in each comparison area. At the same time, the recorded features helped clarify why the examiners reported fit or non-fit for each compared sample. The documentation and examination in the bin-by-bin approach favor objectivity and transparency. The bin justification leads the examiner to make data-driven decisions independent of the previously compared bins.

Moreover, the estimation of a quantitative metric informs the examiner's opinion. When used by practitioners, the relatively high performance of the method demonstrated the value that a systematic documentation method and defined criteria provide when comparing samples for physical fit examinations. In addition, experience and improved training in the method were shown to enhance examiner performance. Overall, this study is anticipated to provide valuable tools for examining, interpreting, and reporting physical fits of trace materials.

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**Physical Fits; Duct Tape; Textiles**

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**B20 A Case Report: Identification of AP-238 and 2-Fluorodeschloroketamine in Internet-Available Powder Samples**

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**Learning Objective:** After attending this presentation, attendees will understand the importance of the analysis of unknown powders sold on the internet.

**Impact Statement:** This presentation will impact the analysis of new synthetic opioids and the risks associated with internet purchases of unknown samples.

Bucinnazine (AP-237) is a synthetic opioid recently discovered in seized heroin samples in the United States and in Europe. It was first synthesized in the late 1960s and has been used for the treatment of cancer-associated chronic pain in China for many years. Bucinnazine is one of the most potent compounds among the series of piperazines, which also include other relevant compounds, such as MT-45, AD-1211, and 2-methyl-AP-237, a methylated derivative of bucinnazine. Bucinnazine is considered a  $\mu$ -selective opioid, binding primarily to the  $\mu$ -opioid receptor. At present, bucinnazine is not scheduled in the United States, as it is not a therapeutic choice for the treatment of pain. Nevertheless, with the advent of cryptocurrency and the easy access to substances on the Darknet, bucinnazine became broadly available in the United States, which presents a public health threat.<sup>1</sup> Powdered samples suspected to contain bucinnazine were obtained and analyzed using DART®-MS and a non-targeted GC/MS analysis approach. Our hypothesis is that the easy order process increases the toxicity risks associated with the use of unknown substances. The aim of this study was to identify the compounds present in two powder samples suspected to contain bucinnazine.

The two powder samples were dissolved in methanol to a concentration of approximately 1mg/mL and then were diluted in methanol to a concentration of 20 $\mu$ g/mL. The samples were then screened on a Jeol JMS-T100LC IonSense® DART® with an AccuTOF™ MS. The samples were wanded in front of the sample injection port five times each. The temperature was 300°C and the DART®-TOF/MS was operated in positive mode. Samples were additionally screened on a Shimadzu® GC/MS QP-2020 instrument. The column used was an Agilent® HP-5MS 30m x 0.250mm x 0.250 $\mu$ m column. The injection temperature was 250°C, the column oven temperature was 70°C for 1 minute, was then ramped to 300°C over 15 minutes, then held for 10.0 minutes. The total run time of the method was 26.33 minutes. The ion source temperature was 250°C. The GC was in splitless mode, and the MS was in SCAN mode with a range of 40.00m/z to 550.00m/z. The screening of the two unknown powders indicated that neither sample contained bucinnazine. The first unknown powder was found to contain 2-fluorodeschloroketamine as its primary constituent, and the second unknown powder was found to contain AP-238 as its primary constituent. The quantification of both powders showed that powder 1 presented 78.0% of 2-fluorodeschloroketamine, and powder 2 presented 88.9% of AP-238.

2-fluoro-deschloroketamine (2F-DCK) is an NPS that has been identified in approximately 20 cases between January and July 2019. In most of these cases, 2F-DCK was identified alongside other ketamine-type drugs. AP-238 is a structural isomer of 2-methyl-AP-237 and an analog of bucinnazine. Little data is available on the health risks of AP-237 or 2F-DCK. They are not currently scheduled in the United States but are advertised as bucinnazine. This data reinforces the evidence that substances sold as one compound frequently contain other compounds, not on the label, which are potentially riskier. The presence of psychoactive substances that are not labeled on the product or not properly described to be present is a public health concern and a significant risk to the consumer.

**Reference:**

<sup>1</sup>. Resnik, K., Brandão, P., Alves, E.A. DARK Classics in Chemical Neuroscience: Bucinnazine. *ACS Chem. Neurosci.* 2021, 12, 3527-3534.

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**NSO; Unknown Powder; Bucinnazine**

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**B21 The Evaluation of Three Derivatization Reagents for the Identification of Eight Aminoindanes by Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Objective:** This presentation will evaluate the effectiveness of three derivatization reagents for improving the gas chromatography and electron ionization mass spectral characteristics of eight aminoindanes.

**Impact Statement:** This presentation will impact the forensic science community by reporting the gas chromatographic retention data and mass spectral characteristics for eight aminoindanes on two different stationary phases using three different derivatizing reagents, N-methyl-bis(trifluoroacetamide) (MBTFA), heptafluorobutyric anhydride (HFBA), and ethyl chloroformate (ECF).

The goal of this project was to evaluate the effectiveness of three different derivatization reagents on their ability to improve the mass spectral properties and the gas chromatographic separation on two different stationary phases of eight aminoindanes.

Aminoindanes are a class of Novel Psychoactive Substances (NPSs) that have become more prevalent over the past decade, and the base structure, 2-aminoindane, can be chemically altered to produce a variety of aminoindane analogs. Structural differences among aminoindanes are very slight, which can challenge crime laboratories in accurately identifying and differentiating these compounds. Minimal analytical data has been reported on these compounds, and limited standards are available for forensic laboratories, which presents a challenge to analysts who lack the necessary information needed for identification and differentiation of aminoindanes. Gas Chromatography/Mass Spectrometry (GC/MS) is often utilized for identifying controlled substances and is well regarded for its ability to separate mixtures. However, GC/MS does have some drawbacks, especially when trying to isolate ring-isomeric compounds. Separation of the aminoindane analogs is difficult due to their very similar structures. Mass spectra via Electron Ionization (EI) are often indistinguishable for these analytes as well. Derivatization techniques are often applied to GC/MS methods for their ability to enhance chromatographic results, providing more selective analysis in seized-drug identification. This study assessed three derivatization reagents, MBTFA, HFBA, and ECF, for the analysis of eight aminoindanes by GC/MS using two different gas chromatographic stationary phases, 5% diphenylpolysiloxane/95% dimethylpolysiloxane (Rxi®-5Sil MS) and 100% dimethylpolysiloxane (Rxi®-1Sil MS). Both systems had the same column dimensions (30m x 0.25mm x 0.25µm). The aminoindanes investigated in this study were 2-aminoindane (2-AI), N-methyl-2-aminoindane (N-methyl-2-AI), rasagiline, 5-methoxy-2-aminoindane (MEAI), 5-methoxy-6-methyl-2-aminoindane (MMAI), 5,6-methylenedioxy-2-aminoindane (MDAI), 4,5-methylenedioxy-2-aminoindane (4,5-MDAI), and 5-iodio-2-aminoindane (5-IAI).

Separation of 4,5-MDAI and MDAI within a mixture of eight total aminoindanes was achieved for the MBTFA, HFBA, and ECF derivatizations on the Rxi®-5Sil MS and Rxi®-1Sil MS columns. Prior to derivatization, differentiation of these isomers was unattainable on either column. Improved chromatography was observed after derivatization, including reduced peak tailing and increased abundance for all the compounds. The most notable finding was separation of 4,5-MDAI and MDAI for all three derivatizations on both columns, which yielded two separated peaks with individual retention times. Prior to derivatization, these two isomers eluted together as one peak and were unable to be distinguished on either column. Mass spectra for the aminoindane-TFA, -HFBA, and -ECF derivatives contained individualizing fragment ions that allowed for further characterization of the aminoindanes. Unfortunately, this excluded 4,5-MDAI and MDAI, since they shared the same characteristic ions for each derivatization, which means they were only distinguishable in this study by gas chromatographic retention time. All three derivatization methods were able to differentiate between the eight aminoindanes, and the data reported provides forensic science laboratories with options for analyzing these compounds.

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**Forensic Science; Aminoindanes; GC/MS**



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**B22 Investigating the Effect of Substitution Location on Fentanyl Analog Identification for Methyl-Substituted Fentanyl Analogs Using Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS)**

*Alexis Pollard, BS\*, Sam Houston State University, Huntsville, TX; J. Tyler Davidson, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will have learned about the effect of substitution location on the identification of methyl-substituted fentanyl analogs using GC/EI/MS. The audience will learn about characteristic differences in EI fragmentation, differing outcomes between the NIST similarity search and NIST hybrid similarity search, and how the location of substitution directly impacts the ability to identify novel analogs.

**Impact Statement:** This presentation will impact the forensic science community by enhancing our understanding about the effect of substitution location on the identification of fentanyl analogs using GC/EI/MS. Furthering our understanding about EI fragmentation patterns and the ability to identify fentanyl analogs using mass spectral search algorithms is directly applicable to the identification of novel fentanyl analogs by forensic practitioners.

GC/EI/MS is one of the most often employed analytical techniques for the identification of controlled substances. The combination of chromatographic separation and the ability to generate structural information provides the enhanced selectivity required for a Category A or confirmatory analysis technique. However, because EI is a hard ionization source, the EI mass spectra of many seized drugs, such as fentanyl analogs, does not contain the presence of molecular ions. Instead, seized drug analysts must rely on the formation of fragment ions to differentiate between closely related fentanyl analogs. Given that fentanyl analogs typically contain only minor structural modifications, such as a single substitution of a methyl group at various locations throughout the core molecule, understanding the effect that substitution location has on fentanyl analog fragmentation and identification using mass spectral search algorithms is important for the identification of novel fentanyl analogs. The central hypothesis of this research is that each methyl-substituted fentanyl analog has a unique EI fragmentation pattern that enables successful identification based on characteristic ion ratios. Additionally, it is hypothesized that the location of the methyl-substitution will impact not only the EI fragmentation pattern, but also the ability to identify the analog based on the NIST Similarity Search and Hybrid Similarity Search algorithms.

An Agilent® GC/EI/MS was used to analyze a series of isobaric methyl-substituted fentanyl analogs that differed only in the location of the methyl-substitution. The fentanyl analogs included butyryl fentanyl, isobutyryl fentanyl,  $\alpha$ -methyl fentanyl,  $\beta$ -methyl fentanyl, ortho-methyl fentanyl, trans-3-methyl fentanyl, 4-methyl fentanyl, and 4'-methyl fentanyl. These analogs include at least a single compound for each common location of substitution to the core fentanyl structure, which is the aniline ring, the amide moiety, the piperidine ring, the alkyl chain, and the monocyclic substituent, in this case a phenyl group. All standards were prepared at a concentration of 10ppm and analyzed to assess the effect of the substitution location on the resulting EI fragmentation. A mixture of the methyl-substituted fentanyl analogs was also prepared and analyzed to determine the ability to achieve chromatographic separation using a standard HP-5MS column.

The isobaric methyl-substituted fentanyl analogs analyzed in this study are constitutional isomers, meaning that they have the same molecular formulas, but different connectivity of their atoms. Specifically, the location of the methyl-substitution to the core fentanyl structure is the only difference between the analyzed series of fentanyl analogs. However, the location of substitution plays a key role in the differentiation and identification of fentanyl analogs based on the formation of unique EI fragmentation and characteristic ion ratios. This study explored the use of unique EI fragmentation, characteristic ion ratios, and the NIST Similarity Search and Hybrid Similarity Search algorithms to enhance our understanding about the effect of substitution location on the identification of novel fentanyl analogs.

The results indicate that the combination of unique fragmentation patterns and characteristic ion ratios, formed through shifts in  $m/z$  values due to the location of substitution and conserved primary  $\alpha$ -cleavages and secondary rearrangement reactions, enable the differentiation and identification of the isobaric methyl-substituted fentanyl analogs. In terms of mass spectral comparison algorithms, the NIST Hybrid Similarity Search was more equipped to handle analogs absent from the NIST library than the Similarity Search algorithm. Finally, although baseline resolution was not achieved for the chromatographic method, the majority of methyl-substituted fentanyl analogs were able to be separated enough to acquire a pure EI mass spectrum from the peak apex.

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**Fentanyl Analogs; EI Fragmentation; Hybrid Similarity Search**

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**B23 Multiple Micro-Abrasions on the Shooter's Hand and the Recharge of Ammunition at Home**

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**Learning Objective:** After attending this presentation, attendees will understand some principles of ballistics, which are fundamental for the forensic pathologist in order to provide a scientifically valid reconstruction of crimes in which firearms have been used.

**Impact Statement:** This presentation will impact the forensic scientific community by serving as a key aspect of ballistic investigations as it can explain the retrograde tattoo phenomenon.

In cases of using firearms, finding a single tattoo on the skin provides some information to the forensic pathologist, mainly regarding the shooting distance. The tattoo is determined by the insertion into the dermis of the largest unburnt granules with greater kinetic energy; unlike smoking, it cannot be removed by skin determination.

The presence of the tattoo alone, therefore, allows us to say, in most cases, that a close-up shot was fired. It is also known that for shots fired on contact, especially at flat bones such as those of the skull, the flame and the gases of the explosion pass under the skin, causing entrance holes of a particular shape (like a star) and with torn edges. In these cases, it is very rare to observe deposits of shot peening residues on the surrounding skin, as gas expansion occurs in the deep layers and therefore the penetration of the unburned granules.

This report will present a case study where the shot was fired in close contact. It was easy to distinguish the entry hole and characterize it as a shot fired in contact both for the typical characteristics of the hole itself and for the finding of the imprint of the spring guide: it was a high-powered 9-caliber semi-automatic pistol.

Smoke deposits were observed around the skin hole, but the dorsal surface of the suicide's right hand showed numerous punctate abrasions, similar to a tattoo. There were no specks of unburned dust under the skin, but the lesions looked like superficial bruises or burns. The presence of the tattoo in a case in which the shot was fired on contact, therefore, posed serious difficulties in formulating a differential diagnosis between suicide and homicide with simulated suicide.

To provide a scientific reconstruction, the judge was asked to be able to examine the ballistic findings: the study of the unexploded cartridges still present in the magazine of the weapon allowed the solving of the enigma and affirmed that the same ammunition was recharged at home by the suicide with almost double the amount of gunpowder normally used. In addition, the gunpowder used was also very progressive, so it tended to burn longer than normal.

The shooting tests were carried out on targets at different distances: this study, therefore, explained the presence of the particular effect on the hand of the suicide. Due to the massive amount of gunpowder, there was a violent expulsion of gas and unburnt gases, even retrograde, starting from the wound that captured the hand of the suicidal subject. The study of the ammunition and the firing tests carried out made it possible to formulate the diagnosis of suicide.

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**Tattoo; Ballistics; Home Reloading of Ammunition**

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**B24 The Sampling of Materials Using Laser Ablation and Electrostatic Capture, and the Analysis of Captured Particles Using Thermal Desorption Electrospray Ionization (TD-ESI) and an Ion Trap Mass Spectrometer**

*Brendan Minick\*, The University of Tampa, Tampa, FL; Kenyon Evans-Nguyen, PhD, University of Tampa, Tampa, FL*

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**Learning Objective:** After attending this presentation, attendees will better understand how laser ablation coupled with TD-ESI/MS can be used for analysis of insoluble materials and adapted for use in the field.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a new, non-destructive, fieldable technique for sampling a variety of trace evidence. Drugs, metals, and inorganic explosives are some examples of samples that can be collected in the field, captured electrostatically, and stored for later analysis via mass spectrometer, SEM-EDS, or other means. TD-ESI is especially useful for MS analysis once the sample is captured on stainless steel mesh for storage.

Analysis of refractory or metallic materials is challenging due to their inability to be easily dissolved in solution. Elemental analysis using mass spectrometry or SEM-EDS can be useful, but sampling objects composed of these materials is difficult, especially for fieldable analysis. Laser ablation is a promising method for freeing material from the surface of an object and has been successfully used in forensic analysis with Laser Ablation Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS). However, LA-ICP/MS is not amenable to field use at the scene. We are working toward using a fieldable laser to ablate material while capturing the material removed from the surface for later off-line MS analysis. Ablated particles are captured electrostatically, using a children's toy (Fun Fly Stick) as a safe and inexpensive high-voltage source. This method allows for sampling of materials in a relatively non-damaging way, relies on very small sample quantities, and can be used in the field for a variety of substances. Once captured, samples can be transferred to an SEM stub or analyzed directly via mass spectrometry. In particular, samples can be thermally desorbed from the site of capture (e.g., metal mesh), ionized using electrospray ionization, and analyzed using an ion trap mass spectrometer. This presentation will discuss this specific methodology for sample identification and its application to fieldable analysis.

Samples were ablated using both a Minilite® Q-switched Nd:YAG laser and a lower-power miniature Nd:YAG laser. Ablated particles were captured on IonSense® OpenSpot® stainless steel mesh cards coated in 2:1:1 glycerol:water:methanol solution. The Fun Fly Stick toy provided the high voltage (~27 kV, minimal current) used for capture. The mesh cards were placed in a 3D-printed thermal desorption apparatus and heated using an oxyhydrogen torch. Desorbed analyte was pulled into an electrospray ionization source interfaced to an ion trap mass spectrometer (Thermo™ LTQ XL™) using vacuum to pull the neutrals into the ion source. Samples were analyzed using this coupled thermal-desorption electrospray (TD-ESI) ion source.

In initial experiments, metallic surfaces were coated with organic samples to test the feasibility of capturing ablated metals and organic solids. Cocaine on metallic surfaces was successfully analyzed by electrostatic capture of the ablation plume, followed by detection with TD-ESI/MS. Current work is focused on the detection of a broad range of inorganic, organic, and elemental analytes to facilitate analysis of a broad range of trace evidence. More specifically, inorganic oxidizers and metallic container components used in improvised explosives as well as the inorganic and organic components of gunshot residue are being tested with the technique. This methodology could facilitate a portable, non-destructive sampling method for on-site collection of trace evidence materials with ready coupling to mass spectrometric analysis.

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**Laser Ablation; Field Sampling; Thermal Desorption**

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**B25 Spectral Trends in Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) Data From the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Library and Literature**

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**Learning Objective:** After attending this presentation, attendees will know how to recognize specific mass spectral trends and characteristics and identify probable sources of diagnostic m/z ions.

**Impact Statement:** This presentation will impact the forensic science community by offering in-depth discussions on observations gathered from GC/EI/MS analysis as well as presenting a more concrete starting point for class identifications.

Rapid identification of drugs of abuse remains a critical challenge in forensic drug chemistry laboratories. Current analytical protocols are well-designed for confirmation of known substances yet struggle when new compounds are encountered. Such compounds, referred to as Novel Psychoactive Substances (NPS), are becoming prevalent because they can produce equivalent psychoactive responses by targeting similar receptors and reaction sites within the body, elude legal definitions of a controlled substance, and evade detection due to identification limitations.

Traditional protocols and techniques found in forensic laboratories use instruments like GC/EI/MS to attempt to classify compounds using data found in curated mass spectral libraries. This effort involves comparing both unknown and known spectra to then hypothesize a compound's identification. With NPSs, however, subtle alterations to core chemical structures can result in nearly indistinguishable spectra, leading to misidentifications. Therefore, understanding how these species break down can help elucidate a class of substances while unearthing other novel characteristics found in a mass spectrum. Therefore, the subject of this presentation revolves around the compilation of literature reports while performing simple exploratory analyses on evaluated GC/EI/MS data to investigate mass spectral trends on the most reported illicit substance classes. To complete this, class identifications obtained from the Cayman Chemical product catalog were matched and appended to entries found in version 3.10 of the SWGDRUG GC/MS library using a custom R script. The generation of this new database resulted in the classification of over 1,500 entries across 7 broad classes and 15 subclasses. These broad classes of substances include barbiturates, benzodiazepines, anabolic steroids, cannabinoids, opioids, stimulants, and hallucinogens. The subclasses include groups such as fentanyls, nitazenes, synthetic cannabinoids, amphetamines, cathinones, and tryptamines.

This presentation captures common core structure substitution and fragmentation pathways while highlighting tables depicting top reoccurring ions characterized by various relative abundance categories such as Base Peak, High, Medium, and Low. To capture other underlying trends, the top reoccurring neutral losses were also investigated to highlight fragmentation commonalities not directly observable in a mass spectrum. The summaries of mass spectral trends shown in this presentation can help an analyst classify new illicit compounds by the patterns observed. Practical examples of mass spectra of varying degrees of difficulties such as feature-poor and rich will also be displayed. The knowledge gained from this information can be swiftly employed in forensic laboratories as a quick reference guide for GC/EI/MS analysis further aiding in characterizations and potential identifications.

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**Novel Psychoactive Substances; Gas Chromatography/Electron Ionization/Mass Spectrometry; Mass Spectral Trends**

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**B26 Statistical Discrimination Methods for Forensic Source Interpretation: The Application to Micromorphometric Feature Measurement of Aluminum Powders Used in Explosives**

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**Learning Objective:** Attendees will better understand two statistical methods currently being tested for application to the interpretation and discrimination of aluminum powder particles, commonly associated with Improvised Explosive Devices (IEDs). Further, attendees will gain a deeper understanding of how automated imaging with measurement of morphological features may aid in forensic science investigations.

**Impact Statement:** This presentation will impact the forensic science community by providing an objective, statistically sound method for the discrimination of source of aluminum powder evidence in IEDs, providing crucial information for forensic science investigations.

Aluminum (Al) powder is often used as a fuel in explosive devices; therefore, individuals attempting to make illegal IEDs often obtain it from legitimate commercial products (like sport shooting targets) or make it themselves using readily available Al starting materials (like Al foil). The characterization and differentiation between sources of Al powder for additional investigative and intelligence value has become increasingly important. Research previously presented at AAFS modeled the distributions of micromorphometric features of Al powder particles within a subsample to support Al source discrimination. Since then, additional powder samples from a variety of different source types have been obtained and analyzed, providing an even larger, more comprehensive dataset for applying the two statistical methods under consideration for interpretation and discrimination of source.

The final dataset consists of 17 micromorphometric parameters measured on Al powder particles from 200 randomly selected fields of view for three aliquots from each of seven subsamples from each of >150 Al powder sources.<sup>1</sup> Here, we compare two different statistical techniques: one using Linear Discriminant Analysis (LDA), and the other using a modification to the method used in ASTM E2927-16e1 and E2330-19.<sup>2,3</sup> The LDA method uses the summary statistics for all 17 micromorphometric parameters in each subsample to compare a questioned subsample to subsamples from many different known sources.<sup>1</sup> This method is designed to recommend the most likely source that gave rise to the trace and results in an Al source classification for each questioned sample, effectively eliminating all other sources besides the “best” one. Alternatively, our modification to the ASTM method compares the 17 micromorphometric parameters for a questioned subsample to those of several known sources using an interval-based match criterion to associate or exclude each of the known sources as the actual source of a trace. Although the outcomes of these two statistical methods are fundamentally different, their performance with respect to the closed-set identification of source problem is compared. Additionally, we explore and discuss the limitation of the ASTM method of only resulting in binary decisions rather than quantifying the strength of the evidence. Therefore, the modified ASTM method will be adapted to provide a vector of scores in lieu of the binary decision as the first step toward a score-based likelihood ratio for interpreting Al powder micromorphometric measurement data.

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- <sup>2.</sup> *E2927-16e1 Standard Test Method for the Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons*, ASTM International, 2022.
- <sup>3.</sup> *E2330-19 Standard Test Method for Determination of Concentrations of Elements in Glass Samples Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for Forensic Comparisons*, ASTM International, 2022.

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**Statistics; Explosives; Image Analysis**

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**B27 A Survey of Mounting Media Used for Hair and Fiber Microscopy**

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**Learning Objective:** After attending this presentation, attendees will understand various mounting media used by microscopists for hair and fiber examination.

**Impact Statement:** This presentation will impact the forensic science community by providing forensic trace evidence examiners with an updated and diverse pool of commercially available media from which to consider alternatives.

Studies seeking alternative mounting media to improve certain aspects of microscopical examination of biological and non-biological samples have been conducted.<sup>1-3</sup> These studies were conducted because microscopists sought improvement in protocols and to address safety and environmental practices. However, these studies have largely been limited to a particular mounting medium and within one laboratory. Because of the limited scope of such studies, inquiries across laboratories could be made and are thus highly desirable. Furthermore, since the majority of previous studies were done well over two decades ago, discovering the newer mounting media that other laboratories may be using gives examiners an updated pool of commercially available media for future consideration. Because of the limited scope of these studies, a larger-scale inquiry across laboratories was undertaken.

A questionnaire developed in conjunction with FBI laboratory hair and fiber forensic examiners consisted of seven free-response questions, which inquired about specific information on mounting media used, challenges with the media, and preparation storage by the respondent. The questionnaire was made available to participants using an online survey tool between October 2017 and November 2017. Participant recruitment involved working with the President of the American Society of Trace Evidence Examiners to reach out to the organization's members. Completion of the questionnaire was made available via a private link.

The survey data revealed there are multiple types of mounting media used in both the United States and abroad. Permount™ was the most popular medium used in the United States, and no other medium usage stood out abroad. Regardless of mounting media type, the vast majority of analyses in a given facility were for both hair and fiber. The most common issues reported by respondents were those affecting mounting medium integrity (e.g., crystallization and yellowing of the mounting media). The outcome of the study could be regarded as a large-scale survey of microscopists concerning the mounting media types they use to prepare hair and/or fiber samples for forensic examination.

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3. Ravikumar, S., Surekha, R., and Thavarajah, R. (2014). Mounting media: An overview. *Journal of Dr. NTR University of Health Sciences* 3(5) 1-8.

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**Fiber Analysis; Microspectrophotometry; Mounting Medium**



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**B28    The Application of Surface-Enhanced Raman Spectroscopy (SERS) for the Detection of Synthetic Cathinones**

*Mario Vendrell-Dones, MS\*, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL*

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**Learning Objective:** Attendees will understand the principles of SERS, how nanomaterials, specifically how colloidal solutions can be applied for forensic science to detect novel psychoactive substances such as synthetic cathinones.

**Impact Statement:** It is our goal to develop SERS-based methods that positively impact society by aiding in the identification and trace detection of those substances that put society at a health risk, in this case focusing on synthetic cathinone analogs.

In the distant past, indigenous people around the horn of Africa began chewing leaves of the khat plant for relief from daily toil. This habit resulted in the discovery of naturally occurring alkaloids with potential medical use known as cathinones and led to the development of countless analogs. Research into their effects is lacking due to the high pace at which new analogs become available, resulting in problems for policing and health agencies. Furthermore, clandestine laboratories synthesize them in numerous ways, with little concern for purity or concentration, increasing the potential for toxic overdose. Therefore, there is a pressing need for a screening method that can assist authorities in detecting cathinone analogs in a rapid, reliable, sensitive, and inexpensive way. Due to its molecular specificity and sensitivity, SERS delivers an ideal and flexible platform that provides Raman scattering enhancements for detection in both seized and biological fluid samples.

This work describes the fabrication of Ag colloidal systems for use as SERS-enhancing substrates to detect the model cathinones: methedrone, 4-chloro- $\alpha$ -pyrrolidinovalerophenone, 3,4-methylenedioxy-5-methylethcathinone, 4-methylethcathinone, and NN-dimethylmethcathinone in liquid form. By varying the functional groups attached to the core synthetic cathinone, we can demonstrate their effects on the obtained signal. Initial work involved Density Functional Theory (DFT) calculations at the B3LYP/6-311G\*\* level to predict Raman frequencies of the studied compounds. Normal Raman measurements on dried solid residues of synthetic cathinones standards were next examined, and the resulting scaled spectra were used to ensure concordance of the DFT-predicted frequencies with experimental values. Subsequent work focused on the synthesis and optimization of Ag nanoparticles, followed by the selection of the aggregating agents such as MgCl<sub>2</sub> and LiCl that can produce high-density hot spots on the nanometallic surface. The SERS signal intensity depends on the proximity of the analyte to the surface and the Local Surface Plasmon Resonances (LSPR), the quality of the analyte adsorption, and its interaction with the surface affects the overall signal enhancement. Our results indicate that higher signal enhancements and lower detection limits are achieved with basic pH conditions. SERS experiments were conducted using a custom quartz well plate.

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**Synthetic Cathinones; Density Functional Theory (DFT); Surface-Enhanced Raman Spectroscopy (SERS)**

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**B29 The Unique Capability of Direct Analysis in Real-Time Mass Spectrometry (DART®-MS) for the Detection of Less Volatile Components in Ignitable Liquids**

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**Learning Objective:** After attending this presentation, attendees will understand the unique capability of DART®-MS for the detection of Ignitable Liquids (IL).

**Impact Statement:** This presentation would impact the scientific community by showing that DART®-MS spectral profiles of the ILs would be reliable for identifying IL.

The detection of IL is critical to the arson investigation process, which may potentially identify the cause of the fire and the ILs used to initiate the fire. The most commonly used technique to analyze IL is GC/MS; however, it has a major limitation of analyzing only the volatile components in IL. The non-volatile or less volatile components in IL are likely to be contained in the fire debris and hence could yield corroborating evidence on the use of specific ILs in the investigation. DART®-MS is an emerging analytical technique in the forensic community and has shown promising capability in drug analysis. But its potential in other forensic fields, such as IL analysis, has not been fully explored. In this study, the ILs such as paint thinner were analyzed by DART®-MS, and the results were compared with those from the traditional GC/MS method.

In the present study, paint thinner was weathered at three different temperatures, 30°C, 90°C, and 210°C, to different degrees of weathering at 30–99% mass reduction of IL prior to being analyzed. To analyze the IL samples on DART®-MS, an automated sample introduction apparatus with Linear Rail Enclosure that holds consumable Quickstrip™ sample cards were used. A 5µL sample volume was spotted on the Quickstrip™ card after diluting the sample in chloroform. For the GC/MS analysis, 20µL of the sample was added to 1mL of chloroform followed by an injection of 1µL with a split ratio of 1:50.

For the DART®-MS data, a unique mass spectral pattern with repeating units of 44 Da was found in the paint thinner samples. The results indicate the presence of the polymeric compounds with ethylene oxide unit (O-CH<sub>2</sub>-CH<sub>2</sub>). Ions with m/z 89.1, 133.1, 177.11, and 221.1 were observed in the DART®-MS spectra, which matched with the product ion patterns of Polyethylene Glycol (PEG)-derived products in the previous publications. A PEG 200 standard solution was analyzed by DART®-MS under the same conditions, and the spectrum was closely matched with the mass spectra from paint thinner samples. In addition, the mass spectral profiles were consistent among all the weathering samples prepared. Our data suggest a mix of glycol ethers or poly-ethylene glycols were present in the paint thinner products, and they could yield reliable marker compounds for the detection of paint thinners. The glycol ethers were not readily identified in the total ion chromatogram of GC/MS.

In comparison to the DART®-MS data, the GC/MS profiles changed more significantly among weathered IL samples because the weathering process alters the relative quantities of the IL. The DART®-MS data of ILs provided less variable profiles and, therefore, may not be suitable for predicting the weathering percentage of IL. However, the capability of DART®-MS in detecting the less volatile IL constituents such as glycol ethers and PEG additives imparts its unique ability to discriminate among different types of ILs.

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**Ignitable Liquids; Paint Thinner; DART®-MS**

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**B30 The Volatile Organic Compounds (VOCs) of Training Aids Used for Human Remains Detection Canines: A Survey on the Impact of Storage Conditions on Odor Signature**

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**Learning Objective:** Attendees will learn about the storage conditions for human remains detection canine training aids. A survey was conducted, asking handlers from around the world how they stored their training aids. This is important to consider because there are no requirements for how handlers should store their aids and previous studies have shown that some conditions can affect the VOCs that produce the odor profile.

**Impact Statement:** The results from the survey will show some major inconsistencies between storage conditions for human remains training aids. Some of the conditions asked about in the survey had a wide spread of answers. In addition, some of the conditions that have been shown in previous studies to affect the odor profile are not considered by handlers. This showcases a need to further study this topic as well as potentially develop procedural guidelines for storage conditions.

This presentation aims to bring awareness to attendees on how Human Remains Detection (HRD) training aids are stored by handlers around the world, as well as highlight inconsistencies among them. This showcases a possible need to provide recommended storage conditions for training aids. This is also important for future research on how all these conditions may affect the volatile VOCs as some conditions have been shown to affect them in other research.

Detection canines are often used to locate a variety of different odors pertinent to forensic investigation, including the detection of human remains (HR). The headspace from HR consists of a complex mixture of VOCs, and there are a variety of factors that could affect the odor signature produced. Because of the complexity, it has yet to be determined which specific compounds the canines use for detection. When it comes to HRD training aids, the storage conditions may affect the aid and therefore modify HR VOC profiles. It has been shown that certain storage conditions, such as temperature and substrate material, can impact the VOCs produced from blood training aids.<sup>1,2</sup> For example, placing the blood on cotton (rather than a non-porous surface), freezing blood (compared to room temperature), and aged blood (compared to fresh blood) have all been shown to impact the VOCs produced.<sup>1-3</sup> Thus, it is important to consider the conditions in which training aids are stored so as to not impact the odor.

It is hypothesized that storage conditions such as container type, humidity, temperature, lighting, and age will affect the VOCs produced over time and that these impacts may not be fully considered by handlers. To bring awareness and to assess the conditions handlers store their aids, a survey relevant to HR training aid storage was conducted. This survey included questions on the types of aids and storage conditions. The survey received a total of 180 responses from predominantly North America and some parts of Europe, as well as several from Asia. This allowed for the collection of data on what the most common storage conditions and types of training aids were among handlers, as well as whether there was a sense of uniformity related to these conditions.

The survey indicated that many diverse types of aids are used by HRD canine handlers, including dry bone, tissue, body parts, decomposition fluid, and dried blood as the top five. For container type, glass is commonly reported, but there was a spread of other containers used by handlers. Most handlers reported that they do not keep track of the humidity when storing their aids, and if they do, it was not consistent among them. When looking at the difference in temperatures, most handlers tended to freeze their aids, and fewer refrigerated or left them at room temperature. As the aids are most commonly stored in the freezer, most handlers store aids in no light and a small fraction in other lighting. Last, many handlers retain old training aids (two years or older) and may never dispose of their aids. The disparity between reported storage conditions from this survey and the limited available research displays the need to test these storage conditions in future research to confirm whether handlers are storing aids in conditions that could affect the VOCs for canine training.

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**Human Remains; Canine Detection; Training Aids**

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**B31 An Assessment of Field-Portable Instrumentation for Pre-Blast Explosives Detection**

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**Learning Objective:** After attending this presentation, attendees will better understand the capabilities of portable Raman spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS) instruments relative to their traditional benchtop counterparts to detect and identify precursors for Homemade Explosives (HMEs).

**Impact Statement:** This presentation will impact the forensic science community by providing empirical data for the effectiveness of field-portable instrumentation relative to benchtop instrumentation for pre-blast explosives detection.

Commercially available field-portable instrumentation has become increasingly prevalent for the presumptive identification of compounds at scenes by law enforcement personnel, including explosives. Tools that can rapidly and reliably detect explosives and their precursors are important for the generation of investigative leads. Given the danger that HME precursors present to investigators, high-quality screening of evidence in the field can improve investigator safety. This study was designed to provide a systematic evaluation and comparison of field-portable and benchtop instrumentation to provide a fundamental performance assessment of field-portable instrumentation for the pre-blast detection of commonly encountered HME precursors using Raman spectroscopy and GC/MS instrumentation.

The explosives analyzed in this study were selected with the aid of the Montgomery County Fire Marshal's Office (MCFMO) and the Bureau of Alcohol, Tobacco, and Firearms (ATF) based on compounds commonly encountered in their current casework and including HME precursors, such as smokeless powder, nitromethane, and Ammonium Nitrate and Fuel Oil (ANFO). These compounds were analyzed using two field-portable Raman spectrometers, one GC/MS portable instrument, and their benchtop counterparts. Method development for the benchtop Raman microscope involved optimizing analytical conditions for each compound. In comparison, the two field-portable Raman instruments required only minimal optimization. The portable and benchtop GC/MS instrumentation were optimized to achieve the necessary chromatographic separation and limits of detection.

Spectral data was acquired from the HME precursors using the benchtop Raman microscope with a 785nm laser wavelength and the two portable instruments, which used a 785nm laser wavelength and 1,064nm wavelength, respectively. A major advantage of both portable Raman instruments is the inclusion of spectral libraries, which provide easy identification without the need for spectral comparisons, assuming the compound is present within the library. In comparison, the benchtop Raman microscope provided higher sensitivity and resolution due to the ability to adjust the microscope and spectrometer parameters.

Likewise, the benchtop GC/MS provided better chromatographic separation and enhanced limits of detection for the HME precursors analyzed in this study. Unlike the benchtop Raman instrumentation, the benchtop GC/MS has several libraries that can be used to assist with the identification of the HME precursors. In addition, the NIST EI library on the benchtop GC/MS instrument contained all HME precursors analyzed in this study, whereas the on-board library for the portable GC/MS did not contain an analyzed HME precursor at least once during this study. However, the portable GC/MS instrumentation has the advantage of being able to be operated in the field by less experienced personnel and provide more rapid investigative leads without the need to collect, transport, and store HME precursor evidence.

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**Pre-Blast Explosives; Raman Spectroscopy; GC/MS**

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**B32 Dating Crime Evidence With Microbes: The Ideal Forensic Clock?**

Josep De Alcaraz-Fossoul, PhD\*, University of New Haven, West Haven, CT; Yue Wang, PhD, Arizona State University, Glendale, AZ; Ruqian Liu, MS, Arizona State University, Phoenix, AZ; Michelle Mancenido, PhD, Arizona State University, Phoenix, AZ; Pamela Marshall, PhD, Arizona State University, Glendale, AZ; Celeste Núñez, BS, Arizona State University, Tucson, AZ; Jennifer Broatch, PhD, Arizona State University's West Campus, Glendale, AZ; Lara Ferry, PhD, Arizona State University, Glendale, AZ

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**Learning Objective:** After attending this presentation, attendees will understand the principle of utilizing the dynamic nature of microbial signatures in fingerprints to estimate time-since-deposition.

**Impact Statement:** This presentation will impact the forensic science community by providing a new approach to characterize activity-level propositions where microorganisms serve as biological clocks based on their relative abundance, diversity, and succession over time.

For decades, friction skin ridge patterns, including latent fingerprints, have been pivotal in relating (crime) evidence with potential suspects. This type of biometric has been traditionally considered for its physical pattern comparisons but, in recent years, the spectrum of potential applications has broadened.<sup>1</sup> Indeed, fingerprints are being regarded as possible means to estimate time-since-deposition, optically and chemically, and as excellent sources for human and microbial DNA.<sup>2,3</sup> With the advent of sensitive technologies for molecular analyses and their application in forensic science, it has become crucial to understand how microbial communities behave when left behind, especially in the plentiful pool of organic and inorganic nutrients that fingerprints provide.<sup>4</sup> This current research investigates how the varied population of microorganisms evolves on non-porous surfaces and how their dynamic and predictable nature might be exploited to assist in dating physical evidence.

In this initial study, 16S rRNA gene amplicons were sequenced from fresh, 7-day-, 14-day-, and 21-day-old fingerprints from three donors with pre- and post-washed hands. Samples were stored in the dark at room temperature on microscope glass slides and swabbed weekly. Time-dependent alterations of the microbial signatures were examined from the perspective of their relative abundance, diversity, and succession. In line with skin microbiome composition, the results revealed the dominance of four major phyla (90–95% of abundance) in freshly deposited fingerprints (i.e., Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes) with little difference across donors and time.<sup>5</sup> The washing treatment had little effect on their relative abundance, implying the resilience and persistence of major resident microbes over time. Interestingly, the presence of minor phyla (~5% of the population) appeared to be time-sensitive and donor-specific with an impact on their diversity and abundance. In fact, these “minor signatures” from fresh fingerprints changed completely by day 14 and their population structure diverged among donors and over time. Of importance, one phylum was recognized as a potential microbial clock: *Deinococcus-Thermus*, which fulfilled all the selection criteria set by the examiners to be considered as a member of the chronomicrobiome. These time-dependent variations should be further studied for dating physical evidence and must be considered when attempting to adopt skin microbiomes as a means for human identification because of their temporality once on a surface.

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**Microbiome; Aging; Fingerprint**

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**B33    The Development of a Self-Assembled Gold Nanoparticle Thin Film to Support Field Detection of Trace Chemicals**

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**Learning Objective:** The goal of this presentation is for attendees to gain a better understanding of the synthesis of gold nanoparticle (AuNP) thin films for use as Surface-Enhanced Raman Spectroscopy (SERS) substrates in the detection of trace chemicals.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating an approach to improve the sensitivity of hand-held Raman spectrometers for field testing using a novel SERS substrate.

With the use of many highly potent controlled substances, such as fentanyl, on the rise, it is more important than ever to be able to detect minute traces of various chemicals. In addition, field testing for chemical threats such as explosives is vital for law enforcement and security agencies. Raman spectroscopy is a valuable tool for identifying unknown substances, but it is not effective on trace or residual amounts of substances due to the relatively weak nature of Raman scattering. Consequently, SERS has been demonstrated to amplify the Raman signal, allowing for more sensitive detection of minute traces of chemicals. This technique uses a SERS substrate, often based on metallic nanoparticles, such as AuNPs, either in solution or fixed to a solid surface. In this work, an AuNPs self-assembled thin film was prepared on several different materials, such as filter paper, scotch tape, double-sided carbon tape, glass, and silicon chips, to serve as SERS substrates. The performance of AuNP thin films' SERS properties was evaluated using 1,2-Bis(4-Pyridyl)-Ethylene (BPE) as the reference material. The experimental procedures for synthesizing AuNPs, forming a thin film, transferring the film onto the desired substrate, and SERS detection using a hand-held Raman spectrometer will be discussed in this presentation.

AuNPs were synthesized in an aqueous solution using the protocol described by Martin et al.<sup>1</sup> The size of the nanoparticles was controlled using 300uL BH4-/OH- in the final synthesis step. First, the size and concentration of the nanoparticles were assessed using a Zetasizer and a UV-Vis spectrometer. Next, the AuNP self-assembled thin film was done using a liquid-liquid interface approach. Five mL of AuNP solution, acetone, and hexane were added to a vial and mixed well by shaking. The solution was then poured into a small petri dish, separating the immiscible liquids into layers. The AuNPs were allowed to aggregate undisturbed at the interface for several minutes before being transferred to the backing materials. The transfer was done by allowing the hexane to evaporate or removing the hexane with a pipette and then using tweezers to relocate the AuNPs films onto the backing materials. Finally, Scanning Electron Microscopy (SEM) was used to visualize the nanoparticle distribution of the self-assembled AuNP thin films.

After adding BPE to the AuNP films using scotch tape as the backing material, a Raman signal enhancement was observed at  $1,200\text{cm}^{-1}$ . When using a double-sided carbon tape as the backing material,  $1,200\text{cm}^{-1}$  and  $1,620\text{cm}^{-1}$  peaks attributed to the BPE were detected. These enhanced Raman scattering signals were absent from the backing materials. The detected Raman signals for BPE demonstrated that the AuNP self-assembled films exhibited SERS capabilities.

Raman-based techniques can produce dependable results utilized in forensic fieldwork. A novel SERS substrate, such as the one discussed in this research, would allow for the detection of trace chemicals. The AuNP self-assembled thin films provide potential SERS capabilities for trace chemical detection in forensic applications.

**Reference:**

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**Self-Assembled Gold Nanoparticles; Surface Enhanced Raman Spectroscopy; Trace Chemical Detection**



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**B34 An Analysis of Polymer-Coated Bullets Using Spectroscopic Methods**

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**Learning Objective:** During this presentation, attendees will learn how polymer-coated bullets differ from traditional non-jacketed bullets. Additionally, attendees will learn how the composition of the polymer-coated bullets were identified by analyzing melting point, solubility, and infrared spectroscopy data. Finally, this presentation will allow attendees to learn about alternative ways of identifying polymer residues when polymer-coated bullets are used.

**Impact Statement:** This presentation will impact the forensic science community by finding alternative ways to identify polymer residues when polymer-coated bullets are used. By finding alternative ways of identifying polymer residues, forensic scientists will be able to determine if polymer-coated bullets were used in the commission of a crime. If a gun recovered from a crime scene or impact holes contain traces of the polymer, these residues left behind can be analyzed and compared to known polymer coatings.

Polymer-coated bullets have gained in popularity in recent years, in part because their use can reduce the user's exposure to heavy metals in the ammunition. A new line of ammunition released by Federal® employs a synthetic jacket, in which the lead core is surrounded by a polymer. The synthetic jacket reduces the wear on the bore of the gun as well as prevents metal-on-metal contact between the bullet and the bore.<sup>1</sup> The composition of two polymer-coated bullets (American Eagle® Syntech [red polymer] and Syntech Defense 9mm Luger® [blue polymer]), were determined by studying the solubility, melting point, and molecular vibrations of the polymers. The results indicated that the blue and red polymers studied had very different solubilities, melting points, and molecular vibrations. Infrared spectroscopy revealed that the blue polymer had similar functional groups to dimethyl iso phthalate while the red polymer had similar functional groups to poly(ethylene glycol terephthalate). These results confirmed that both polymers have different compositions as evident by the vast differences in solubility, melting point, and their infrared signatures. In addition, since polymer-coated bullets do not retain individualizing minutiae like standard bullets do, the next steps in the research are to determine if traces of the polymers can be detected on impact marks as well as in the barrel of the gun. This will be done by swabbing and rinsing the barrel of the gun as well as analyzing targets of various compositions shot with these bullets. This is significant because it can help determine if polymer-coated bullets were used in the commission of a crime, if a gun recovered from a crime scene has traces of the polymer. If any residue is present, it can then be compared to known polymer coatings to verify that the residue did in fact come from a polymer-coated bullet.

**Reference:**

- <sup>1</sup>. Hutchcroft, Joel J. Federal Syntech Ammunition. *Shooting Times*, Feb. 2017, p. 58. Gale General OneFile, [https://link-gale-com.ez.lib.jjay.cuny.edu/apps/doc/A473692095/ITOF?u=cuny\\_johnjay&sid=ITOF&xid=e5ae68f3](https://link-gale-com.ez.lib.jjay.cuny.edu/apps/doc/A473692095/ITOF?u=cuny_johnjay&sid=ITOF&xid=e5ae68f3).

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**Polymer-Coated Bullets; Spectroscopy; Polymer Residue**

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**B35    Differentiation of Delta-9-Tetrahydrocannabinol (Delta-9-THC) and Cannabidiol (CBD) Using Silver-Ligand Ion Complexation and Electrospray Ionization Tandem Mass Spectrometry (ESI-MS/MS)**

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**Learning Objective:** After attending this presentation, attendees will have learned about the differentiation of  $\Delta$ 9-THC and CBD using silver (Ag)-ligand ion complexation for the formation of unique MS/MS product ion spectra. Attendees will learn how differences in binding affinities between Ag-ligand complexes and cannabinoids lead to the formation of unique product ions, as well as the enhanced selectivity, solubility, and stability compared to the use of simple silver salts.

**Impact Statement:** This presentation will impact the forensic science community by providing a method for the differentiation of  $\Delta$ 9-THC and CBD using Ag-ligand ion complexation to produce unique MS/MS fragmentation. Specifically, this method will enable the differentiation of  $\Delta$ 9-THC-rich and CBD-rich cannabis based on the presence of unique MS/MS fragmentation without the need for lengthy chromatographic separations.

The 2018 Farm Bill defines marijuana as *Cannabis sativa* L. or any derivative thereof that contains more than 0.3%  $\Delta$ 9-THC, with anything containing 0.3%  $\Delta$ 9-THC or less being considered hemp. Due to the classification of marijuana, or  $\Delta$ 9-THC-rich cannabis, as a Scheduled I controlled substance with no accepted medical or recreational use on a federal level, the differentiation between hemp and marijuana has become crucial within the seized drug community. CBD is the main cannabinoid present in hemp, which is often referred to as CBD-rich cannabis.  $\Delta$ 9-THC and CBD are structural isomers, meaning that they have the same chemical formula, but a different structural arrangement, which complicates the differentiation of hemp and marijuana. The central hypothesis is that Ag-ligand ion complexation will lead to the formation of unique MS/MS fragmentation for  $\Delta$ 9-THC and CBD. The ability to observe unique MS/MS fragmentation will enable the differentiation of the two main chemical constituents of *Cannabis sativa* L. without the need for chromatographic separation, as well as provide a mechanism for the differentiation of  $\Delta$ 9-THC-rich versus CBD-rich cannabis. Additionally, it is hypothesized that the Ag-ligand complexes will be more stable and soluble than simple silver salts alone. An Agilent® 6530 quadrupole Time-Of-Flight (qTOF) mass spectrometer with direct injection Electrospray Ionization (ESI) was used for the analysis of the Ag-ligand ion cannabinoid complexes.  $\Delta$ 9-THC and CBD were analyzed individually and as a mixture at concentrations of 50ppm, both without the presence of any Ag complexes and with pre-formed Ag complexes. Product ion spectra were collected for any abundant precursor ions present in the full scan spectrum with collision energies of 15eV, 25eV, 35eV, and 45eV, with a 4 Da isolation width and a 250ms/spectrum acquisition time. Spectral comparisons were used to identify unique product ions capable of differentiating between  $\Delta$ 9-THC and CBD, as well as providing information about the binding of the Ag-ligand to the cannabinoid.

The Ag-ligand complexes were synthesized in simple 1-2 step reactions between silver salts containing weakly coordinating anions and the desired ligand, followed by purification through recrystallization, and characterization using <sup>1</sup>H and <sup>31</sup>P NMR spectroscopy. Several complexes were also characterized by X-ray crystallography. A variety of monodentate and bidentate ligands were chosen to examine the selectivity of binding between the Ag ion and alkene functionality of the cannabinoids. Ag ions are known to reversibly bind to carbon-carbon  $\pi$ -bonds with different affinities depending on the alkene structure, and the different affinities are dependent on the steric profile of the supporting ligand as well as its electronic donor ability and chelate bite angle. The results indicate that Ag-ligand ion complexation can be used to differentiate  $\Delta$ 9-THC and CBD based on differences in the binding affinity between the Ag-ligand and the cannabinoids. Ag complexes are bound more tightly to CBD than  $\Delta$ 9-THC, leading to the formation of unique fragmentation through the loss of the weakly bound Ag-ligand for  $\Delta$ 9-THC and cleavage within the terpene moiety and the retention of the Ag-ligand for CBD. Without the formation of the Ag-ligand ion complexes, the product ion spectra of  $\Delta$ 9-THC and CBD are nearly identical, which necessitates the inclusion of lengthy chromatographic methods for cannabis analysis. The developed method provides a mechanism for the differentiation of  $\Delta$ 9-THC-rich versus CBD-rich cannabis and improves upon the selectivity, solubility, and stability due to the incorporation of the ligand(s).

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**Cannabinoid Differentiation; MS/MS Fragmentation; Metal Ion Complexation**

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**B36 Assessing the Individual and Joint Use of Ultraviolet/Visible (UV/VIS) Microspectrophotometry and Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS) to the Differentiation of Colored Spray Paint**

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**Learning Objective:** After attending this presentation, attendees will gain knowledge about the discrimination potential of two routinely encountered analytical techniques for the forensic analysis of paint when applied individually and jointly to the detection of paint components and in the context of comparative examinations.

**Impact Statement:** This presentation will impact the forensic science community by providing information about the complementary and redundant use of methods typically included in a typical analytical scheme for the forensic examination of paint.

Currently, the analytical scheme for paint is applied sequentially with microscopical examinations being first, followed by instrumental analysis techniques. When differentiation cannot be achieved between questioned and known samples, the data are typically evaluated to address a question about their common source. This study intended to compare the ability of the methods of UV/Vis Microspectrophotometry (UV/Vis MSP) and SEM/EDS to detect the relevant features used during comparative examinations. While MSP is used for comparisons, SEM/EDS also enables the potential to identify the components of paint; though these two techniques can be regarded as dependent since they both primarily inform about their pigment content.

A sample set of spray paints from different brands as well as of automotive paint collected from various autobody shops were analyzed. The colors analyzed included blue, black, red, silver/gray, and white. For the blue samples, copper was observed in all EDS spectra confirming that C.I. Pigment Blue 15 is the main pigment used for this end-use; titan was observed in four of the five brands considered and was attributed to the rutile form of titanium dioxide, used as an extender. Other elements such as chlorine, calcium, and silicon, iron, barium, and sulfur were detected in the various samples which enabled their differentiation. The MSP spectra of these samples presented similarities in the visible region of the spectrum, but the UV range (240–380nm) showed clear differentiating features. Each of the red samples presented few elements in addition to the obvious carbon and oxygen; these were titan, aluminum, silica, calcium, titan, and chlorine. Most of them indicated the presence of extenders (i.e., aluminum silicate, calcium carbonate, and rutile) but it was posited that the presence of chlorine was attributed to a pigment (to be subsequently verified using micro Raman spectroscopy). The MSP spectra allowed to differentiate all the red samples by consideration of both the UV and visible spectra regions. For the black color block, the detected elements of calcium, titan, sulfur, and barium allowed to differentiate the five brands based on the use of different extenders (i.e., calcium carbonate, rutile, and barium sulfate); it was concluded that carbon black was the pigment present in the chemical formulation of all the black samples. All silver samples contained aluminum, as expected. In one sample, titan and silicon were also detected, indicating the presence of rutile and aluminum silicate as extenders. Another sample included chromium in its elemental composition. The white samples exhibited the least between-brand variation: titan, aluminum, and silicon were detected in all of them, indicating the presence of rutile as the main pigment and aluminum silicate as an extender. The visible region of the MSP spectra (400–700nm) was relatively featureless for all the black, silver, and white samples; the UV region, however, exhibited absorption bands that contributed to their differentiation.

The qualitative evaluation of the spectra was supplemented using PCA. For MSP, the variables from 240–770nm were used for data analysis; the full pattern from 0 and 20keV of the energy spectra was used for the EDS data. Their joint use consisted of datasets resulting from the juxtaposition of the two data frames of the single methods after normalizing them to a scale of 0 to 1. Overall, PCA reflected the qualitative evaluation of the obtained data, and, except for the white samples, the score plots of the principal components of the data collected from the individual techniques showed substantial separation of the various groups; the level of separation was higher when the data of both techniques were combined.

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**Paint; Microspectrophotometry; SEM/EDS**

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**B37 Tool Mark Matching Using 3D Structures**

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**Learning Objective:** Attendees will learn the possibilities provided by using 3D measurements for tool mark matching such as visualizing the structural differences or a more accurate description of the evidence structure.

**Impact Statement:** This presentation will help in improving the analysis of tool marks by showing a case for implementing 3D measurements for impression marks.

As technology improves, new and advanced methods for forensic examination are implemented to improve their accuracy and objectivity. Since matching tools with marks found at a crime scene is an important process in recreating the scene, the area of tool mark matching has not been an exception to this change. Tool mark matching has usually been done by human examiners via image, evidence, or cast comparison, using a comparison microscope. However, this process has possibilities of human error, as the process depends on the judgment of trained human examiners, as well as limitations when obtaining the samples. Images can be distorted due to lighting or surface conditions, making casts from marks can end up incomplete or deformed due to bubbles or contamination, and processing the evidence has a possibility of damaging it.

One method of improving this procedure is utilizing 3D imaging, as it can add an additional factor of depth, increasing the reliability of the measurements. Due to this advantage, there have been studies on matching striation marks using 3D imaging by measuring the cross-correlation and likelihood ratio of the cross section of the 3D structure. In this study, we intend to implement 3D imaging to compare tools with indentation marks using 3D structures to determine whether a mark was made by a given tool or not. With a 3D microscope, we created structures of screw drivers and the indentation marks made using them. After creating the 3D structure, we first aligned the structures, converted the 3D structure into a matrix, then calculated the normalized 2D cross correlation between each tool and mark. Afterward, we overlapped each structure, matching the tool over the mark, calculated the difference and standard deviation, and created a color map to visualize the overall difference.

The results of the experiment were that the tools used to make the mark had a greater correlation value and smaller mean difference compared to the other tools, showing the possibility of using 3D microscopy and structures when matching tools with impression marks.

In the future, we intend to increase the size of the experiment to see the reliability of this differentiation procedure and implement additional feature extraction and machine learning to enhance its performance.

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**Tool Marks; 3D Structure; Matching**

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**B38 Standards Development Activities in Human Forensic Biology**

*Beth Ordeman, BS, MS\*, Pinellas County Forensic Laboratory, Largo, FL*

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**Learning Objective:** 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.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to human forensic biology. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards. During this presentation, updates related to standards development in Human Forensic Biology 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 Academy Standards Board (ASB) pending Registry approval process
4. Documents currently in development at the OSAC or by the ASB:
  - Training standards from the training standards suite
  - Standard for Internal Validation of Human STR Profiling on CE Platforms (and BPR)
  - Standards for Prevention, Monitoring, and Mitigation of DNA Contamination
  - Assigning Propositions for Likelihood Ratios in Forensic DNA Interpretations
  - Best Practice Recommendation for Validation of Forensic DNA Software
  - Standard for Internal Evaluation of a Laboratory's DNA Mixture Interpretation Protocol
  - Standard for Reporting DNA Conclusions
  - Standards for Determining Analytical and Stochastic Thresholds for Application to Forensic DNA Casework Using Electrophoresis Platforms
  - Standards for Validation and Implementation of Familial Searching for Forensic Purposes
  - Forensic Autosomal STR DNA Statistical Analyses—General Protocol, Protocol Verification, and Case Record Requirements
  - Best Practice Recommendations for Testimony Regarding DNA Activity Propositions
  - Technical Report: Appendix Examples for Biology/DNA Reports

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.

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**Forensic DNA; Standards; Forensic Biology**

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**B39 DNA Testing: Looking Back and Looking Forward**

*Robin Cotton, PhD\*, Boston University School of Medicine, Boston, MA*

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**Learning Objective:** Attendees will develop an appreciation for how different the technology was when DNA testing was first introduced. Using differential extraction as an example, attendees will gain an appreciation for the number of scientific advances contributing to the method as it is used today and how research is continuing to further improve this commonly used method.

**Impact Statement:** This presentation will impact the forensic community by providing historical context and up-to-date research information about commonly applied differential extraction methods.

In 1985, Alec Jeffreys and colleagues published one of their three “first papers” on the use of DNA for human identification.<sup>1</sup> Included in that paper was the description of the procedure for using a “differential extraction” method to separate the sperm and non-sperm cells found in sexual assault samples. That same year, a paper describing the Polymerase Chain Reaction (PCR) was published by Cary Mullis and colleagues.<sup>2</sup> A few years later, in 1987, the first RFLP evidence was presented at trial in the United States, and the United States application of DNA analysis to criminal cases began. Federal, state, local, and private laboratories began to validate RFLP testing and incorporate the procedure for differential extraction into their protocols since evidence from sexual assault cases represents a large portion of samples. The differential extraction chemistry described by Gill, Jeffreys, and Werrett, takes advantage of the need to incorporate a reducing agent to lyse sperm cells.<sup>1</sup> In comparison to current techniques, about 100 to 500 times as much DNA would be needed to obtain a result via RFLP testing.

Time passed and in the early 1990s, the PCR began to be applied to forensic DNA analysis. Those early tests had very low discrimination potential and, thankfully, the use of Short Tandem Repeats (STRs) began in the late 1990s. Immediately, the amount of DNA needed to produce a result was dramatically reduced. However, although more than 10 years have elapsed, the original differential extraction has not been substantially changed and was put to use on increasingly smaller samples.

The PCR amplifications require control of the input amount of sample DNA. At first, the amount of DNA, post-extraction, is measured using the “slot blot” methodology, but again, research comes to the rescue. Molecular biologists, studying gene expression were measuring the concentration of specific RNA transcripts in different cell types or under different developmental conditions using quantitative PCR (qPCR) measurements.<sup>3</sup> The input RNA concentration was measured in “real-time” by examination of the rate of PCR product accumulation in each PCR cycle. For forensic DNA applications, the qPCR, originally designed to measure the concentration of human DNA in an extract, was rapidly developed to also detect both total human and total male DNA (and later, other reaction components). These measurements allowed assessment of the success of the differential extraction, prior to producing DNA profiles, as the amount of male and female DNA could be estimated in the epithelial cell and sperm cell fractions prior to STR amplification. Decisions on how to move forward with analysis could now be made in advance of seeing the DNA profile result.

Research in the areas of proteomics, developmental biology, and assisted reproduction are providing additional information on sperm structure and biochemistry. Microfluidics, temperature-controlled enzymes, new filtration capture methods, and other advances have the potential to replace the multiple steps of differential extraction. Anyone testifying to DNA evidence has had to describe, in simple terms, the steps in a differential extraction. Like many problems, the question seems simple until one begins to look closely at the detail. Like all the other areas of DNA analysis, and forensics in general, important changes, based on new scientific information, will continue. However, the underlying requirement, Do the Science Well, remains the same.

**References:**

- <sup>1.</sup> Gill P, Jeffreys A, Werrett D, 1985. Forensic application of “DNA Fingerprints.” *Nature*. 318:p577.
- <sup>2.</sup> Saiki R., Scharf S, Faloona F, Mullis K, Horn G, Erlich H, Arnheim N, 1985. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science*. 230:p1350.
- <sup>3.</sup> Higuchi R, et. al. 1993. Kinetic PCR analysis: Real-time monitoring of DNA amplification reactions. *Nature Biotechnology*. 11:p1026.

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**DNA Analysis; Non-Forensic Research Areas; Differential Extraction**



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**B40 “Back to the Future”—Bayes’ Theorem Goes Around, Comes Around**

*Patrick Buzzini, PhD, Sam Houston State University, Huntsville, TX; Tim Kalafut, PhD\*, Sam Houston State University\*, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the change from the frequentist approach to the Bayesian approach in the DNA community, and how such an approach can extend beyond both the common DNA subsample (Who?) statistic, and the DNA discipline.

**Impact Statement:** This presentation will impact the forensic science community by reminding practitioners that a Bayesian approach was once championed by non-DNA disciplines, is being adopted by DNA practitioners now, and can benefit other forensic disciplines.

One of the biggest changes in the United States Forensic DNA community has been the adoption of Probabilistic Genotyping (PG) to give weight to our DNA findings when a Person Of Interest (POI) is included as a possible source of a DNA profile. In order to use a PG approach, practitioners have had to procure bespoke software and undergo specific training in its theory and use. This has also necessitated the adoption of the Likelihood Ratio (LR) and retiring the binary approach to mixture deconvolution.

As it turns out, the adoption of the LR and Bayes’ theorem may end up having the greater impact than the specific switch from the binary to the probabilistic DNA statistic. This change from a frequentist approach to a Bayesian approach allows for the characterization of the uncertainty in the probability of the evidence given propositions of interest. These propositions represent the parties’ theories, views, scenarios, or arguments of the case.

In the DNA arena, the questions asked at trial are more and more related to “how the DNA got there,” rather than “whose DNA is there.” This “how” question can be described as the evaluation of the DNA findings given activity level propositions, or sometimes simply “activity level.” Although such questions are nothing new, the inherent uncertainty in the evaluation of DNA findings given such propositions is addressed in DNA literature by the use of Bayes’ theorem.

The Bayesian approach has been used in various disciplines such as glass and serology (blood transfer problems), although it seems to have fallen out of favor, at least in the United States. However, the use of Bayes’ theorem can be adopted by numerous disciplines. The medical examiner could give an opinion that, “These findings are more likely if the manner of death was homicide than if it was suicide.” The trace evidence examiner could state that, “The findings are on the order of 8,000 times more likely if the suspect was near the window when it was smashed than if he was never near this window.” The firearms examiner could state that, “These findings are more likely if the two cartridge cases were fired by two different guns than if they were fired by the same gun.” The hair examiner could give an opinion that, “Finding a clump of hair with roots in this phase of growth is more likely if they were forcibly removed than if they were naturally shed.”

The language of the DNA expert addressing these questions would be, “The DNA evidence is more likely if the person of interest used the item, than if the person of interest did not use the item, but previously interacted with someone that did.” Note that the example statements are all opinions about the evidence, not the propositions, as only the fact finder (typically the jury) should evaluate the propositions as one step in their ultimate decision on the innocence or guilt of the accused.

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**Uncertainty; Bayes' Theorem; Testimony**

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**B41 When and “Y” to Screen With QIAGEN® Investigator® Casework GO!**

*Julia Wang, MS\*, Sam Houston State University, Huntsville, TX; Grace Rutledge, MS, Sam Houston State University, Houston, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX; Sheree Hughes, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will have gained practical insight on applications of Y-screening in sexual assault evidence processing.

**Impact Statement:** This presentation will impact the forensic science community by presenting support for alternative screening methods in sexual assault evidence DNA workflows that can greatly reduce processing time.

Serological screening and differential extraction workflows have been the standard approaches to sexual assault evidence processing. Due to advances in DNA quantification chemistries, Y-screening methods have now become an alternative to serological screening tests for detecting semen. Additionally, direct-to-DNA workflows that bypass DNA purification and quantification in conventional forensic DNA practices have been developed. These direct-to-DNA methods can also be applied to sexual assault evidence to reduce DNA loss due to sample processing that occurs during preferential lysis or other differential extraction techniques. As recognized by documents such as *NIJ National Best Practices for Sexual Assault Kits: A Multidisciplinary Approach* and *SWGDM Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits*, Y-screening methodology can delay or eliminate sample consumption during preliminary screening.

In this study, various Y-screening methods combined with the QIAGEN® Investigator® Casework GO! kit were compared to classic differential extraction and traditional serological tests. From mock sexual assault evidence samples, Y-screening methods using Casework GO! were equivalent or more sensitive at detecting semen on swabs compared to differential extraction, P30, and confirmatory microscope slide searching with Christmas Tree staining. Y-screening with one-eighth of the semen-spiked vaginal swab was as sensitive as screening with one-half of the swab. Although high human-to-male concentration ratios were detected in samples using Y-screening, where no differential washes were applied to the sample during the screening process, the presence of male DNA was successfully screened. The Y-pellet screening with QIAGEN® Casework GO! was the most successful method of the screening processes and most closely matched results observed after full differential extraction and best predicted autosomal STR profile recovery. In swabs that were screened negative by serological testing or at low concentrations by quantitative Y-screening, Y-STR profiles were still generated using QIAGEN® Investigator® Argus Y-28 QS. The most effective Y-screening methods were then applied to genuine post-coital vaginal swabs of various time intervals to demonstrate success with more forensically relevant samples.

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**Y-Screening; Sexual Assault; Differential Extraction**

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**B42 An Interlaboratory Comparison of SpermX™ and Conventional Differential Extractions for DNA Analysis of Sexual Assault Samples**

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**Learning Objective:** Attendees will be introduced to SpermX™, a novel method for differential extraction of sexual assault evidence designed to capture sperm more efficiently. The performance of SpermX™ compared to the conventional differential extraction method was evaluated by three different laboratories using the same prepared female and male mixtures. Participants will gain an understanding of how SpermX™ compares to standard differential extraction methods used in different laboratories and how improving sperm recovery in sexual assault samples can provide more CODIS up-loadable STR profiles.

**Impact Statement:** Current differential extraction methods have been shown to only recover 5–30% of the sperm from a sexual assault swab, and 30–50% of SAK samples that screen positive for male DNA do not produce a complete CODIS profile. SpermX™ can improve the likelihood of obtaining a CODIS up-loadable DNA profile in cases that previously had insufficient DNA for analyses. The method is also capable of high throughput automation on a Hamilton® liquid handling platform for high throughput processing of SAK kits to reduce current backlogs.

An interlaboratory mixture study was conducted by three laboratories. InnoGenomics®, the University of Central Florida, and the Center for Forensic Science Research and Education processed eight female:male mixtures (A-H) in triplicate using the InnoGenomics® manual SpermX™ (SX) method and their in-house conventional Differential Extraction (DE) method.<sup>1-2</sup> The eight mixtures were prepared with the same female (saliva) and male (semen) donors. The results from all three study sites indicated that SX recovered significantly more sperm DNA than Differential Extraction (DE) from mock evidentiary swabs with as low as 100 cells (swab set G-330pg) and 25 cells (swab set H-82.5pg). The use of the SpermX™ method in routine casework workflows will provide probative CODIS uploadable STR profiles from sexual assault cases with low sperm evidence, whereas current DE methods may give insufficient or no discriminatory results for a CODIS search.

Quantification data, culled from the sperm fractions, were used to make comparisons of the total Y DNA recovered, the total human-to-Y ratios, the percentage recovery of male in the sperm fraction relative to the theoretical amount, and the percentage of female carryover. On average, SX recovered 5 times more male DNA than did the DE method. The average S/Y ratios were lower with SX than DE, indicative of a better separation of the male component from the female component. The DE S/Y ratios ranged from 5.4 to 183 times higher than SX indicating more female DNA carry over in the sperm fraction. The percentage recovery of the male DNA in the sperm fraction relative to the theoretical amount of semen placed on the swabs was higher for SX. On average, SX recovered from 1.5- to 10-fold more DNA in the sperm fraction in mixtures A-D and from 20- to 1,533-fold more male DNA in the lower end sperm series, E-H. The percentage of female carryover in the sperm fraction was calculated by the amount of female DNA in the sperm fraction divided by the total female in all fractions (not compared to the theoretical amount) for each method. The DE exhibited higher average percent female carryover for all swab sets from all three labs, ranging from 2.8- to 10-fold more than SX. STR data comparisons of the sperm fraction profiles include the percentage of unshared male alleles recovered, the male percentage observed in the STR profile, and statistical probabilities of inclusion. On average, SX had higher recovery percentages of male alleles in the STR profiles than DE by 7-fold. STRmix™ generated male profile proportions that demonstrated SX recovered higher percentages of the male component for all samples, ranging from 2- to 36-fold more than the DE. The likelihood ratios from STRmix™ showed SX had comparable or higher LR's, ranging from 2 to 23 orders of magnitude.

In conclusion, the interlaboratory study indicated the SpermX™ method provided higher quantities of male DNA with less female carryover compared to the DE methods for the sexual assault sample processing by all three participating labs.

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- <sup>2.</sup> <https://www.ojp.gov/pdffiles1/nij/grants/238500.pdf>.

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**Sexual Assault Sample; Differential Extraction; SpermX™**

**B43 Modified Differential Cell Lysis Using a Combined prepGEM™ and Alkaline Approach**

Brittany Hudson, MS\*, Virginia Commonwealth University, Richmond, VA; Tracey Dawson Green, PhD, Virginia Commonwealth University, Richmond, VA

**Learning Objective:** After attending this presentation, attendees will have a better understanding of an alternative differential cell lysis technique that reduces the time and costs associated with processing sexual assault samples.

**Impact Statement:** This presentation will impact the forensic science community by presenting data that demonstrate the potential for a combined enzymatic (prepGEM™) and alkaline lysis technique to reduce time and costs while providing high-quality STR profiling results, which could help analysts more efficiently process sexual assault samples.

Research aimed at improving the traditional differential extraction procedure and tackling the Sexual Assault Evidence Collection Kit (SAECK) backlog has been ongoing for ≥20 years. While funding efforts have provided great relief in reducing the current backlogs nationwide, new legislation has also mandated the submission and testing of all SAECKs in many states, likely producing future backlogs. Thus, ongoing research is necessary to reduce the time and costs associated with processing sexual assault samples, as well as to provide improved separation of their associated male and female contributions.

The traditional differential extraction procedure, which uses proteinase K, SDS, and DTT to isolate sperm and non-sperm components within a sample, is often inefficient at fully separating these fractions; it is also time and labor intensive.<sup>1-5</sup> Thus, this study evaluated a combined enzymatic (prepGEM™) and alkaline technique for cell lysis with the goal of developing a method that could efficiently lyse epithelial cells and spermatozoa within sexual assault samples, omit lengthy purification steps, and consistently generate high-quality STR profiles, as well as reduce reagent consumption, time, and associated costs.

Semen swab cuttings ( $n=10$ ) containing ~6,000–19,000 spermatozoa, vaginal swab cuttings ( $n=10$ ), and unique mock semen-vaginal mixtures ( $n=10$ ) were prepared. All eluates were subjected to enzymatic treatment using prepGEM™ following manufacturer recommendations (at half volume) to accomplish non-sperm cell lysis. Intact sperm cells were then pelleted via centrifugation at 17,000 x g for five minutes and the entire supernatant was removed as the non-sperm fraction. Sperm and non-sperm fractions were then subjected to alkaline solution to lyse all remaining cells.<sup>6</sup> A subsequent set of semen, vaginal, and mixture samples was also tested using twice the amount of prepGEM™ enzyme in the same final reaction volume.

Quantification using Investigator® Quantiplex™ HYres revealed that this technique retained  $72.0 \pm 18\%$  of DNA within sperm fractions for semen eluates and  $15.8 \pm 14\%$  of DNA within sperm fractions for vaginal eluates. Amplification of lysates from processed mixture samples was performed with Promega™ PowerPlex® Fusion 6C, and separation of amplicons was conducted on the ABI® 3500 Genetic Analyzer. Analysis of resulting STR profiles revealed that this procedure improved male:female (M:F) ratios in sperm fractions by  $3.2 \pm 2.4$ -fold and recovered  $5.9 \pm 7.8$  additional male contributor alleles not observed in the corresponding unseparated controls. Implementing additional enzyme for non-sperm cell lysis generated a  $4.1 \pm 5.0$ -fold improvement in M:F ratios but only improved recovery of additional male contributor alleles by  $3.6 \pm 4.4$  compared to that observed with corresponding unseparated controls. For both data sets, while the male contributor was notably enriched, alleles from the female contributor remained. In fact, dropout of some female contributor alleles was only observed in 30% of sperm fractions.

Overall, this study demonstrates the potential for a combined enzymatic and alkaline approach as a quick, cheap, and reduced volume technique for processing sexual assault samples. Although optimization is still needed to isolate cleaner sperm fractions, this method was capable of improving M:F ratios in resulting STR profiles and required approximately 20 minutes of overall processing time, which is a prominent improvement over existing, traditionally used methods. After testing modifications such as two rounds of prepGEM™ treatment and other alternative non-sperm cell lysis approaches to maximize fractional separation efficiency, future studies should evaluate postcoital samples and the transition of this technique onto a microdevice to enable automation.

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**Sexual Assault; PrepGEM™; Alkaline Lysis**

**B44    The Use of “Carrier” Sperm to Improve the Yield of Human Sperm Cell DNA During Differential Extraction Analysis**

*Constance Lansdale, Oklahoma State Bureau of Investigation\*, Edmond, OK; James Creecy*

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**WITHDRAWN**

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**B45 Evil Under the Sun: An Assessment of Body Fluid Identification and DNA Profiling After Exposure to Tropical Weather Conditions**

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**Learning Objective:** After attending this presentation, attendees will understand the impact of environmental conditions, in this case tropical weather, on Body Fluid Identification (BFID) and DNA profiling.

**Impact Statement:** This presentation will impact the forensic science community by providing an extensive study of the effects of sun and tropical storms over time on the identification of blood, semen, saliva, and their mixtures, also assessing the possibility of DNA profiling.

The identification of body fluids is of paramount importance in criminal investigations to characterize potential biological evidence. This step normally precedes DNA profiling. Current methods for BFID are based on chemical, enzymatic, and serological methods. The latter is more specific toward the identification of body fluids as it is based on antigen-antibody reactions binding to a specific protein, unique or almost unique for the body fluid. In this regard, there are serological tests for blood, menstrual blood, semen, and saliva. In spite of the widespread use of these serological tests, there are no studies assessing the impact of environmental conditions on BFID.

The present work assessed the effect of tropical weather conditions on body fluid identification and posterior DNA profiling overtime. Blood (20ul), semen (20ul), and saliva (50ul) samples, and mixtures of blood and semen, and semen and saliva were deposited on polyester samples. Three samples per body fluid, mixture, and time were exposed to open air tropical weather conditions during the month of June in Miami, FL, and subjected to high temperature and humidity (average temperature 29.3°C, average humidity 73°C, UV index 7), as well as tropical storms for 19 days. Immunochromatographic tests (SERATEC®) Lab and Crime Scene (CS) test versions were used for BFID (one per each body fluid type) 1, 2, 14, and 30 days after deposition. DNA extraction was performed on two blood, semen, and saliva samples after 14 and 30 days after deposition, followed by quantification and DNA profiling.

It was possible to detect body fluids and their mixtures up to 14 days after deposition, except for saliva, which showed very light bands, being almost undetectable when mixed with semen. Thirty days after deposition, with variability and mostly showing light bands, it was possible to identify blood and semen but not saliva in the mixtures.

DNA was extracted from the BFID buffer. One sample (blood at 14 days) from the lab tests retrieved quantifiable DNA. From CS tests, quantifiable DNA was obtained from one blood sample at 14 days and one blood sample at 30 days; two saliva samples at 14 days and two saliva samples at 30 days; and two semen samples at 14 days. Full DNA profiles were obtained from 14-day-old blood samples (Lab and CS tests), and partial profiles were obtained from the remaining samples, except from one semen sample at 14 days, and one blood sample at 30 days. In order to improve identification from these partial profiles, mtDNA sequencing was carried out, successfully obtaining a good quality mtDNA in all samples, and being able to identify the mtDNA haplogroups.

In conclusion, this study demonstrated for the first time the possibility of BFID and DNA profiling after exposure to tropical weather conditions over time. Success depends on the type of body fluid, as well as the length of exposure. Future research could aim to evaluate this identification in other conditions, also trying to optimize DNA retrieval and improve profile quality.

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**Body Fluid Identification; Immunochromatography Tests; Tropical Weather**



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**B46 The Impact of Various Storage Temperatures and Times on the Microbiome of Forensically Relevant Biological Samples**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how microbial communities useful in forensic body fluid identification are impacted under various storage conditions (time and temperature).

**Impact Statement:** This information will impact the forensic science community by helping forensic scientists overcome the unknown variables often encountered in microbial research, where the impact of storage conditions has not been clearly assessed.

Previous studies have demonstrated that body fluids have a distinct, distinguishable microbial signature. Identifying these indicative microbial taxa within forensically relevant fluids not only has the advantage that previously indistinguishable samples, such as venous and menstrual blood, may be separated, but these markers can also be easily implemented into comprehensive High-Throughput Sequencing (HTS) panels used in the forensic workflow alongside the typical human phenotypic marker characterization. Our foundational work investigated the microbial signatures associated with 812 forensically relevant body fluid samples; however, investigations into the robustness and stability of the taxa over storage time and temperature are an important part of the developmental validation, as previous studies have suggested that bacterial patterns can begin to vary in samples within as little as one week.<sup>1</sup> According to the Technical Working Group on Biological Evidence Preservation, biological materials should be collected and stored according to the short- and long-term best practices. However, these standards were created with human DNA in mind, and do not consider how storage conditions impact the microbial evidence. Identifying how rare and/or indicative taxa are impacted not only affects the accuracy of the microbial body fluid classification method, but this can also aid the forensic community in understanding storage requirements for microbial evidence.

Using IRB-approved collection methods, ten human biological samples from each body fluid were stored at four temperatures (-20°C, 4°C, 25°C, 37°C) for three time periods (30 days, 60 days, and 90 days) ( $N=720$ ; 10 individuals \* 6 body fluid types \* 4 storage temperatures \* 3 storage). Briefly, semen was collected into sterile containers and dried onto sterile cotton swabs. Venous blood was collected onto cotton swabs using a finger prick and dried at room temperature. Saliva, vaginal secretions, menstrual secretions, and feces were collected onto cotton swabs and dried at room temperature. After appropriate time and temperature intervals, DNA was isolated using the QIAGEN® DNA Investigator® kit according to the manufacturer's protocols. Following extraction, all samples were quantified using an in-house developed 16S rDNA specific bacterial DNA quantification method, followed by sequencing of the V4 region of the 16S rDNA on the Illumina® MiSeq® FGx sequencing platform following the dual-indexing protocol as described by Kozich et al.<sup>2,3</sup> Sequences were then analyzed using the open-source microbial analysis platform, mothur version 1.47, and statistical analysis was performed using R version 4.2.1.<sup>4,5</sup>

As expected, the body fluid type had a significant impact on the bacterial DNA yield ( $p$ -value < 0.001), with fecal samples having an average bacterial DNA yield of 6,000ng, and varying significantly from all other body fluids ( $p$ -value < 0.001). Storage time (up to 90 days) did not significantly impact the bacterial DNA yield of body fluids. Storage temperatures had a significant impact on the bacterial DNA yield ( $p$ -value < 0.05), with 37°C and -20°C being most significant ( $p$ -value < 0.05).

The preliminary results suggest that while storage time did not significantly impact our bacterial DNA yields, the storage temperature does. Findings from this study will help minimize errors associated with our microbial signature-based body fluid identification method, while enhancing current forensic protocols.

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**Body Fluid ID; Microbial Evidence Storage; 16S rDNA**

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**B47 Forensic Body Fluid Classification Using a Panel of MicroRNAs in DNA Extracts**

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**Learning Objective:** After attending this presentation, attendees will understand how microRNAs (miRNAs) can be of significant value for body fluid identification in forensic casework. Attendees will understand that specific miRNA markers can distinguish different body fluids, and that miRNAs may be a better molecular-based method for the identification of body fluids rather than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating that the evidence for miRNAs as molecular markers for body fluid identification continues to build strength.

There is significant interest in the use of miRNAs for forensic casework because of their short length and high resistance to degradation.<sup>1</sup> They have also been shown to co-extract and be detectable in DNA extracts, which could make the use of miRNAs a more streamlined and easily implementable molecular body fluid identification method than other described methods.<sup>2</sup>

Foundational work by Seashols-Williams et al. reported a reverse transcription-quantitative PCR (RT-qPCR) panel of eight miRNAs that classified venous and menstrual blood, feces, urine, saliva, semen, and vaginal secretions through analysis of differential gene expression.<sup>3</sup> This panel of miRNAs includes a pair of endogenous reference markers that provide normalization of miRNA expression without evaluation of the RNA quality or known input quantity. In a subsequent report, Rhodes et al. conducted a developmental validation of the RT-qPCR miRNA panel using Quadratic Discriminant Analysis (QDA) for the classification of forensically relevant body fluids with 93% accuracy in RNA extracts.<sup>4</sup>

In this project, the previously mentioned miRNA panel and QDA method was evaluated in DNA extracts from a population of 50 donors of each body fluid (venous and menstrual blood, feces, urine, saliva, semen, and vaginal secretions). Initially, an overall correct classification rate of 87% was obtained, which increased to 92% when three additional miRNA markers were incorporated to the panel. Identification of the biological fluids was found to be reliable across population samples of mixed ages, ethnicities, and sex, with 72–98% of the unknown samples classified correctly.

The model was then tested against other body fluid sample replicates over time and biological cycles, as well samples exposed to compromising conditions. To evaluate the robustness of the miRNA panel optimized for DNA extracts, dried body fluid samples (blood, urine, saliva, and semen) were subjected to harsh treatments including heat exposure, Ultraviolet (UV) radiation, simulated environmental chamber, or chemical treatments with either dish soap, glacial acetic acid, 1:10 bleach, or full-strength bleach prior to extraction. The classification rate of each body fluid after treatment depended on both the body fluid and the miRNA marker. These data suggest that miRNAs are comparatively detectable in both RNA and DNA extracts and therefore eliminates the need for an RNA extraction, which would greatly reduce evidentiary sample consumption if miRNA analysis were to be implemented into forensic laboratories.

In conclusion, the panel of nine miRNA markers were shown to have high specificity for classifying biological fluids in DNA extracts, but additional work is important to improve classification of saliva and female intimate secretions before implementation in forensic casework. In future studies, this assay could benefit from adding markers of different types, such as microbial DNA markers, and using high-throughput sequencing to increase multiplexing ability, which would decrease required analyst time.

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**Body Fluid Identification; MicroRNA; MiRNA**

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**B48 A Field-Forward Microfluidic Method for Transcriptomic Serological Analysis**

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**Learning Objective:** After attending this presentation, attendees will have been introduced to a novel rotationally driven sample in-answer out microdevice that integrates mRNA extraction, multiplexed RT-PCR amplification, electrophoresis, and fluorescence detection to detect and identify forensically relevant body fluids.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating progress toward an integrated platform for rapid detection of messenger RNA (mRNA) targets for forensic serological analysis.

The identification of body fluids is a critical component of forensic science that guides subsequent DNA analysis and contextualizes crime scenes; however, this process is often difficult as many body fluid stains are present in small quantities or mixtures.<sup>1</sup> Traditional colorimetric/enzymatic presumptive assays, though rapid and simple, exhibit limited sensitivity and/or specificity and are incompatible with DNA profiling as body fluid identification consumes the limited sample.<sup>2</sup> Transcriptomics, or RNA analysis, provides a promising alternative to conventional body fluid identification as it does not compromise the compatibility of DNA for downstream analysis and is amenable to integration in the traditional forensic workflow.<sup>3</sup> Unfortunately, existing transcriptomic techniques involve time-consuming, laborious analysis that requires trained analysts to operate complex instrumentation in a centralized laboratory. To overcome these limitations, we describe significant progress toward a portable sample in-answer out microfluidic platform that integrates multiple steps (e.g., sample extraction, amplification, electrophoretic separation, and detection) for rapid body fluid identification at the point of need.

Microfluidic systems offer multiple advantages over traditional laboratory workflows, including rapid analysis times, reduced reagent and sample volume requirements, and enclosed formats that minimize the risk of contamination and reduce user intervention.<sup>4</sup> Here we describe a centrifugal microdevice that controls fluid flow via rotation, eliminating the need for cumbersome external hardware, such as pumps and syringes, and thus promoting portability.

The reported rotationally driven microdevice represents significant progress toward the development of a miniaturized Total Analysis System (uTAS) capable of performing integrated nucleic acid extraction, multiplexed RT-PCR, and electrophoretic separation with minimal user intervention. We demonstrate successful co-extraction of DNA, ready for genetic analysis, and RNA for downstream body fluid identification. Confirmatory body fluid identification from mRNA is complete in under 1 hour, with enzymatic co-extraction, multiplexed RT-PCR, and electrophoresis consuming < 15min, ~30min, and 10min, respectively. A previously validated panel of mRNA targets were selected to probe for forensically relevant body fluids, including saliva, venous blood, menstrual blood, vaginal fluid, and semen.<sup>5</sup> Ongoing work is underway to automate all on-disc processes and incorporate on-board reagent storage for ease of analysis. The presented work illustrates progress toward a field-forward, sample in-answer out uTAS for a rapid, confirmatory body fluid identification instrument.

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**Body Fluid Identification; mRNA; Microfluidics**

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**B49 My Results are Unintuitive, Now What? Casework Studies on Unintuitive STRmix™ Results and the Approaches Utilized for Reporting**

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**Learning Objective:** In this presentation, several cases in which unintuitive STRmix™ results were obtained will be highlighted. Issues noted include mixture proportions that do not line up with the data observed in the electropherogram and multiple individuals with the same contributor assignment, in addition to contributor assignments with multiple flags upon assessment of the genotype weights observed in the deconvolution report. The method by which these profiles were evaluated to identify the issue will be discussed in addition to the propositions that were considered. Finally, the reported results will be reviewed with emphasis on court testimony explanations.

**Impact Statement:** The consideration of alternate propositions for unintuitive probabilistic genotyping results and the appropriate explanation of their limitations can provide the stakeholder with a full picture of the results based on the information that was available at the time of reporting.

Probabilistic genotyping software is an incredible resource for forensic DNA laboratories, allowing for the interpretation of complex mixture profiles that were previously considered inconclusive. In the application of software programs such as STRmix™, it is critical that the validation and subsequent implementation address the limitations of the system and that the end users (e.g., the DNA analyst) are properly trained to evaluate the results to ensure that they are intuitive. When unintuitive results are obtained, there is a range of approaches that may be reasonable. While it may be appropriate to consider a deconvolution and/or the likelihood ratio generated for a person of interest inconclusive; the consideration of other propositions may be warranted, particularly when a familial component could be impacting the results.

Signature Science, LLC (SigSci), a private Forensic DNA Laboratory based in Austin, TX, implemented STRmix™ for casework use in May of 2018 with the Applied® GlobalFiler® system. Following GlobalFiler®, the QIAGEN® Investigator® 24plex QS system was validated and implemented in April of 2020 with additional work performed for the new formulation of the QIAGEN® Investigator® 24plex QS system in June of 2022. In the four years since its employment at the laboratory, hundreds of DNA profiles have been interpreted with the aid of STRmix™ and the subsequent results closely evaluated by the DNA analysts on the SigSci team. As is the case for many laboratories that utilize probabilistic genotyping software, problematic samples have ultimately crossed an analyst's desk on more than one occasion.

Careful evaluation is key for confidently reporting probabilistic genotyping results. It is critical that additional considerations be taken when unintuitive results are observed. Reporting these results without proper investigation could mean false support for an inclusion or exclusion due to an over- or underestimated likelihood ratio. On the other hand, deeming these results unreportable can also fail to provide the stakeholder with important information.

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**DNA; Probabilistic Genotyping; Testimony**

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**B50 Probabilistic Genotyping With Microhaplotypes**

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**Learning Objective:** Attendees of this presentation will learn about the probabilistic genotyping using microhaplotypes, which are a new forensic DNA marker that can complement conventional STR markers enhancing complex mixture deconvolution capabilities.

**Impact Statement:** Microhaplotypes are promising, and potentially impactful, forensic DNA markers made available thanks to the advances in sequencing technology. This presentation will demonstrate that they are amenable to probabilistic genotyping, which is an additional step towards casework implementation.

Microhaplotypes (MHs) are an emerging forensic DNA marker characterized by sets of Single Nucleotide Polymorphisms (SNPs) within a short distance of each other and displaying multiple allelic combinations. Although less polymorphic than Short Tandem Repeat polymorphisms (STRs), they have some advantages, including alleles all have the same size within a locus, absence of stutter artifacts, and mutation rates are significantly lower than those of STRs. Additionally, MHs carry more discriminating ancestry informativeness than STRs. Several MH-multiplex panels have been reported in the past five years, including the 74-locus panel developed in our laboratory. Casework implementation of such large panels is only feasible if paired with Probabilistic Genotyping (PG), as manual deconvolution of complex mixtures would be excessively time consuming and not compatible with conventional forensic DNA laboratory operations.

To evaluate the feasibility of this project, both EuroForMix and DNA•View® Mixture Solution were adapted to processing MH data from 74 loci analyzed on the Ion S5 Massively Parallel Sequencing (MPS) platform. Relative Fluorescence Unit (RFU) values were replaced by allele-sequence coverage and tested on a set of DNA mixtures. Twenty-five mixtures of varying number of contributors up to five and their relative contributor ratio were generated and tested with both STR/CE using conventional STR 24-plex assays and the MH 74-plex assay. Likelihood ratios for the known ground truth were compared between the different methods and the MH 74-plex assay was found to always outperform STR/CE by several orders of magnitude. Non-contributor tests also yielded results consistent with the ground truth. For the forensic community to accept and implement a new genetic marker, several steps are required; these include, but are not limited to, demonstrating an added value compared to the current methods and their adaptability to currently used methods. Results from this project demonstrate that MHs are amenable to PG making this novel promising type of forensic marker one step closer to casework implementation.

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**Microhaplotypes; Probabilistic Genotyping; Mixture Deconvolution**

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**B51 Identifying Victim Remains Using Kinship Genotype Inference**

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**Learning Objective:** After attending this presentation, attendees will understand how kinship information is used to construct a missing person's probabilistic genotype. Comparing this genotype with an unknown evidence genotype can calculate a statistical association.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the real-world utility of kinship inference in disaster victim identification. We show how kinship is used to infer missing persons' genotypes, even with sparse or unknown biological relationships.

Following a horrific fiery bus crash in Komatipoort, the South African Police Service (SAPS) recovered victims' bodies that were burned beyond recognition. Identification records did not exist for these rural passengers. Although the police collected DNA from the victim remains, it was not helpful—there were no victim reference profiles on file for comparison. But the police also collected DNA from 15 people who thought their relative may have been on the bus. The police wanted to use the biological relatives' DNA to identify the victim remains, but their efforts were unsuccessful.

In 2007, Cybergenetics had previously used their TrueAllele® Technology (TA) to identify victim remains from the World Trade Center Disaster.<sup>1</sup> This computer-based DNA interpretation technology reconstructed missing person kinship genotypes (up to probability). The reconstruction used the DNA of relatives, such as parents, children, and siblings. TA database comparison of these kinship genotypes with remains data helped identify victim remains.

In 2009, SAPS reached out to Cybergenetics for help. The police sent them the STR DNA profile data from 15 victim remains and 15 possible relatives. They provided the assumed biological relationships between relatives and missing people. None of the missing were related to one another; thus, each kinship genotype would be constructed using just one family reference. For one reference, the biological relationship was unknown, so there was no kinship information.

Child probabilistic genotypes can be inferred when both parents' genotypes are known (e.g., by using a Punnett square). This genotype is comprised of allele pair possibilities, and their probabilities. When only one parent genotype is available, a computer can use population probability for the other parent's genotype to reconstruct the child's genotype. Probabilistic kinship genotypes for missing parents and siblings are constructed similarly.

In the Komatipoort case, the TA analyst input relative profile and kinship information into the TA computer. For the one reference lacking kinship information, the analyst asked the computer to construct the various parent, child, and sibling genotypes. Within minutes, the computer had created the corresponding kinship genotypes. The expected values of their match statistics (Kullback-Leibler divergence) were 2 to 3 ban, as predicted when using 9 loci of STR data.

The analyst compared the 15 kinship genotypes to the 15 victim remains to calculate match statistics. Nine of the 15 remains were statistically associated with a kinship reference (including the one without kinship information). The likelihood ratios ranged from hundreds to tens of thousands. No family association was found for the other six remains. Perhaps there was no available biological relative. Or, in this rural area, a "relative" may not be biologically related, since children there may be raised by distant relatives honored as "parents."

This presentation demonstrates the real-world utility of kinship inference in disaster victim identification. We show how kinship is used to infer missing persons' genotypes, even with sparse or unknown biological relationships.

**Reference:**

- <sup>1</sup>. Perlin, M. W. (2007). Mass casualty identification through DNA analysis: Overview, problems and pitfalls. *Forensic Investigation and Management of Mass Disasters*. M. I. Okoye and C. H. Wecht. Tucson, AZ, Lawyers & Judges Publishing Co: 23-30.

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**Kinship Inference; Probabilistic Genotype; Missing Persons**



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**B52 DNAmix 2021—Inter-laboratory Variation in Interpretations, Comparisons, and Statistical Analyses of DNA Mixtures**

*R. Austin Hicklin, PhD\*, Noblis, Vienna, VA; Nicole Richetelli, PhD\*, Noblis, Inc, Glen Burnie, MD; Brandi Emerick, PhD, Noblis, Sterling, VA; Jonathan Davoren, MSc, Bode Technology, Lorton, VA*

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**Learning Objective:** Attendees will learn about the accuracy and inter-laboratory variability of interpretations, comparisons, and statistical analyses conducted by different laboratories on DNA mixtures.

**Impact Statement:** This presentation will communicate the accuracy and inter-laboratory variation in interpretations, comparisons, and statistical analysis of DNA mixtures. These results provide estimates that may be used by laboratory managers, standards organizations, and the legal community to assist in decision making, improving procedures and training, and highlighting areas for potential standardization.

The purpose of this presentation is to discuss the results collected and variation observed in assessments of suitability, assessments of the number of contributors, categorical comparison conclusions with respect to the Person Of Interest (POI), and statistical analyses (statistical values with respect to the POI) reported by forensic laboratories on DNA mixture profiles provided as electropherograms. These results were collected as part of the NIJ-funded study entitled Inter-laboratory Variation in Interpretation of DNA Mixtures (hereafter referred to as “DNAmix 2021”) (NIJ Grant # 2020-R2-CX-0049).

DNAmix 2021 is a rigorous, large-scale study conducted to evaluate the extent of consistency and variation among forensic laboratories in interpretations and statistical analyses of DNA mixtures and to assess the effects of various potential sources of variability. The study was conducted in four phases. This presentation will discuss the results of the final two phases: the Number of Contributors (NOC) Subtest and the Interpretation, Comparison and Statistical Analysis (ICSA) Subtest. Each participating laboratory that completed DNAmix 2021 submitted interpretations on 20 distinct mixtures; participants also conducted comparisons and statistical analyses for 8 of these mixtures. All mixtures were created under controlled conditions to allow for ground truth attribution. Mixtures were created to be broadly representative of the range of attributes encountered in casework. All DNA profiles used in this study (mixtures and references) were from real people; no profiles were simulated or contrived.

This presentation will specifically report on two sets of results from the overall study:

**Set 1:** Assessments of suitability and number of contributors—data analyzed include over 2,100 assessments of suitability and number of contributors reported by 134 participants: Participants submitted responses regarding whether an assigned mixture was suitable, any factors that indicated the mixture was not suitable, the estimated number of contributors in a mixture, and any factors that affected this estimate. During this presentation, we will report on: (1) the variability of assessments of suitability, (2) the accuracy of assessments of the number of contributors, (3) the variability of assessments of number of contributors, and (4) the extent to which any variation can be reasonably explained by differences in laboratory standard operating procedures.

**Set 2:** Comparison and statistical analysis results—data analyzed include over 650 comparisons and statistical analyses (if applicable) reported by approximately 100 participants: Participants submitted responses regarding their comparisons (the categorical conclusion that would be reported with respect to the POI) and their statistical analyses with respect to the POI (the statistical values computed using each population database routinely used in operational casework). During this presentation, we will report on: (1) the accuracy of reported categorical conclusions, (2) the prevalence of adventitious likelihood ratios and other misleading statistics obtained, (3) the observed level of variation in the statistical responses, and (4) the degree to which sample-specific and/or procedure-specific factors explain any observed variation.

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**DNA Mixture; Mixture Interpretation; Inter-Laboratory Variation**

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**B53     Selectively Analyzing and Interpreting DNA From Multiple Donors With a Full Single-Cell Strategy**

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**Learning Objective:** This presentation will discuss the potential associated with single-cell pipelines for solving the complex DNA mixture problem. This research could eventually help the forensic science community tackle the interpretation of mixtures from casefiles with complex or uncertain contexts.

**Impact Statement:** Developing a single-cell forensically relevant pipeline will help solve the DNA mixture conundrum in that it can provide full mixture deconvolution without the unwanted effects associated with delineating what range of propositions to apply. Not only can it, theoretically, provide full-profile information without interference effects from the other contributors, but it also negates propositional or contextual effects since in no case is a co-contributor assumed.

Interpreting mixture samples from a bulk pipeline is arduous since the signal is a cacophony of low-fidelity fluorescence from noise, artifact, and allele signal from an unknown number of contributors. The resultant Electropherograms (EPGs) are sometimes so complex as to require significant computational power to complete the interpretation. An alternate to the bulk-processing pipeline is a single-cell one, where the sample is collected, and each is sequestered. The DNA is then extracted, amplified, and electrophoresed. The result is n single-source single-cell EPGs from n cells. Efficient direct-to-PCR treatments compatible with downstream processes are needed if single-cell forensics are to translate to operations.

First, we improved single-cell EPG's (scEPGs) qualities—measured by peak height, allele drop-out, and scEPG sloping—by applying a direct-to-PCR laboratory pipeline that incorporated phosphate buffer saline, Proteinase K, and DTT at concentrations significantly less than those applied to bulk procedures. At optimal conditions, a significant 77% of the scEPGs exhibited at least a 50% allele detection rate and a median peak height of 550 RFU. In addition to examining the effects of candidate laboratory treatments, we confirmed the signal quality is unchanged between manual and semi-automated processes while exploring signal quality with respect to cell type.

Next, we explored a two-stage interpretation strategy that clusters scEPGs into distinct groups and calculates a Likelihood Ratio (LR) for all scEPGs in that cluster. Since this LR assumes the group of scEPGs are of the same individual, effective clustering and good scEPG quality are paramount. Gaussian Mixture Models (GMM) as implemented in Mclust was found to be an effective unsupervised learning approach since it simultaneously determined the number of clusters and assigned a group index to each scEPG. We report on the efficacy of GMMs to accurately cluster on a set of blood or epithelial cell admixtures consisting of two to five persons. Once EPGs with poor total signal were excluded, GMM was deemed fit-for-purpose, offering high-fidelity clustering into groups, with early results showing that the propensity for GMMs to render clusters with scEPGs from more than one contributor is rare (< 0.1% across all mixtures tested).

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**Single-Cell Analysis; DNA Mixture Interpretation; Predictive Modeling**

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**B54 Using a Non-Destructive Method to Predict DNA Yield and the Presence of a Mixture in Touch DNA**

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**Learning Objective:** Attendees will learn how anti-testosterone/DHT and anti-cytokeratin staining patterns could be used as an indicator of a mixture sample and estimate DNA yield prior to STR analysis in touch evidence. Informative data and interpretation of results will be presented, including histograms of fluorescence shift of single source and mixture samples, correlation coefficients for DNA yield vs. fluorescence intensity, and STR profiles of mixture samples to compare to their respective histograms. An emphasis will be placed on the objective data obtained and how it can be used for future research and implementation in the forensic science community.

**Impact Statement:** This study will aid in the development of a novel technique to non-destructively estimate DNA quantity in touch DNA samples as well as determine if there is more than one contributor prior to STR analysis. This method would provide additional information to the analyst while preserving valuable evidence to determine the most effective method of extraction, purification, and analysis. Additionally, this method could assist in sample triaging in order to spend time and resources only on the most probative evidence available.

Due to advancements in DNA analysis technology and an increased ability to detect trace quantities of low template DNA, “touch” DNA has become a prevalent form of evidence for forensic investigations. “Touch” or trace DNA is biological material left on a surface from skin cells after being touched or handled. Applying a method to non-destructively screen “touch” DNA to predict DNA yield and whether multiple contributor cell populations are present in the evidence prior to STR analysis could improve the success rate of casework and prevent the consumption of limited sample that would fail with contemporary technologies. Current methods of touch DNA analysis require quantitation of the sample with qPCR followed by STR analysis, however, the low template DNA may yield allele and locus drop-out or complex mixtures resulting in an unusable profile or reduce the probative value of the evidence. Androgens, such as Testosterone (T) and Dihydrotestosterone (DHT), make a feasible biological marker for pre-DNA analysis touch sample characterization. This is due to the epidermis being a target tissue for testosterone action and detected levels varying between individuals. Additionally, the high expression of Cytokeratin (CK) proteins in epithelial cells makes them a suitable candidate for human individualization. CK proteins and the hormones, testosterone and DHT, are exceptionally stable, proving to be a robust and reliable target to study and characterize touch evidence.

This study evaluated the following aspects of “touch” sample pre-DNA characterization: (1) variation in antibody binding efficacy among ten individuals using fluorescently tagged probes targeting either CK proteins or testosterone and DHT; (2) the correlation between the quantity of DNA measured in the “touch” samples versus the binding efficiency of T/DHT or CK probes; (3) the ability to distinguish between a single source “touch” donor versus mixtures of two, three, or four donors using antibody staining patterns; and (4) correlation between antibody probe staining patterns and contributor ratio in two person mixtures.

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**Touch DNA; Flow Cytometry; Antibody Hybridization**

## **B55 Is This Fingerprint Suitable for Dactyloscopy or Genotyping? It Depends on the Minimum Surface Requirement (MSR)!**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the educated option of favoring the retrieval of DNA from a fingerprint over collecting it for dactyloscopic examination.

**Impact Statement:** This presentation will impact the forensic science community by providing a new methodology to determine the minimum latent fingerprint surface extension required for successful DNA genotyping.

The simultaneous, and successful, collection of a fingerprint ridge pattern and the respective isolation of its human DNA may be a difficult undertaking at a crime scene. This dual procedure can become a challenging endeavor due to the destructive nature of the DNA recovery methods and the potential contamination from the fingerprint visualization technique employed. For these reasons, crime scene investigators are usually encouraged to recover both types of evidence from different sites or are inclined to favor the recovery of the fingerprint pattern over the isolation of DNA. Oftentimes, fingerprints hold little discriminatory power due to the insufficient extension of individualizing ridge pattern areas or the inadequate low visual quality of such patterns. In these cases, a proper DNA collection, an efficient DNA extraction, and a powerful target Short Tandem Repeat (STR) amplification could lead to a high-quality DNA profile suitable for yielding a genetic identity.<sup>1-3</sup> Indeed, it has been proven that a complete STR genetic profile, appropriate for forensic purposes, can be obtained from a touch DNA sample.<sup>4,5</sup>

To date, several experiments have been conducted to investigate how fingerprint visualization techniques may affect the recovery of human touch DNA. However, no research has yet explored the (potential) quantity of DNA that could be isolated per area extension of a ridge pattern. In this current study, the authors have standardized the fingerprint deposition and DNA collection methods to determine the Minimum ridge Surface extension Requirement (MSR) to be able to obtain a meaningful human STR genotype. A total of ten participants, both male and female, were asked to provide ink prints of their index, middle, and ring fingers from both hands on a white paper. The goal was to calculate an average finger size to create a standard area of analysis for all fingerprints. Because of the elasticity of the skin, three pressure ranges were applied based on literature: low (180–220g), middle (480–520g) and high (780–1,000).<sup>6-8</sup> Each participant's ink prints were deposited in triplicate for each pressure range using an electronic scale. This process allowed the fingerprint area of analysis to be normalized across all donors. Then, a cutout paper mold was prepared that could hold every participant's fingers, letting the same size of fingerprints be imprinted onto any surface placed underneath it.

After area standardization, participants were asked to wash their hands with water and soap and allowed to naturally "recharge" for five minutes while applying gentle finger, neck, and forehead tapping to homogenize skin/sweat compounds. Fingerprints from each hand were deposited onto glass through the cutout mold in a cumulative fashion where a half, one, and three finger impressions were made applying middle pressure for 60s. DNA extraction was conducted by using the Qiagen® QIAamp® DNA mini kit.<sup>9,10</sup> DNA quantitation was run on a 7500 Real-Time PCR System by using the Human Quantifiler® kit by Applied Biosystems™. DNA concentration per cm<sup>2</sup> of deposited fingerprint was calculated before samples were amplified by AmpFLSTR™ Identifier™ 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 from AppliedBiosystems®. This method for DNA collection was an improvement from the most commonly used protocol: the double swabbing technique.<sup>11</sup> Preliminary results showed that human DNA quantities increased by combining up to three finger depositions. In addition, meaningful genetic STR profiles were produced, although they are still under examination. The final results of this experiment will provide key knowledge on the MSR and how it may assist crime investigators in prioritizing dactyloscopic, genotyping, or both analyses concurrently when examining latent fingerprints.

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### **DNA; STRs; Fingermarks**

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**B56 Using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) to Characterize Circulating Free DNA (cfDNA) Interactions With Metal Surfaces to Optimize the Collection and Processing of Touch DNA Samples**

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**Learning Objective:** The goal of this presentation is threefold: demonstrate to attendees the conformational changes to the DNA helix when bound to copper, brass, and stainless steel, as demonstrated via ATR/FTIR spectroscopy; evaluate the collection efficacy of swabbing, tape-lifting, and the Microbial Vacuum (M-Vac®) Wet-Vacuum System on cellular and cfDNA; and evaluate a separation method for cellular and cfDNA fractions of touch samples for more effective extraction and purification. After attending this presentation, attendees will better understand the nature of touch DNA and its interactions with transition metals and alloys thereof, as well as gain a better understanding of effective collection and extraction methods for touch DNA on metal surfaces.

**Impact Statement:** This presentation will impact the forensic science community by proposing a decision matrix for collecting and extracting touch DNA from metal surfaces, partially fulfilling a need identified by OSAC as of May 2021. By comparing three metals and three collection techniques, investigators can better evaluate the nature of the evidentiary items left behind at the scene of a crime to determine what approach needs to be taken in order to collect the maximum quantity of DNA possible. Additionally, this presentation will evaluate a novel extraction method for touch DNA samples by separating the cellular and cell-free components for downstream processing such as quantitation, amplification, STR separation and detection, and subsequently, interpretation. The proposed workflow for each sample and metal type takes into consideration the nature of the biological material deposited and its relationship to the substrate and can therefore increase DNA recovery and STR profile quality for those typically more challenging evidentiary items thought to contain trace amounts of DNA.

While several improvements have been made in recent years to optimize the recovery of touch DNA, relatively little research has been conducted to understand the relationship between trace amounts of DNA and the binding affinity of that DNA to metal substrates. Furthermore, characterization of cell-free DNA's (cfDNA) contribution to touch samples and those cfDNA-metal interactions from objects commonly identified at crime scenes, such as cartridge casings, knives, and doorknobs, have been lacking. Research has identified the tendency of transition metals to bind with the nitrogenous bases and the phosphate backbone of DNA, specifically demonstrated in the intercalation of metal ions into the B-DNA helix, ultimately degrading the sample, as well as other damaging conformational changes resulting from a lengthened phosphate backbone. These effects have been observed in aqueous solutions under physiological conditions; yet virtually no examples of this phenomenon exist out of solution. In recent years, collection techniques, such as the double-swab method and the microbial vacuum (M-Vac®) wet-vacuum DNA collection system, have been developed to optimize the recovery of trace DNA from various substrates. Tape-lifting and soaking methods have also been used to retrieve DNA from small metal items (e.g., cartridge casings and projectiles); however, large metal items have still proven to be problematic due to their size, shape, and the volume of buffer required to retrieve sufficient DNA for analysis. It persists, however, that these techniques have been developed in order to retrieve as much cellular material, and therefore, nuclear DNA (nDNA) as possible; yet, it has recently been demonstrated that circulating cfDNA comprises the majority of DNA in a touch sample. Therefore, maximizing the amount of cfDNA acquired from metal surfaces presents itself as an additional consideration in the collection and extraction process.

To generate an optimized workflow regarding collection and extraction procedures for touch DNA from copper, brass, and stainless-steel surfaces, the binding effects of metal ions with cfDNA, as well as the composition of touch DNA samples, need to be further evaluated. As such, a three-part study was developed to illustrate the structural effects of the metal ions on the DNA helix (out of solution), as well as to characterize the composition of touch DNA, including its cellular and cfDNA fractions. Each fraction's contribution to an STR profile was also evaluated. The first was conducted to determine if cfDNA experienced the same binding phenomenon to copper, brass, and stainless-steel sheet metals as observed *in vitro*, evaluated via a PerkinElmer® Spectrum Two™ FTIR with the resulting spectra processed using RStudio®. Next, the efficacy of three DNA recovery methods (swabbing, tape-lifting, and the M-Vac® wet-vacuum DNA collection system) was tested for both cellular and cfDNA deposited on the copper, brass, and stainless-steel sheet metal. The collected and extracted DNA was quantitated using Quantifiler® Trio. Finally, a pseudo-differential extraction was performed separating cellular DNA from cfDNA components of touch samples on glass slides to evaluate the profile quality of each fraction of the sample. The cellular fraction was extracted and purified using the Qiagen QIAamp® DNA Investigator Kit and the cfDNA was purified using QIAGEN® QIAamp® MinElute ccfDNA Mini Kit. Upon extraction and purification, the DNA was quantitated using Quantifiler® Trio and amplified using the GlobalFiler® PCR Amplification Kit. All profiles were evaluated using GeneMapper® ID-X v1.3. This study contributes to the ongoing research throughout forensic biology concerning the efficient collection of DNA at the scene and from evidentiary items and provides investigators with a framework from which to collect and process touch DNA from commonly encountered metals.

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**ATR/FTIR; cfDNA; DNA Collection and Extraction**



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**B57    Updating Research Data on Firearms Cases Using Expanded Autosomal Short Tandem Repeat (STR) Kits**

*Rachel Oefelein, MS\*, DNA Labs International, Boca Raton, FL*

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**Learning Objective:** This presentation will discuss the efforts made to expand the body of published research pertaining to firearms as it relates to the transfer, persistence, prevalence, and recovery of DNA using current autosomal STR typing systems.

**Impact Statement:** This presentation will impact the forensic science community by being of interest to laboratories looking to implement evaluative reporting.

A large body of data exists on Transfer, Persistence, Prevalence, and Recovery (TPPR) of DNA; however, much of this data is outdated, uses DNA profiling systems not used in the United States, or DNA profiling systems pre-dating the expansion of the CODIS core loci, and employs scenarios not classified as “real world.” Furthermore, there is a lack of data pertaining to TPPR in relation to several different case types, one of which is cases where an alleged firearm was handled. Multiple European bodies outline methods for evaluative reporting that would be useful in the United States for examining evidence with relevant TPPR arguments. However, the lack of data specifically relating to firearms and “real world” scenarios limits the ability of the American DNA analyst to employ evaluative reporting in casework. Firearms cases in particular are often scrutinized for the possibility of secondary transfer DNA as an explanation for the DNA profile obtained from the firearm itself.

Starting in 2020, DNA Labs International sought to conduct a series of experiments utilizing firearms specifically. Additionally, existing criminal casework data was evaluated to compare internal validation data against uncontrolled casework data. Experimental scenarios covered widely encountered hypotheses presented in court, such as firearm was wrapped in a pillowcase, firearm was wrapped in clothing, firearm was briefly handled to render it safe, the firearm was handled by the individual but at a date prior to the alleged incident, and the firearm belonged to a consensual partner. The active casework data looked at mixture proportions of contributors in cases where a firearm was an item of evidence and there was data regarding when the firearm was handled and by whom.

A summation of the final firearm experimental data will be presented. Additionally, research data being generated for evaluative reporting in other casework-type areas will be noted. Finally, the plans for database growth for evaluative reporting, an example of where this statistical analysis has been employed, and future implementation of evaluative reporting goals will be discussed.

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**Evaluative Reporting; Firearms; TPPR**

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**B58 Generating Short Tandem Repeat (STR) Profiles on a Porous Substrate Using Nile Red Detection**

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**Learning Objective:** This presentation will showcase a novel way to use Nile Red dye as an indicator for touch DNA on white cotton fabric. Additionally, a new method of processing touch DNA samples to increase DNA concentrations and final percent profiles will be presented.

**Impact Statement:** This presentation will provide a more efficient method of processing touch DNA samples to get the most cellular material off the substrate during extraction.

A consensus method for locating touch DNA on items of physical evidence does not currently exist. This poses potential difficulties for crime laboratories that receive evidence for which touch DNA needs to be located and extracted. This study utilized Nile Red dye to visualize touch DNA on white cotton fabric while simultaneously evaluating three collection methods: traditional cutting method, combination cutting method, and the M-Vac<sup>®</sup> wet vacuum method. Nile Red proved to be a viable method of visualizing touch DNA on cotton for most samples tested (50 of 60). The traditional method consistently yielded the lowest quantity of DNA (Median=0.00445ng/μl), produced the lowest percent profiles ( $\mu$ =58.35%), and had the most allelic dropout of the three collection methods in 16 of 20 samples. The combination and M-Vac<sup>®</sup> methods both outperformed the traditional method with the M-Vac<sup>®</sup> method providing the best data regarding DNA quantity, percent profiles, and allelic dropout. The combination method had the second highest quantity of DNA (Median=0.0305ng/μl), the second highest percent profiles ( $\mu$ =93.83%), and the second lowest number of total dropped alleles in 4 of 20 samples. The M-Vac<sup>®</sup> method produced the greatest quantity of DNA (Median=0.0447ng/μl), highest percent profiles ( $\mu$ =95.78%), and the lowest number of total dropped alleles in 5 of 20 samples. Conversely, the M-Vac<sup>®</sup> method produced the most non-donor alleles (94 alleles), followed by the combination method with 12 non-donor alleles and the traditional method with 8 non-donor alleles.

This study shows that utilizing Nile Red as a detection method for touch DNA in conjunction with improved collection methods is viable for detecting touch DNA on cotton fabric. Crime laboratories can immediately implement the combination method for touch DNA sample collection in lieu of traditional cutting methods without the need for additional materials.

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**Touch DNA; Nile Red; DNA Collection**

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**B59 An Automated Genotype Database for Associating Counterfeit Drug Evidence**

*Mark Perlin, PhD\*, Cybergenetics, Pittsburgh, PA; William Allan, MS, Cybergenetics, Pittsburgh, PA; Matthew Legler, BS, Cybergenetics, Pittsburgh, PA*

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**Learning Objective:** After attending this presentation, attendees will understand the utility of a probabilistic genotyping system and associated genotype database in finding associations among counterfeit drug packaging.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the application of an automated genotype database to a complex real-world dataset.

Counterfeit pharmaceuticals are a growing problem that can harm, and even kill, unsuspecting patients. Identifying counterfeits and catching counterfeiters is an important task that requires the ability to review thousands of evidence samples. Drug manufacturers have launched forensic service programs to combat the rising incidence of drug counterfeiting. However, relying on older data interpretation methods that cannot extract all identification information from DNA data does not yield useful results. Instead, an automated and informative DNA database capable of comparing hundreds or thousands of DNA items is required. Cybergenetics TrueAllele® database is an automated probabilistic genotyping system that can infer and genotype.

A drug manufacturer provided Cybergenetics with a drug packaging dataset. The study reassessed evidence items collected from November 2018 through May 2020. On May 27, 2020, Cybergenetics received the dataset of 103 package evidence items. Using the TrueAllele® system, Cybergenetics automatically separated the genotypes in each mixture and uploaded them to a TrueAllele® database. The TrueAllele® database then automatically compared the genotypes to produce DNA match statistics. On June 3, 2020, after completion of genotype production and comparison, the forensic company sent a spreadsheet of the log(LR) match statistics to the manufacturer. The database comparisons produced 408 item-to-item DNA connections between the 103 package evidence items, for a total of 723 DNA matches. Each connection showed a match statistic of at least a million. Further review of the match statistics revealed an informative cluster of package-to-package DNA matches for a specific cancer drug. On one specific package (Item A), a profile was seen with an expected match statistic of a nonillion (1,030). Previous analysis had deemed the item as “Not Searchable Mixture.” TrueAllele® calculated an association between this item (Item A) and another item (Item B) of a nonillion (1,030). Items A and B were both found to be associated with a third Item C with match strengths of 10 octillion (1,028) and 100 sextillion (1,026), respectively. All three packages were prepared by different lab scientists, and all three contained DNA from the same unknown person. The extent of this cluster proved to be 25 packages of the specific cancer drug, spanning 21 counterfeiting cases, with 265 package-to-package DNA matches. From this cluster, joint analysis developed a CODIS-searchable profile with an expected database match statistic of ten billion (1,010). Reviewing all DNA matches for informative clusters revealed four additional clusters.

Cybergenetics ultimately produced six DNA profiles that were suitable for searching on current CODIS-style allele databases. To summarize, Cybergenetics was able to fully interpret one-and-one-half years’ worth of data once a week. The TrueAllele® database found 723 counterfeit package DNA matches and produced six CODIS-searchable profiles. This automated processing did not require much manual data review. All DNA genotype inference and comparison was conducted automatically on the TrueAllele® database. Previously unsolved mixtures yielded informative solutions. An automated and informative DNA database can catch drug counterfeiters and save patients’ lives. Moreover, this technology can be extended to other big DNA data problems, such as organized retail theft and illicit drug production.

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**DNA Database; Genotypes; Counterfeit Drugs**

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**B60 Standards Development Activities in Firearm and Toolmark Examination**

*Robert Sanger, JD\*, Sanger Swysen & Dunkle / Santa Barbara College of Law, Santa Barbara, CA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards related to the examination of firearms and toolmarks.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to firearms and toolmarks.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created by the National Institute of Standards and Technology (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 Academy Standards Board (ASB) was formed by the American Academy of Forensic Sciences (AAFS) and is recognized as an SDO to accomplish the task of completing and publishing Standards, Best Practice Recommendations, and Technical Notes.

The Firearms and Toolmarks Consensus Body (CB) was created by the ASB. The Firearms and Toolmarks Consensus Body is comprised of representatives of the various stakeholders in the firearms and toolmarks forensic discipline. It includes analysts, academics, prosecution and defense lawyers, industry representatives, and related forensic scientists.

The Firearms and Toolmarks Consensus Body meets regularly and has done so over the past several years. The CB does most of its work through committees that meet on specific work proposals. Those proposals generally are originated by the Firearms OSAC, which promulgates draft standards. The Consensus Body reviews the proposed draft standards, submits them for rigorous discussion and review by the relevant committee members and by the Consensus Body itself. The proposed standards are then circulated for public comment and often return for several rounds of discussion and revision.

During this presentation, updates related to firearms and toolmark evidence will be discussed. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC proposed standards; (3) published standards from the (ASB) that have yet to go through the Registry approval process; (4) documents currently in development at the OSAC or by the ASB; and (5) priorities for new documents or work proposals, and other highlights.

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**Firearms and Toolmarks; Academy Standards Board; Consensus Body**

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**B61 Standards Development Activities in Trace Materials**

*Celeste Grover, MSFS\*, Oregon State Police\*, Clackamas, OR*

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**Learning Objective:** 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.

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.

During this presentation, updates related to standards development activities in trace materials will be discussed (standards were placed in categories based on their status projected for February 2023. Thus, the status of a given document may differ at the time of the presentation). These include:

1. Recent standards that have been added to the OSAC Registry (published)
  - a. E3294 Standard Guide for Analysis of Forensic Geological Materials by Powder X-Ray Diffraction
  - b. E2809 Standard Guide for Using Scanning Electron Microscopy/X-ray Spectrometry in Forensic Polymer Examinations
  - c. E3296 Standard Guide for Using PyGC-MS in Forensic Polymer Examinations
2. Recent standards that have been added to the OSAC Registry (proposed—pending SDO approval)
  - a. Standard Practice for a Forensic Fiber Analysis Training Program
  - b. Standard Guide for Forensic Fiber Analysis and Comparison
  - c. Standard Guide for Forensic Analysis of Fibers by Microspectrophotometry
  - d. Standard Guide for Forensic Physical Fit Examinations
3. Published ASTM standards going through the Registry approval process
  - a. E2224 Standard Guide for Forensic Analysis of Fibers by IR
  - b. E2225 Standard Guide for Forensic Examination of Fabrics and Cordage
  - c. E2227 Standard Guide for Forensic Examination of Non-reactive Dyes in Textile Fibers by Thin Layer Chromatography
  - d. E2228 Standard Guide for Microscopic Examination of Textile Fibers
  - e. WK70035 Standard Practice for Determination and Comparison of Color by Visual Observation in Forensic Soil Examination
  - f. WK72932 Standard Guide for Forensic Glass Analysis and Comparison
  - g. WK56743 Standard Practice for Training in the Forensic Examination of Hair by Microscopy
  - h. WK72597 Standard Guide for the Forensic Examination of Hair by Microscopy
  - i. WK74138 Standard Guide for Using X-ray Fluorescence in Forensic Polymer Examinations
4. Standards going through ASTM process (five-year review)
  - a. E2926 Standard Test Method for Forensic Comparison of Glass Using Micro X-ray Fluorescence (u-XRF) Spectrometry
  - b. E2927 Standard Test Method for Determination of Trace Elements in Soda-Lime Glass Samples Using Laser Ablation Inductively Coupled Plasma Mass Spectrometry for Forensic Comparisons
  - c. E3085 Standard Guide for Fourier Transform Infrared Spectroscopy in Forensic Tape Examinations
5. Standards going through STRP/non-STRP process
  - a. Standard Practice for a Forensic Glass Analysis Training Program
  - b. Standard Guide for Interpretation and Reporting in Forensic Comparisons of Trace Materials
6. Documents currently in development with the subcommittee
  - a. Standard Guide for Using Scanning Electron Microscopy for Morphology and Energy Dispersive Spectroscopy in Forensic Soil and Geological Materials Analysis
  - b. Standard Guide for the Use of Polarized Light Microscopy in the Forensic Examination and Comparison of Sands and Soils
  - c. Standard Guide for Physical Fit Training Program
  - d. Standard Guide for Using X-ray Diffraction in Forensic Polymer Examinations
  - e. Standard Guide to Raman Spectroscopy in Forensic Polymer Examinations
  - f. Standard Guide for Assessing Physical Characteristics in Forensic Tape Examinations
  - g. Standard Guide for the Recovery of Trace Evidence
7. Priorities for new documents or work products
  - a. Development of technical guidance related to fluorescence microscopy of fibers
  - b. Interdisciplinary development of technical guidance related to the examination of General Unknowns

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**OSAC; Trace Materials; ASTM**

## B62 Standards Development Activities Related to Ignitable Liquids, Explosives, and Gunshot Residue

*Brenda Christy, MS\*, Virginia Department of Forensic Science, Norfolk, VA*

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards related to Ignitable Liquids (IL), Explosives (E), and Gunshot Residue (GSR).

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities related to IL, E, and GSR.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, IL, E, and GSR standards development activities will be discussed. These include

1. Recent standards that have been added to the OSAC Registry
  - a. ASTM E2451-21 Standard Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples (added April 5, 2022)
  - b. ASTM E3196-21, Standard Terminology Relating to the Examination of Explosives
  - c. ASTM E3253-21, Standard Practice for Establishing an Examination Scheme for Intact Explosives (added July 5, 2022)
  - d. ASTM E3329-21, Standard Practice for Establishing an Examination Scheme for Explosive Residues (added July 5, 2022)
  - e. ASTM E3309-21, Standard Guide for Reporting of Forensic Primer Gunshot Residue (pGSR) Analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray Spectrometry (SEM/EDS)
2. OSAC proposed standards
  - a. Standard Practice for the Classification for Ignitable Liquids Encountered in Fire Debris Analysis
  - b. Standard Test Method for Interpretation of Gas Chromatography-Electron Ionization Mass Spectrometry Data for the Identification of Ignitable Liquid Classes in Fire Debris Analysis
  - c. Standard Practice for the Analysis of Organic Gunshot Residue (oGSR) by Gas Chromatography - Mass Spectrometry
  - d. Standard Practice for the Analysis of oGSR by Liquid Chromatography - Mass Spectrometry
  - e. Standard Practice for the Collection of Primer Gunshot Residue (pGSR) Particles from Clothing, Vehicles, and Other Objects using Adhesive Lifts
3. Published standards from the ASTM that have yet to go through the Registry approval process
  - a. ASTM E2881-18 Standard Test Method for Extraction and Derivatization of Vegetable Oils and Fats from Fire Debris and Liquid Samples with Analysis by Gas Chromatography-Mass Spectrometry.
  - b. ASTM E2998-16 Standard Practice for Characterization and Classification of Smokeless Powder
  - c. ASTM E2999-17 Test Method for Analysis of Smokeless Powder by Gas Chromatography-Mass Spectrometry and Fourier-Transform Infrared Spectroscopy
  - d. REVISION to ASTM E1386 Standard Practice for Separation of Ignitable Liquid Residues from Fire Debris Samples by Solvent Extraction (under revision as WK78319)
  - e. REVISION to ASTM E2997-16 Standard Test Method for Analysis of Biodiesel Products by Gas Chromatography-Mass Spectrometry (under revision as WK78732)
4. Documents currently in development at the OSAC or by the SDO
  - a. WK75150 Standard Practice for Training in the Forensic Examination of Fire Debris
  - b. Standard Practice for the Forensic Analysis of Explosives by Polarized Light Microscopy
  - c. Standard Practice for the Characterization of Solid Oxidizer/Fuel Explosive
  - d. WK72856 Standard Practice for Collection and Preservation of oGSR
  - e. WK72526 Standard Practice for Expert Opinions on the Interpretation of pGSR by SEM/EDS
  - f. WK58457 Standard Practice for Training in the Forensic Examination of GSR Using SEM/EDS
5. Priorities for new documents or work products
  - a. Fire Debris and Explosives annexes for ASTM E2549
  - b. Standard Practice for Validation and Verification of Analytical Methods for Forensic Science Service Providers Performing Forensic Chemistry Analysis
  - c. Standard Guide for the Performance of Automated pGSR Analysis by SEM/EDS
  - d. Standard Guide for Reacquisition of pGSR Particles by SEM/EDS
  - e. Standard Guide for the Classification of pGSR particles by SEM/EDS analysis and other highlights

### **Ignitable Liquids; Explosives; Gunshot Residue**



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**B63 Standards Development Activities Related to the Examination of Friction Ridge Detail**

*Heidi Eldridge, PhD\*, The George Washington University, Washington, DC*

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**Learning Objective:** 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:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to the examination of friction ridge detail.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was 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.

The Academy Standards Board (ASB) is an SDO created by the American Academy of Forensic Sciences (AAFS) to provide standards development activities related to standards and best practices in the forensic sciences. Its Friction Ridge Consensus Body currently receives draft standards and Best Practice Recommendations (BPRs) from OSAC to undergo this process prior to being released as standards to the community. The ASB Friction Ridge Consensus Body can also choose to work on New Work Products of their own devising.

During this presentation, updates related to friction ridge standards development activities will be presented. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC proposed standards that are currently published on the OSAC website in draft form; (3) published standards from the Academy Standards Board (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 products.

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, what the near future looks like for the promulgation of friction ridge standards and guidelines, and a brief review of some of the main points of the most impactful documents currently in development.

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**Friction Ridge; Standards; OSAC**

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**B64 Current and Future Standards Development Activities in Seized Drugs**

*Agnes Winokur, MS\*, DEA/Southeast Laboratory, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the current status and future path of discipline-specific standards pertinent to the examination of seized drugs. Attendees will gain awareness of how the development of standards for seized drugs are prioritized and how gaps in the standard development process are identified.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to the examination of seized drugs. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding of the role that standards in seized drugs have in laboratories.

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 drafts standards that are forwarded to the standards developing organization, ASTM International, to further develop and publish them. The OSAC Seized Drugs Subcommittee 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.

During this presentation, the presenter will show how the newly created process map for the analysis of seized drugs is utilized to not only prioritize the development of specific standards, but to identify gaps in the standards development process for seized drugs.

In addition, updates related to the standards development in seized drugs will be presented. These include: (1) recent standards that have been added to the OSAC Registry or as OSAC proposed standards (Standard Practice for the Differentiation of Marijuana and Hemp in Seized Drug Analysis and Standard Guide for Court Testimony in Seized-Drug Analysis); (2) documents currently in further development at ASTM (Practice for Education and Training of Seized-Drug Analysis, Guide for the Analysis of Seized Drugs Using Fourier Transform Infrared [FTIR] Spectroscopy, Guide for Analysis of Seized Drugs Using Gas Chromatography and Electron Ionization Mass Spectrometry, Guide for The Development of Electron Ionization-Mass Spectral Libraries for the Analysis of Seized Drugs, Interdisciplinary Standard for Validation and Verification, Practice for the Uncertainty Assessment in Qualitative Measurements in the analysis of Seized-Drugs); (3) new and on-going documents currently in development at the OSAC seized drugs subcommittee (Guide on Reporting in Seized-Drug Analysis, Practice in Evidence Handling of Seized Drug Evidence, Guide for Recognizing New Psychoactive Substances and True Unknowns in Seized Drug Analysis, Test Method for the Analysis of Seized Drugs Using Color Tests, Guide for the Assessment of Structural Similarity of Substances); and (4) 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.

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**Seized Drugs; Standards; Forensic Science**

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**B65 The Development of a Standard Guide for the Use of X-Ray Diffraction in the Forensic Examination of Geological Materials**

*Libby Stern, PhD\*, FBI Laboratory - CFSRU, Quantico, VA; Ian Saginor, PhD, FBI Laboratory, Quantico, VA; Alastair Ruffell, PhD, Queen's University, Belfast, Belfast, NORTHERN IRELAND*

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**Learning Objective:** This presentation will summarize the newly published ASTM standard guide for use of powder X-ray diffraction in forensic examination of geological materials (E3294). The presentation will review the standard development process and summarize the recommended adaptations of established geoscience methods toward characterization geological material examined as trace evidence in criminal investigations.

**Impact Statement:** E3294 is the first ASTM-International (ASTM) standard published to recommend laboratory methods for the examination of geological materials as trace evidence in the context of criminal investigations. While XRD is only one of an array of methods commonly used in the characterization examination of forensic geological materials, this guide describes an approach to the interpretation of Powder X-Ray Diffraction (XRD) patterns of soil evidence within an examination scheme aimed at sample comparison.

ASTM E3294-22 Standard Guide for Forensic Analysis of Geological Materials by Powder X-Ray Diffraction originated from the Organization of Scientific Area Committees (OSAC) for Forensic Science task group on geological materials and was further developed in ASTM-International E30.01/E30 and with input from the International Union of Geological Sciences-Initiative on Forensic Geology (IUGS-IFG). At the time of drafting this abstract, ASTM E3294 had not yet been placed on the OSAC Registry of approved standards.

The goals of examination of geological materials in a criminal forensic context include identification of an unknown material, comparison of geological material (typically soil) on an object or person to geological material at a crime scene, and examination of geological material to provide geographic constraints of region of origin. Mineral identification in geological materials by XRD can assist in any of these three goals but is typically employed in parallel with a range of additional characterization methods.

XRD is a widely used technique for the characterization and identification of crystalline materials alone or in multi-component mixtures. XRD has been in use for over a century for mineral identification, and there have been many studies of the reliability of its use. Because of the strong foundational science in XRD, the new guide, ASTM E3294-22, recommends adaptations of sample preparation suitable for samples of limited quantity typically encountered with forensic soil evidence and describes approaches to interpreting XRD patterns within the context of a forensic comparison of materials. This guide recommends minimum criteria for mineral identification by comparison to reference diffraction patterns, in the absence of supporting information.

**Sample preparation:** The guide describes ideal sample preparations for XRD, but because typical forensic samples are insufficient for optimal sample preparation, the guide recommends adaptations, including placement of trace material on “zero background” substrates; *in situ* analyses (material adhered to fabric or item), potential use of parallel beam optics, or using specialized sample holders for micro XRD.

**Mineral ID criteria:** This guide recommends minimum criteria for mineral identification by XRD but permits provisional identification when these criteria are not fully met. Mineral ID from diffraction patterns not meeting the full ID criteria can be confirmed by orthogonal methods, including SEM-EDS, PLM, or Raman spectroscopy.

**Framework for sample comparisons by XRD:** Examinations of forensic geological materials typically employ multiple analytical techniques. When a technique demonstrates differences between two samples being compared that cannot be explained by the history of the samples or the representativeness of the known exemplar, these differences can be used to exclude a common source of the two samples. XRD, in isolation, can be used to document exclusionary differences (typically the presence of a mineral of moderate abundance present in one sample but absent in another or major minerals present in substantially distinct relative abundance). Unconsolidated geological materials can be fractionated, altered, or mixed prior to analysis, so some observable differences may be explainable and may not be exclusionary.

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**X-Ray Diffraction; Geological Materials; Standards Development**

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**B66 Seized Drugs Process Map**

*David Gouldthorpe, MS, LVMPD, Las Vegas, NV; Melissa Taylor, BS, MD\*, National Institute of Standards and Technology, Gaithersburg, MD; Heather Waltke, MFS, MPH\*, National Institute of Standards and Technology/Stratavia\*, Crompond, NY*

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**Learning Objective:** The National Institute of Standards and Technology (NIST) through its Organization of Scientific Area Committees (OSAC) has been working with practitioners in various forensic science disciplines to create process maps that identify key decision points during the forensic examination process. These process maps enable laboratory management to better understand how their protocols compare with those of other laboratories and to provide a framework for developing standard operating procedures, best practice documents, and quality assurance measures. In this presentation, we will discuss the development process and guide the attendees through the steps involved in the seized drugs process map. The development of the Seized Drugs process map was accomplished by a task group that included representatives from federal, state, and local forensic laboratories.

**Impact Statement:** Attendees will understand the purpose and value of process mapping, how process maps can be used to inform contextual information management and cognitive bias reduction strategies, and how they can aid in the standardization of forensic processes and terminology.

In this presentation, we will discuss the methodology used to create process maps, guide the participants through the various decision points that are essential to the forensic discipline of Seized Drugs, and go into detail on how and where to start the creation of a process map in reference to the analysis of seized drugs. This process map will portray the workflow from when the suspected seized drugs evidence is received by the analyst all the way to the disposition of the evidence and generation of a formal laboratory report. Additionally, we will discuss the different analytical schemes associated with seized drugs analysis in a forensic laboratory and how they are represented on the process map. We will review the steps that were considered for these varying analytical schemes and explain how the task group worked through the differences between laboratories to find a universal process map design that can be used by any laboratory. We will also discuss the challenges that arose when creating a general process map designed to be applied to different analytical schemes.

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**Process Mapping; Seized Drugs; NIST**

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**B67    An Inter-Laboratory Study to Assess Practical Utility of the Organization of Scientific Area Committees (OSAC) -Proposed Standard 2021-S-0006 (Standard for the Use of GenBank for Taxonomic Assignment of Wildlife)**

*Khushi Patel, North Carolina State University, Garner, NC; Melissa Scheible, MSF, North Carolina State University, Raleigh, NC; Kelly Meiklejohn, PhD\*, North Carolina State University\*, Raleigh, NC*

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**Learning Objective:** After attending this presentation, attendees will understand (1) the main steps involved in completing DNA-based taxonomic assignment for wildlife forensic applications, (2) the criteria outlined in OSAC proposed standard 2021-S-0006 for taxonomic assignment, and (3) the outcomes of the inter-laboratory study.

**Impact Statement:** The goal of OSAC is to develop technically sound standards and promote their adoption through the forensic community. This presentation will identify the practical successes and potential shortcomings of 2021-S-0006 for the taxonomic assignment of diverse species encountered in wildlife forensic casework. More broadly, it will provide the forensic science community with a framework for conducting inter-laboratory studies centered around an OSAC proposed standard.

Wildlife forensic laboratories are frequently asked by law enforcement to confirm the taxonomic identity of material submitted as evidence. When possible, taxonomic assignment based on morphology is preferred. However, in scenarios where an incomplete specimen is submitted, diagnostic features are lacking, or suitable comparative reference materials are not available, amplification and Sanger sequencing of informative regions of the genome is often employed. Unlike human forensic DNA analysis, a single curated reference database does not exist for comparisons for the diverse range of species encountered in wildlife forensic casework. Practitioners often rely on comparing their unknown evidence sequence to public sequence databases such as GenBank to permit taxonomic assignment.

To address the use of GenBank in forensic casework, the OSAC Wildlife Forensic Biology subcommittee developed 2021-S-0006: Standard for the Use of GenBank for Taxonomic Assignment of Wildlife (currently an OSAC Proposed Standard). To address the practical utility of 2021-S-0006, an inter-laboratory study was conducted. To execute this, Sanger sequence data for informative mitochondrial loci were generated from 40 diverse wildlife species encountered in forensic casework (primarily encompassing mammals, avians and reptiles). Eleven wildlife forensic laboratories from across the globe were recruited to participate and were sent ten unknown sequences to analyze using 2021-S-0006. Taxonomic assignments generated by each laboratory were compared: (1) to the true identity to determine correctness, and (2) across laboratories to assess congruence (each sample was analyzed by at least two laboratories). Results were also independently evaluated to determine whether laboratories were interpreting the criteria of the standard correctly and reporting to the appropriate taxonomic level (i.e., species level or higher). When following 2021-S-0006, laboratories that reported to the species level were correct in 98.4% of cases and high congruence was observed between laboratories.

In this presentation, details on the outcomes of the inter-laboratory study will be presented along with lessons learned for conducting inter-laboratory studies.

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**Inter-Laboratory Study; Wildlife Forensics; OSAC Proposed Standard**

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**B68 An Update on the Status of the Department of Justice, Office of Justice Programs, National Institute of Justice (NIJ), National Center on Forensics (NCF) Grant**

*Joseph DiZinno, DDS\*, George Mason University, Alexandria, VA*

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**Learning Objective:** After attending this presentation, attendees will understand the progress that has been made to address the goals of the NIJ NCF grant listed below:

- Providing medicolegal learning opportunities for medical students to train as deputy medical examiners/coroners in underserved rural areas
- Providing forensic science and legal training to prosecutors, judges, and law enforcement
- Developing opportunities as appropriate among the designated partners to benefit current and future practitioners in the field

**Impact Statement:** This presentation will present detail on the medico-learning opportunities created and the forensic science training topics most requested by prosecutors, judges, and the law enforcement community to include forensic laboratory personnel.

George Mason University and its partners, the National Association of Attorneys General, the American Society for Clinical Pathology, and the Montana Forensic Science Division along with our affiliates at the University of Washington Medical & Law Schools were awarded the three-year, \$2 million NIJ NCF grant in January 2021. Since that time, the grant team has distributed and collected several surveys, conducted numerous focus group sessions, collected and interpreted data from the surveys and focus groups, and created programs. The team is currently developing forensic science trainings to address the goals of the grant listed above. The presentation will present details on the medico-learning opportunities created and the forensic science training topics most requested by prosecutors, judges, and the law enforcement community, including forensic laboratory personnel.

The forensic science topics that prosecutors, judges, and the law enforcement community requested include, but are not limited to, digital forensics, basic forensic science concepts, legal issues, including pre-trial, general forensic science, and admissibility issues, crime scene processing, forensic genetic genealogy, firearms/toolmarks examinations, the cold case investigation processes, forensic document examinations, forensic toxicology examinations, blood spatter examinations, the collection and preservation of DNA evidence, DNA mixtures, DNA statistical analysis/probabilistic genotyping, basic serology, and an overview of DNA databases (CODIS) and molecular genealogy databases. In addition, the presentation will inform attendees about a recently created NIJ NCF website that will serve as a link to NIJ NCF forensic science training sessions and a repository of forensic science and legal information for the medical examiner, legal, and law enforcement communities.

As a result of this presentation, attendees will be made aware of medico-legal learning opportunities for medical students and trainings for judges, prosecutors, and law enforcement personnel. Information will also be given about plans for future grant activities, including an in-person training in 2023.

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**NIJ; National Center on Forensics; Grant Update**



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**B69 Revisiting the Essence of Forensic Science: The Sydney Declaration Coming of Age?**

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**Learning Objective:** Attendees will learn about the Sydney Declaration and appreciate how it can positively impact on the practice, education, and research and development in forensic science. Attendees will also learn about the feedback received from the forensic science community to date and the future directions of the Sydney Declaration so it can be more generally promoted and accepted.

**Impact Statement:** The presentation will impact the forensic science community by encouraging attendees to reflect on the fundamental principles enunciated in the Sydney Declaration and how they underpin the practice of forensic science and guide education and research directions by providing a solid foundation and shared understanding between all practitioners and stakeholders.

While forensic science is usually seen as a mainstay of the criminal justice system, its effectiveness and reliability have been consistently challenged over at least the past decade. The debate mainly focuses on validity, backlogs, quality management, bias mitigation, evidence evaluation, and the communication of forensic findings. Interestingly, most issues have been presented through organizational lenses (legal or various scientific disciplines) rather than from the perspective of forensic science as a discipline. Several papers and commentaries recently argued that this fundamental gap may be a root cause for many of the challenges. Reclaiming forensic science and refocusing on its foundation, including a shared understanding and broad acceptance of its essence, purpose, and fundamental principles, is necessary.

The most recent and significant effort in this area is the Sydney Declaration, which attempts to define forensic science through its foundational basis, beyond organizations, techniques, or protocols.<sup>1</sup> The Sydney Declaration has been shared with the forensic science community through conference and association presentations and workshops worldwide since its launch and publication in the peer-reviewed literature.<sup>1,2</sup> This allowed us to promote a shared understanding of forensic science and gather feedback essential for the broader acceptance and implementation of these principles.

This presentation will provide an overview and update of the Sydney Declaration, including the current feedback from the worldwide forensic science community. It will identify areas in the declaration that require refinements. It will also examine the current state of many forensic practices and map them against the Declaration (i.e., how far are we from the principles of the declaration?) Finally, it will discuss the challenges and opportunities regarding its potential operationalization. It is argued that the principles proposed in the Sydney Declaration should underpin the practice of forensic science and guide education and research directions by providing a solid foundation and improved shared understanding between all practitioners and stakeholders.

**References:**

- <sup>1</sup> Roux, C., Bucht, R., Crispino, F., De Forest, P., Lennard, C., Margot, P., Miranda M. D., NicDaeid, N., Ribaux, O., Ross, A., Willis, S. (2022). The Sydney Declaration – Revisiting the Essence of Forensic Science through its Fundamental Principles, *Forensic Science International* 332, 111182, <https://doi.org/10.1016/j.forsciint.2022.111182>.
- <sup>2</sup> <https://iafs2023.com.au/sydney-declaration/>.

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**Sydney Declaration; Forensic Science Principles; Forensic Science Practice**

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**B70 The Sydney Declaration: Should We Care About Forensic Science Principles?**

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**Learning Objective:** Attendees will learn about the Sydney Declaration and appreciate its relevance, particularly why its principles are critical to the development of forensic science in an ever-changing and complex world.

**Impact Statement:** This presentation will impact the forensic science community by encouraging attendees to reflect on the fundamental principles enunciated in the Sydney Declaration and why and how they transcend the practice of modern forensic science.

An international group of forensic scientists from various backgrounds recently revisited what forensic science encompasses and shared seven principles with the forensic science community.<sup>1</sup> While attempting to formalize the forensic science building blocks, the aim was to develop principles that could be used as anchors for a discipline that often evolves in a fragmented and uncoordinated way.

The group has been presenting these principles at various conferences to promote discussion about the principles to the broader forensic science community. A seemingly obvious question is consistently raised from the generally positive feedback received: Why and how should we practically care about these principles?

One of the arguments against them is that the practical reality in day-to-day crime scenes and laboratory operations is responding to legal requests. As a result, technical expertise, adherence to standard operating procedures, quality management, certification, and accreditation are often presented as sufficient to guide the laboratory process. Other comments suggest that the underlying scientific principles belong to core disciplines (e.g., biology, chemistry, computer science), which define specializations. These are understandable concerns in an operational environment that strives to meet demands and customer satisfaction daily. So, what is the added value of the principles expressed in the Sydney Declaration to forensic science practice? In straightforward terms, why bother?

To address this question, it is necessary to consider recent societal changes (e.g., digital transformations) and how they impact investigations and forensic science practice. Scales are changing: the traceability of human activities has increased by orders of magnitude, and the traditional framework can no longer account for these changes and respond to current and future challenges. It is hypothesized that the current vision of the forensic laboratory is becoming outdated.

The frailty of the current situation becomes evident when considering critical questions such as: Can we still claim value for the forensic science laboratory as policing and investigative environments rapidly change? If the use of instruments can be decentralized, is there still a role for the forensic science laboratory?<sup>2</sup> Does it make sense to keep all specialties under the same roof in a siloed forensic science laboratory? How do we educate to integrate people into forensic career paths that give them broader perspectives?<sup>3</sup> Why hold forensic science conferences when participants remain encapsulated in separate specialist sessions? Does research around current frameworks take into account digital transformations?

We argue that there is an urgent need to go back to fundamental principles to “re-fit” forensic science in a framework that is more fit for purpose and can facilitate the integration of emerging areas within a coherent forensic science discipline. Beyond apparent imperfections, the principles expressed in the Sydney Declaration are critical as they bring momentum and unity to the entire debate.

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1. Roux, C., Bucht, R., Crispino, F., De Forest, P., Lennard, C., Margot, P., Miranda M. D., NicDaeid, N., Ribaux, O., Ross, A., Willis, S. (2022). The Sydney Declaration – Revisiting the Essence of Forensic Science through its Fundamental Principles, *Forensic Science International* 332, 111182, <https://doi.org/10.1016/j.forsciint.2022.111182>.
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**Sydney Declaration; Forensic Science Principles; Forensic Science Purpose**

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**B71 An Exercise in Scientific Problem Solving**

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**Learning Objective:** After attending this presentation, attendees will have learned why observation and reasoning are vital parts of forensic science and how the identification, recognition, and signification of traces at the crime scene by a generalist forensic scientist ensured that the right questions were being asked in the case presented, leading to a coherent event reconstruction.

**Impact Statement:** This presentation will impact the forensic science community by illustrating how the principles of the Sydney Declaration are relevant to forensic science practice.

The Sydney Declaration articulates a set of principles that apply generally to all forensic science problems, especially complex cases.<sup>1</sup> In the most general sense, forensic science is tasked with investigating and understanding the physical aspects of events, whether they are connected with crimes or accidents. Approaches and techniques of scientific problem-solving are necessary, especially for those cases where the scientist needs to be involved in the definition of the problem—that is, cases where the question of interest is not pre-defined. The principles of the Sydney Declaration are particularly helpful in introducing the relevance and value of scientific problem-solving in this context.<sup>1</sup> The purposes of this presentation are to highlight the importance of learning about events from careful consideration of less obvious traces; to demonstrate that in order to engage in scientific problem solving, it is necessary to apply methods of observation and reasoning in forensic investigations in a manner that can be clearly articulated; and finally, to illustrate how the Sydney Declaration principles are revealed in real-life practice.<sup>1</sup>

This presentation utilizes a case involving the shooting death of a police officer. In this case, the only account of what took place was provided by the defendant, a scenario that could not be ignored or clearly refuted by the original investigation and subsequent laboratory analyses conducted. It became apparent that the problem originally defined by investigators at the scene and later by prosecutors assigned the case was precluding what ultimately became the correct trace analysis and interpretation, further hampering efforts towards an accurate reconstruction of events. Moreover, the investigatory focus on a particular trace (blood) at the scene and an absence of asking the right questions resulted in key traces being overlooked. After careful observation and consideration of additional traces and objects suggestive of suspected activities at the event scene by a consulting generalist forensic scientist, different questions (alternative hypotheses) were presented that eventually led to a coherent reconstruction of the investigated event through the assignment of meaning (relevance) to traces that originally went unrecognized by investigators.

The case demonstrates the perils of: (1) not considering seemingly unimportant traces in favor of obvious traces; (2) requesting analyses on objects recovered from the scene without clearly defining the problem/questions to be answered; and (3) tunnel vision.

This presentation draws attention to this case with the goal of asserting the importance of the scientist at the scene at the outset to ensure a scientific approach to crime scene investigation and reconstruction, which is marked by observation and reasoning (e.g., abductive and inductive). As a complex scientific endeavor, examination of the event scene and careful evaluation of the traces contained therein required consideration of the whole and its parts—the traces and the object within the scene and their relationship to one another—with context being crucial to the critical thinking process. As such, the consulting expert was not only able to refute the account of the defendant but was able to aid in demonstrating premeditation through the assignment of meaning to the traces and objects within the event scene.

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**Sydney Declaration; Crime Scene Investigation; Generalist Forensic Scientist**

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**B72    *Ficta Vivos Docent: Learning From Detective Fiction***

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**Learning Objective:** After attending this presentation, attendees will have learned about the role that detective fiction could play in teaching a scientific approach to crime scene investigation through the study and signification of traces.

**Impact Statement:** The presentation will impact the forensic science community by encouraging attendees to reflect on how crime scene investigation is a demanding intellectual endeavor requiring the expertise of the generalist forensic scientist.

Forensic science has seen a marked evolution, both in fiction and the real world. In some instances, the expression “life imitates art” has blurred the reality and perceived capability of forensic science disciplines. The burgeoning world of true crime and “based on a true story” disclaimers at the outset of television and film narratives has fueled interest in forensic science and crime scene investigation, cultivating the idea of the real-world, amateur armchair detective. The concept of the fictional detective using clues and techniques characteristic of forensic investigations to solve crimes and identify perpetrators is hardly novel—it would be unsurprising to find that many forensic scientists and criminal investigators of the 20<sup>th</sup> and 21<sup>st</sup> centuries were influenced by some of the most well-known fictional detectives, from Edgar Allan Poe’s Dupin to Kane and Finger’s superhero Batman.

The introduction and success of Sir Arthur Conan Doyle’s consulting detective Sherlock Holmes paved the way for those murder mystery and detective fiction writers that became part of the Golden Age of Detective Fiction. The “formula” for detective fiction in this era was based on unraveling a mystery through detection of a series of subtle clues and a grand denouement by the (oftentimes) main character. Attention to detective fiction persists and remains in high demand today, with iterations of characters like Holmes and Batman starring in box-office hits through modern-day interpretations of their classic casework and investigative prowess.

Exploring and analyzing historical detective fiction through a forensic science lens is crucial to appreciating the history and development of forensic science, and such works are important to understanding the complexity of forensic investigations and the importance of events and traces (pre-event and activity-produced) in reconstructing such events. Most notably, select detective fiction from the 19<sup>th</sup> and early 20<sup>th</sup> centuries can and should be utilized to convey the idea that crime scene investigation and reconstruction (and the broader criminal investigation) are demanding intellectual endeavors that require the expertise of a generalist forensic scientist—one who is properly educated, trained, and experienced—to undertake such endeavors. By carefully reading, dissecting, and interpreting such texts, it becomes apparent that there is a complex scientific approach to crime scene investigation facilitated by the study, recognition, and signification of traces.

This presentation will draw attention to such assertions by focusing on one such short story—*The Case of Oscar Brodski* by Dr. Richard Austin Freeman, featuring fictional forensic investigator Dr. John Thorndyke. Dr. Freeman (a physician-cum-writer like Doyle) utilized his scientific knowledge to cultivate Thorndyke, a character that uses clues, reasoning, and scientific tools to investigate and reconstruct events. Moreover, Freeman has been credited with creating the inverted detective story (howcatchem as opposed to the whodunit?), another formulaic approach to crime-solving that is still used today.

This presentation will begin by describing the inverted detective story and then it will explore *The Case of Oscar Brodski* to demonstrate how the story supports a scientific methodology, or approach, to post-event reconstruction through the detection and assignment of meaning to traces (what Freeman refers to as the “mechanism of detection”). It will conclude by highlighting the importance of traces (beyond blood and fingerprints!); analytical reasoning; and the generalist forensic scientist at the scene at the outset of an investigation. This presentation will also address the pedagogical importance of detective fiction and provide suggestions for utilizing such stories to educate the modern forensic scientist, whether fledgling or experienced. The overarching goal of this presentation is to reflect on the above expression (now stated conversely) that “art imitates life,” and the scientific sleuth should be the cornerstone of real-world forensic science and scene investigations.

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**Traces; Generalist; Crime Scene Investigation**

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**B73 A Study Comparing the Use of Agilent® QP Gas Chromatography/Mass Spectrometry (GC/MS) for Screening and Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS) for the Confirmation of Smokeless Powders**

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**Learning Objective:** This presentation will demonstrate the application of the Agilent® QuickProbe® GC/MS for rapid screening of smokeless powder residues and the use of Gas Chromatography-Triple Quadrupole (GC-QQQ) MS for sensitive confirmation of trace smokeless powder residues.

**Impact Statement:** This presentation will impact the forensic science community by providing a typical Electron Ionization/GC/MS (EI/GC/MS) method for screening of smokeless powders and a sensitive GC-QQQ method for the detection of smokeless powder traces.

The goal of this project is to evaluate the use of two GC/MS techniques in a workflow for screening and confirmation using smokeless powders as the material of interest.

Analytical workflows for most chemical analysis involve a screening technique followed by a confirmation technique. The most common screening tests used for drugs of abuse and explosives are based on colorimetric detection. They do not provide structural identity and the results can be confounded in complex matrices. When sensitive mass spectrometry techniques are used, the resulting mass spectra are not searchable against existing GC/MS libraries. GC/MS is the current mainstay for separation and identification of complex mixtures. However, traditional GC/MS lacks the sensitivity required for trace analysis of some materials such as explosives. GC Tandem Mass Spectrometry (GC/MS/MS) is a powerful technique that elevates the sensitivity and selectivity obtained by traditional GC/MS instruments. This presentation discusses the analysis of smokeless powders two GC/MS techniques—one for rapid screening and the other for confirmation of trace residues.

The QuickProbe® is housed on top of the gas chromatograph on a traditional GC/MS system. Samples in liquid or solid form are introduced in the QuickProbe® injector using a direct probe or a variety of sampling techniques. The vaporized sample is carried into a short column that is rapidly heated for fast separations followed by traditional single quadrupole SIM/Scan mass spectral analysis. The resulting scan mass spectral data can be easily analyzed using existing electron ionization libraries for identification. The Selected Ion Monitoring (SIM) data helps to lower the limits of detection. Therefore, this technique could serve as a rapid and reliable screening GC/MS technique requiring little-to-no sample preparation. GC-QQQ with Single Reaction Monitoring (SRM) allows for highly sensitive and selective analysis of trace residues, as would be expected with post-blast recovery of explosives. The low detection levels also help improve the detection of thermally labile explosives such as nitroglycerin that are difficult to detect in traditional GC/MS instruments at trace levels.

Smokeless powders are low explosives that are the most commonly used propellants in firearm ammunition. They have also been used in improvised explosive devices. Smokeless powders are commonly recovered and analyzed as burnt or partially burnt particles, or as residues. Gas chromatography and infrared spectroscopy are used to analyze these materials. In this presentation, we discuss the analysis of 15 smokeless powder additives, including nitroglycerin, diphenylamine, and its nitrated derivatives, centralites, dinitrotoluenes, and phthalates. The additives were analyzed individually as chemical reference standards, and the method was verified against ten smokeless powders. The GC-QQQ method was developed with a scan method to determine precursor ions, then with product ion scan for each of the 15 additives. The method validation includes the determination of limits of detection, quantitation, repeatability, reproducibility, and selectivity. The QuickProbe® GC/MS method and the GC-QQQ methods were evaluated by analysis of a single burnt and unburnt smokeless powder particle and residues recovered from cartridge cases and hand swabs of individuals who handled a firearm.

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**Smokeless Powders; QuickProbe® GC-MS; Triple Quadrupole (QQQ)**

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**B74 Assessing the Evidentiary Value in Fire Debris Analysis: A Proposed Workflow**

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**Learning Objective:** After attending this presentation, attendees will understand a proposed workflow and method for assessing the evidentiary value of fire debris samples.

**Impact Statement:** The presentation will impact the forensic community by demonstrating one approach to assigning discrete scores to forensic samples and establishing an evidentiary value threshold for categorical reporting.

When forensic reporting requires categorical statements, as opposed to probabilistic statements, the analyst must relate their decision to the strength of the evidence. For example, in fire debris analysis, the analyst must decide if the evidence for ignitable liquid residue in the sample is strong enough to support a positive categorical statement. Each analyst will have a different assessment of the strength of the evidence, which can be reflected in a personal score that represents their belief regarding how strong the evidence is in support of their decision. Each analyst will also have their own threshold score where the evidence is deemed strong enough to support a positive statement. Different strength of evidence assessments (scores) for a set of known ground truth samples is required to create a Receiver Operating Characteristic (ROC) curve and establish an operational decision threshold for an analyst. The convex hull of the ROC curve allows the analyst to simply convert their scores into calibrated probabilities reflective of whether a sample contains ignitable liquid residue. In a binary system, where ignitable liquid residue is either present or it is not, the calibrated probability leads directly to a likelihood ratio when coupled with a knowledge of the prior odds.

This simple procedure will be demonstrated for the analysis of 20 ground truth fire debris samples by three analysts following the sample classification guidelines of ASTM E1618-19 and ignitable liquid and substrate databases. The analysis is coupled with a simple scoring method and the workflow is implemented following the concepts of linear sequential unmasking. The methodology is then applied to two large-scale burn samples that are representative of structure fire casework. Results from each analyst's performance validation and the application to casework-relevant samples will be presented.

*This research was supported by Award Number 2017-IJ-CX-0023 awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this presentation are those of the authors and do not necessarily reflect those of the Department of Justice.*

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**Fire Debris Analysis; Linear Sequential Unmasking; Evidentiary Value**



## **B75 The Detection of Gasoline in Fire Debris Using Artificial Intelligence: Image Transformation of Gas Chromatography and Mass Spectrometry Data and Deep Learning**

*Ting-Yu Huang, MSc\*, Sam Houston State University, Huntsville, TX; Jorn (Chi-Chung) Yu, PhD, Sam Houston State University, Huntsville, TX*

**Learning Objective:** After attending this presentation, attendees will understand how deep learning is applied to recognize the features of gasoline in 2-dimensional pseudo-color heatmaps that were transformed from Gas Chromatography/Mass spectrometry (GC/MS) data.

**Impact Statement:** The impact of this study is to introduce Artificial Intelligence (AI) to assist GC/MS data interpretation in fire debris analysis. The presentation will introduce a GC/MS data transformation for AI development using a deep learning approach. The AI-powered workflow of data interpretation could strengthen and assist human interpretation of chromatogram patterns.

Arson has been a notorious crime that claims hundreds of lives annually in the United States. Between 2014 and 2018, 59% of the annual average of deaths and injuries were caused by arson fires ignited by Ignitable Liquids (ILs).<sup>1</sup> Currently, the standard test method for identifying an IL in fire debris is to adopt ASTM E1618-19 using GC/MS.<sup>2</sup> However, new materials in household items derived from petroleum have been constantly developed. The substantial contributions from those pyrolysis products in the chromatograms cause matrix interferences and can even mask the presence of an IL.<sup>3</sup> The issue has made detecting and classifying ILs in fire debris samples challenging. The objective of this presentation is to develop an automated system with high performance to increase the efficiency and accuracy of fire debris data analysis.

Deep Learning (DL) is a subbranch of machine learning and has been a popular research topic in recent years. DL is designed to automatically extract and generalize representative features from the input data by using multiple artificial neuron layers.<sup>4</sup> One of the dominant DL-based architectures is the Convolutional Neural Network (CNN), known as a powerful technology for classifying imageries without handcrafted engineering.<sup>5</sup> Across a variety of domains, CNN has demonstrated superior performance in its classification capabilities. Instead of training a CNN from scratch, transfer learning enables a more efficient learning approach by fine-tuning an existing CNN to fit a new classification task with a small data set, which is common in operational and academic laboratories.<sup>6</sup>

In this work, a novel AI-assisted framework is proposed to analyze GC/MS data for identifying gasoline residues in fire debris using transfer learning of a pre-trained CNN. Four brands of gasoline (87 grade) were purchased from local gas stations in Huntsville, TX. To increase the throughput, a headspace sampling approach was adopted to extract the characteristic profiles of gasoline analytes before using GC/MS for chemical identification. The GC/MS data were first resampled into 547×2125×1 arrays. Next, the retention time, mass to charge ratio (m/z) regions, and signal intensities of the gasoline target analytes were transformed into a pseudo-color heatmap representing the sample headspace gasoline signature. Subsequently, a pre-trained CNN, GoogLeNet, was re-trained using the transformed images as inputs to recognize the characteristic features in the pseudo-color heatmaps. A total of 327 GC/MS data were utilized for the learning task, with 252 “gasoline present” data and 75 “gasoline absence” data.

The results indicated that the proposed AI-assisted framework performs well in classifying test samples containing gasoline or without gasoline. The detection limits were 0.4 and 3.0µg of gasoline residues in a 20mL headspace vial for neat and simulated fire debris samples (300mg of spiked Nylon 6,6 carpet), respectively. The classification accuracy was successfully achieved at 100%. The proposed framework demonstrates potential improvements in interpreting GC/MS data of complex mixtures. Moreover, the statistical-based analytical results provided by the framework may strengthen the decision-making process in a legal proceeding. In conclusion, the integrated scheme combining image transformation and deep learning is a promising tool to improve the accuracy and efficiency in the forensic analysis of gasoline evidence.

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### **Fire Debris Analysis; Deep Learning; GC/MS Pseudo-Color Heatmap**

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**B76 Method Optimization and Validation for a Confined Thermal Desorption-Direct Analysis in Real-Time Mass Spectrometry (TD-DART®-MS) Sampling Interface Developed for Ignitable Liquid Analysis**

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**Learning Objective:** After attending this presentation, attendees will learn about the application of a modified DART®-MS interface for fire debris applications, involving method development, validation, and extension to ignitable liquids and simulated fire debris samples.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the ability to detect ignitable liquid components directly from sample containers using a modified Thermal Desorption (TD) DART®-MS approach.

Analysis of fire debris samples to determine the presence of ignitable liquids is critical in understanding if a fire was intentionally set. Currently, Gas Chromatography/Mass Spectrometry (GC/MS) is the most commonly used technique for such analyses, as it enables sample separation and compound identification. However, sample preparation procedures can be time-consuming (e.g., 4hr to 24hr) and require the use of hazardous solvents. Instrumental analysis methods can also be lengthy (~30min/injection), which can result in extensive instrument sequences.

The use of ambient ionization mass spectrometry for forensic analysis has been of interest in recent years. Specifically, the utility of DART®-MS has been studied for screening purposes, as it allows for rapid analysis (e.g., < 5s), requires minimal-to-no sample preparation, and is capable of detecting forensically relevant compounds. Furthermore, DART®-MS can be coupled with a TD interface to tailor the types of compounds that are desorbed (e.g., volatile compounds). Use of temperature-programmed TD, in place of static TD, can provide an added benefit of time-resolved spectra in lieu of a separation component. Such an approach can be useful for samples containing compounds of ranging volatilities, such as ignitable liquids. However, current (TD)-DART®-MS interfaces are not amenable to fire debris samples, as they require the sample to be removed from its container for analysis. Given the variation in size and form of such samples (e.g., solid flooring, burned debris particles), removal from the container is not always possible.

To modify (TD)-DART®-MS for fire debris applications, a novel sampling chamber was previously developed in our laboratory to enable direct sampling from containers and real-time analysis of sample contents. The interface was determined to effectively heat and sample the contents of pint-sized paint cans. In this work, the performance and utility of the interface for screening of fire debris samples was investigated. Optimization of instrumental parameters (source gas and gas temperature, ion mode, tee temperature, Vapor® flow rate, and chamber temperature) was performed to develop an analysis method best suited for representative ignitable liquid components. The method was then validated by assessing accuracy, reproducibility, and sensitivity. Validation was performed using a standard mixture containing aliphatic and aromatic compounds commonly found in ignitable liquids. Performance of the sampling interface was further expanded to the analysis of ignitable liquids (i.e., gasoline and diesel fuel), both neat and evaporated (30%, 50%, 70%, and 90% by volume). Peaks associated with compounds commonly used for the respective liquid classifications were identified in the corresponding spectra. Simulated burn samples were then prepared by spiking common substrates (e.g., carpet, wood) with each liquid. The samples were analyzed using the chamber setup to investigate the ability to identify ignitable liquid-specific components in the presence of substrate and matrix interferences.

This work demonstrates the utility of the modified (TD)-DART®-MS interface for direct sampling and detection of ignitable liquids. Despite the complexity of such samples, this novel approach mitigates the need for lengthy and potentially hazardous sample preparation procedures and provides a fast, sensitive, and informative screening tool for fire debris applications.

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**Fire Debris; Ignitable Liquids; DART®-MS**

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**B77 Population Statistics and a Reference Population Database of Firearm Toolmarks**

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**Learning Objective:** Attendees will learn about the current statistical framework being developed to report the weight of evidence for firearm and toolmark analysis. Four populations of firearms were used to generate ground truth comparisons used to develop statistical models to test the performance of the system. This framework is being implemented into the Reference Population Database of Firearm Toolmarks, which is a tool that will enable forensic laboratories in the future to supplement examiner testimony with objective degree of similarity and weight of evidence reports.

**Impact Statement:** This presentation will describe state-of-the-art research projects, which forms the foundational requirements to ultimately achieve the ability to report a statistical weight of evidence for firearm toolmark comparison conclusions.

The 2009 Report by the National Academies on forensic science in the United States recommended development of precisely specified, and scientifically justified, protocols that yield an objective determination of a match or non-match with well-characterized confidence limits.<sup>1</sup> The Forensic Toolmark Analysis Project (FTAP) at the National Institute of Standards and Technology (NIST), with support from National Institute of Justice (NIJ) has been making significant progress to address the technological and validation gaps within firearm and toolmark examinations. Objective algorithms and statistical analysis have been the top research priority at NIST for the past decade. Application and validation of the NIST algorithms and statistical framework requires the use of relevant population distributions. A population distribution describes the frequency distributions of a similarity score for, respectively, same-source comparisons and different-source comparisons. Like DNA analysis, these distributions are required to establish a statistical foundation for the estimation of identification confidence limits and false positive error rates.

NIST sampled four specific firearm manufacturers with 100+ firearms from each to test the systematic error rates associated with the developed protocols. These include Ruger®, Glock®, S&W®, and Sig Sauer®. Each firearm was used to test fire a minimum of two test fires using Remington® UMC 9mm ammunition with brass case and nickel primers. For each population of firearms, all available Known Matching (KM) scores, and 10,000 Known Non-Matching (KNM) scores were calculated. These were used to establish the statistical distributions for further analysis of Cumulative False Positive (CFP) and False Negative (CFN) error rates. These error rates describe the systematic error rate of the NIST analysis protocols. The results show low false positive and false negative error rates using the NIST analysis protocols across all four populations. Also, the research demonstrates the importance of utilizing a relevant population for statistical analysis due to differences observed between the four populations. Statistical methods such as the Likelihood Ratio (LR) are especially sensitive to these differences.

NIST, the Federal Bureau of Investigation (FBI), and the Netherlands Forensics Institute (NFI) have been collaborating to implement these algorithms and statistical framework into the Reference Population Database of Firearm Toolmarks (RPDFT). RPDFT is a system that consists of a reference database of firearm toolmarks (impressed and striated), data processing modules, quantitative similarity metrics, statistical weight of evidence calculation protocols, and a user interface. RPDFT is nearing the end of its initial development cycle. Currently, raw data can be submitted, processed, correlated, and statistical weight of evidence reported. Considerable effort has been taken to ensure that the methods, statistics, and population database are applicable to the evaluation of firearms evidence. Reference population database acquisitions have begun at the FBI and NFI, which will enable parallel casework pilot testing to be conducted this year. This presentation will end by introducing the audience to the design of RPDFT, limitations, demonstrate the workflow, reporting examples, results of initial pilot studies conducted, and future implementation strategies.

**Reference:**

- <sup>1</sup>. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.

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**Population Statistics; Weight of Evidence; Firearm Toolmarks**

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**B78 Reporting Likelihood Ratio (LR) for Case Work in Firearm Evidence Identification**

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**Learning Objective:** In this presentation, an LR procedure is established using a reference dataset established from a set of firearms, each of which test fired for two cartridge cases. The Congruent Matching Cells (CMC) method was used to generate CMC scores, which are used to fit in the Known Matching (KM) and Known Non-Matching (KNM) statistical models for LR estimation. The initial results were performed using the reference dataset consisting of 8 firearm manufacturers, 130 firearms that produced 260 granular images of breech faces impressions, resulting 130 KM and 8,378 KNM image pairs. The initial results showed that the KM and KNM distribution intersect at  $CMC=2$ , which is equivalent to  $LR=1$  (equally to observe  $CMC=2$  from either the KM or the KNM population). When the CMC threshold is increased to six or more, the LR values are higher than a million, which can provide extremely strong support to the prosecutor propositions in case work and court testimony.

**Impact Statement:** This presentation will provide ballistics examiners with a powerful tool for automatic and objective firearm evidence identifications and reports of likelihood ratios that will support their case work and court testimony.

Firearm evidence identification using microscopic side-by-side image comparison has a history of more than 100 years. However, the scientific foundation of firearm identification has been recently called into question by several government-funded reports. As stated in the National Academies Report in 2009: “The validity of the fundamental assumptions of uniqueness and reproducibility of firearms-related tool marks has not yet been fully demonstrated...” and in the PCAST Report in 2016: “If firearms analysis is allowed in court, the scientific criteria for validity as applied should be understood to require clearly reporting the error rates seen in appropriately designed black box studies.”<sup>1,2</sup>

LR plays an important role in estimating the weight of evidence in firearm evidence identification. LR is computed from a statistical model, including the distribution of the known-matching (KM or within) and known-non-matching (KNM or between) comparison scores. Current LR procedures rely on KM/KNM scores from existing reference firearm toolmark datasets or, alternatively, from generating a set of test fires using multiple firearms. Both procedures may contain theoretical or practical issues that may hinder LR estimations in case work.

**References:**

- <sup>1.</sup> National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.
- <sup>2.</sup> President’s Council of Advisors on Science and Technology (PCAST). *Report to the President: Forensic Science in Criminal Courts: Ensuring Scientific Validity Of Feature-Comparison Methods*. (2016). Washington, DC: Executive Office of the President of the United States.

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**Forensics; Firearm Evidence Identification; Likelihood Ratio (LR)**

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**B79 The Role of Adsorption on the Detectability of Contained Explosives and Drugs**

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**Learning Objective:** This presentation will provide insight into a fundamental process that impedes the success of vapor-based detection methods in locating illegal substances. The susceptibility of Volatile Organic Compounds (VOCs) from different explosives and drugs to adsorb to commonly used containment materials will be discussed.

**Impact Statement:** The results presented will identify situations in which the loss of certain VOCs to certain containment material is more likely to hinder detection efforts. This information can be considered for detection teams seeking these types of concealed or contained targets.

Explosives, drugs, and other contraband are frequently packaged or confined with the intent of concealing their presence. These attempts to avoid detection are often ineffective when up against well-trained detection canines who are able to sniff out the VOCs emanating from the target. The transport of VOCs from a hidden target is obstructed by the products used to wrap or contain it, due to both limited transport through the material, as well as losses due to adsorption. This research focuses on losses due to adsorption, in which the degree of loss is impacted by the chemical properties of both the containment material and the target analyte involved. Our work sought to gain a better understanding of the adsorptive properties of commonly used materials—cardboard, plastic, and stainless steel—as they were exposed to certain explosives and drugs. 2,4-Dinitrotoluene, ammonium nitrate, triacetone triperoxide, and cocaine each have a single dominant analyte in their headspace, thus classifying them as simple vapor profile, whereas hexamethylene triperoxide diamine and fentanyl have a mixture of VOCs comprising their headspace, ranking them complex vapor profiles.<sup>1-6</sup> For the simple vapor profiles, vapor adsorption would merely result in a lower concentration of analyte found in the headspace than expected based on the quantity of the bulk material. However, for the complex vapor profiles, preferential adsorption of certain VOCs can alter the expected concentration ratios that makes up their distinctive and anticipated headspace causing more severe detection complications.

Complementary methods of adsorption/desorption affinity through the use of a Quartz Crystal Microbalance (QCM) and headspace analysis by way of Solid Phase Microextraction with Gas Chromatography with either a Mass Spectrometer or Electron Capture Detector (SPME-GC/MS or SPME-GC/ECD) were implemented. The QCM allowed for the measurement of nanogram level mass changes as vapor from each analyte was flowed over and interacted with sensors coated with cellulose, polystyrene, and stainless steel. The use of SPME-GC/MS(ECD) revealed losses of the headspace concentration of each analyte in the over time when different amounts of cardboard, polystyrene, and stainless steel were introduced. The results confirm previous knowledge about the tendency for analyte vapors to interact with certain materials. In particular, cardboard demonstrated higher levels of adsorption, leading to a diminished concentration of the headspace, and cellulose, its QCM counterpart, had cases of analyte absorption to the sensors. In contrast, the trials in which stainless steel was used resulted in minimal variation of the headspace and a limited increase in the mass on the QCM sensor. Polystyrene, on the other hand, was more varied. In addition to validating the anticipated behavior of containment material when exposed to specific explosives and drugs, the results also offer useful information for consideration for detection teams seeking these types of concealed or contained targets.

**References:**

1. Jenkins, TF; Leggett, DC; Ranney, TA. Vapor signatures from military explosives: Part 1. Vapor transport from buried military-grade TNT. *Special Report 99-21, U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory*, December 1999.
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**Vapor Transport; Headspace Analysis; Contraband**

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**B80 The Combined Solid Phase Extraction Procedure for the Analysis of Organic and Inorganic Explosives Residue**

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**Learning Objective:** Attendees of this presentation will gain an understanding of an improved method for explosives analysis including extraction and processing of organic and inorganic explosives via Solid Phase Extraction (SPE) with screening analysis via Gas Chromatography/Electron Capture Detection (GC/ECD) and Ion Chromatography/Conductivity Detection (IC/CD).

**Impact Statement:** This presentation will impact the forensic science community by providing a single extraction and SPE cleanup procedure that can be applied to the combined recovery of organic and inorganic explosives residue on both cotton balls and cotton applicators. This procedure may also decrease the amount of time and money devoted to routine instrument maintenance and improve performance in explosives detection with dirty matrices.

Post-blast explosives residue may contain organic and/or inorganic explosives. Current methods employed involve splitting the sample swab between analytical methods for each explosive type (acetone for organics, water for inorganics). Analysis is then performed utilizing GC/ECD and IC/CD. This method of splitting swabs may result in loss of target analytes, resulting in the explosives residue extract falling below the Limit Of Detection (LOD).

The goal of this project is to develop a sample cleanup procedure for the combined recovery of organic and inorganic explosives residue on cotton swabs. By keeping the sample whole and using a single extraction, the proposed method could save time, provide a more representative sample, and increase the possibility of detecting explosives that may be present on collection swabs. Thus, improving recoveries for one class of explosives without sacrificing recoveries of another analyte or explosives class in the process. Organic explosives investigated include nitramines and nitrosamines (RDX, HMX, R-salt), nitrate esters (EGDN, NG, ETN, PETN), nitroalkanes (DMDNB), nitroaromatics (tetryl, 4-nitrotoluene, 2,4-dinitrotoluene, 2,4,6-trinitrotoluene), and organic peroxides (TATP and HMTD). Inorganic analytes include both anions (Cl<sup>-</sup>, ClO<sub>3</sub><sup>-</sup>, ClO<sub>4</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, SCN<sup>-</sup>) and cations (NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Li<sup>+</sup>, Mg<sup>+2</sup>, Ca<sup>+2</sup>, Sr<sup>+2</sup>, Ba<sup>+2</sup>).

In this method, a deionized water:acetone solution is utilized to extract organic and inorganic explosives from collection swabs. The extract is then loaded onto a conditioned SPE cartridge for cleanup and pre-concentration. This load fraction is collected to analyze the inorganic explosives by IC/CD. The cartridge is washed with water and the organic explosives elute with acetone into an autosampler vial for analysis by GC/ECD.

Extraction recoveries for the proposed SPE method and current methodology (syringe extraction) were evaluated. Organic and inorganic explosives standards on cotton balls and cotton applicators were investigated. The SPE method is comparable to the syringe method, with cotton balls having higher recovery than cotton applicators. Additionally, the proposed method is being applied to field samples and burned bulk powders (Red Dot Smokeless Powder, IMR Smokeless Powder, Black Powder, Pyrodex, Potassium Perchlorate/sugar/sulfuric acid mix). Extracts were screened with GC/ECD and IC/CD for explosives residue.

In conclusion, a method for the combined extraction of organic and inorganic explosives residue was developed. Recovery efficiencies were evaluated, and the method utilized to analyze field samples from intact and burned explosives. The described method allows for the processing of a single swab for analysis by GC/ECD and IC/CD, resulting in decreased processing time and elimination of splitting collection matrices.

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**Organic Explosives; Inorganic Explosives; Solid Phase Extraction**



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**B81 The Statistical Characterization of the Discriminating Power of Trace Elemental Concentrations of Aluminum Foils Used in Improvised Explosive Devices**

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**Learning Objective:** Attendees of this presentation will gain an understanding of the utility of trace elemental profiles via Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) and statistical methods for assessing discrimination power of these data from aluminum foil sources commonly used to produce powders for Improvised Explosive Devices (IEDs).

**Impact Statement:** Research presented will help the community understand the value of using ICP/MS to quantitate trace element compositions to further characterize IED component sources beyond identification of constituents. The approach used for statistical characterization of the discriminating power of the analytical method may be suitable for other multivariate data and evidence types encountered in forensic science.

IEDs often contain inexpensive materials such as aluminum (Al) powder, which can be obtained directly from legitimate manufacturers or produced by amateur methods using commercially available sources (e.g., foils). At prior AAFS meetings, analytical method development and preliminary results were presented.<sup>1,2</sup> Here, new data using a refined ICP/MS analytical method and statistical characterization of the discriminating power of the trace element concentrations in Al foils will be presented.

Previously reported intra-roll homogeneity tests showed that the roll was likely not statistically significantly heterogeneous and that the trace element profile was independent of the sampling location.<sup>1,2</sup> Preliminary tests also confirmed that trace element profiles were distinct among at least two manufacturers, allowing for between-source discrimination. Based on these results, a statistical subsampling and analysis scheme was implemented for the Al foil collection of ~164 rolls, including a variety of global manufacturers and products, and rolls from both the same and different production lots of the same manufacturer. From each roll of foil, we collected nine subsamples. The trace element concentrations were measured on three aliquots/replicates from each subsample, resulting in 27 measurements of 29 trace element concentrations for each source. For each source, all aliquots from six subsamples were analyzed on one day, with all aliquots from the remaining three subsamples for that source analyzed on the next day. To ensure and verify accuracy and stability, external calibrations were used, a multi-element internal standard covering the analyzed mass range was added to all samples to correct for instrumental drift, and a NIST-certified standard reference material was analyzed with all samples.

Our first statistical question is whether “n” is 27 (aliquots) or 9 (subsamples) for each source. An analysis-of-variance approach was used to test if the six subsamples (all analyzed on the same day) explain a significant amount of the variability between the aliquots for corresponding subsamples. If the resulting distributions of the p-values significantly depart from a uniform distribution, then the number of subsamples (not aliquots) determine the statistical sample size.

This analysis of the sources of variance suggested that we had some outlier concentrations among the three aliquots for some of the subsamples, which was spiking the aliquot variance. This suggests that not only would the smaller sample size ( $n=9$ ) have to be used, but a strategy for summarizing the “average” concentration among the three aliquots that is robust to outliers would also need to be developed. For this, we have chosen to use a median summary of the three aliquot concentrations. When comparing the median to the mean of the replicate concentrations, some elements appear to have a high degree of replicate variability. A modified ASTM score rule based on the median summary, mean summary, and using all 27 replicates was implemented.<sup>3</sup> For each of the three methods, we characterized the different discriminating powers for differing choices of the number of sigma thresholds, including the traditional 4-sigma threshold, providing a U-statistic-based confidence interval.<sup>4</sup> We then replicated this analysis by comparing only same-day comparisons, only cross-day comparisons, then within-day same-source comparisons versus different-day between-source comparisons.

By decomposing the analysis of the discriminating power, we gain an understanding of the effect of using the replicates as if they were independent pieces of information and the effect of measurement day on the resulting perception of the discriminating power of the proposed methods.

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**Trace Elements; Statistics; Explosives**

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**B82 Gunshot Suicides Caused by Cane-Gun and Alarm Garden Gun Cannon: Two Case Reports**

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**Learning Objective:** After attending this presentation, attendees will better understand the importance of the forensic pathologist's fieldwork, especially in complex or atypical suicides. Attendees will hear about two cases that reported a suicide with an original historical firearm as the cane-gun, a walking stick which conceals a firearm, and a suicide with a modified alarm cannon, which is a small cannon, that fires blanks to scare away garden animals. The aims of the study were to describe the scene, the corpse external examination, and the autopsy to understand the death mechanism.

**Impact Statement:** This presentation will impact the forensic science community by illustrating the added value of a thorough investigation of the scene by the medical examiner. Detailed documentation of the death scene is necessary to support the autopsy findings and to determine the cause and circumstances of death, especially in the absence of a suspicious crime scene. This article encourages the medical examiner to examine the entire scene of a firearm death, especially if it is not typical, and allows the medical examiner to expand his or her knowledge of atypical cases to fit any crime scene.

Suicide is a frequent type of unnatural death seen frequently by forensic scientists. Among them, gunshot wounds are one of the most frequent causes of death (15%). In each case, one of the forensic pathologist's main missions is to attempt to distinguish death by suicide from other forms of violent death such as accidents or homicides. In gunshot suicide cases, the majority of cases show injuries related to a touching shot or a shooting from a very short distance (print on the barrel of the weapon, for example), with the weapon still present near the victim. Obtaining a firearm is not always easy, which is why some firearms that are antique or whose use is not intended to kill are modified for suicide purposes. However, the use of unusual firearms might create a complicated and unsettling situation for the forensic pathologist who does not often come across antique, concealed, or handmade firearms. Unusual or atypical firearm injuries must be able to be recognized as such by forensic medical experts. This presentation shows two uncommon suicides by "unusual" guns. In this presentation, the first case presents the use of a cane-gun, a walking stick which conceals a firearm, that was used by a 57-year-old man. The second case involves the use of an alarm garden gun cannon, which is a small cannon that fires blanks to scare away garden animals. It was remodeled by an 86-year-old man. These cases illustrate the added value of a thorough investigation of the scene. Detailed documentation of the death scene is necessary to support the autopsy's results and to ascertain the cause and the circumstances of death. This article encourages the medical examiner to consider the entire scene of a firearm death, especially if it is not typical, and allows any forensic scientist to enrich his knowledge of unusual cases.

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**Atypical Firearms; Alarm Garden Gun Cannon; Cane-Gun**

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**B83 The Application of the Expert Algorithm for Substance Identification (EASI) on Synthetic Cathinones Using Mass Spectrometry (MS)**

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**Learning Objective:** After attending this presentation, attendees will understand how correlated ion abundances within replicate mass spectra can be used to improve the confidence in compound identification for synthetic cathinones with high structural and spectral similarities.

**Impact Statement:** This presentation will provide the forensic community with a flexible and robust regression-based mass spectral algorithm that can minimize the risk of false-positive and false-negative identifications. With EASI, forensic chemists have reduced rates of false-positive identifications while having increased confidence in their identifications and testimonies.

Our central hypothesis is that relative ion abundances of replicate mass spectra are not independently variable but highly correlated, as confirmed through correlation matrices for each substance. This hypothesis is supported by robust statistical theories of unimolecular fragmentation that have described mass spectral fragmentation patterns since the 1950s. Current mass spectral algorithms use an exemplar or consensus approach that often struggles to distinguish between compounds with high structural and mass spectral similarities. The novel regression-based EASI has successfully differentiated other NPS, such as fentanyl analogs, with false negative error rates of ~9% compared to the consensus-based approach of ~26%.

For this presentation, ten synthetic cathinone isomers were analyzed in replicate using Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) on at least five different instruments in two different laboratories. The database contained more than 2,000 replicate spectra of the ten cathinones. For each compound as the model, the spectra were randomly divided into a training and a test set. The ion abundances for each spectrum were first normalized to the base peak, then the 20 most abundant ions for the training set were used to build 20 General Linear Regression Models (GLM) by sequentially using the abundance of each ion as the dependent variable and the abundance of the 19 remaining ions as the covariates. Spectra from 20 additional cathinones from NIST/SWGDRUG databases were also used as extra validation KNs.

The models for each compound were then used to predict the ion abundances for all the KPs and KNs in the database. The predicted abundances were compared to the measured abundances using various similarity and dissimilarity metrics, like the Pearson Product-Moment Correlation (PPMC) and mean absolute residual. Each metric was then used as a binary classifier to determine the true-positive and false-positive rates over a range of threshold values. These classifications were used to plot a Receiver Operating Characteristic (ROC) curve from which we could calculate the Area Under the Curve (AUC) to determine the strengths of each model. PPMC values between the measured and predicted spectra of KPs exceeded 0.9910 for the training sets. For each cathinone model, most of the KNs had smaller PPMC values than the smallest PPMC value of all the KPs, thus enabling successful classification. EASI consistently outperformed the consensus approach with AUCs of 1.000 in the ROC plots for binary classification: i.e., no errors. The residuals (MAR) in the predictions for the KPs typically improved by a factor of 3 over the traditional consensus approach. In short, the new algorithm outperforms existing algorithms for accurately identifying cathinone analogs from their mass spectra.

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**Cathinones; Seized Drugs; Mass Spectrometry**

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**B84 The Application of the Expert Algorithm for Substance Identification (EASI) to the Mass Spectral Identification of Fentanyl Analogs**

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**Learning Objective:** By the end of this presentation, attendees will have learned how the correlation of ion abundances within replicate mass spectra of a drug can be used to improve confidence in compound identification, especially for fentanyl analogs sharing structural and spectral similarities.

**Impact Statement:** This presentation will provide the forensic community with a robust algorithm that minimizes the risk of false positive and false negative identifications and enables confident identifications without the need to analyze contemporaneous drug standards. The goal of the algorithm is to provide adequate confidence in substance identifications while obviating the need for laboratories to purchase and analyze their own drug standards with casework.

**Hypothesis:** Our central hypothesis, supported by an extensive database of >57,000 replicate fentanyl analog spectra, is that relative ion abundances of replicate mass spectra are not independently variable, as has been assumed for more than 70 years, but are in fact, highly correlated. Therefore, we use the measured correlations between spectra in a series of general linear models to make accurate predictions of ion abundances within questioned spectra.

**Methods/results:** Current search algorithms for Electron Ionization/Mass Spectrometry (EI/MS) data use a “consensus” approach when making unknown identifications. These algorithms compare unknown spectra to a library’s discrete, fixed spectra of standards. However, the inter-day or inter-laboratory variance in the abundance of each fragment in a spectrum is known to vary by  $\pm 20\%$ , so compounds with similar EI mass spectra, like fentanyl analogs, can be difficult to distinguish using only the EI spectral comparison. EASI takes a more informed approach that effectively interpolates between and extrapolates beyond replicate measured spectra and provides a continuously variable model of ion abundances for each compound in the database. This model can account for the variance between instrument vendors and different laboratories.

A database containing approximately 57,000 replicate mass spectra of 76 fentanyl analogs was compiled from GC/MS data from nine different laboratories by extracting every mass spectrum across the eluting chromatographic peaks of interest. The ion abundances for each spectrum were normalized to the base peak in each spectrum. As examples, 23 fentanyl analogs with varying R-substituted groups were chosen as Known Positives (KPs) for model building, and the remaining 75 analogs served as Known Negatives (KNs). The 20 most abundant ions for each analog were extracted, and the abbreviated spectra were randomly divided into training and testing sets. Twenty General Linear Regression Models (GLM) were built for each compound by sequentially using the abundance of each ion as the dependent variable and the abundance of the 19 remaining ions as the independent variables. The models for each compound were then used to predict the ion abundances for various KPs and KNs. The predicted abundances were compared to the measured abundances using various similarity and dissimilarity metrics like the Pearson product-moment correlation (PPMC) and mean absolute residual. Each metric was then used as a binary classifier to determine the true-positive and false-positive rates over a range of threshold values. These classifications were used to plot a Receiver Operating Characteristic (ROC) curve from which we could calculate the Area Under the Curve (AUC) to determine the strengths of each model.

The residuals in the predictions for the KPs typically improved by a factor of 3 over the traditional consensus approach, and at a zero false positive rate, EASI had an average false negative error rate of  $\sim 12\%$  compared to the consensus approach at  $\sim 31\%$ . In short, the new algorithm outperforms existing algorithms for accurately identifying the most structurally similar fentanyl analogs in the absence of retention time data or any contemporaneously analyzed standards.

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**General Linear Model; Mass Spectrometry; Fentanyl Analogs**

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**B85    The Development and Validation of a Quantitative Method for the Analysis of Fentanyl Containing “Dope” Samples**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the design, validation, and implementation of a multi-component quantitative assay for solids and powders suspected to contain fentanyl. Through their attendance, attendees will be able to explain quantitative drug methods, parameters to include in their validation, some applications, and what challenges to anticipate during development.

**Impact Statement:** This presentation will impact the forensic science community by describing a novel, multi-component assay to quantitatively analyze drug material. Benefits to the forensic community are support for public health initiatives, medicolegal death investigations, and prosecutions based on the purity of drugs found on overdose victims. The method also supports forensic investigations involving sample comparisons and intelligence on sample profiling.

Illicit fentanyl is the primary contributor or co-contributor to overdoses and overdose deaths in the United States. However, purity of drugs in exhibits in criminal casework are rarely determined, inhibiting investigations of drug overdoses and adverse event outbreak investigations. Moreover, the drug supply in the United States has entered a “poly-drug” phase where drugs (especially opioids) are found in combination with other substances of varying pharmacological activity. For example, in Philadelphia, PA, fentanyl is the primary opioid in “tranq-dope” samples, which are largely adulterated with xylazine, a non-controlled animal tranquilizer not intended for use in humans.

For this study, drug material samples were collected for qualitative and quantitative analysis by the Philadelphia Department of Public Health. Analysis was performed at the CFSRE by Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). This presentation will describe the development and validation of the GC/MS method for the measurement of fentanyl, xylazine, 4-ANPP, para-fluorofentanyl, methamphetamine, cocaine, levamisole, and lidocaine. Methamphetamine and cocaine were included in the assay so that they could be measured if found in combination with fentanyl. This presentation will describe the method, its design, the validation plan, the validation results, and application to authentic samples.

A single-step, basic drug extraction was selected due to superior chromatography compared to methanol dilution, yet still providing an efficient workflow for measuring the selected analytes and identifying other basic drugs. Validation was performed over five days and included evaluation of calibration model, accuracy, precision, carryover, limit of detection, dilution integrity, recovery, process efficiency, and matrix effects. The method was successfully validated based on the 2019 SWGDRUG recommendations for validating analytical methods and ASB recommendations for validating quantitative toxicology methods.<sup>1,2</sup> Initial results show that mean fentanyl purity in powder was 11.5% (interquartile range=8.2–15.2%, *n*=58), 4-ANPP had a mean concentration of 2.2% (interquartile range from 1.0–2.7%, *n*=54), and xylazine being a major component with a mean of 31.8% (interquartile range from 21.6–41.3%, *n*=52).

Timely data on street drug potency allows public health agencies to decide how best to communicate vital information concerning drug purity, potency, and combinations to prevent overdoses and promote harm reduction. Potency data and the concentrations of adulterants and reaction intermediaries and by products can allow development of drug signatures, allowing comparisons of drug exhibits from different seizures for assessment of common origin, consistency of the contents of different “stamps,” or temporal changes in drug potency in the street drug supply.

**References:**

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2. AAFS Standards Board 2019, Standard Practices for Method Validation in Forensic Toxicology, American Academy of Forensic Sciences, [https://www.aafs.org/sites/default/files/media/documents/036\\_Std\\_e1.pdf](https://www.aafs.org/sites/default/files/media/documents/036_Std_e1.pdf).

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**Fentanyl; GC/MS; Quantitation**

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**B86 Distinguishing Piperazine and 12 Analogs Using Raman Microspectroscopy and Chemometrics**

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**Learning Objective:** After attending this presentation, attendees will better understand the ability to distinguish piperazine and 12 of its analogs using Raman microspectroscopy and multivariate statistics.

**Impact Statement:** This presentation will impact the forensic science community by reporting the Raman microspectroscopic data of piperazine and 12 of its analogs as well as providing an objective analysis of the spectra through the use of chemometrics.

Novel Psychoactive Substances (NPS) are new drugs created through chemical modification of existing illicit substances. One common class of NPS, known as piperazines, contain a cyclic structure with two nitrogen atoms in opposite positions. Substitutions among this piperazine ring result in a variety of structurally similar piperazine analogues and isomers that are difficult to distinguish and identify. Gas Chromatography/Mass Spectrometry (GC/MS) is a widely used technique for analyzing piperazine analogs. Although this technique provides adequate separation, it lacks the ability to distinguish isomers due to similar fragmentation patterns. Raman microspectroscopy, however, offers a solution to this problem. This technique in combination with multivariate statistics provides a rapid, objective method for the identification of piperazine analogues and isomers.

In this study, Raman microspectroscopy was used to identify piperazine and 12 analogs. This includes the most common analogs, 1-benzylpiperazine (BZP), 1-(3-trifluoromethylphenyl)piperazine (3-TFMPP), and 1-(3-chlorophenyl)piperazine (3-CPP), as well as their isomers 2-benzylpiperazine (2-BZP), 1-(2-trifluoromethylphenyl)piperazine (2-TFMPP), 1-(4-trifluoromethylphenyl)piperazine (4-TFMPP), 2-(3-trifluoromethylphenyl)piperazine (2,3-TFMPP), 1-(4-chlorophenyl)piperazine (4-CPP), and 2,3-dichlorophenylpiperazine (2,3-DCPP). Additionally, phenylpiperazine, 1-(3,4-methylenedioxybenzyl)piperazine (MDBP), and 1-(4-methoxyphenyl)piperazine (4-MeOPP) were analyzed. The standards were first analyzed in triplicate to determine which Raman microspectroscopic parameters produced the highest resolution and peak intensity for each drug. To do so, laser powers of 10, 15, and 20mW were utilized at six different number of scans: 4, 16, 32, 64, 128, and 256. In general, there was an increase in resolution and intensity as the laser power and/or number of scans increased for each drug, with 20mW of laser power at 128 or 256 scans being the most common. This laser power and number of scans combination produced quality spectra with noticeable differences between all drugs, including isomers.

Additionally, no degradation of any of the piperazine analogs was observed with increasing laser power. Multivariate analysis was then completed on the three different categories of isomers and one group of single drugs that did not fit any category by using the peak position and intensity data obtained from the best combination of parameters for each drug. Principal Component Analysis (PCA) was first employed to reduce dimensionality, followed by Linear Discriminant Analysis (LDA) of the first five principal components to separate the samples. Upon analysis of the principal component scores, 99% of the total variance was explained by PC2 for the group containing two benzylpiperazine isomers, by PC3 for the group containing three chlorophenyl piperazine isomers and the group containing four trifluoromethylphenyl piperazine isomers, and by PC4 for the group containing 4-MeOPP, MDBP, piperazine, and phenylpiperazine. Upon visual inspection of the LDA plots, there was good separation between all groups analyzed, including those that contained isomers. These results suggest that Raman microspectroscopy is a suitable method for producing high-quality spectra of piperazine analogs that can be utilized in objective analysis techniques such as multivariate statistics.

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**Piperazine; Raman Microspectroscopy; Multivariate Statistics**



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**B87     Untargeted Headspace/Solid-Phase Microextraction Coupled to Gas Chromatograph/Mass Spectrometer (HS/SPME-GC/MS)? An HS/SPME-GC/MS Analysis of Terpenes and Cannabinoids for the Geographical Sourcing of Marijuana Using Multivariable Data Analysis**

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**Learning Objective:** After attending this presentation, attendees will gain an awareness of the use of Volatile Organic Compounds (VOCs) for the untargeted analysis of marijuana to determine characteristic features using a combination of headspace chemistry and multivariate data analysis.

**Impact Statement:** This presentation will impact the forensic science community by introducing an optimized headspace analysis and sampling approach that allows for a practical field sampling of marijuana odor and by the presentation of the use of common chemometric tools for the analysis of complex chromatographic data.

There have been many uncertainties related to the canine detection of marijuana due to the widespread, ongoing legalization of *Cannabis sativa* (i.e., marijuana) throughout the United States. This sociolegal issue has resulted in an increasing interest regarding the generalization and specification within canine detection of marijuana. More specifically, canine trainers have expressed concerns on whether canines can generalize on the odor of marijuana regardless of the origin of their training materials. This study aimed to differentiate multiple marijuana samples from a variety of regions in the United States based on the VOCs found in their odor profile. Some of the features examined for each sample group were the location of origin and whether the marijuana was confiscated by law enforcement at the time of sampling. Previous work in this field showed promise toward the potential differentiation of several marijuana varieties.

In this research, an HS/SPME technique was implemented for the collection of both terpenes and cannabinoids from marijuana. The HS samples were analyzed using two full-scan, optimized methods on a GC/MS, and a variety of chemometric tools were applied to the data to enable differentiation and potential classification between sample populations. Principal component analysis in addition to multiple supervised analysis methods employed in this study demonstrated a disparity between marijuana varieties using the VOCs extracted from their odor profile. With this information, it is possible to also determine specific biomarkers that are common between samples and develop a targeted analysis for differentiation. Subsequently, if necessary, adjustments could be made to canine detection training of marijuana to improve the generalization of their target. Moreover, this work expands on the foundation already established in SPME/GC/MS research regarding the differentiation of similar-like substances of forensic and agricultural interest such as hemp and marijuana or distinct varieties of foodstuffs.

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**Geographical Sourcing; Marijuana/*Cannabis sativa*; Headspace Analysis**

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**B88    The Development and Validation of a Method for the Differentiation of Hemp and Marijuana by Gas Chromatography/Mass Spectrometry (GC/MS)**

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**Learning Objective:** Attendees will be able to describe the development and validation of a method for the analysis of cannabinoids in various seized drug matrices by GC/MS, including challenges with quantitating cannabinoids, such as decarboxylation of THCA, differentiation of THC isomers, and the sample preparation required for testing complex matrices.

**Impact Statement:** This presentation will impact the forensic science community by presenting a validated method used to meet the legal requirements for reporting marijuana. The discussion of method development and the unexpected findings that arose from initial experimentation will alert practitioners to potential confounding issues and will help ensure that methods developed for this analysis have been fully characterized to confirm their strengths and limitations in the quantification of cannabinoids in complex matrices.

The passage of the 2018 Farm Bill created a legal distinction between hemp and marijuana based on the delta-9-THC content of the plant or derived products. This required forensic laboratories performing seized drug testing to rapidly adapt to the new requirements of the law, shifting from the general identification of cannabinoids to the quantitation of delta-9-THC. In addition, sample types submitted became more diverse as the popularity and prevalence of CBD products increased, and as states legalized marijuana and THC products. To respond to this challenge, a decision-point method and a full quantitative method were developed for delta-9-THC, delta-8-THC, THCA, and CBD in matrices including plant material, liquids and oils, waxes, edibles, and bath and body products. The decision-point method is used to screen samples for those containing concentrations of total delta-9-THC greater than 1%. For samples close to the 0.3% legal limit, quantitation can be performed.

Method development included an investigation into the decarboxylation of THCA, optimization of solvent for cannabinoid extraction, use of plastic versus glass test tubes, and derivatization conditions. The instrumental method was optimized to separate isomers of THC and provide an analytical measurement range that encompassed the legal threshold. Validation was performed on the final method to assess imprecision/bias, acceptability of the calibration model, recovery, interferences, limit of detection, matrix matching, carryover, accuracy, and an assessment of CBD conversion to delta-9-THC.

The finalized method consisted of six main steps: homogenization, sampling a weighed aliquot, extraction of cannabinoids into solvent, liquid/liquid extraction, derivatization, and GC/MS analysis. Most steps for the decision-point and quantitative analysis were the same. Samples were ground with a mortar and pestle where possible. Matrices not amenable to grinding at room temperature such as edibles were homogenized using a cryogenic grinder. Cannabinoids were extracted from the matrix through dilution in methanol. The samples were then prepared using liquid/liquid extraction, partitioning between salt water and a hexane/ethyl acetate mixture, to remove matrix components that could hinder derivatization. Finally, samples were derivatized with BSTFA to prevent interconversion of cannabinoids (e.g., CBD to delta-9-THC) and decarboxylation of cannabinoid acids (e.g., THCA to delta-9-THC). A five-point calibration curve and three controls were used. A single calibrator and control were prepared for decision-point analysis. A CBD conversion control was included in all runs to monitor conversion. Samples were analyzed on a scan GC/MS method. This provided full mass spectral data and retention time data for qualitative identification of cannabinoids in the sample including, but not limited to, the cannabinoids that were quantitated.

The quantitative method and the decision-point method developed were able to successfully analyze delta-9-THC, delta-8-THC, THCA, and CBD in various matrices. The decision-point method was successfully propagated and verified at laboratory sites in multiple states within the NMS network of labs for use in casework.

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**Cannabinoids; Quantitation; Validation**

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**B89 The Commercialization of a Universal Method for Body Fluid Identification for Forensic Purposes**

*Alexis Weber, MS\*, SupreMEtric LLC, Albany, NY; Igor Lednev, PhD, Department of Chemistry, University at Albany, SUNY, Albany, NY*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the tumultuous process between conducting laboratory research and commercialization of the work.

**Impact Statement:** This presentation will impact the forensic science community by presenting a rapid, non-destructive, and confirmatory method to replace front end serological testing.

The ability to identify body fluid traces at crime scenes, while preserving any DNA present, is critically important in forensic science. Currently in forensic science laboratories, this identification can be difficult and many of the current techniques are specific to one body fluid. Additionally, typical biochemical methods are destructive—preventing any further analysis. When there is a problem within the scientific field, research laboratories are the main group to solve this problem. After conducting research in the laboratory, the next step in the process is to commercialize the research. Commercialization is bringing a product to market and selling it for financial gain. Within the Lednev Laboratory, in order to develop a universal, confirmatory, non-destructive approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling.

All six forensically relevant body fluids (blood, semen, saliva, sweat, urine, and vaginal fluid) were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and non-destructive, offering substantial advantages over the current techniques used to identify body fluids. This development of this product has occurred over several years to prepare it for sale, with the culmination of this being the creation of the start-up company SupreMEtric LLC. SupreMEtric's mission is to streamline the forensic analysis of biological stains by creating a universal non-destructive method for the identification of all main body fluids. This presentation will discuss the process from researching the problem in an academic laboratory to the commercialization process of this technology. Additionally, this presentation will highlight the values and potential of SupreMEtric's product for potential users, including forensic biologists and serologists in both public, federal, and private laboratories. The goal of this presentation is to facilitate discussions of our technology and consider areas of improvement in SupreMEtric's product.

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**Body Fluid Identification; Chemometrics; Raman Spectroscopy**

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**B90 The Evaluation of Demineralization Parameters for DNA Extraction Methods From Skeletal Remains**

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**Learning Objective:** This presentation will provide an overview of the comparison of total versus partial demineralization methods and the impact of incubation temperature on DNA extraction methods from skeletal samples.

**Impact Statement:** It is important to achieve successful DNA recovery from skeletal remains, as these samples are frequently the only evidence available for many human identification cases. Skeletal samples often have limited DNA quantity and quality, and therefore determining an extraction method that can successfully recover DNA from these samples without further degrading the DNA is essential for downstream processing.

Skeletal samples are often used for human identification purposes in cases involving mass disasters, missing persons, and unidentified remains. The inherent nature of these samples allows DNA to be preserved within the hard hydroxyapatite matrix of bone, protected from the environment. However, to successfully extract DNA from these samples, a demineralization step is required to break down this matrix, since it encases the osteocytes containing the DNA of interest.<sup>1</sup> Established extraction methods for skeletal samples involve either partial demineralization, requiring less time but resulting in unused skeletal material; or total demineralization, which includes overnight incubation, but often results in total dissolution of the skeletal sample.<sup>1,2</sup> In addition to the length of incubation, resulting in either a total or partial demineralization, another variable that requires examination is the incubation temperature. Forensic skeletal extraction methods utilize a 56°C incubation; however, there is speculation that this higher temperature may be detrimental, further degrading DNA and hindering successful recovery. Ancient DNA extraction methods utilize lower temperatures (e.g., 37°C) to prevent additional DNA degradation, but the digestion process may be limited at these lower temperatures due to incomplete activation of the Proteinase K enzyme.<sup>3,4</sup>

To investigate these variables, four extraction methods were performed on six skeletal samples in duplicate. Partial demineralization methods were completed using the PrepFiler® BTA extraction kit. This method was first performed following the manufacturer's protocol with a two-hour incubation at 56°C. The samples were also extracted with the same protocol but using a lower incubation temperature of 37°C for one hour before additional Proteinase K was added and incubated for another hour at 56°C. Total demineralization methods were performed following Loreille et al.<sup>1</sup> Samples were incubated at both 56°C and 37°C overnight. Following the 37°C incubation, additional Proteinase K was added, and the samples were incubated for another hour at 56°C prior to MinElute® purification. Extracts from all four methods were compared by examining the overall DNA quantity and quality utilizing real-time PCR quantification methods and the Agilent® 2100 Bioanalyzer system. Samples were also processed using traditional STR typing, and the success of each method was examined by comparing allele call rates.

The quantification results identified no significant trend when comparing the demineralization temperature. However, when examining the STR allele calls, total demineralization using the traditional 56°C incubation had a 55.35% increase compared to incubation at 37°C. An obvious decrease in DNA yield was identified for PrepFiler® BTA samples compared to total demineralization; however, the partial demineralization method utilized only a fifth of the bone powder used in the total demineralization method. When adjusting for this difference, DNA yields were more comparable. Incubation temperature did not appear to affect STR call rates for the partial demineralization method.

Overall, total demineralization at 56°C indicates more successful DNA recovery leading to applicable human identification methods. However, if limited by time or sample quantity, proceeding with a partial demineralization method may allow for successful recovery but appears to be sample dependent.

**References:**

1. O.M. Loreille, T.M. Diegoli, J.A. Irwin, M.D. Coble, T.J. Parsons, High efficiency DNA extraction from bone by total demineralization, *Forensic Sci. Int. Genet.* 1 (2007) 191–195. <https://doi.org/10.1016/j.fsigen.2007.02.006>.
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3. N. Rohland, M. Hofreiter, Comparison and optimization of ancient DNA extraction, *Biotechniques*. 42 (2007) 343–352.
4. M.P. Evison, D.M. Smillie, A.T. Chamberlain, Extraction of single-copy nuclear DNA from forensic specimens with a variety of postmortem histories, *Journal of Forensic Science*. 42 (1997) 1032–1038.

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**Skeletal Remains; Human Identification; DNA Extraction**

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**B91 Improving Allele Call Rates for Challenging Samples With ePCR1 Buffer**

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**Learning Objective:** By attending this presentation, attendees will see the impact of the Verogen™ enhanced PCR1 (ePCR1) buffer on sequencing results from challenging bone samples. These bone samples encompass a range of degradation quality and inhibition.

**Impact Statement:** This presentation will demonstrate how implementing the ePCR1 buffer in the Verogen™ ForenSeq™ DNA Signature Prep kit for Next Generation Sequencing (NGS) applications can aid in improving allele call rates.

NGS offers many benefits over the current method of Capillary Electrophoresis (CE). Some of these benefits include enhanced sensitivity, the ability to sequence smaller amplicon sizes, SNP generation, and greater levels of multiplexing. NGS also allows the analysis of isometric alleles—STRs that are the same length but contain a variation in their sequence. SNP analysis allows for hair, eye color and ancestry prediction to become an available—and desirable—tool in the forensic field. NGS also comes with its own set of challenges: samples need to meet input quantity and quality thresholds to have sequencing success; PCR inhibitors, especially those found in bones, will affect sequencing if they are present in the sample; and large multiplexes with an optimal size for STR and SNP amplicons can be difficult to develop.

This study was initiated during our internal validation, where a number of bone samples showed poor sequencing results compared to those from CE. Eleven out of 21 mock casework samples were composed of bones and teeth; some of the bone samples resulted in fewer than expected, or no, sequencing reads. These results were unexpected as they had a relatively high allele call rate with CE. The study was undertaken to see if implementing the new ePCR1 buffer would improve sequencing results for challenging samples such as bones and teeth.

The DNA was extracted from bone and tooth powder using the ICMP demineralization method.<sup>1,2</sup> The extracted DNA was quantified using the Quantifiler® Trio kit and then sequenced on the Verogen™ MiSeq® FGx Sequencing System. Samples were sequenced using the original ForenSeq™ DNA Signature Prep kit and sequenced a second time using the enhanced PCR1 buffer procedure.

Fourteen bone and teeth samples were chosen that covered a range of success through CE as well as what year they were recovered. The DNA was freshly extracted from these samples and prepared for sequencing following the protocols listed above. 1ng of large autosomal DNA was targeted for these sequencing runs, however some samples had lower input amounts due to a lower amount of DNA being extracted from the sample. Initial data showed that the ePCR1 buffer only improved read count in 6 out of the 14 samples, with the average read count being approximately 37,000 reads higher for the regular PCR1 buffer. When looking at the data more closely, we observed the samples using the ePCR1 buffer resulted in higher allele call rates across all markers. Autosomal STRs showed an increase of 9%, YSTRs by 10%, XSTRs increased by 13%, and iSNPS saw a 2% increase.

In order to use the same procedure for all samples, the ePCR1 buffer would need to be verified with non-challenging samples. The BCIT Forensic DNA Lab is currently evaluating the ePCR1 buffer to see if it would be effective for standard casework samples or other challenging samples other than bone and teeth.

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2. Huel et al. (2012). DNA extraction from aged skeletal samples for STR typing by capillary electrophoresis. In: *DNA Electrophoresis Protocols for Forensic Genetics* (pp. 185-198). Humana Press.

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**Next Generation Sequencing (NGS); Challenging Samples; Forensic DNA Analysis**

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**B92 The Differentiation of *Cannabis Sativa* Crop Type and Biogeographical Origin Using Nine Novel Chloroplast Single Nucleotide Polymorphism (SNP) Markers**

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**Learning Objective:** After attending this presentation, attendees will gain insight regarding the benefits of analyzing chloroplast SNP markers that may help to differentiate crop type and biogeographical origin in *Cannabis sativa* samples.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the applicability of nine new chloroplast SNPs markers that may help to differentiate legal from illicit crops, to determine the biogeographical origin of *C. sativa* samples and to aid law enforcement in drug trafficking intelligence.

*C. sativa* can be broadly classified into two main types according to psychotropic cannabinoid  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) content: the drug-type cannabis (marijuana) and the fiber-type cannabis (hemp). According to the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), the majority of European Union countries treat possession of cannabis, for personal use, as a minor offence with the possibility of incarceration. Despite the model of medical use (i.e., Italy) or legal supply (i.e., Spanish cannabis club, Netherlands coffee shops), cannabis continues to be a growing problem for forensic investigations while remaining the most trafficked illicit plant in the European Union. Differentiating cannabis crops or tracing the biogeographical origin is crucial for law enforcement purposes. Chloroplast DNA (cpDNA) markers may assist in determining biogeographic origin and in differentiating hemp from marijuana. In this research, nine novel *C. sativa* cpDNA polymorphic SNPs were identified and evaluated to differentiate crop type and to provide information about its biogeographical origin. These regions were discovered aligning five different sequences varieties of *C. sativa* chloroplast genome.

This study developed five SNaPshot™ assays (three multi-plex and two single-plex) for nine chloroplast markers, and samples from Spain, Italy, Chile, and the United States-Mexico border were analyzed. All samples yielded full profiles for the five assays. Four haplotypes groups were identified through the analysis of 106 *C. sativa* samples. Haplotype 1 was the most common one observed in 58.5% of all analyzed samples. Haplotypes 3 and 4 were observed only in hemp samples. Haplotype 2 was found in some Chilean and United States-Mexico border samples, confirming past research on these samples. Despite 60% of samples sharing the same haplotype, some genetic differences were observed between marijuana and hemp samples. Principal Component Analysis (PCA) revealed relationships among Spanish and Italian hemp samples, as well as for United States-Mexico border and Chilean marijuana samples. Although PCA analysis suggested that these markers may be useful in differentiating crop type and biogeographic origin, a more extended number of samples and genetic markers is needed to provide detailed information about crop differentiation and biogeographical origin.

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**Forensic Plant Science; *Cannabis sativa*; Single Nucleotide Polymorphisms**



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**B93 Maximum DNA Recovery From Cold Case Victims Using Ancient and Forensic DNA Extraction Methods**

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**Learning Objective:** This research provides a comparison of methods of genomic and mitochondrial DNA isolation as well as the Next Generation Sequencing (NGS) approaches used to address limitations in DNA recovery in cases of severely degraded remains.

**Impact Statement:** This presentation will impact the forensic community by establishing best practices for the recovery of sufficient DNA for the identification of individuals in cases where tissue degradation prevents the use of traditional approaches.

Currently, there are over 100,000 unidentified remains in the United States alone, with nearly 4,400 additional individuals recovered every year. The identification of these remains poses significant technical challenges for researchers and government agencies due to the degradation of biological tissues. This degradation results in low yields of highly fragmented DNA molecules. Technical advances generated for use in isolating and sequencing endogenous genomic DNA stemming from ancient remains have the potential to greatly increase the DNA recovery efficacy from modern degraded tissue. Specifically, techniques used to isolate DNA from archaeological skeletal material have been optimized to capture and isolate the highly degraded DNA typical of ancient remains (e.g., highly damaged, short DNA fragments) more efficiently. The application of these techniques in more modern but still degraded forensic skeletal tissues has the potential to significantly increase DNA yields and quality in these challenging samples, aiding in their identification. These technical optimizations may be especially useful in cold cases where traditional PCR-based applications have failed.

Presented here is preliminary data comparing the efficacy of the ancient DNA extraction protocol outlined in Dabney et al with that of the commonly used forensic bone sampling protocol outlined in Lorielle et al regarding DNA recovery, STR profile generation, mitochondrial haplotyping, and diagnostic Single Nuclear Polymorphism (SNP) analysis.<sup>1,2</sup> This study included 42 individuals, represented by 75 degraded bone samples provided by the Maricopa County Office of the Medical Examiner. The DNA extracted from these two protocols was also used to create double- (dsDNA) and single-stranded (ssDNA) DNA libraries for mitochondrial DNA using the mito capture *H. sapiens* Representative Global Diversity Panel (197 mtDNA sequences) and SNP enrichment utilizing a panel of ~4700 diagnostic nuclear SNPs. Analyses of these generated mitochondrial enriched dsDNA libraries have yielded 25 high-coverage mitochondrial genomes (average of 92.25x coverage), with continued analyses of additional mitochondrial and SNP enriched libraries is in progress. In addition, 20 complete STR profiles (X loci verified in triplicate), representing 12 (28%) individuals have currently been recovered from these samples. Preliminary results demonstrate that by optimizing protocol efficiency to recover DNA from highly degraded bone samples we can enhance the probability of identifying individuals from challenging forensic contexts.

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**NGS; Degraded DNA; Forensic DNA**

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**B94 Mitochondrial DNA Sequence Variation in the Lebanese Population**

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**Learning Objective:** After attending this presentation, attendees will understand the important applications of mitochondrial DNA (mtDNA) in forensic investigations, the process of databasing mtDNA using Next Generation Sequencing (NGS), the analysis of haplotypes of mtDNA, and will have an example of a population established using this process.

**Impact Statement:** This presentation will impact the forensic science community by contributing a population sample to available mtDNA reference data, which allows for the weight of the mtDNA evidence to be determined in forensic cases.

Now that mitochondrial genome (mitogenome) sequencing is becoming more widely used in forensics, it is necessary to augment the available population reference data for meaningful haplotype frequency estimations. To this end, mitogenome data from 94 Lebanese individuals were generated using massively parallel sequencing. The 94 saliva samples were obtained from males and females in Beirut, Lebanon, who have at least one parent born in Lebanon. The DNA extraction was performed using a salting-out approach that yielded highly concentrated DNA. Then, long-range PCR amplification of two 8,500 base pair targets was performed to enrich the complete mitogenome. All post-PCR processing was automated on a robotic liquid handling platform to minimize the opportunity for user error. The DNA concentration of each amplicon was determined to normalize by concentration. After purification of the combined amplicons, library preparation with a target input of 150ng was performed in which a unique barcode was attached to each sample in each well. The concentration and quality of the DNA libraries were checked prior to equal-volume pooling, followed by sequencing on an Illumina® system. The data were then compiled and analyzed using a validated forensic mitogenome workflow and checked by forensic technicians.

The sample success rate was 98.9%, as 93 out of 94 samples produced complete mitogenome sequences. The average coverage for the 93 passing samples of the Lebanese population was approximately 1,400X, ranging from 640X to 2,980X. Eighty-three haplotypes were found to be unique in the dataset (observed once), while five haplotypes were shared by two samples each when indels and heteroplasmy were ignored, corresponding to a random match probability of 1.19% and haplotype diversity of 0.9988. Of note, one of the samples displayed a 16-bp insertion in the third hypervariable region, representing a novel polymorphism for this H14a haplotype. There were 32 Point Heteroplasmies (PHPs) observed across the total dataset using a 5% variant frequency threshold. Twenty-four samples exhibited PHP, with a maximum of three PHPs in a single haplotype and a pre-sample rate of 25.8%. Of the 32 observed PHPs, 11 (34.4%) were located within control region 21 (65.6%) within the coding region, which is consistent with other high-quality mitogenome datasets. The mitochondrial haplogroups were predominantly West Eurasian lineages (96.8%), with only three haplogroups associated with other biogeographical regions (one African, one East Asian, and one Native American). This population dataset can be used to further expand the use of mitogenome sequences within forensic investigations, as well as be used in haplotype frequency estimations for a previously underrepresented population.

*The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.*

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**mtDNA; Next Generation Sequencing; Populations**

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**B95 Utilizing Environmental DNA (eDNA) from Geologic Materials for Sample-to-Sample Comparisons**

*Teresa M. Tiedge, MPS\*, North Carolina State University, Raleigh, NC; Kelly Meiklejohn, PhD, North Carolina State University, Raleigh, NC*

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**Learning Objective:** After attending this presentation, attendees will understand: (1) how soil and dust from mock evidence items were collected for downstream DNA analysis, (2) the processing steps of the DNA workflow, including isolation, amplification, and next generation sequencing, and (3) the bioinformatic analysis of sequencing data.

**Impact Statement:** If the goals of this research are met, DNA from biological taxa present in soil and dust may be used to provide additional information to strengthen the evidentiary weight of geologic evidence.

Soil and dust are often submitted to crime laboratories as trace evidence and can be used to link an individual to a crime scene or to determine an evidentiary sample's origin. Methodologies that are routinely applied to analyze these geologic materials aim to characterize their physical properties (e.g., color and pH) and inorganic components (e.g., mineral content). However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information from evidence.

DNA metabarcoding is a commonly used approach to identify the biological taxa present in various environmental samples by amplifying and sequencing short, informative regions of the genome and is not restricted by sample amount. The goal of this research was to determine the utility and stability of environmental DNA (eDNA) from four biological taxa associated with soil and dust for sample-to-sample comparisons and sample origin. In this study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a one-year period. Mock items included: (1) soil removed from t-shirts, boot soles, and trowels; (2) exposed dust collected from brick pavers using polyurethane swabs; and (3) dry dust from air filters (~1" X 1" area used). DNA was isolated from mock geologic evidence using the PowerSoil® Pro Kit and DNA metabarcoding was applied to characterize bacteria (16S), fungi (ITS1), arthropods (COI), and plants (ITS2, trnL) associated with each sample (n, 1026). Libraries were generated using custom-indexed primers and were subsequently sequenced using the Illumina® MiSeq®. Raw sequencing reads were processed through a bioinformatic pipeline that removes primer sequences, identifies amplicon sequence variants (ASVs) via DADA2, and searches the ASVs against GenBank for taxonomic identification.

This presentation will focus on the experimental design and workflow and will include a preliminary assessment of temporal and spatial variables on the recovery of bacteria, fungi, arthropods, and plants from mock geologic evidence.

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**Forensic Geology; eDNA; DNA Metabarcoding**

## B96 Optimizing DNA Extraction in Highly Degraded Bone

Michelle Woo, BS\*, Virginia Commonwealth University, Richmond, VA; Daniela Frausto, BS\*, Virginia Commonwealth University, Richmond, VA; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA; Joseph Jones, PhD, William & Mary University, Williamsburg, VA; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA

**Learning Objective:** This presentation will provide attendees with a better understanding of DNA extraction methods maximizing yields of amplifiable human DNA from compromised bone samples for downstream applications, such as STR typing.

**Impact Statement:** This presentation will impact the forensic science community by providing information on the optimization of DNA recovery from highly degraded human skeletal remains.

During construction on the Virginia Commonwealth University medical campus in 1994, 19<sup>th</sup>-century human remains and artifacts were discovered in what is now referred to as the East Marshall Street Well (EMSW).<sup>1</sup> In the context of 19<sup>th</sup>-century medical education practices in Richmond, VA, institutions were not legally allowed to acquire cadavers for medical training procedures, and grave robbing was widely practiced. The “well” was actually an “anatomy pit” used to discard the remains from surgical practices. The East Marshall Street Well Project (EMSWP) was established upon the excavation of the commingled skeletal elements.<sup>2</sup> A seven-year community consultation concluded with the election of a Family Representative Council providing recommendations on research, commemoration, and dignified reburials to honor the individuals whose remains resided in the ESMW. As part of the EMSWP’s effort to advance future reburial process for the ancestral remains and learn more about individual life histories, pair-matched limbs (previously done by anthropologists at the Smithsonian using osteometric analysis) are being subjected to DNA extraction and profiling.

In this study, two bone elements, the femur and humerus, were used in the optimization of DNA recovery from degraded human remains. Three different bone sampling methods were tested, including scalpel scraping to produce fine bone pieces and Dremel® rotary grinding tools for obtaining bone powder, and Dremel® rotary cutting tools for cutting bone fragments that were then homogenized using the QIAGEN® TissueLyser II. Several different DNA extraction methods were tested and compared for human DNA quantity and quality. Organic phenol-chloroform extraction protocol (organic method) adapted from a previous study by Cartozzo et al., consisted of a lysis buffer containing 10mM Tris-HCl, 10mM EDTA (pH 8.0), 50mM NaCl, and 2% SDS, along with 15µL of 20mg/mL proteinase K.<sup>3</sup> DNA was precipitated by ethanol precipitation as well as by using Microcon®-30 concentrator. Additionally, one sample from each Dremel® and scalpel sample collection extracted with organic method and eluted with Microcon® filters were then processed with DNeasy® PowerClean® Pro Cleanup Kit from QIAGEN®. A modified lysis buffer was also tested with 50 µL proteinase K (20 mg/mL) using Microcon®-30 concentrator. An InnoXtract™ Bone DNA Extraction & Purification Kit was tested with bone powder collected using the Dremel® rotary cutting tools and homogenized cut bone samples. An additional lysis buffer developed by Loreille et al. was also tested.<sup>4</sup> For this method, the lysis buffer contains 0.5M EDTA and 1% SDS, along with 200µL of proteinase K (20mg/mL).

All samples were quantified using Qubit® 2.0 Fluorometer to obtain total double-stranded DNA yield. Hypervariable region 1 and 2 of mtDNA was amplified using primer and protocol as described by Nelson and Melton.<sup>5</sup> The amplified PCR products were visualized on 2% agarose gel. Those samples that were amplified using mtDNA primers were then quantified for human nuclear DNA using Quantifiler® Trio DNA Quantification Kit.

To date, a total of three samples have shown visible mitochondrial DNA bands on agarose gel. These samples are all collected using Dremel® rotary cutting tools, extracted using the organic method with 15µL of 20mg/mL proteinase K, eluted with Microcon®-30 filter, and followed by DNA cleanup using the PowerClean® kit. Of the three samples, only one sample showed a Y-chromosome DNA concentration of 0.001ng/µL, with a cycle threshold (ct) value of 35.762.

In conclusion, according to results obtained to date, collecting bone powder using Dremel® tools, followed by organic extraction with 15µL of proteinase K (20mg/mL) and the PowerClean® kit is most likely the best method to recover amplifiable human DNA.

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### Bone; DNA Extraction; Human Identification

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**B97 The Development of a Screening Tool to Determine Optimal Sampling Sites for DNA Recovery From Human Skeletal Remains**

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**Learning Objective:** Attendees will learn how Raman spectroscopy may be used to further streamline the DNA recovery process within skeletal remains casework.

**Impact Statement:** It is hypothesized that the addition of Raman spectroscopy to skeletal remains casework could serve as an effective non-destructive screening tool to determine the optimal sampling sites on the diaphysis of a long bone for forensic DNA testing. This method could reduce time, labor, costs, and the degree of destructive sampling necessary to obtain a DNA profile. Furthermore, it could help maximize DNA recovery as well as improve identification success rates in Unidentified Human Remains (UHR) investigations.

Forensic experts estimate the number of unidentified dead in the United States to be between 40,000 and 60,000. Numerous challenges exist with forensic genetic testing of human skeletal remains due to diagenesis patterns in bone microstructure, DNA degradation, and the presence of PCR inhibitors. Diagenesis is the microscopic breakdown of the bone matrix, which consists primarily of mineralized calcium hydroxyapatite and collagen. The process of diagenesis occurs in a heterogeneous, non-uniform manner along the diaphysis of a long bone and determining the region with the most intact bone microstructure is not possible with the naked eye. Therefore, taking cuttings from the diaphysis for DNA testing is a blind process, and decades of research and casework have demonstrated that differences in DNA recovery do exist between cuttings along the shaft of the same long bone. An additional consideration is that forensic genetic testing of bones is a time-consuming and labor-intensive process. Development of an effective screening method to determine the optimal sampling site(s) on the diaphysis could reduce time, labor, costs, and the degree of destructive sampling necessary to obtain a DNA profile. This approach could help maximize DNA recovery and improve success rates in unidentified human remains (UHR) investigations.

Non-destructive Raman spectroscopy could serve as a reliable screening tool to obtain information about bone microstructure and stage of diagenesis which, according to previous research, often correlates to the quantity and quality of endogenous DNA within that region of bone. In the first phase of this research, Raman spectroscopy was evaluated for its effects on known quantities of human DNA extracted from buccal swabs. This step was implemented to determine if exposure to the Raman laser would damage endogenous DNA, which would preclude the use of spectroscopy in genetic casework involving human skeletal remains. Additionally, a fresh non-human (mammal) bone was scanned to serve as a reference for high-quality (non-degraded) bone microstructure. In the second phase of this research, Raman spectroscopy was used to scan various pre-marked sections of the diaphysis of long bones from three sets of human skeletal remains with varying postmortem intervals (9 months, 5 years, 50 years). Compositional analysis of each scanned section provided information about the degree of diagenesis within the bone microstructure. The scanned regions of each long bone diaphysis were subsequently sectioned with an autopsy saw (Mopec), pulverized into fine powder using liquid nitrogen and a SPEX SamplePrep 6770 Freezer/Mill, and the associated bone powder fractions were then extracted for DNA. DNA extraction from buccal swabs and bone powder were performed using the QIAamp® DNA Investigator Kit and a modified organic extraction method, respectively. Total DNA recovery and a Degradation Index (DI) were determined using the Quantifiler® Trio Human DNA Quantification Kit and the QuantStudio™ 5 Real-time PCR System. Data on both DNA quantity and quality were compared to the Raman spectroscopy data to evaluate the correlation between bone diagenesis and DNA recovery.

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**Raman Spectroscopy; DNA Recovery; Skeletal Remains**

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**B98 The Generation of High-Quality Libraries for the Prediction of External Visible Characteristics and Biogeographical Ancestry Using a Low-Cost Automation Platform**

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**Learning Objective:** After attending this presentation, attendees will understand the ability to automate library preparation for a phenotypical and biogeographical ancestry predictive assay that can produce comparable next generation DNA sequencing results to manual library preparation.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the ability to automate an assay workflow that can help generate investigative leads by prediction of external visible characteristics and biogeographical ancestry of unknown DNA donors to crime scene evidence.

Massively Parallel Sequencing (MPS), also known as Next Generation Sequencing (NGS), enables greater detection power and forensic insights in contrast to Sanger sequencing, especially with degraded and limited-quantity samples. The MPS workflow involves the preparation and sequencing of libraries that include small, targeted regions of the genome, such as Short Tandem Repeats (STRs), Single Nucleotide Polymorphisms (SNPs), and mitochondrial DNA (mtDNA). Implementation of laboratory automation for the preparation of the MPS libraries can help reproducibly generate high-quality data that maximizes the benefits of MPS, while also increasing throughput and eliminating manual repetitive pipetting operations that are prone to human error. However, the purchase of an automation platform can strain a laboratory budget and can consume valuable laboratory space. Scripts for a liquid handler, the Verogen™ PrepStation, were developed to address the need for automation of NGS library preparation kits. The Verogen™ PrepStation is a small, high-precision liquid handler that includes pre-programmed protocols that are optimized for use with the MiSeq® FGx™ Sequencing System and the Universal Analysis Software (UAS). This integrated workflow provides a cost- and size-effective entry point to laboratories considering automating MPS workflows for forensic applications.

This presentation will demonstrate the ability of the Verogen™ PrepStation to automate Verogen's™ ForenSeq™ Imagen assay kit workflow for library preparation while maintaining the assay's sensitivity, reliability, and specificity. The assay employs two primer mixes: one for the interrogation of 55 phenotype-informative SNPs (piSNPs) for external visible characteristic prediction and another that includes the 55 piSNPs with the addition of 56 ancestry-informative SNPs (aiSNPs) for biogeographical ancestry prediction. Once prepared, the libraries are sequenced on the MiSeq® FGx™ Sequencing System, and the sequencing data is analyzed using UAS to generate Hiris Plex-S reports that are compatible with online prediction tools.<sup>1</sup>. These predictions are then able to be used to generate investigative leads.

Results will be presented from the sensitivity, stability, accuracy, and precision studies performed using the ForenSeq™ Imagen Library Prep Kit on the Verogen™ PrepStation. Furthermore, this presentation will demonstrate the use of automated scripts for the target enrichment, purification, normalization, and pooling steps of library preparation. Additionally, the results will show the PrepStation's automated workflow performs comparably to manually prepared libraries, with column-to-column reproducibility and no detectable sample cross-contamination.

The ForenSeq™ Imagen Kit can provide useful phenotype and biogeographical ancestry information to aid as an investigative tool, particularly for instances in which the STR DNA profile obtained from crime scene evidence does not match any reference database profiles. The automation of the library preparation workflow of this assay kit will provide ease and efficiency that is invaluable for time-sensitive investigative work.

**Reference:**

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**Next-Generation Sequencing; Automation; Crime Scene Investigation**



## **B99 The Optimization of Single Nucleotide Polymorphism (SNP) SNaPshot™ Multiplexes to Distinguish Fiber and Drug-Type Cannabis Sativa**

Natalia Czado, MSFS\*, Sam Houston State University, League City, TX; Sheree Hughes, PhD, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the ability to use genomic information to differentiate the crop-type of *C. sativa* plants.

**Impact Statement:** This presentation will impact the forensic science community by providing an alternative to traditional chemotyping of drug and fiber-type *C. sativa* using a modified SNP assay and the ability to generate some information about the biogeographical origin of the plants.

*Cannabis sativa* is a highly trafficked and cultivated plant, mainly for its intoxicant properties. The plant can be classified into two forms, fiber-type (hemp) or drug-type (marijuana). In the United States, drug-type *C. sativa* remains federally classified as a Schedule I drug under the Controlled Substances Act. In contrast to drug-type *C. sativa*, hemp production and sales have been legalized since the passage of the 2018 Agricultural Improvement Act. For *C. sativa* to be classified as hemp in the United States, it must contain less than 0.3% THC by weight. This established threshold has made it difficult for law enforcement agencies to prosecute drug cases due to most seized drug labs lacking a validated quantitation method. Furthermore, detection of the presence of THC does not provide information on the origin of the plant material; therefore, law enforcement cannot effectively mitigate illegal drug trafficking. Due to the limited intelligence provided by chemical analysis, researchers have been investigating molecular methods to distinguish and associate *C. sativa* samples.<sup>1-5</sup>

Recently, a study described a 23 SNP panel that was able to differentiate crop-type and provide biogeographical information.<sup>6</sup> The panel used a custom KASP assay to examine 22 nuclear SNPs and one mitochondrial SNP.<sup>6</sup> Although the assay is useful for determining the genetic diversity and cluster association of *C. sativa*, it requires separate reactions for each SNP. This requires the consumption of larger volumes of DNA extract, which may not always be possible. Therefore, it would benefit the forensic community to convert the assay to a technique that requires less input and utilizes the traditional instrumentation found in crime laboratories, such as a SNaPshot™ assay, which utilizes capillary electrophoresis. In this study, DNA sequences provided by Henry et al. were used to design PCR and Single-Base Extension (SBE) primers to create a 3-multiplex SNaPshot™ assay.<sup>6</sup> After the multiplexes were optimized, DNA previously extracted from known hemp and marijuana samples using the DNeasy® Plant Mini kit were examined. Marijuana samples were obtained from seizures at the United States–Mexico border by Customs and Border Patrol; seizures by Policia de Investigaciones in southern Chile; and extracts from Chilean collaborators. Hemp samples were purchased from Manitoba Harvest in Canada; Navitas™ Organics and American Hemp Harvest in the United States; and The Original Hemp and CBD Hemp Direct Buds in the United States. All extracts were quantified using the Qubit® dsDNA High-Sensitivity (HS) assay on the Qubit® 2.0 Fluorometer. Custom SNP panels were created in GeneMapper® ID-X v.1.4 Software. SNP and haplogroup data were recorded and genetic data was compared with the corresponding cannabinoid data. Principal Component Analysis (PCA) was conducted on the SNP results of all samples. Overall, results demonstrated the utility of the multiplexes in differentiating crop-type, as well as determining cluster origin.

### **References:**

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### ***Cannabis sativa*; Single Nucleotide Polymorphism; Crop-Type**

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**B100 Creating a Forensic Reference Ladder and Multiplex to Be Used in Animal Abuse Cases**

*Vallarie Ostenson, BS\*, Florida International University, Homestead, FL; DeEtta Mills, PhD, Florida International University, Miami, FL*

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**Learning Objective:** Attendees will learn about the science behind creating a forensic reference ladder and how to design and optimize a multiplex PCR protocol for multiple species. Attendees will also learn the optimization techniques used when creating protocols and validation techniques. There is a large gap in the current standards of species identification in the forensic setting, and this project provides a novel, stream-lined solution.

**Impact Statement:** This project provides a stream-lined presumptive identification process of unknown domestic species that can be utilized in a variety of cases involving animals. Forensic cases that are directly involved with animals, and cases where this project is relevant, include: animal abuse cases, sport-fighting of animals, illegal slaughter, and food fraud. The large gap that exists in current standards for species and multi-species identification leaves analyzers and researchers no choice but to go through the rigorous process of identification. This project optimizes the process of domestic species identification and allows for presumptive results to be achieved where current standards call for repeat testing, and a process of trial and error.

Animal abuse cases have gained traction in the media in recent years and have prompted government officials to increase penalties for animal abuse cases, to amend existing laws, and write more stringent statutes that surround animal abuse cases.<sup>1</sup> Currently, there are no commercial forensic kits available on the market that can discriminate between domestic species and allow for rapid species determination in collected evidence. Current protocols require extensive testing with a process of elimination to garner which species may be present.<sup>2</sup> This project aimed to create a reference (allelic) ladder for nine domestic animal species as well as a protocol for a multiplex PCR for presumptive species identification using the Cytochrome-b gene in mitochondrial DNA. The nine species include horse, sheep, goat, pig, cow, chicken, and turkey, as well as domestic dog and cat. The reference ladder and the multiplex that was designed provided a quick, robust species identification of unknown sourced evidence collected from animal fighting rings, illegal slaughter cases, crime scenes involving animal abuse, or food fraud cases.

Whole blood from each species was obtained from a commercial supplier of banked animal blood and DNA was extracted using a spin column kit, quantified and then amplified using PCR. The PCR primers were created to target specific regions of the Cyt-b gene in the mtDNA.<sup>3</sup> A universal forward primer and species-specific reverse primers were used to differentiate species by amplicon length differences.<sup>3</sup> Amplicon lengths for individual species are variable enough to give clear results. For instance, the fragment achieved when amplifying chicken is 227 BP, and the fragment achieved when amplifying horse is 437 BP. The same primer sets were used to create the species reference ladder that were in the multiplex reactions. DNA from each individual species was run against all other species to ensure no cross-amplification and that distinct individual species identification could be achieved. The multiplex for all species was optimized so it provided clear results and when the amplicon was compared against the reference ladder, the unknown was properly identified.

The current standards in the field of forensics do not allow for discrimination of an unknown species in a stream-lined process without multiple steps, repeated testing, and a process of elimination to discern a species identity. The creation of a multi-species reference ladder and multiplex protocol allows for a stream-lined analysis process and rapid species identification.

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**Mitochondrial DNA; Multiplex PCR; Animal Abuse**

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**B101 The Creation of a Presumptive Multiplex Kit to Test for the Presence of Domestic Animal Mitochondrial DNA in Cases of Presumed Animal Abuse**

*Lindsey Kaufman, BA\*, Florida International University, Coral Springs, FL; DeEtta Mills, PhD, Florida International University, Miami, FL*

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**Learning Objective:** Attendees will learn how to build a ladder and optimize a multiplex. After attending, they will understand the significance of being able to test for animal DNA quickly and efficiently.

**Impact Statement:** By creating a presumptive forensic multiplex kit, users will be able to quickly distinguish between common domestic pets in cases involving animals. For instance, this multiplex kit would be helpful in determining the unknown species present in evidence taken from a sport-fighting scene or other instances of abuse. There currently isn't anything on the market that allows users to distinguish between multiple animals using only a single kit. This kit will also benefit the scientific community by reducing the amount of time and reagents spent on unknown evidence testing.

Currently, forensic identification techniques used in cases involving pets at crime scenes often rely on blood or shed hair analysis as it might relate to the victim or suspect. However, these analyses can be difficult since there are only a few non-human species-specific "kits" available for domestic animals. Most of these STR kits, like the Meowplex used for identifying cats, focus on one species' identification.<sup>1</sup> While autosomal STRs may provide a means for specific identification of an animal, mitochondrial DNA degrades slower than nuclear DNA and is much more plentiful within cells; therefore, this type of DNA can often be useful when evidence from an unknown source is collected at a crime scene.

When hair, tissue, or blood are found at a crime scene and no animal is present, a method to identify the unknown species as the source of the evidence is needed before moving to STR analysis. The objective of this project is to use mitochondrial DNA markers to create a reference ladder and design a multiplex kit that can simultaneously detect dog, cat, rabbit, donkey, goat, and human, all possible domestic animals associated with the human environment and could be associated with a crime scene. A labeled universal forward primer and species-specific reverse primers are used for simultaneous amplification of six species in one multiplex.<sup>2</sup> These species are distinguished by the length heterogeneity of the amplicons. Successful single-plex amplification of dog and cat mtDNA has been done and the others are underway. The individual species' PCR products are column cleaned, then mixed together to form a reference ladder. Then by amplifying any unknown evidence samples, the contributing species can quickly be identified when aligned with the known reference ladder using the genetic analyzer or gel electrophoresis. The multiplex still needs to be validated and optimized but this "kit" will provide a quick presumptive identification of the species of origin for unknown crime scene evidence.

**References:**

- <sup>1.</sup> Butler, J. M., David, V. A., O'Brien, S. J., & Menotti-Raymond, M. (2002, September). *Profiles in DNA*.
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**Animal Abuse; PCR Multiplex; Mitochondrial DNA**

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**B102 The Potential of the 165 Ancestry-Informative Single Nucleotide Polymorphisms (SNPs) in Further Discriminating Asian Populations**

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**Learning Objective:** After attending this presentation, attendees will understand the potential of the 165 Ancestry-Informative SNPs (AISNPs) included in the Applied Biosystems® Precision ID Ancestry panel for further discriminating Asian populations, and the patterns of genetic structure in Asian populations based on those SNPs.

**Impact Statement:** This presentation will impact the forensic science community by serving the genetic characteristics of the large number of Asians from northeast, southeast, and southwest of Asia investigated using 165 AISNPs, particularly including Korean and Myanmar populations that have barely been studied, which can be importantly considered for forensic genetics.

Ancestry-informative genetic markers are valuable in forensic investigation by providing investigative leads such as specific ancestral genetic origins when no reference to be compared to is available. Multiple ancestry-informative markers or SNP panels for this purpose have been developed, and ancestral prediction currently can be achieved at the continent level using the commercial panels. However, in practice, it is more useful to distinguish ethnic groups within a continent in detail, which can be more informative. Asia is the largest continent with diverse ethnic groups, but it is difficult to classify regions of Asia based on their residing geographical area or nations, as well as it is not clear that this division can accurately reflect their ancestral origins. In addition, more data is still needed to better understand the genetic structure of Asian populations.

Here, we evaluated 165 AISNPs in further discriminating Asian populations for samples of 794 Asians obtained from Korea, China, Vietnam, Myanmar, Bangladesh, India, Sri Lanka, and Pakistan. Genotyping of the SNPs was performed using the Precision ID Ancestry panel and the IonS5xl system according to the manufacturers' instruction. Calculation of the population likelihood and admixture prediction was performed using the HID SNP Genotyper® plugin of the Torrent Server provided by the manufacturer. Genetic structures of populations were investigated using STRUCTURE software v2.3.4.

In the result, using the HID SNP Genotyper® plugin with its reference database, 246 Korean samples were all predicted to be East Asians with high likelihood values, and 9 Chinese residing in Southern region of China were predicted to be East Asians or Southeast Asians (Lao Loum); 199 samples from Myanmar were predicted to be admixed with East Asians and Southeast Asians, with a higher portion of East Asians; 199 samples from Vietnam were also predicted to be admixed with East Asians and Southeast Asians, but with a higher portion of East Asians compared to those of samples from Myanmar; 141 samples from Southwest Asian populations exhibited distinct patterns from East or Southeast Asian populations. Most of the samples tested were predicted to be South Asians with high likelihood values and were partially admixed with Europeans or Southwest Asians.

Genetic structures of Asian populations were investigated for 794 Asians tested, together with more data of 750 individuals from our previous study and of 492 from Snipper reference.<sup>1</sup> Populations were divided into three groups based on their geographical region in this study, 761 Northeast Asians (Chinese, Korean, and Japanese), 498 Southeast Asians (Vietnamese and Myanmar), and 777 Southwest Asian (Bengali, Indian, Sri Lankan, and Pakistani from Pathan and Punjabi). A total of 2,036 samples were analyzed with varied assumed populations (K) from K=1 to K=10. In the result, an optimum K=2 was observed, and SWA was the most distinguished from other populations at K=2. Three NEA populations showed a pattern distinct from populations of Vietnam and Myanmar at higher K values, and a different genetic structure for Vietnam and Myanmar was revealed at > K=4. Myanmar presented a unique genetic structure. Koreans and Japanese were identical in their genetic structure, and Chinese populations were close to both of two populations but similar to Vietnam populations as well. NEA and SEA populations were closer genetically compared to SWA populations. We believe that the 165 AISNPs have the potential to discriminate Asian population at a finer scale and be informative to understand genetic structures within Asia.

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**Ancestry Inference; Single Nucleotide Polymorphism; Asian Population**

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**B103 The Use of Optical Trapping Coupled With a Microfluidic Device for Cell Separation**

*Brittney Hackworth, BS\*, Department of Forensic Science, Virginia Commonwealth University, Lexington, KY; Samantha Pagel, MSFS, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA; Sarah Seashols-Williams, PhD, Department of Forensic Science, Virginia Commonwealth University, Richmond, VA; Joseph Reiner, PhD, Virginia Commonwealth University, Richmond, VA; Mackenzie Lally, MSFS, Greenwood Genetic Center, Richmond, VA; Tracey Dawson Green, PhD, Virginia Commonwealth University, Richmond, VA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how small-scale cell separation of forensic mixtures using optical trapping on a microfluidic device can rapidly and efficiently separate cells with minimal sample preparation and time.

**Impact Statement:** This novel cell separation method will impact the forensic science community by providing an efficient alternative solution for forensic mixtures.

Biological mixtures are prevalent in many forensic science evidentiary samples and make interpreting DNA profiles difficult. Currently, differential extractions are used to separate a mixture into a spermatozoa fraction and non-spermatozoa fraction; however, this separation method can result in ambiguous allele calls, masking of alleles, and persistent mixtures in the DNA profiles. There are no established methods of cell separation for other types of mixed evidentiary samples.

Optical trapping, also known as optical tweezing, is a front-end separation technique that utilizes a highly focused laser beam capable of manipulating dielectric particles such as cells. The optical tweezer method paired with a microfluidic device can be used to isolate cells of interest from a mixed sample and minimize interpretation discrepancies that occur with differential extraction. The microfluidic device layout was created using AutoCAD LT® 2018 software and cut with a nitrogen laser. The five layers of the microfluidic device are composed of PET, a heat-sensitive adhesive, and a coverslip. Spermatozoa were optically trapped with a 700mW, 1,064nm continuous-wave laser in an equal volume mixture of neat vaginal fluid and 1:20 seminal fluid. Between 5 to 25 spermatozoa were isolated in triplicates with the optical tweezers.

Transition to a 5W ytterbium linearly polarized laser provided the capability of beam splitting into two optical traps, allowing for capture and transport of two cells at once. The new laser and dual trapping mechanism decreased the trapping time by 43 percent on average. The optically trapped cells were excised from the microfluidic device and carried through the DNA workflow. 97.37± 2.63% of the expected alleles were observed with few to no alleles from the female contributor, and a full DNA profile was observed with as few as 25 sperm cells. The average degradation index from the quantification stage was calculated to be 1.00± 0.52 for the 1:20 seminal fluid samples and 1.16±0.52 for the mixture containing equal parts of 1:20 seminal fluid and vaginal fluid, corresponding to a negligible amount of degradation.

The continuation of this research will demonstrate that vaginal epithelial cells containing nuclear DNA can be optically trapped and processed into the DNA workflow and the flow within the microfluidic device optimized for faster cell separation. In conclusion, this novel cell separation method shows promise for use in differentiating cells in forensic mixtures—not only in sexual assault samples, but in mixed samples of other types.

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**Optical Trapping; Cell Separation; Microfluidic Device**

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**B104 The Effects of the Composition of Whole Saliva/Oral Fluids on the Development of Epigenetic Markers**

*Nicole Fernandez-Tejero, MS\*, Florida International University, Miami, FL; Mirna Ghemrawi, PhD, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will understand the effect of the collection method on the DNA methylation levels and the importance of validating epigenetic markers on various fluid depositions.

**Impact Statement:** This presentation will impact the forensic science community by providing the DNA analyst with an alternative method to identify saliva markers based on buccal or spit collection.

In forensic casework, DNA is extracted from biological exhibits and compared to databases. Yet, in cases where an offender has regular access to a victim, a DNA profile may not be probative unless the source of the biological stain is known. One of the commonly found body fluids at a crime scene is saliva. In terms of its cellular composition, saliva is known to be heterogenous, and a mix of epithelial, leukocytic, and bacterial cells may be present. Thus, the differentiation between deposition methods could provide insight into the type of scenario at a crime scene.

One of the epigenetic modifications most extensively studied is DNA methylation, which involves the reversible covalent binding of a methyl group to a cytosine followed by a guanine (CpG) in a DNA sequence. Bisulfite Conversion is the gold standard for differentiating between methylated and unmethylated cytosines. Pyrosequencing is the method used to analyze the CpG sites. Identifying different body fluids is typically based on finding genes that are differentially expressed in different tissues/cells. Naturally, some body fluids may consist of multiple cell types, with each type varying in DNA methylation level. From the literature, four CpG markers within/around four genes, BCAS4, SLC12A8, SOX2OT, and FAM43A, were identified as “saliva” markers based on either buccal swabs or spit collection. None have explored the differences in methylation percent between the two collection types.

For these experiments, buccal, lip, tongue, spit, chewing gum, and nasal swabs were collected from ten individuals ( $N=60$ ), DNA was extracted, bisulfite converted, amplified using in-house designed assays, and pyrosequenced. The observed methylation levels were then compared to other body fluids (semen, blood, vaginal epithelial, and menstrual blood [ $N=20$ ]). A total of 370 pyrosequencing results across multiple loci demonstrated that sampling location and collection method could greatly influence the level of methylation, statistically significant differences between the various deposition methods.

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**Body Fluid Identification; Pyrosequencing; DNA Methylation Markers**



**B105 A Snapshot of Current Opinions on Sequencing Technology in Forensics**

Megan Foley, MSFS, The George Washington University, Washington, DC; Fabio Oldoni, PhD\*, Arcadia University, Glenside, PA

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the opinions of forensic DNA researchers/practitioners on the application of sequencing technologies in forensics and its forthcoming implementation in casework.

**Impact Statement:** This presentation will impact the global forensic science community by investigating the current status and further requirements for implementation of sequencing technologies into both research and development and crime laboratories based on a global Qualtrics® survey.

Next Generation Sequencing (NGS) has introduced a big wave of innovations in forensics and is undeniably shaping the future of forensic DNA testing.<sup>1</sup> Albeit an increasing amount of forensically relevant NGS data have been documented in the literature over the past few years, only a minority of forensic laboratories worldwide have started to validate and implement this revolutionary sequencing technology in casework.<sup>2,3</sup> Moreover, the acceptance and implementation of NGS in casework within the global forensic DNA community has been limited, in particular in the United States.<sup>4</sup> NGS has an enhanced multiplexing capability in comparison to the traditional and widely accepted Capillary Electrophoresis (CE) system. This novel sequencing technology allows for analysis in parallel of hundreds of biomarkers for different forensic applications, including, among others, human identification, prediction of biogeographical ancestry and phenotypic physical traits, kinship, and human microbiome identification.<sup>5</sup> In addition, sequences of conventional short tandem repeats polymorphisms are determined by sequencing, which empowers further the deconvolution of contributors in high-order mixed profiles as well as the separation of true alleles from artifactual data.<sup>6</sup>

In this study, a Qualtrics® survey was distributed to 6,001 forensic DNA researchers and practitioners worldwide who are current members of the American Academy of Forensic Science (Criminalistics #2,630, Pathology/Biology #901, and General #727 section) and the International Society for Forensic Genetics (#1,743). The survey was approved by the Arcadia University's IRB board and included a total of 20 questions. These were divided into background demographics, training and experience of personnel, ongoing applications, current concerns, and future technology use.

A total of 367 participants returned the survey. In particular, the majority of respondents were from North and South America (69.8%), Europe (21.2%), Asia (5.5%), Oceania (2.5%), and Africa (1%). Among the respondents, 50% were forensic DNA practitioners, 31% forensic DNA researchers, and 19% were both researchers and practitioners. Approximately 38% of participants presently have an NGS platform in their laboratory and 13% are intending to purchase one. The percentage of these respondents varies based on the location of the laboratory. Overall, the data from this global survey showed that there is an extensive knowledge of NGS in forensics even though there are yet many laboratories that are currently coping with major issues, including limited funding and personnel and time-consuming and cost-ineffectiveness of the sample processing workflow. Among the technical concerns raised by respondents, including those laboratories who currently have an NGS system, is the lack of training, limited statistical applications and bioinformatics support, lack of rigorous recommendations and guidelines, and also acceptability of the legal system. Nonetheless, most of respondents envisage that a technology shift from CE to NGS may occur within a 5–10-year period. In conclusion, this work outlines current analytical challenges experienced by the global forensic DNA community and addresses different strategies for the implementation of NGS technologies in casework.

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**Qualtrics® Survey; Next Generation Sequencing; Forensic DNA Applications**

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**B106 A Side-by-Side Evaluation of Presumptive Blood Identification Tests**

*Hillary Culbertson, PhD\*, Defense Forensic Science Center, Forest Park, GA*

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**Learning Objective:** After attending this presentation, attendees will better understand the benefits and limitations of five different presumptive blood tests.

**Impact Statement:** This presentation will inform the forensic community about the benefits and limitations of five specific presumptive blood tests currently used in the field.

Blood is one of the most important biological traces that is often found at crime scenes. Not only can it provide insight as to who was present at the scene, but its pattern may also contribute to understanding the circumstances under which the blood was deposited. Therefore, collection of any and all blood at crime scenes is critical to an investigation.

Rather than collect all substances that could be blood, field agents routinely use presumptive tests at crime scenes to establish if a substance is or is not blood. While presumptive tests are typically portable, inexpensive, and provide results quickly, they do have limitations. Some of these limitations include short shelf lives, specific storage conditions, false positives, and false negatives. While some of these limitations are listed on the device's specification sheet, not all the provided information aligns with what is observed in real-world scenarios.

This work evaluated the Limit Of Detection (LOD), reaction time, accelerated aging, and ability to withstand acute storage conditions for five presumptive blood tests (Hemastix™, Hemident™, Hexagon OBTI®, QuickCheck™ Bloodstain Green, and phenolphthalein). While all tests produced positive results within five minutes, Hexagon OBTI® and Hemastix™ had the lowest observed LODs at 16µg/mL heme (1:104 of blood in TE buffer) and 160µg/mL heme (1:103). All other presumptive blood tests had observed LODs of 1,600µg/mL heme (1:102). It was also noted that the order of ampule breakage for the Hemident™ greatly influenced the prominence of the test's color change. While physical changes were observed during the accelerated aging at 75°C for most of the tests, only Hexagon OBTI® failed the accelerated three-year shelf life test. All presumptive blood tests provided positive results at -20°C, ambient temperature, 42°C, and 75°C when stored at these temperatures for 28 days.

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**Blood; Presumptive Test; Comparison**

**B107 The Development of Carbon Nanotubes-Assisted Solid-Phase Microextraction for Fire Debris Analysis**

Ting-Yu Huang, MSc\*, Sam Houston State University, Huntsville, TX; Jorn (Chi-Chung) Yu, PhD, Sam Houston State University, Huntsville, TX

**Learning Objective:** Attendees will understand how a Carbon Nanotube (CNT) -based solid Phase Microextraction (SPME) fiber is made and how the fiber performs when extracting the alkane and aromatic compounds in gasoline. A new experiment designed to investigate the carry-over effect of an SPME fiber will also be demonstrated.

**Impact Statement:** The impact of this study is to introduce nanotechnology to extract Ignitable Liquids (ILs) in fire debris analysis. As nanomaterials have unique properties in their surface areas and specific affinity toward target compounds, selectivity for ILs could be improved.

According to the National Fire Protection Association (NFPA), an estimated 52,260 intentionally set fires were reported annually to United States municipal fire departments between 2014 and 2018.<sup>1</sup> Because gasoline is readily available on the market, it has become the most widely used IL by arsonists. The current gold standard to identify the presence of gasoline in fire debris samples is the test method using Gas Chromatography/Mass Spectrometry (GC/MS).<sup>2</sup> Before GC/MS analysis, pre-concentration of the analytes is essential since the evidence is easily destroyed by combustion or water during fire extinguishment.

Headspace/SPME (HS/SPME) is a simple, reusable, and solvent-free technique that extracts various volatile organic compounds from complex matrices.<sup>3</sup> HS/SPME has been standardized for separating a very low concentration of an IL in the sample with high sensitivity.<sup>4</sup> Because the extraction performance is strongly affected by its fiber, the development of new sorbent chemistry and coating methodology has become a popular research interest for scientists to improve the extraction efficiency of target compounds in the samples. The aim of this work is to propose a straightforward SPME fiber assembly using a stainless-steel wire and Single-Walled Carbon Nanotubes (SWCNTs) as a novel coating to extract gasoline residues in fire debris samples efficiently.

CNTs have aroused extensive attention in a broad range of applications because of their high surface area, strong mechanical strength, and excellent chemical stability.<sup>5</sup> Based on their nanoscale, tube-shaped structure and non-covalent interactions with hydrophobic compounds, it was hypothesized that CNTs may be a suitable adsorbent to extract the target analytes in gasoline. Attempts were made to prepare a SPME fiber coated with polydopamine, epoxy resin, and SWCNTs, layer by layer, through a dipping procedure. The effects of various parameters on the efficiency of the HS/SPME process, such as incubation temperature and extraction time, were studied. To validate the optimized conditions, spiked nylon carpet samples were prepared by adding 5  $\mu$ L of the gasoline reference (unleaded premium grade, 20mg/mL methanol) with serial dilution ( $n=8$ ) to 300mg of the burned matrix. Sixteen aromatic compounds were selected as gasoline target analytes for the investigation.

The results proved the reliability of the proposed CNTs fiber and the optimized HS/SPME/GC/MS method for determining gasoline residues in simulated fire debris samples at trace levels, obtaining the detection limit at 3  $\mu$ g in a 20mL headspace vial. Good precision with relative standard deviations lower than 9% was found among triplicate analyses in the linear range (3–50  $\mu$ g in a 20mL headspace vial). Furthermore, no carry-over effect was observed under a repeated desorption process ( $n=6$ ). The significance of the proposed CNTs fiber coating is that it provides a novel headspace extraction adsorbent with high affinity toward gasoline target analytes in fire debris samples. In conclusion, the proposed CNTs fiber performs well in extracting gasoline in fire debris. The great potential for forensic analysis of gasoline evidence using the proposed fiber is also demonstrated.

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3. Michael J. Swierczynski, Grau K, Schmitz M, Kim J. Detection of gasoline residues present in household materials via headspace-solid phase microextraction and gas chromatography–mass spectrometry. *J. Anal. Chem.* 2020;75(1):44–55. doi:10.1134/s1061934820010153.
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**Carbon Nanotubes (CNTs); Solid-Phase Microextraction (SPME); Fire Debris Analysis**

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**B108 Trace Metal Profiling of Polymer Filaments Used in 3D Printing**

*Thomas Gluodenis, PhD, Lincoln University, Lincoln University, PA; John Stallworth\*, Lincoln University, Philadelphia, PA*

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**Learning Objective:** Attendees will learn about metal additives used during polymer manufacturing, which metal profiles can be used for the purpose of chemically differentiating the same polymer type produced by two different manufacturers, and how elemental fingerprinting can provide an enhanced level of differentiation beyond physical properties and organic composition.

**Impact Statement:** Forensic identification of 3D printers and their products are inhibited by the lack of applied research and validated test procedures. This presentation will demonstrate that it is feasible to chemically differentiate polymer filaments commonly used in 3D printing based on their trace elemental “fingerprint.” This, in turn, opens the possibility of associating a polymer filament to a 3D printed object and perhaps even to the printer on which it was manufactured.

The 3D printing process simplifies manufacturing and enables virtually limitless customization of products. It also enables an individual to print practically anything without the boundaries of societal controls, thereby attracting counterfeiters, terrorists, and organized crime. Samples of black Polylactic Acid (PLA) filament from five different commercial vendors were analyzed using XRF and Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). PLA was chosen as the test sample as it is compatible with consumer printers and has properties commensurate with the manufacture of 3D printed weapons and other illicit items. Samples (0.24g) of black PLA filament were cut into approximately 2mm pieces in order to maximize surface area then digested in 1ml of concentrated nitric acid using a Milestone UltraWAVE microwave digestion system. The resulting digests were diluted 1:50 with deionized water, spiked with a 20ppb internal standard mix, then analyzed for all elements on the periodic table using an Agilent® Technologies 7700 ICP/MS. A series of pairwise comparisons was performed using a *t*-test (95% confidence level) in order to determine which elements were present above the limit of quantification and distinguishable between the various sample pairs. Twenty-five elements were found to be distinguishable, thereby creating an elemental “fingerprint” for the PLA samples analyzed. These results suggest that the trace metal profiling would enhance a forensic examiner’s ability to make determinations regarding association or exclusion between recovered evidence and a reference sample.

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**Polymers; 3D Printing; Metals**

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**B109 The Elemental Analysis of Recently Manufactured Automotive Glasses and a Comparison of Data Processing Programs**

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**Learning Objective:** After attending this presentation, attendees will understand the importance of the automotive glass database in forensic glass analysis.

**Impact Statement:** This presentation will discuss current trends of recently manufactured automotive glasses and the performance of data processing programs.

Glass fragments can play an important role in reconstructing crime scenes in various criminal cases, including car accidents, theft, and burglaries. The value of glass evidence increases in the absence of biological evidence and digital records such as DNA, fingerprints, and surveillance cameras. To determine whether questioned glass fragments originated from the same source as known samples, it is necessary to examine the elemental profile of glass fragments. The analysis of glass fragments has been greatly developed in the past ten years with advances in technology. Laser Ablation-Inductively Coupled Plasma/Mass Spectrometry (LA-ICP/MS) is considered a gold standard technique allowing for the analysis of small glass fragments (< 500µm) in practice. Recently, there have been many studies to determine the rarity of glass fragments with statistical approaches that require a database for elemental profiles of different types of glass.

This study aims to investigate the elemental profiles of recent automotive glasses in addition to the previously obtained glass database up to 2009. The construction of a database is important to determine the rarity of questioned glass fragments collected from crime scenes. Recently manufactured automotive windshield glass samples were collected from vehicles manufactured between 2016 and 2020 in collaboration between the NFS and Korea Automobile Testing & Research Institute (KATRI). Elemental analysis of these glass samples was performed using a NWR 213 laser ablation system (Electro Scientific Industries, Inc.) coupled to 8800 Triple Quad ICP/MS (Agilent Technologies). During the data processing of elemental analysis by LA-ICP/MS, many laboratories utilize the GLITTER program whereas our laboratory uses the developed program called LA-Quant 15 data analysis program. To determine the performance of LA-Quant 15, the comparison of two data processing programs was also performed in this study. Based on the elemental profiles of the recent automotive glasses, the difference in elemental composition between car manufacturers is discussed.

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**LA-ICP/MS; Forensic Glass Analysis; Laser Ablation**

### **B110 Femicide During the COVID-19 Pandemic: The Italian Context**

*Gianluca Rossi, MD\*, University of Turin, Italy, Torino, Piemonte, ITALY; Alice Porceddu, MD, University of Turin, Italy, Torino, Piemonte, ITALY; Camilla Bonci, MD, University of Turin, Italy, Torino, Piemonte, ITALY; Francesca Capello, MD, University of Turin, Italy, Torino, Piemonte, ITALY; Giancarlo Di Vella, PhD, University of Turin, Italy, Turin, Piemonte, ITALY*

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**WITHDRAWN**



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**B111 The Forensic Analysis of 3D-Printed Polymers Using Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)**

*Jenna Covey, MSFS\*, University of New Haven, New Haven, CT; Brian Musselman, PhD, Bruker, Saugus, MA; Maria-Isabel Carnasciali, PhD, University of New Haven, West Haven, CT; Brooke Kammrath, PhD, University of New Haven & Henry C. Lee Institute of Forensic Science, West Haven, CT*

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**Learning Objective:** After attending this presentation, attendees will have insight into how DART®-MS can be used for the source identification and discrimination of polymers used in the manufacturing of 3D-printed objects.

**Impact Statement:** This presentation will impact the forensic science community by introducing and demonstrating the value of employing DART®-MS for the source identification and discrimination of polymers used in the manufacturing of 3D-printed objects.

Additive manufacturing, or 3D printing, is a burgeoning industry with examples of its products existing in all aspects of everyday life, including automotive and other mechanical parts, house and bridge construction, medical and emergency equipment, food and pharmaceuticals, and items of aesthetic value (e.g., jewelry, clothing, and shoes). As 3D printer technologies continue to evolve with concerted improvements in quality and decreased costs, its ease of access for providing parts for nefarious endeavors has been exploited. A variety of 3D-printed parts have been used in criminal activities, including firearm components, knuckle dusters, pipe bomb components, and ATM skimmers. In order to assess the evidentiary significance of 3D-printed materials, the nature and variability of polymer materials used in their assembly must be investigated and understood.

The goal of this research was to evaluate the ability of DART®-MS for the source identification and discrimination of polymers used in the manufacturing of 3D-printed objects. DART®-MS is a rapid, non-contact, and non-destructive ambient ionization technique that enables near instantaneous determination of sample composition when paired with a mass spectrometer. DART®-MS is a proven technique for the identification of a range of samples of forensic interest, such as explosives and drugs. There is also concerted interest in evaluating its utility for the identification and discrimination of other materials, including ink, paint, and polymer fibers.<sup>1</sup> DART®-MS has been demonstrated to differentiate polymers from different manufacturers due to its ability to detect a diversity of its complex components. A variety of chemical additives (e.g., dyes, pigments, UV absorbers, and plasticizers) may be added to a polymer product in order to produce a certain chemical or physical property of the final material. Although it is known that differences in chemical components of polymers exist between manufacturers, analytical techniques that may be used for their discrimination need to be evaluated. In particular, this research focused on understanding the advantages and limitations of DART®-MS as it applies to the brand classification and source identification of 3D-printed objects made using Polylactic Acid (PLA). PLA filament spools of different colors within the same brand and different brands were analyzed using DART®-MS. Intra-sample variability was assessed for pre-manufactured samples through analysis at several locations along the filament. To evaluate brand classification and source identification, seven different colors of PLA filament from three different manufacturers were analyzed by DART®-MS. Last, the ability to associate a 3D-printed part to an unused spool of polymer was assessed through a comparison of the DART®-MS analysis of a PLA polymer filament and its post-3D-printed part. It was ultimately concluded that DART®-MS is a rapid and reliable tool for the forensic analysis of 3D-printed PLA polymers that provides chemical information that can be used for its classification and discrimination.

**Reference:**

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**3D Printing; Polymers; DART®-MS**

### **B112 Improving the Delta-9-Tetrahydrocannabinol (delta-9-THC) Quantitative Measurements in Forensic Laboratories by The National Institute of Standards and Technology (NIST) CannaQAP**

*Walter Wilson, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Andrea Yarberry, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Jerome Mulloor, MS, National Institute of Standards and Technology, Gaithersburg, MD*

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**WITHDRAWN**

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**B113 The Investigation and Quantitation Using Ultraviolet/Visible (UV/Vis) Spectrophotometry of the Products of the 4-Aminophenol Reaction With Cannabinoids**

*Juliet Pearsall, BS\*, Cedar Crest College, Milford, NH; Marianne Staretz, PhD, Cedar Crest College, Allentown, PA; Matthew Wood, PhD, Ocean County Sheriff's Office, Toms River, NJ; Jeanne Berk, PhD, Cedar Crest College, Allentown, PA*

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**Learning Objective:** This presentation will examine the visible absorption spectra of the product of various cannabinoids with 4-aminophenol and investigate the use of the visible absorbance of the THC/4-AP product as a potential method for quantifying THC in the presence of other cannabinoids.

**Impact Statement:** This presentation will impact the forensic science community by enhancing knowledge of the 4-aminophenol/cannabinoid reaction and introducing a potential method of quantifying THC by analyzing the product of the THC/4-aminophenol reaction using UV/Vis spectrophotometry.

The goal of this project is to perform UV/Vis spectral analysis of the product formed in the reaction between various cannabinoids and 4-aminophenol and determine whether UV/Vis spectrophotometry of this product is an effective method for quantifying THC in the presence of other cannabinoids.

Delta-9 Tetrahydrocannabinol (THC) and Cannabidiol (CBD) are two major cannabinoids often derived from the plant *Cannabis sativa* L. Uses of cannabinoids range from recreational drug use to medical uses. A new test being utilized by law enforcement and forensic science analysts is the "Swiss test" or the 4-aminophenol (4-AP) test, which is a presumptive color test used to determine if a sample potentially contains THC or CBD, which is important in distinguishing a legal hemp sample from a marijuana sample. In the current study, a visible spectrophotometric analysis of the products of various cannabinoids with 4-aminophenol was performed. The wavelength maxima for the products of the 4-AP reaction with delta-9 THC, CBD, cannabinol (CBN), delta-8 THC were 650nm, 525nm, 685nm, and 650nm, respectively. The kinetics of the reaction was studied at a THC concentration of 159.0µM and the time required for maximum formation of product was observed to be 15 minutes. Most of the other cannabinoids had a similar kinetic profile. The formation of products was found to be linear with increasing concentration of starting cannabinoid for all cannabinoids. Standard curves were generated using the absorbance of the 4-AP reaction products at the respective maximal wavelengths using a concentration range of 1.590µM to 159.0µM for CBD, 1.590µM to 119.2µM for both delta-8 and delta-9 THC, and 1.611µM to 80.54µM for CBN. The ability to quantify THC in the presence of CBD using visible spectrophotometric analysis of the 4-AP product was also investigated. Delta-9 THC and CBD were combined at the following ratios (THC: CBD) 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, and 1:10. THC was effectively quantitated at each ratio using the maximum absorbance of the THC/4-AP product with biases ranging between -7% and 22%.

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**Cannabinoids; 4-Aminophenol; Ultraviolet-Visible Spectrophotometry**

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**B114 An Investigation of Organic Gunshot Residues by Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)**

*Thomas Ledergerber, BS\*, West Virginia University, New Martinsville, WV; William Feeney, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Luis Arroyo, PhD, West Virginia University, Morgantown, WV; Tatiana Trejos, PhD, West Virginia University, Morgantown, WV*

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**Learning Objective:** Attendees of this presentation will understand the validation process for common organic gunshot residue components using DART®-MS and the capabilities and limitations of DART®-MS for rapid screening of authentic gunshot residue samples.

**Impact Statement:** This presentation will impact the forensic community by bringing attention to a rapid screening method for detecting organic gunshot residue.

Gunshot Residue (GSR) analysis is crucial in investigating and reconstructing specific events centered around firearm-related incidents. When a firearm is discharged, a plume of gaseous and solid traces is released and deposited onto surrounding surfaces. This plume typically contains particles of Inorganic (IGSR) and Organic (OGSR) compounds. The current practice for GSR identification follows the ASTM E1588-20 guideline, which outlines Scanning Electron Microscopy and Energy Dispersive Spectrometry (SEM/EDS) to determine single-particle IGSR morphology and elemental composition. While effective, this technique is lengthy, further contributing to mounting backlogs in forensic laboratories and relatively slow turnaround time in the criminal justice system.

This guideline does not account for OGSR analytes which can improve the evidentiary value of samples. Hence, rapid techniques like DART®-MS can complement current methodologies and provide additional information for scientists. Although DART®-MS has been previously demonstrated to analyze components associated with OGSR, there has been limited research using authentic samples. The goal of this study was to develop, optimize, and validate a DART®-MS method for OGSR analysis and demonstrate its feasibility in casework by analyzing authentic specimens simulating various case scenarios (e.g., residues from hands of individuals of interest, spent cartridges, and residues from clothing and around bullet holes).

Standard solutions of compounds commonly associated with OGSR, including 2-nitrodiphenylamine (2-NDPA), 2,4-dinitrotoluene (2,4-DNT), 4-nitrodiphenylamine (4-NDPA), akardite II (AKII), diphenylamine (DPA), ethyl centralite (EC), methyl centralite (MC), and nitroglycerin (NG), were under investigation and were used to determine optimum instrument parameters. A full factorial design screened variables including ionization gas temperature, exit grid voltage, and orifice 1 voltage, which were optimized using a Box Behnken design. After optimization, an extensive validation study was conducted to evaluate the instrument's performance and its fit for purpose. Figures of merit such as accuracy and precision, reproducibility, specificity, sensitivity, and method robustness were also investigated. The LODs determined by ASTM Method E2677 were in the low nanograms, ranging from 75pg for DPA to 12ng for NG. The targeted compounds' reproducibly fell within the mass tolerance of  $\pm 5$ mmu over a series of days by multiple examiners. Finally, the method was validated using authentic specimens. A total of 300 specimens were collected from West Virginia University's indoor ballistics range and consisted of six subsets, each composed of 50 specimens. Clothing samples shot from 6 inches were stubbed in the immediate areas surrounding the bullet hole (set 1). Hands of the shooters were sampled following the firing event (2), whose signatures were compared to a subset of background (non-shooters) hand samples collected from the surrounding student population at WVU (3). Paired samples were also collected from the respective spent cartridges by washing the inner walls with dichloromethane to extract organic components (4). Finally, samples of 5 types of partially burnt propellant particles were collected from the fired clothing substrates (5) and compared to unburnt smokeless powder originating from the respective brands of ammunition (6). A subset of these samples was corroborated and tested by an LC-MS/MS method. Low false positive and false negative rates show the efficiency of this method for OGSR detection while also demonstrating its versatility for residue identification from skin and clothing, identification of a questioned orifice as an entrance bullet hole, and OGSR characterization of unburnt and partially burnt smokeless powder flakes.

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**Gunshot Residue; DART®-MS; Validation**

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**B115 A Mass Spectral Interpretation of Pinaca-Type Novel Psychoactive Substances (NPS)**

*Christopher Poulos, BS\*, West Virginia University, Morgantown, WV; Glen Jackson, PhD, West Virginia University, Morgantown, WV*

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**Learning Objective:** After attending this presentation, attendees will understand how compounds fragment in mass spectrometry and how unknown compounds are characterized when a reference standard is not available. Attendees will understand the fragmentation pathway for the synthetic cannabinoid 5F-APP-PINACA, also known as PX-2.

**Impact Statement:** This presentation will impact the forensic science community by providing a better understanding for how PX-2 and related PINACA class synthetic cannabinoids fragment. This will aid the characterization and identification of PINACA class designer drugs, which are designed to mimic the effects of synthetic cannabinoids and circumvent laws against scheduled substances. This understanding will assist analysts in identifying synthetic cannabinoids, especially when a reference standard or reference spectrum is not available and will enable analysts to better present and defend the reliability of mass spectral identifications to triers of fact.

The proposed fragmentation pathway for PX-2 uses a combination of high mass accuracy for elemental determinations, MSn for pathway determinations, and rational electron pushing mechanisms to identify diagnostic fragments and demonstrate how the fragments form.

Mass spectral characterization of PX-2 in its protonated form was performed on a variety of LC/MS instruments. Analysis involved injecting the PX-2 into a high-resolution qTOF mass spectrometer and a Velos Pro Linear Ion Trap (LIT) mass spectrometer, the latter of which has multistage tandem mass spectrometry capabilities (MSn) and a heated electrospray (HESI) source. The Normalized Collision Energy (NCE) was optimized for the sample at each stage of MSn. Synthetic cannabinoids with similar structures (PX-1, PX-3) were also analyzed to assess the role of different groups in the synthetic cannabinoid structure. PX-1 and PX-2 have the same n-pentyl tail, carboxamide linker, and amide-substituted benzene ring linked groups and differ only by having different core groups. PX-1 has an indole core and PX-2 has an indazole core, which provided differences in a dominant R-group transfer observed in PX-2. PX-2 and PX-3 have the same core, linker, and linked groups and only differ in that PX-3 has an ethylcyclohexane tail group as opposed to the n-fluoropentyl tail group of PX-2.

Accurate mass measurements were collected using an Agilent® Technologies 6538 UHD Accurate-Mass quadrupole Time-of-Flight (qTOF) mass spectrometer with a dual Electrospray Ionization (ESI) source. The accurate mass measurements provided confidence in the elemental compositions of the proposed fragments. The scan range and collision energy were optimized for each sample.

Fragmentation of the molecular ion of PX-2 at m/z 397 yields product ions at m/z 352, 251, 233, and 213. The base peak is observed at m/z 251 and defies simple explanations through normal cleavages and known pathways and requires significant rearrangement of the molecular skeleton. The proposed identity for the ion at m/z 251 (C<sub>13</sub>H<sub>16</sub>FN<sub>2</sub>O<sub>2</sub>) is within 0.7ppm error of the expected accurate mass and is a more accurate, if less-obvious, candidate than the previously proposed identity of (C<sub>16</sub>H<sub>15</sub>N<sub>2</sub>O) with 3.8ppm error. The simple loss of water from the intermediate at m/z 251 yields the proposed ion for the major peak at m/z 233 (C<sub>13</sub>H<sub>14</sub>FN<sub>2</sub>O). The difference in 20 Da between m/z 233 and m/z 213 is consistent with the simple loss of Hydrofluoric Acid (HF) from the alkyl chain, thus explaining the four major ions in the product ion spectrum.

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**Synthetic Cannabinoids; Multi-Stage Mass Spectrometry (MSn); Fragmentation Pathways**

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**B116 The Application of the Expert Algorithm for Substance Identification (EASI) on N-Alkanes**

*Alexander Pfeffer, BS\*, West Virginia University, North Attleboro, MA; Alexandra Adeoye, MS, West Virginia University, Morgantown, WV; Glen Jackson, PhD, West Virginia University, Morgantown, WV*

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**Learning Objective:** After attending this presentation, attendees will know how to use correlated ion abundances within replicate mass spectra to more accurately identify substances from their mass spectra. Here, we use n-alkanes as an example because existing algorithms are known to struggle to distinguish them.

**Impact Statement:** This presentation will impact the forensic science community by providing a new algorithm to identify substances. This model will minimize the false identification rate and enable forensic practitioners to have more confidence in their identifications and testimonies. The specific application to n-alkanes could assist fire-debris analysts in assigning peak identities to alkanes in petroleum distillates.

Our central hypothesis is that the relative abundances of replicate mass spectra are not independently variable because of the fundamental kinetics of ion fragmentation. Further, measured linear correlations between fragment ion abundances can be used to help identify any given substance more accurately than algorithms that assume a static exemplar or consensus spectrum.

A majority of existing search algorithms for Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS) use a Consensus (CNS) approach when identifying an unknown substance. The unknown spectra produced for any given compound are compared to the database of known spectra. However, compounds with similar EI/MS spectra can be challenging to distinguish from one another. For alkanes, problems are pronounced for larger n-alkanes, such as those larger than C<sub>16</sub>H<sub>34</sub>, because they tend not to give molecular ions, so their fragmentation patterns are almost indistinguishable to the human eye and most algorithms. The multivariate algorithm developed in this work is termed Expert Algorithm for Substance Identification (EASI). EASI provides models that account for the correlation in ion abundances and therefore explains most of the variance in replicate mass spectra and allows for more confident identifications.

A database of 3344 n-alkane EI-mass spectra was compiled. The abundance and m/z values of the 20 most abundant ions were then extracted to a database, filtered to ensure a minimum of 1,000 counts at each m/z, and randomly divided into a training set and a test set. Peak abundances from dozens of n-alkane spectra from other laboratories served as an external validation set. Twenty general linear models were built for each n-alkane by sequentially using the abundance of each ion as the dependent variable and the abundance of the remaining 19 as the independent variables. The linear models were then used to predict the ion abundances, and the 20 predictions were compared to the 20 measured values in each spectrum using the Pearson Product-Moment Correlation (PPMC), Euclidean distance (EUC), and Mean Absolute Residual (MAR).

The accuracy of predictions between measured values and EASI models were compared to the accuracy of the consensus approach of the same spectra using a Receiver Operating Character curve (ROC curve), which assesses the true positive and false positive rates over a range of threshold values. On average, EASI outperformed the consensus approach and had improved (i.e., smaller) residual errors by a factor of >3. When used as a binary classifier to identify n-alkanes such as C<sub>20</sub>H<sub>42</sub>, the EASI algorithm had an error rate of less than 3%, whereas the consensus approach had an error rate greater than 20%. EASI also outperformed the consensus method in all three different metric measurements of model validity: MAR, EUC, and PPMC. With EASI, scientists can accurately identify more substances and forensic practitioners can have more confidence in their testimonies with fewer false identifications.

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**Gas Chromatography/Electron Ionization/Mass Spectrometry (GC/EI/MS); Multivariate Linear Regression Model; Drug Identification**



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**B117 The Influence of Instrument Parameters on Replicate Mass Spectra and Spectral Comparison Algorithms**

*Isabel Galvez Valencia, BS\*, West Virginia University, Morgantown, WV; Glen Jackson, PhD, West Virginia University, Morgantown, WV*

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**Learning Objective:** In this presentation, attendees will learn how the Electron Ionization (EI) conditions, such as ion energy, ion focus, and repeller voltage, influence the ion abundances of replicate mass spectra and how this spectral variance affects the traditional approach to mass spectral comparison algorithms.

**Impact Statement:** This presentation will provide the forensic community with a flexible multivariate algorithm capable of dealing with the effects of instrument conditions on relative ion abundances, which will help inform analysts' decisions when identifying a substance using GC/EI/MS data. The goal of EASI is to improve confidence in identifications while limiting the need for samples to be analyzed on the same instrument.

Current search algorithms based only on electron ionization-mass spectra provide the correct identification as the top result approximately 80% of the time. One contributing factor to the ~20% error rate is that traditional algorithms work by comparing the unknown spectrum to an "ideal" or consensus spectrum of each reference compound. The inclusion of replicate reference spectra in a database has been shown to improve the probability of ranking the correct identity in the number one position. However, the variance in ion abundances caused by different conditions or different instruments remains an intractable problem and a major source of uncertainty in spectral identification.

To assess the relative contributions of different factors to the spectral variance of replicate spectra, this study considered repeller voltage, ion focus voltage, and EI energy as the primary parameters. A three-factor, three-level, full-factorial design of experiments was conducted using cocaine as a model compound. A library of 1,163 cocaine spectra was collected with a Gas Chromatography/Electron Ionization/Mass Spectrometer (GC/EI/MS) by extracting each spectrum across the eluting chromatographic peak. The 20 most abundant ions in the database of cocaine spectra were extracted to conduct a Multivariate Analysis of Variance (MANOVA). Results showed that the repeller voltage and the ion focus were responsible for most of the variance observed, with each explaining 41% and 32% of the variance in ions abundances, respectively.

To determine the effect of spectral variance on spectral identifications, this project also compared the traditional approach, which uses a consensus spectrum, to a novel algorithm, called the Expert Algorithm for Substance Identification (EASI), in their abilities to distinguish between replicate spectra of cocaine and non-cocaine spectra, which included >300 cocaine spectra from more than 20 laboratories, 11 replicates of cocaine diastereomers: allococaine, pseudococaine, and pseudoallococaine, and >1,000 other known negatives. EASI uses multivariate linear modeling to predict the ion abundances of the 20 ions in each spectrum. EASI assumes that each of the 20 ion abundances in the cocaine database is continuously dependent on a subset of the other 19 ion abundances, and general linear modeling enables ion abundances to be modeled or predicted and then compared to the measured values. Common measures of spectral similarity and dissimilarity—like Pearson Product-Moment Correlation coefficients (PPMC), mean absolute residuals, and Euclidian distances—were calculated between each test spectrum and its respective predicted spectrum using EASI and the consensus models. Analysis of Receiver Operating Characteristic (ROC) curves using the mean absolute residuals concluded that the EASI model has a sensitivity of 96.2% when no false positives are allowed, whereas the consensus approach only has a sensitivity of 84.5% under the same conditions.

**Reference:**

- <sup>1</sup>. Samokhin, A.; Sotnezova, K.; Lashin, V.; Revelsky, I. Evaluation of Mass Spectral Library Search Algorithms Implemented in Commercial Software. *J. Mass Spectrom.* 2015, 50 (6), 820–825. <https://doi.org/10.1002/jms.3591>.

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**General Linear Model; Mass Spectrometry; Multivariate Analysis**

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**B118 Boar Hunt or Manhunt? Fatality During Boar Hunting Expedition**

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**Learning Objective:** Hunting-related shooting fatalities are an important issue in the forensics world. After attending this presentation, attendees will comprehend the importance of exhaustive forensic investigations, comprehensive radiological, and ballistic and genetics analysis, including the autopsy of the wild animal in order to give an accurate evaluation of the events.<sup>1</sup> This presentation will depict a complex case in which the question “accident or homicide?” will be answered by using multiple forensic techniques and with the collaboration of multiple specialists.

**Impact Statement:** If a hunter voluntarily discharges their weapon and kills a human, the event can be classified as a homicide; however, if they fire a weapon and the projectile strikes an animal, ricochets, and kills a human being, the event can be classified as accidental.<sup>2</sup> Given the insufficient and heterogeneous literature on accidental shooting and the often-unclear circumstances regarding hunting accidents, this case shows the importance of a multidisciplinary approach to accurately assess ambiguous crime scenes.

During a wild boar hunting expedition, a 40-year-old man died of blood loss after a bullet wound to the abdomen. The expedition started in the morning when the victim and a group of 18 hunters decided to stake out the woods to hunt wild boars. He was posted in a clearing, waiting for the animals, when the other hunters spotted a wild boar and started firing their weapons. The boar was initially injured by two bullets and chased by hounds, then a third hunter fired his hunting rifle, striking the boar in the right humerus, killing him. A few minutes after the kill, the victim was found agonizing in the clearing. Resuscitation techniques were performed, with no results, until the arrival of medical personnel, who declared him dead.

A CT scan of the body was performed prior to the autopsy, revealing a 1.5cm bullet lodged in the soft tissue between the peritoneum and the vertebral spine. The external examination of the corpse did not show any injuries other than an oval wound (2.8x1cm) located 3.2cm from the medial line, 13cm from the right superior iliac crest, and 103cm from the right foot. This lesion appeared to be irregular, with uneven and jagged margins, and compatible with the entry wound. The internal examination revealed a penetrative wound of the right rectus abdominis muscle, a hemoperitoneum, a laceration of the posterior peritoneum, and a laceration of the right external iliac artery. Thoracic and abdominal organs appeared to be intact. Histological examination of the abdominal wound and the right external iliac artery revealed the presence of significant red blood cell infiltration and incongruous materials, similar to plant organisms. The cause of death was, therefore, represented by meta-hemorrhagic shock secondary to significant blood loss because of the right external iliac artery laceration. The wild boar examination, performed by a veterinary, exposed the presence of five bullet wounds, four of which had a trajectory from left to right, while the fifth ran in the opposite direction. Ballistic analysis, lastly, proved to be critical to reconstructing the dynamics of the events. Microscopic analysis of the bullet confirmed that the victim was hit by a 12-caliber Gualandi bullet, which first hit the humerus of the boar. The impact of the bullet with bone determined a deformation of its apical portion and determined a deviation of its trajectory resulting in the death of the hunter, posted in the clearing. Furthermore, genetics analysis performed on the bullet, recovered during the autopsy, confirmed the presence of biological traces compatible with the boar's DNA. Eventually, this multidisciplinary approach allowed the investigators to provide an accurate reconstruction of the event and to certify this death as an accident.

**References:**

1. Cascini F, Tartaglione T, Oliva A, Marchetti D. A case of hunting death due to an overpenetrated bullet. *Int J Legal Med.* 2009 Mar;123(2):151-3. doi: 10.1007/s00414-008-0270-2. Epub 2008 Aug 6. PMID: 18682968.
2. Spagnolia, Alessandra BS; Kemp, Walter L. MD, PhD Fatality While Bear Hunting, *The American Journal of Forensic Medicine and Pathology*: June 2019 - Volume 40 - Issue 2 - p 144-146 doi: 10.1097/PAF.0000000000000467.

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**Hunting; Firearm; Accident**

### **B119 The Quantitative Analysis of $\Delta^9$ -Tetrahydrocannabinol ( $\Delta^9$ -THC) in Cannabis Plants Using the Fast Blue BB Colorimetric Test**

*Nicole Valdes, MS\*, Florida International University, Miami, FL; Roberta Gorziza, PhD, Florida International University, Miami, FL; Jose Almirall, PhD, Florida International University, Miami, FL*

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**WITHDRAWN**

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**B120 Open Fire**

*Abigail Wilson, BS\*, John Jay College of Criminal Justice, Edgewater, MD; Peter Diaczuk, PhD, John Jay College, New York City, NY*

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**Learning Objective:** This presentation will provide attendees with knowledge regarding wound ballistics, specifically surrounding the behavior of hollow point bullets.

**Impact Statement:** After attending this presentation, attendees will have a better understanding of the expansion characteristics of a hollow point bullet.

Bullets used in ammunition manufacture can be broadly divided into two categories, full metal jacketed and jacketed hollow points. These can be further divided into numerous subsets within each group, including some specialty groups, such as frangible. This research examines a representative brand of jacketed hollow point bullets used in a common caliber cartridge, the 9mm Luger®. A jacketed hollow point bullet is designed to open or expand when it comes into contact with a viscous medium such as water, tissue, or ballistic gelatin. This research used ballistic gelatin as a tissue simulant with a chamois overlay as a substitute for skin. The 9mm Luger® caliber jacketed hollow point bullets were fired and subsequently recovered from cotton waste. Cotton waste was used as the recovery medium so any bullet expansion, or lack thereof, would not take place post-gelatin. After recovery from the cotton waste, the bullets were carefully weighed, and their expansion measured. As a positive and negative control, a series of bullets were fired into a full-size block of gelatin to achieve complete expansion and into just cotton waste to resist expansion. There are six petals that compose the tip and ogive of the hollow point bullet used in these tests. The distance between all the opposing petals of each bullet were measured, resulting in three measurements per bullet. There were five bullets fired for gelatin thicknesses of 3 inches, 2 inches, 1.5 inches, 1 inch, and 0.5 inches, as well as for the negative and positive controls. The positive control averaged 12.97mm and the negative control averaged 6.24mm at maximum width. Bullet expansion through the respective gelatin slices averaged 12.47mm for 3 inches, 12.23mm for 2 inches, 11.83mm for 1.5 inches, 11.84mm for 1 inch, and 11.49mm for 0.5 inches. The differences between the average distances of the expanded petals can be explained by the differences in the thickness of the gelatins. As the gelatin slices get thinner, the width of the expanded bullets gets narrower. Velocity data was collected both from the muzzle of the firearm and as the bullets left the varying thicknesses of the chamois-covered ballistic gelatin. The velocity loss decreased as the gel thickness decreased.

This research is intended to provide an understanding of how hollow point bullets behave and increase knowledge of those who are not familiar with wound ballistics.

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**Ballistic Gelatin; Hollow Point Bullet; Wound Ballistics**

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**B121 Accounting for Household Substrates in a Model for Predicting the Weathering of Ignitable Liquids**

*Max Denn, MS, BS\*, West Virginia University, Arnold, MD; Glen Jackson, PhD, West Virginia University, Morgantown, WV*

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**Learning Objective:** Following this presentation, attendees will better understand the impact that household substrates have on the weathering changes of ignitable liquids and how the different components of ignitable liquids are affected by the heat of a fire.

**Impact Statement:** This presentation will provide an increased understanding of the behavior of components of an ignitable liquid at elevated temperatures. This understanding will assist analysts with the creation of predicted chromatographic data for comparison to casework samples, aiding in the investigation of arson involving the use of ignitable liquids.

The hypothesis is that modifying the surface area-to-volume ratio of a sample of an ignitable liquid will impact the relative evaporation rates of the components in an ignitable liquid in a manner that correlates with the presence of a porous substrate. Additionally, the mathematical model used to predict the extent to which the ignitable liquid has been weathered can be altered using empirically determined Henry's law coefficients to account for non-ideal behavior.

A synthetic gasoline mixture was created using nine different compounds encompassing a wide range of volatilities and representative of several different classes of chemicals found in gasoline. Experimental weatherings were performed by placing 1mL aliquots of the artificial gasoline into aluminum weigh boats that had been preheated to the desired temperature (e.g., 210°C) inside a vacuum oven. The artificial gasoline was then left on the weigh boat for different times to achieve different extents of weathering (between 50–99.5% by mass) before removing the boat from the oven and immediately cooling on a second, cold aluminum block to prevent further weathering.

This same weathering process was repeated in the presence of a substrate with an additional step providing time for the artificial gasoline to be absorbed by the substrate before weathering. Four different substrates were used: pinewood, plywood, nylon carpet, and cotton cloth. The pre-weathering absorption time varied from 0–30 minutes. The ignitable liquid residues were collected via solid/liquid extraction in a sealed container with a minimal headspace to prevent unwanted evaporative losses.

The porosity of a substrate is known to affect the evaporation of ignitable liquids through the combined effects of absorption, adsorption, and entrapment. To simulate the effect that substrates of different porosities have on the weathering of the artificial gasoline, weatherings were performed in glassware of various sizes with known surface area:volume ratios. The results show a strong correlation between the relative distributions of the nine components in more porous substrates like plywood and glassware with a lower surface area:volume ratio.

The introduction of substrates, real or simulated, causes the experimentally determined relative composition of the artificial gasoline to deviate further from the relative composition predicted by an evaporation model that uses Raoult's law to calculate iterative evaporative losses of each component relative to that component's vapor pressure. Deviations from ideality can be compensated for through the use of empirically determined estimates of Henry's law constants for each component. Preliminary results show that the inclusion of Henry's law constants results in more accurate predictions of the experimentally determined fractional composition with an ~18% decrease in the root mean squared error of predictions in predictions made using optimized coefficients versus predictions made using predicted vapor pressures.

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**Ignitable Liquids; Arson; Weathering**

**B122 The Qualitative Identification of Delta-8-Tetrahydrocannabinol (delta-8-THC) and Delta-9-Tetrahydrocannabinol (delta-9-THC) Using Thin-Layer Chromatography (TLC)**

*Sara Moreno, BS\*, Marshall University, Lost Creek, WV; Emily Kell, BS, Indiana State Police Crime Laboratory, Fort Wayne, IN; Kimberly Burrow, BS, Indiana State Police Laboratory, Fort Wayne, IN; Lauren Richards-Waugh, PhD, Marshall University, Huntington, WV*

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WITHDRAWN



**B123 The Quantitative Analysis of Mitragynine and 7-Hydroxymitragynine in Kratom Samples Commercially Available in Richmond, VA, Using Gas Chromatography/Mass Spectrometry (GC/MS)**

*Jade Smith, BS\*, Virginia Commonwealth University, North Chesterfield, VA; Hunter Fleming, MS, Washington, DC, Office of the Chief Medical Examiner, Simpsonville, SC; Emanuele Alves, PhD, Virginia Commonwealth University, Richmond, VA*

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**WITHDRAWN**

### **B124 The Raman Spectroscopic Analysis of Drugs in Contaminated Fingermarks: The Effect of Temperature and Time Since Deposition**

*Mohamed Amin, MSc\*, Kuwait University, Khaldyia, Al Asimah, KUWAIT; Entesar Al-Hetlani, PhD, Kuwait University, Khaldyia, Al Asimah, KUWAIT; Igor Lednev, PhD, Department of Chemistry, University at Albany, SUNY, Albany, NY*

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**WITHDRAWN**

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**B125 The Tools, Resources, and Collaborative Research Used to Address Seized Drugs Measurement Challenges**

*Edward Sisco, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Objective:** After attending this presentation, attendees will understand the ongoing efforts at the National Institute of Standards and Technology (NIST) to collaboratively develop tools and resources that address research questions or challenges identified by drug chemists and are freely available to the community.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how researchers at NIST are available to assist the community in tackling pressing questions. This presentation will also provide the community with an understanding of how to access the tools and resources that have been developed so far as well as describe what tools and resources are under development.

The current drug landscape presents a number of challenges to forensics laboratories, from identification of new psychoactive substances or emerging drugs to differentiation or isomeric species to ensuring safety of chemists in the face of increasingly potent materials. When faced with these, or other, challenges, many laboratories don't have the time or resources to dedicate to researching and developing solutions. To assist laboratories who have identified challenges, researchers at NIST have established a collaborative research pipeline. Through this pipeline, NIST researchers and forensic chemists work side-by-side to develop meaningful solutions that are both practical and implementable. Once a solution is identified—typically resulting in an instrument method, software tool, or other resource—it is made freely available to the entire community.

This presentation will highlight some recent collaborative efforts that have resulted in the development of resources or tools now available to the community. Three examples that will be highlighted include: (1) the development of weighing practices that lower potential exposure hazards and lower the spread of powder that could increase the drug background in laboratories, (2) the creation of software tools to simplify the analysis of Ambient Ionization Mass Spectrometry (AIMS) data, and (3) efforts to increase the objectivity of Gas Chromatography/Mass Spectrometry (GC/MS) analyses through rigorously developed methods and spectral databases containing replicate measurements. Research efforts that span the analysis chain—from point of seizure through to reporting—will be highlighted. In addition to discussing tools and resources available today, ongoing and near-future research efforts will be discussed. These include tools for identification of unknown compounds analyzed by AIMS, methods for emerging screening technologies, developing measurement platforms for forensic intelligence purposes, and upcoming interlaboratory studies. Opportunities for the community to engage in collaborative research will also be highlighted.

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**Seized Drug; Gas Chromatography/Mass Spectrometry (GC/MS); Ambient Ionization Mass Spectrometry (AIMS)**

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**B126 One Laboratory's Experience With Next Generation Sequencing (NGS) Using the Verogen™ MiSeq® FGx System and Signature Prep Kit: The Decision, the Validation, and the Implementation**

*Mandi Van Buren, MPharm\*, Kern Regional Crime Laboratory, Bakersfield, CA; Brooke Ramirez, BS, Kern Regional Crime Laboratory, Bakersfield, CA*

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**Learning Objective:** This presentation will provide attendees with information pertaining to Next Generation Sequencing and the decision to validate the system for use in a forensic DNA laboratory. The presentation will review one lab's decision-making process, why was NGS the best option, what factors were considered, and how did we get stakeholders on board with this project. Additionally, the presentation will discuss in brief the validation completed, what kits, what instrument, and the timeline of the project. To finish, the presentation will provide insight into implementation, consideration for case acceptance, recommended training program, and testimony preparation.

**Impact Statement:** NGS has been a hot topic in the forensic community and how it will impact DNA testing in criminal cases. Our laboratory is the first lab to validate the NGS instrument in conjunction with robotics for a fully automated system. Additionally, the laboratory is moving toward all cases in the county being processed with NGS through a systematic case triage review. We plan to introduce multiple sequencing kit options, as each one has a benefit for certain cases and evidence items.

The Kern Regional Crime Laboratory is a division of the Kern County District Attorney's Office in Bakersfield, CA, providing forensic services to 47 law enforcement agencies and completing over 6,700 requests annually across all disciplines. The DNA Analysis Unit is staffed with five criminalists and two forensic technicians, averaging approximately 700 requests for service, including biological screening, DNA analysis, Cold Case processing, Kinship Analysis, and Familial Searching. The unit's current methodologies and testing platforms include Hamilton Robotics for automation and the Applied Biosystems® 3130 genetic analyzer for typing Autosomal and Y-STRs.

When the laboratory's Capillary Electrophoresis (CE) platform was end-of-lifed, the DNA unit's goal was to replace it with a Next-Generation Sequencing (NGS) workflow that would reduce sample processing time, maximize the amount of data obtained, and improve mixture interpretation in complex mixtures for all casework samples. The laboratory's vision was to validate and implement NGS for all casework samples and integrate the data with the current probabilistic genotyping system. The DNA unit was awarded grant funds in 2019 from the DNA CEBR grant to fund this project.

The decision for the laboratory to make the leap from CE to NGS for all casework samples involved many considerations and support from various stakeholders. This presentation will take a further look into why the laboratory made the decision to move toward NGS as the standard for casework, what was considered, what challenges we faced throughout the validation process, and recommendations for other laboratories considering NGS. Additionally, this presentation will fast forward to where the laboratory is today, discussing implementation, training, case acceptance and triage, integration with our existing probabilistic genotyping and local databasing system, and testimony preparation.

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**Next-Generation Sequencing; DNA Analysis; Validation**

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**B127 The Internal Validation of Verogen™ ForenSeq™ DNA Signature Prep Kit Primer Set B Using the Hamilton Nimbus HD and MiSeq® FGx**

*Mandi Van Buren, MPharm\*, Kern Regional Crime Laboratory, Bakersfield, CA; Brooke Ramirez, BS, Kern Regional Crime Laboratory, Bakersfield, CA*

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**Learning Objective:** This presentation will be an in-depth review of the validation of the MiSeq® FGx system using Hamilton Robotics for a fully automated sequencing process for DNA analysis. We will review the kits tested, the studies completed, and the examination of the data. Additionally, concordance studies were completed on various samples using CE technology and sequencing. These results will be presented and evaluated to demonstrate possible benefits of using NGS for mixture deconvolution.

**Impact Statement:** This is the first completed validation using the MiSeq® FGx system and Hamilton Robotics for a fully automated process for DNA testing. Many laboratories are still in the decision-making stage for NGS, and this presentation will provide insight into the benefits of the technology, the validation process including deciding what kits to validate and why. The additional concordance runs will impact the community by providing casework examples processed with traditional CE workflows and how using NGS helped provide additional information for an analyst.

Internal validation of Verogen™ ForenSeq™ DNA Signature Prep Kit Primer Set B using the Hamilton Nimbus HD and MiSeq® FGX Next generation sequencing is the future of forensic DNA analysis. The ForenSeq™ DNA Signature Prep Kit Primer Set B from Verogen™ allows forensic laboratories to detect more than 200 forensically relevant markers in a single reaction. Forensic evidence is often limited in nature and having the ability to type autosomal, Y-STR, X-STR, and identity-informative single nucleotide polymorphisms in one reaction allows the forensic scientist to obtain all existing information in a sample, instead of having to determine which kit would result in the most probative information for the case.

The Kern Regional Crime Laboratory performed an internal validation of the Verogen™ ForenSeq™ DNA Signature Prep Kit Primer Set B using the Hamilton Nimbus HD and MiSeq® FGX. Precision and accuracy studies, sensitivity and stochastic studies, mixture studies, mock evidence sample studies, and a contamination assessment study were performed during the validation. Male and female two-person mixtures with a range of concentrations were also evaluated to determine the sensitivity of the MiSeq® FGX and illustrate the advantage of obtaining autosomal and Y-STR alleles in a single reaction. In addition to performing studies following the QAS validation requirements, the Kern Regional Crime Laboratory was able to evaluate the methods using robotics for the setup of library purification, normalization, and pooling. During this validation, the robotics method was optimized, allowing for greater efficiency. A contamination study was also performed on the Hamilton Nimbus HD to evaluate the reliability of the instrument for NGS set up. All samples were run with Applied Biosystem® GlobalFiler® kit using capillary electrophoresis as well as ForenSeq™ DNA Signature Prep Kit Primer Set B using the MiSeq® FGX. The concordance between the profiles were evaluated as well as the intensity of the alleles between the two systems. Mixtures were evaluated to determine if sequencing information allowed for more accurate determination of number of contributors to a profile than CE data allows.

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**Next-Generation Sequencing; DNA Analysis; Validation**

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**B128 The Evaluation of the ForenSeq™ MainstAY Kit for Mixed Forensic DNA Samples**

*Damani Johnson, BS\*, Sam Houston State University, Huntsville, TX; Lucio Avellaneda, BS, Sam Houston State University, Houston, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX; Tim Kalafut, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of a plexity in next generation sequencing, how commonly encountered mixtures and challenging sample types may affect flow cell capacity, and recommendations for maximizing run efficiency.

**Impact Statement:** This presentation will impact the forensic science community by addressing a potential obstacle for labs interested in implementing NGS for casework and by providing suggestions aimed at making that transition easier and more accessible.

Next Generation Sequencing (NGS) has been widely considered to be the future of forensic casework, with increasing numbers of labs beginning to implement it into their workflows alongside, or even in place of, traditional Capillary Electrophoresis (CE). There are numerous advantages to NGS such as shorter amplicons, increased sensitivity, the potential detection of isoalleles, and the massive number of markers and marker types that can be run simultaneously. One main limiting factor, though, is plexity, the number of samples that can be run at once. Plexity is affected by the physical capacity of the flow cell, which facilitates the formation of clusters that each represent a distinct PCR target (DPT). Since only so many DPTs can fit on the flow cell, the read coverage at each locus in an NGS run is highly dependent on the flow cell's capacity, the number of markers in the kit, and the number of DPTs generated during the PCR process. The total number of DPTs includes things like forward and back stutter as well as the alleles. More loci and higher numbers of contributors result in higher DPTs.

Verogen's™ recently released ForenSeq™ MainstAY kit is specifically aimed at a forensically relevant set of 27 autosomal STR and 25 Y-STR markers. It uses the MiSeq® FGx Reagent Micro Kit, with a flow cell capable of five million reads. This combination of markers and flow cell capacity should allow full coverage of a run of 96 single-source samples. However, forensic casework samples are rarely this simple. Labs regularly process samples that contain a mixture of two or more contributors, often with low-level trace donors. Mixed samples can multiply the number of DPTs present, causing a 96-sample run that should fall within the capacity of the flow cell to exceed it, reducing the effective coverage of each locus. In addition, the increased sensitivity promised by NGS has not yet been fully explored for mixed samples, especially for batched samples such as sexual assault swabs, which commonly have very high female-to-male mixture ratios. Such samples, if included in a full run of 96 libraries, must compete for space on the flow cell for detection.

The study presented here aimed to test the limits of MainstAY's plexity by simultaneously running a variety of sample types and mixtures that would be typically encountered in forensic casework. The presentation will explore the trade-off of more rare genotypes due to isoalleles against detection challenges due to extreme plexity and competition for a spot on the flow cell. Additionally, properties unique to NGS such as sequence-specific stutter models will be explored. Last, the presentation will provide recommendations for optimal batching of samples for an efficient NGS case processing workflow.

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**Next Generation Sequencing; Plexity; Isoalleles**



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**B129 The Identification of Trends of Sequenced-Based Allelic Drop-Out in Autosomal Short Tandem Repeats (STRs)**

Megan Foley, MSFS\*, Oklahoma State University/The George Washington University, Washington, DC; Gerwald Koehler, PhD, Oklahoma State University-Center for Health Sciences, Tulsa, OK; Jun Fu, PhD, Oklahoma State University-Center for Health Sciences, Tulsa, OK; Robert Allen, PhD, Oklahoma State University, Tulsa, OK; Jarrad Wagner, PhD, Oklahoma State University-Center for Health Sciences, Tulsa, OK

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of trends seen in allelic drop-out of sequenced autosomal STRs.

**Impact Statement:** This presentation will impact the forensic science community by identifying trends in allelic-drop out for the sequencing of autosomal STR analysis in order to better create models of this stochastic phenomenon for statistical analysis.

Current STR analysis uses Polymerase Chain Reaction (PCR) in conjunction with Capillary Electrophoresis (CE) to develop a length-based genetic DNA profile. If an association is made between a crime scene profile and a reference sample, the weight of the evidence must be determined in order to report and testify to these findings in court. Currently, the field is utilizing probabilistic genotyping software to calculate a likelihood ratio based on the crime scene evidence profile. Probabilistic genotyping performs complex statistical analysis of the crime scene profile by using algorithms and biological modeling of DNA data. It is crucial to determine if allelic drop-out could have occurred, and this is expressed as the probability of drop-out. Allelic drop-out occurs during amplification of low-level or poor-quality DNA samples due to stochastic effects and unequal sampling of the extracted biological material.<sup>1-3</sup> Allelic drop-out is not a new consideration in the interpretation and statistical evaluation of STR profiles.

As crime laboratories are introduced to technologies and assays that allow for the sequencing of autosomal STRs, challenges and hurdles are uncovered that must be dealt with before implementation, including access to statistical applications that can evaluate sequenced-based STR data. In order to develop appropriate models for these data, profile parameters, like the probability of allelic drop-out, must be well understood. The main objective of this research was to identify and evaluate trends in allelic drop-out in single source DNA samples sequenced using the MiSeq<sup>®</sup> FGx. A series of dilutions were prepared using a control DNA source containing values ranging from 7.82pg to 4,000pg of total DNA. Libraries were prepared using the ForenSeq<sup>™</sup> Signature Prep amplification kit with Primer set A, following manufacturer's guidelines. Sequencing was performed using the MiSeq<sup>®</sup> FGx, and STR profiles were developed and analyzed using the Universal Analysis Software from Verogen<sup>™</sup>. Allelic drop-out was evaluated considering locus information and various DNA parameters (concentration, allelic length, read count, etc.). Heterozygous and homozygous locations were analyzed separately. Overall, allelic drop-out was observed at 125pg at one locus and was seen more consistently across loci starting around 62.5pg. D8S1179 displayed the highest amount of drop-out occurrence across samples, followed by D2S441, and D13S317. Loci with the least amount of drop-out included D1S1656, D9S112, D19S433, and D20S482. No allelic drop-out was observed with D16S539. Trends could be seen in various loci when looking at the total reads of a sample, amount of DNA per flow cell, and allelic length. Locus drop-out, homozygous drop-out, and a statistical comparison of DNA parameters will also be included in the presentation.

**References:**

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2. Tvedebrink, T., Eriksen, P. S., Mogensen, H. S., & Morling, N. (2009). Estimating the probability of allelic drop-out of STR alleles in forensic genetics. *Forensic Science International: Genetics*, 3(4), 222–226. <https://doi.org/10.1016/j.fsigen.2009.02.002>.
3. Andersen, M. M., Mogensen, H. S., Eriksen, P. S., Olofsson, J. K., Asplund, M., & Morling, N. (2013). Estimating Y-STR allelic drop-out rates and adjusting for interlocus balances. *Forensic Science International: Genetics*, 7(3), 327–336.
4. <https://doi.org/10.1016/j.fsigen.2013.01.005>.

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**STR Sequencing; Allelic Drop-Out; Forensic Science**

**B130 Determining Linkage Patterns for the Identity of Single Nucleotide Polymorphisms (SNPs) Found in Common Forensic Sequencing Assays**

*Isabella George, BS\*, The George Washington University, Arlington, VA; Daniele Podini, PhD, Department of Forensic Science, Washington, DC; Megan Foley, MSFS, Oklahoma State University/The George Washington University, Washington, DC*

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**WITHDRAWN**

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**B131 Mitochondrial Genome Sequences From 6,025 Population Samples**

*Charla Marshall, APhD\*, FMES-AFDIL and SNA International, LLC, Dover Air Force Base, DE; Kimberly Andreaggi, PhD, AFMES-AFDIL and SNA International, LLC, Dover, DE; Jennifer Higginbotham, MFS, AFMES-AFDIL and SNA International, LLC, Dover Air Force Base, DE; Elise Anderson, MFSF, Amentum/AFDIL, Dover Air Force Base, DE; Walther Parson, PhD, Institute of Legal Medicine/Medical University of Innsbruck, Innsbruck, Tirol, AUSTRIA*

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**Learning Objective:** After attending this presentation, attendees will understand the methods utilized to generate reference-quality mitochondrial genome (mitogenome) sequences using Next Generation Sequencing (NGS) methods, the need for augmenting publicly available population reference data for meaningful statistical analysis of mitogenome profiles, and the application of pathogenic variant filtering to mitigate genetic privacy issues around the usage of the coding region mitochondrial DNA (mtDNA) information.

**Impact Statement:** This presentation will impact the forensic science community by characterizing mitogenome population datasets from 6,025 samples. The myriad applications for mitogenome sequence analysis in the age of single nucleotide polymorphisms (SNPs) and NGS will be explored.

This report will summarize some of the results from two National Institute of Justice (NIJ) -funded projects to expand reference-quality mitogenome datasets for forensic applications. The project aimed to produce a minimum of 6,000 United States and 2,000 global mitogenome haplotypes using a high throughput, automated NGS workflow. This automated workflow involves PCR amplification of two overlapping 8,500 base pair targets, followed by enzymatic fragmentation and library preparation. Samples with DNA that are too degraded for this enrichment approach undergo PCR amplification using either a four-amplicon or mini-amplicon method, followed by library preparation with or without fragmentation, respectively. Paired-end, 300x300 cycle sequencing is performed on an Illumina® instrument. Data analysis begins with an automatic bioinformatic pipeline followed by manual review completed by two independent DNA analysts. The resulting haplotypes undergo a series of quality assurance checks before submission to publicly available DNA databases.

Here, the haplotypes from 6,025 population samples are described. The samples originated from anonymized, de-identified donors representing 58 populations throughout the United States, Sweden, Afghanistan, Guatemala, Honduras, and Japan. Suspected maternal relatives with matching haplotypes were assessed using Short Tandem Repeat (STR) analysis. Only one of each matched mitogenome sequence was included in the final dataset when a 1<sup>st</sup>- or 2<sup>nd</sup>-degree relationship was predicted. All samples are therefore presumed to be no closer than 3<sup>rd</sup>-degree relatives. Excluding variation due to heteroplasmy, a total of 4,929 different haplotypes were observed in the 6,025 samples. There were 591 haplotypes observed two or more times in the dataset, and these were found in 1,687 (28%) individuals. A total of 4,338 (72%) haplotypes were observed only once (as singletons). Thus, the probability of observing a singleton in the dataset is high. This indicates that further expansion of the mitogenome dataset is needed for haplotype frequency estimations. Of the 6,025 complete mitogenome haplotypes, only 38 (0.63%) exhibited any clinically recognized pathogenic variants. These results reinforce the general findings that pathogenic variants are rarely observed in randomly sampled mitogenome sequences. Pathogenic variant filtering can be applied to remove these affected variants from a haplotype with no effect on match statistics, as previous studies have shown. The next step for these NIJ-funded projects is to facilitate an external review of the data prior to inclusion in the EDNAP MtDNA Population (EMPOP) database. Then the haplotypes will be publicly available for practitioner use.

*The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.*

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**Mitochondrial DNA; Next Generation Sequencing; Population Dataset**

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**B132 Perceptions of Forensic Scientists on Statistical Models and Ethical Implications in DNA Evidence Evaluations Using Sequence Data**

*Sanne Aalbers, MSc\*, University of Washington, Seattle, WA; Bruce Weir, PhD, University of Washington, Lake Forest Park, WA*

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**Learning Objective:** After attending this presentation, attendees will have gained insight into the current perceptions of forensic scientists on statistical models and ethical implications relating to the use of sequence data for forensic DNA evidence evaluations.

**Impact Statement:** This presentation will impact the forensic community by providing insight into the perceptions of forensic scientists regarding the use of statistical modeling and sequence data, which will provide valuable information in the move toward implementing sequencing methods for DNA evidence evaluations.

DNA typing is a mature field and overwhelmingly seen as the gold standard in forensic science. The interpretation of DNA evidence, however, is far from straightforward and challenges arise when evaluating complex profiles and assessing the statistical weight of the evidence. Accurate representation of forensic evidence in court is crucial to avoid misinterpretations and, ultimately, to reduce the possibility of a miscarriage of justice. This not only requires sensible models that can handle the complexity associated with DNA profiles, but also an understanding of the used methods by forensic scientists who will be writing the reports and potentially serve as an expert witness in court.

With the introduction of sequence data, it will be of interest to assess if forensic scientists feel equipped to interpret and present DNA evidence for such data. Early research has focused on the perceptions of sequencing technologies through surveys and highlighted opinions on current use, future views, and challenges in forensics.<sup>1</sup> To get a more in-depth understanding of the current situation, we conducted a qualitative study involving interviews with U.S. forensic scientists to assess their perceptions on statistical models as well as some ethical implications for DNA evidence evaluations based on sequence data. This study illuminates the views of, and barriers faced, by these professionals and can serve as guidance for the implementation of statistical models when transforming current approaches to incorporate sequence data.

We present the results of a cross-sectional study involving United States forensic scientist working with DNA evidence. Semi-structured interviews have been conducted with 16 professionals with a varying degree of work experience in the forensic field, ranging from less than 1 year to over 33 years of employment. Open-ended interview questions were used to explore participants' views on both the application of statistical models and use of sequence data in forensic DNA evidence evaluations. Some ethical topics concerning these concepts have also been assessed. Recordings of the ~45-minute one-time interviews have been obtained with consent and were transcribed for subsequent qualitative analysis. A conventional content analysis was carried out in ATLAS.ti software.

Results will highlight important themes and subthemes concerning statistical concepts, sequence data, ethical implications, and their interactions. We show to what extent forensic scientists feel comfortable with statistical concepts and sequence data for forensics, and to what extent they feel they need to be proficient in statistics and have an understanding of the statistical models used, to be able to perform their work. We also identify experienced barriers and needs in light of feeling better prepared to work with statistical models and sequence data, as well as to present such results in a court setting. Finally, we discuss the perceived impact of sequence data on the forensic field and reveal some contrasting views on whether or not the benefits outweigh potential risks.

**Reference:**

- <sup>1</sup>. Foley M., Oldoni F. An Evaluation of the Current Opinion of Next Generation Sequencing (NGS) Technology in Forensics Through a Global Survey. *Proceedings of the American Academy of Forensic Sciences, 74<sup>th</sup> Annual Scientific Conference*. Seattle, WA 2022.

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**Qualitative Research; Next Generation Sequencing; Statistics**

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**B133 Trends in Profiles of Offenders Caught Using Forensic Genetic Genealogy (FGG)**

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**Learning Objective:** This presentation will help to illustrate how FGG is being applied in the forensic community and more specifically what types of offenders it is most commonly being used to identify in terms of previous offenses and criminal records (as well as some data on gender, age, etc.)

**Impact Statement:** FGG is a relatively new technique in the forensic science community, and it is extremely important to understand not only how it is being used, but who it is being used to identify. This is because the better these concepts are understood, the more effectively FGG can be used. With more information about FGG, it will also be easier eventually to work towards effective legislation to allow and govern the practice.

FGG is a new tool in the forensic science community that allows violent offenders to be identified by genetic similarities their relatives share with a DNA sample. Since FGG is so new and limited in its use, it is important to examine the types of offenders FGG is successfully identifying in terms of previous criminal record and whether or not a sample is in CODIS. This type of information is important to have so that more effective legislation can be developed to regulate the practice.

Currently, there are no studies effectively analyzing FGG with regard to trends in cases where it has been successfully used. In order to address this topic, a way to analyze the cases that provided insight into variation in the offenders was developed. The first step was to research uses of FGG that resulted in success to identify potential cases to be used as part of the study. These cases were then analyzed to gain basic information such as dates, locations, and victims of the crime as well as the name of the offender and several other pieces of information. This research also entailed analyzing the case to determine the criminal record of the offender and whether they were in CODIS, as well as if DNA was taken or should have been taken. From there, potential cases involving violent offenders were identified by research and Freedom of Information Act (FOIA) requests were subsequently filed. Through these, the case files for each individual case were obtained that allowed criminal history, the date of the crime, and other key information to be identified. In cases where this information needed to be supplemented, certain detectives on these cases were contacted and interviewed. The research is vital because it helps to provide more insight into a fast-growing field of forensic science and one that will surely be integrated into law enforcement techniques as it becomes more fully understood.

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**Genealogy; Forensic Genetics; Cold Cases**

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**B134 Solving More Cases Using Search Keys and Expanded DNA Indirect Matching (EDIM)**

*Ray Wickenheiser, DPS, MBA\*, New York State Police Crime Lab System, Albany, NY*

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**Learning Objective:** After attending this presentation, attendees will understand some principles of DNA databases, use of search keys for indirect matching, the EDIM technique, and its associated bioethical considerations. Business case examples will also be provided to demonstrate the cost savings.

**Impact Statement:** This presentation will impact the forensic community by serving as a key aspect of increasing DNA database effectiveness by increasing the number of investigative leads, particularly for those cases that have not previously been solved with direct matches.

DNA databases are remarkably effective crime-solving tools. Currently, many forensic laboratories utilize DNA databases to compare profiles they obtain from crime scenes (forensic profiles) to develop suspects through direct matching. That is, the forensic profile exactly matches the known profile. Additional investigative leads can be provided by increasing the number and type of qualifying offenses and by including arrestees in the DNA database. A business case will be used to demonstrate the cost effectiveness of the database size expansion. Concepts evaluated include increasing database size incrementally, expanding to the largest size possible through a universal database, and the relationship of DNA database size to the number of investigative leads.

Conversely, the size of a DNA database can be increased scientifically through indirect matching. Indirect matching utilizes the known sharing of DNA between biologically related individuals to link forensic to known samples. Currently used indirect matching techniques include Partial Matching, Familial Searching, and Investigative or Forensic Genetic Genealogy (IGG or FGG). Indirect matching compares the forensic profile to known profiles from individuals that have not committed the instant crime, but rather are potentially biologically related to the perpetrator. Once indirectly matched, candidates are further examined for biological relatedness through kinship analysis. Investigative leads are developed through building of family trees and comparison of family members to particulars of the crime. Finally, suspects are developed for direct comparison to the forensic sample.

EDIM is an innovative new technique that utilizes the DNA profile of the forensic samples beyond the core Combined DNA Index System (CODIS) autosomal loci. This expanded profile permits the strategic use of search keys. Search keys are areas of DNA that are inherited in known biological and statistical patterns, thereby enhancing the location of biologically relative individuals. Potentially related candidate profiles are then further evaluated through kinship analysis to determine whether the candidate is a close or distant relative or merely a coincidental search key link.

Y-STRs (Short Tandem Repeats), X-STRs, mtDNA (mitochondrial DNA), and SNPs (Single Nucleotide Polymorphisms) are potential search keys, which are enabled by expanding the areas of DNA analyzed. A case flow for EDIM analysis will demonstrate various strategies to maximize the value of forensic evidence, which is adaptable to forensic laboratories' current workflows. Expanded use of UHRs (Unidentified Human Remains) is an additional mechanism to resolve outstanding forensic cases. An identical twin model business case will demonstrate the additional crime solving potential of EDIM, by estimating the number of relatives in a DNA database without compromising database security and privacy. Recommendations for improving the effectiveness of DNA databases and EDIM implementation will be presented, along with pertinent bioethical considerations.

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**Search Key; Indirect Matching; DNA Database**



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**B135 The Impact of Manually Degraded Single Nucleotide Polymorphism (SNP) Microarray Data on GEDmatch Top Genetic Matches for Forensic Genetic Genealogy Purposes**

*Justin Rivera, BS\*, University of New Haven, Chicago, IL; Claire Glynn, PhD, University of New Haven, West Haven, CT*

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**Learning Objective:** After attending this presentation, attendees will have gained insight into how degraded DNA/SNP data files may affect the top genetic matches generated by uploading to GEDMatch. As this is a crucial step in the forensic genetic genealogy process, this study aims to assess how the top genetic matches are impacted and changed through manually degraded SNP files.

**Impact Statement:** This presentation will impact the forensic science community by introducing and providing empirical findings on how degraded DNA samples can affect a critical step in forensic genetic genealogy and an understanding of how to assess the information that comes from an individual's matches on GEDmatch at various levels of degradation.

Forensic Genetic Genealogy (FGG) has recently become a valuable tool in the forensic science community and is having a great impact on the resolution of unresolved cases, including homicides, sexual assaults, and Unidentified Human Remains (UHRs) cases. In forensic investigations, following traditional Forensic DNA (STR) analysis and CODIS upload (within the United States), and failure to produce a candidate match in CODIS, FGG could produce investigative leads to identify an unknown individual. FGG employs SNP sequence data uploaded to genetic genealogy databases (i.e., FamilyTreeDNA® and GEDmatch PRO®) to identify genetic relatives (i.e., genetic matches) of the unknown individual. Family tree(s) are then constructed using the genetic matches to reach a possible candidate identity of the unknown individual. SNP sequencing (i.e., SNP microarray) typically requires high-quality/high-quantity DNA samples. Degraded DNA samples, however, are regularly encountered in forensic investigations. Therefore, a critical analysis of the impact of degraded DNA/SNP data is necessary to investigate the downstream effects this may have on the subsequent FGG analysis within the genetic genealogy databases. Addressing this potential issue, this study investigates how manually degraded SNP DNA data files affect the top ten genetic matches generated in GEDmatch.

Following informed consent, three volunteers provided their own downloaded raw DNA SNP microarray data. Once received by the principal investigator, the data files were anonymized and subjected to a randomized manual deletion protocol using Microsoft® Excel®. This process is composed of increasing increments of deletion percentages from the overall SNP data profile with a total of nine modified files for each donor (minus 5%, 10%, 15%, 20%, 25%, 30%, 40%, and 50% deletion), each file was uploaded to GEDmatch as “Research Files,” and a list of the top ten genetic matches based on shared DNA (total shared cM value) was produced. Each modified file was examined using autosomal One-to-Many matching, autosomal One-to-One Q-Matching, and Segment Searching, to investigate how values and top matches were altered with increased deletion of data. Currently, this specific protocol and analysis is being completed for SNP profiles generated through Whole Genome Sequencing (WGS), which has been valuable in FGG. Once there is data for the modified WGS data files, it will be analyzed and compared with the completed SNP microarray data analysis.

The results highlight various changes among top matches, including, but not limited to: matches that decrease/increase in total shared cM value, decrease/increase in quality scores of matching segments on a one-to-one basis, and changes to percentage confidence in predicted relationships. Additionally, the ranking of each donor's top ten genetic matches became altered with increasing deleted percentages, with some moving up in rank, some moving down in rank, and some lost completely (from the top ten list) when compared to the original full DNA SNP data file. Practically, these findings highlight potential issues for match assessment as typically the top ten genetic matches are the most valuable starting point in an FGG investigation. As FGG use grows, it is important to understand how to assess the information coming from a subject's matches, particularly when dealing with degraded DNA samples. Overall, this research emphasizes the need for further empirical research to assess the impact of degraded DNA samples in FGG investigations.

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**Genetic Genealogy; SNP Microarrays; GEDmatch**

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**B136 Experimental Maceration on Human Bones: How Does It Impact the “Forens-OME”?**

Noemi Procopio, PhD\*, University of Central Lancashire, Chorley, England, UNITED KINGDOM; Andrea Bonicelli, PhD, University of Central Lancashire, Preston, England, UNITED KINGDOM; Sheree Hughes, PhD, Sam Houston State University, Huntsville, TX

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**Learning Objective:** This presentation will inform attendees on the impact of maceration procedures on multiple biomolecules in human bones and on the consequent impact on the use of such molecules for Human Identification (HID), Age-At-Death (AAD) and Postmortem Interval (PMI) estimation.

**Impact Statement:** This presentation will provide guidelines for human bone treatment in order to maximize the amount of information that can be obtained from skeletal remains for human HID, PMI, and AAD estimation.

Forensic analyses on human remains for HID purposes often involve the cleaning and processing of bone material prior to anthropological evaluations and genetic analyses. The same is also true for Human Taphonomy Facilities (HTFs) where bones are usually treated prior to long-term storage in multiple ways depending on the needs of the skeletal remains and on the preference of the operators. In addition to routine analyses, such as anthropological evaluations, “-omics” analyses (such as proteomics and metabolomics) have also been explored more recently as additional tools to estimate the PMI and AAD of the cadaver.<sup>1-3</sup> However, how cleaning affects the integrity of the bone biomolecules, and the downstream “-omics” analyses aimed at performing HID, AAD, and PMI is unknown. Previous results on animal models showed how detrimental bone maceration is on DNA, proteins, metabolites, and lipids, and confirmed that higher temperatures damage the biomolecules more than lower ones.<sup>4</sup> However, its effect on human bones has not been investigated yet.

Here two maceration protocols currently used by HTFs have been tested on  $n=30$  human tibiae, and full untargeted proteomics and metabolomics analyses have been conducted via LC-MS/MS on both non-treated (left) and treated (right) tibiae. Maceration protocols differed for the temperatures (55°C and 87°C) and for the detergents type and concentration used. Untargeted proteomic and metabolomic analyses were conducted with high-sensitivity LC-MS/MS instruments and data were compared between fresh and macerated samples.

Preliminary results show that maceration does significantly affect the “Forens-OME.” In particular, the alteration in the metabolome extracted from the bones may result in severe consequences that impede the use of this methodology for accurate PMI estimation. The type of cleaning protocol affects the “Forens-OME” in different ways, highlighting the importance of adopting consistent procedures across forensic laboratories and HTFs that use minimally invasive cleaning procedures and limit their usage only to circumstances where maceration cannot be avoided.

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**Bone Maceration; Omics; Bone Biomolecules**

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**B137 The Application of Ancient DNA Methodologies to Badly Burned Forensic Samples and Their Potential to Aid in the Identification and Analysis of Difficult Samples**

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**Learning Objective:** This presentation is a generalized overview of the experimental design and preliminary data related to potential gains expected from incorporating methodologies adopted from the analyses of ancient DNA to the isolation and analyses of degraded DNA from badly burned forensic samples

**Impact Statement:** This research will serve as an optimal laboratory strategy guide for basic sampling strategies and locations, DNA extraction methods, STR profiling, and DNA library preparation when dealing with burned skeletal samples.

The ability to identify human remains using DNA analyses has revolutionized forensic science and become an important component of forensic investigation. However, these analyses generally rely on the isolation of high-concentration, quality-endogenous DNA that may not always be available. This is especially true when considering environmentally degraded source material that may have significantly less DNA retention and preservation due to various factors, including time since death and/or thermal alteration.

For temporally degraded remains, the decomposition of DNA molecules can alter not only the amount of DNA retained in tissue, but also decrease the fragment length and alter base composition. As such, the field of ancient DNA (aDNA) analyses has developed highly optimized protocols for sampling, extraction, and analyses of DNA recovered from skeletal remains to maximize the amount and quality of informative DNA recovered. In forensic contexts, studies have shown that adopting these techniques for use in modern, degraded, skeletal samples can greatly increase the likelihood of DNA identification.

The exposure of tissue to extreme temperatures, such as when burned, similarly affects DNA degradation in that much of the DNA is destroyed outright and the fragment length of recovered DNA may be drastically reduced. Presented here is a systematic investigation comparing forensic and ancient DNA laboratory protocols (the Dabney 2019 DNA extraction protocol vs. the Loriele 2007 protocol as well as the Meyer and Kircher 2010 single-stranded library preparation vs. the Troll et al. 2019 double-stranded preparation) across multiple skeletal elements and levels of thermal alteration.

Ten donor cadavers were burned under similar conditions at the University of Tennessee Anthropology Research Center. From each of these individuals, as many of the same skeletal elements as possible were collected and sent to Arizona State University's School of Human Evolution and Social Change (SHESC). All samples were then examined and assigned a burn scoring on a 1–5 scale, with one being the least thermally altered and five being the highest (i.e., cremains). DNA extractions using both test protocols will be performed using the same source material for each sample. Each extraction will then be assessed for STR profile recovery before being converted into both double- and single-stranded DNA libraries. Samples will be analyzed in terms of raw DNA yield, STR profile recovery, mitochondrial haplotyping, and Single Nucleotide Polymorphism (SNP) profiling. In addition, case samples provided by the Office of the Medical Examiner, Maricopa County, AZ, will be processed alongside the controlled samples to give a better sense of practical expectations. Preliminary results indicate that, while the Loriele DNA extraction protocol generally results in higher overall DNA recovery when successful, the Dabney ancient DNA extraction protocol is more consistently successful across all categories of thermal alteration with 96% (24 of 25) extraction attempts yielding measurable quantities of DNA and 88% (22 of 25) yielding sufficient DNA for downstream STR analysis as opposed to a 56% (14 of 25) success rate for both criteria using the Loriele protocol. When completed, the resulting manuscript will act as a generalized procedural guide for sampling, DNA extraction, DNA library preparation, and practical analyses of badly burned samples.

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**Forensic DNA; Degraded Remains; Skeletal Sampling**

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**B138 The Recovery of Human DNA From Fly Larvae**

*Cesar Cantu, BS\*, Sam Houston State University, Huntsville, TX; Sibyl Bucheli, PhD, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** Attendees will learn about the recovery of human DNA from fly larvae (maggots) by gaining an understanding of maggot sample preparation and their use for Short Tandem Repeats (STRs) and mitochondrial DNA analysis.

**Impact Statement:** The use of maggots as a potential sample for human identification has been shown to be possible by recovering human DNA for STR and mitochondrial DNA analysis from maggots. This study helps to implement fly larvae as a potential sample type that can be useful in unique cases where a body is not present, or other samples are not available.

Forensic entomology is the use of insects in criminal investigations and is primarily associated with the estimation of the Postmortem Interval (PMI). Another potential use of insects is the recovery of human DNA, for example, from the digestive tracts of larvae of Diptera found on decomposing bodies. Studies have shown that human DNA can be recovered from the crops of fly larvae (maggots), making them useful in the identification of individuals when other tissue is not available or in establishing a link between a scene and a victim. Previous research shows that preferred extraction methods should have purification steps to remove inhibitors that could be found within fly larvae.<sup>1</sup> This study investigates the use of two extraction methods, EZ1&2 DNA™ Investigator and DNeasy® Powersoil® Proto recover human DNA that can undergo several forensic genotyping methods. Maggots were collected at the Southeast Texas Applied Forensic Science (STAFS) Facility from various cadavers to process crops and whole maggots, as well as fly larvae collected from burned cadavers and fly larvae at different life stages. The extracts were quantified for human DNA using Quantifiler® Trio DNA Quantification Kit and STR typing with Investigator® 24plex QS. Mitochondrial DNA analysis of hypervariable regions (HV) 1 and 2 was performed using BigDye™ Direct Cycle Sequencing Kit. Powersoil® Pro with manual purification yielded full and partial profiles from crop and whole fly larvae samples, while EZ2 and Powersoil® Pro with automated purification on the QIAcube® Connect yielded partial profiles or were unsuccessful to produce a STR profile. mtDNA analysis was successful, producing haplotypes for several samples in which STR amplification itself was unsuccessful. Using fly larvae as a potential sample for human DNA can allow laboratories to attempt STR and mitochondrial DNA, which can aid investigations and potentially lead to CODIS-eligible profiles and haplotypes.

**Reference:**

- <sup>1</sup>. Cantu C, Bucheli S, Houston R. Comparison of DNA extraction techniques for the recovery of bovine DNA from fly larvae crops. *J Forensic Sci* 2022;67(4):1651–9. <https://doi.org/10.1111/1556-4029.15010>.

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**Forensic Entomology; Fly Larvae; Human DNA**

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**B139 The Progress on Obtaining Nuclear DNA Profiles From a Human Hair Shaft**

*Elaine Lewis, PhD, The George Washington University, Silver Spring, MD; Daniele Podini, PhD, Department of Forensic Science, Washington, DC; Arely Parra López, BS\*, The George Washington University, Washington, DC*

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**Learning Objective:** Attendees of this presentation will learn about the progress made by our group in extracting nuclear DNA from hair shafts and generating a Single Nucleotide Polymorphism (SNP) profile suitable for human identification.

**Impact Statement:** The ability to obtain a nuclear DNA SNP profile from hair found at crime scenes or from unidentified individuals would improve our ability to identify the contributor through DNA analyses.

Hair strands are a frequent component of materials found at crime scenes, yet their use as a source of genetic identification has been limited. While hair strands have both nuclear and mitochondrial DNA, the DNA is degraded and in low quantity, and the hair shaft usually has no intact nuclei. Results from attempts to use standard STR analyses of recovered hair shaft DNA have been inconsistent.

To obtain DNA sequence information from rootless hair, we have developed a protocol using massively parallel sequencing and capture hybridization to generate genotype profiles that could contribute toward the identity of a suspect or victim. Hair DNA was extracted using modifications of methods originally developed for ancient DNA. A panel of 225 single nucleotide polymorphisms and 18 microhaplotypes were targeted. To enrich the hair DNA for the targeted variants, extracted DNA was hybridized to an oligonucleotide array representing the panel, and subsequently amplified and sequenced. The genotypes at the targeted markers were then compared to the references from buccal swabs obtained from hair donors and processed separately using a different sequencing platform. The read coverage, and subsequent number of alleles recovered, varied between hair donors. The best sample elicited a probability of inclusion of approximately one in greater than  $9.6 \times 10^{39}$  in the major United States population, showing the potential of this approach to generate a nuclear DNA profile from hair shaft that can be used for reliable human identification. For the same sample, using a subset of ancestry informative SNPs included in the panel, a biogeographical ancestry prediction was calculated determining that it is at least  $2.6 \times 10^{15}$  times more likely to observe this AISNP profile if it is from an individual of European descent rather than of EA, AA, or LA descent. This is consistent with the ancestry of the donor of the hair sample. Our results demonstrate the potential of capture hybridization to generate nuclear DNA profiles from hair strands collected as forensic evidence.

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**Hair Shaft; SNPs; Microhaplotypes**

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**B140 The Development of a Next Generation Sequencing Panel Targeting Cannabinoid Synthase Genes to Distinguish Between Marijuana and Hemp**

*Ya-Chih (Jessica) Cheng, MS\*, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will understand the advantages behind utilizing Next Generation Sequencing (NGS) techniques to analyze three cannabinoid synthase genes and their pseudogenes in order to reveal novel polymorphisms that could potentially aid in the differentiation method of *Cannabis sativa* crop types, as well as provide more information about the relationship between chemotype and synthase gene copies.

**Impact Statement:** *Cannabis sativa*, which can be cultivated as hemp or marijuana, has been one of the most controversial plants in the United States. The passage of the 2018 Agriculture Improvement Act that legalized hemp charges law enforcement with differentiating the two crop types. This presentation will impact the forensic science community by demonstrating the process using the NGS panel to discover novel polymorphisms that could potentially assist law enforcement agencies in distinguishing legal hemp and illicit marijuana and also aid in the linkage of illegal cases and drug trafficking. The study also contributed to the expansion of the genetic database of synthase genes and pseudogenes, as well as the knowledge about the relationship between the synthase genes, pseudogenes, and chemotype of *Cannabis sativa*.

Genetic markers are desirable for determining crop type in sample types that are inconducive to chemical analysis, such as trace residues, small leaf fragments, immature crops, aged samples, seeds, and root material. Cannabinoid synthase genes are considered the key enzymes that determine the chemical composition or chemotype of a particular cultivar. The use of cannabinoid synthase genes, specifically Tetrahydrocannabinolic Acid Synthase (THCAS) and Cannabidiolic Acid Synthase (CBDAS), to predict the chemotype of *C. sativa* has been proposed in several studies. However, existing methods for crop type differentiation based on past theory could lack specificity and precision, and a wider range of cannabis varieties must be considered when examining cannabis-based genetic markers. The success of cannabis genome assemblies has broadened our knowledge of cannabinoid synthase genes, which are found to be highly repetitive in the cannabis genome. Most homologs were pseudogenes consisting of incomplete coding sequences or contained stop codons in the reading frame, and their sequences can have 91–95% nucleotide identity to the functional synthase genes. Thus, the risk of amplification of pseudogenes cannot be overlooked in all synthase-gene targeting methods for genetic testing of cannabis. Furthermore, targeting only active THCAS or active CBDAS genes, the unique genotypes of some samples may have been missed by conventional methods. Therefore, the targets of this study also include another commonly seen synthase gene, Cannabichromene Acid Synthase (CBCAS), and the synthase-like genes (pseudogenes) with an expanded sample variety that can not only improve the identification method but also provide more information about the relationship between chemotype and synthase gene copies.

A custom NGS panel was developed that allowed us to target all synthase genes (THCAS, CBDAS, and CBCAS) and tease out the amplification of pseudogenes. Additionally, with targeted sequencing, NGS platforms can also sequence a high throughput of samples. In this study, a variety of sample types were included, including reference *C. sativa* samples from the National Institute on Drug Abuse (NIDA) and the National Institute of Standards and Technology (NIST), CBD and CBG hemp samples from the United States and Canada, and marijuana samples from the United States–Mexico, Chile, and around the United States. Novel polymorphisms, mostly SNPs, were found between the different sample types, and the observed polymorphisms may also be related to the biogeographical origin of the plant. The results also demonstrated that the presence or absence of pseudogenes may potentially be related to the cannabinoid content in the cannabis plant. This study also discovered several synthase-like gene sequences that have not been reported before and can assist in the expansion of the database of synthase genes and pseudogenes. Overall, this presentation highlights the usage of the custom targeted NGS panel that serves as a comprehensive analysis of the three synthase genes and their pseudogenes to discover novel polymorphisms between crop types and/or strains, and the relationship between chemotype and gene copies. The large-scale evaluation of synthase genes in this study can help researchers understand the variety both within and between crop types.

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***Cannabis sativa*; Cannabinoid Synthase Gene; Pseudogenes**



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**B141 The Intraspecies Variation of Commercially Available Kratom Products in Common DNA Barcode Regions**

*Kari Graham, DPhil\*, UNTHSC Center for Human Identification, Venus, TX; Cesar Cantu, BS, Sam Houston State University, Huntsville, TX; Rachel Houston, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the ability to use DNA barcode information to differentiate *Mitragyna speciosa* (kratom) products.

**Impact Statement:** This presentation will impact the forensic science community by providing the intraspecies variation of four barcoding markers for kratom. This information could be used for species identification and sourcing and tracking of illegal kratom samples in the United States.

*Mitragyna speciosa*, also known as kratom, is a narcotic plant that is used for its unique mood-enhancing and pain-relieving effects. It is marketed in the United States as a “legal high” and has gained popularity as an alternative to opioids. However, there has been an increase in accidental overdoses involving kratom, especially among polydrug users. The Drug Enforcement Agency (DEA) and the Food and Drug Administration (FDA) have advised consumers against consumption, but it is not yet federally illegal. However, kratom is banned in six states, making the monitoring of illegal kratom difficult. There is a need to identify and monitor drug trafficking routes in states where kratom is illegal. Common forensic techniques using morphology or chemical analysis are beneficial in some cases but are not useful in source attribution because most seized kratom is powdered and the alkaloid content of samples can vary within products, making sourcing unreliable. DNA is independent of these factors, and DNA barcoding is a reliable technique that has been used for the accurate identification of plant species.

This is the first study to evaluate the intraspecies variation of *M. speciosa* in multiple barcode regions for use in the development of a biogeographical tool for species identification and sourcing and tracking of illegal kratom samples in the United States. This study evaluated one nuclear barcode region, ITS, and three chloroplast barcode regions, matK, rbcL, and trnH-psbA, for their utility in biogeographical source attribution. Fifteen commercial kratom products were sequenced. Successful sequencing of the ITS region, matK, and trnH-psbA was achieved. Novel polymorphisms were discovered and reported to the GenBank® database, and the ITS region showed the greatest intraspecies variation. Two haplotypes were discovered, but there was no correlation with biogeographical sources, as commercial products originated from a similar geographical area. However, the sample database needs to be further expanded to help identify more potential regions that can be used to discover trends and be used in a comprehensive forensic tool.

The lack of *M. speciosa* sequencing data available in databases makes it difficult to develop forensic intelligence tools. Investigation of the chloroplast barcode regions trnL-trnF, rpl14-16, and petA-psbJ may help uncover polymorphisms that this limited study may have missed. Additionally, these proposed barcode regions and developed *M. speciosa*-specific primer sequences are reported in this study.

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***Mitragyna speciosa*; DNA Barcoding; Kratom**

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**B142 An Investigation of Forensic Applications for Nanoplate-Based Digital Polymerase Chain Reaction (PCR)**

*Mirna Ghemrawi, PhD\*, Florida International University, Miami, FL; Nicole Fernandez-Tejero, MS, Florida International University, Miami, FL; Alyssa Sanchez, BS, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will understand the difference between qPCR and digital PCR. They will be made aware of potential applications of nanoplate-based digital PCR, including species identification, fecal contamination of food and surface waters, and human mtDNA quantification.

**Impact Statement:** This presentation will impact the forensic science community by providing the DNA analyst with alternative methods to identify commonly found species, detect fecal contamination, and quantify mtDNA with higher resolution without the need for standards.

Digital PCR is a novel method for detecting and quantifying low levels of DNA. Unlike standard real-time PCR, this procedure does not require comparison to calibration curves. Because it isolates and amplifies each DNA molecule in a separate small volume on a nanoscale, it can detect trace levels of DNA while being less sensitive to PCR inhibition.<sup>1</sup> In this project, we examined several potential applications for digital PCR, including species ID and fecal contamination of food and surface waters. The procedures also have potential applications for quantifying human mtDNA and mixtures of tissues and/or body fluids from different species.

In this study, a nanoplate-based digital PCR assay for species identification as well as fecal contamination was created. The first assay was designed with two multiplexes focusing on the identification of human (D-loop), canine (ATP6), feline (D-loop), cattle (D-loop), swine (Cyt-b), fish (tRNA-Val), and chicken (D-loop). The second assay focused on the detection of fecal contamination from humans (*B. vulgaris* and HF183), canines (BacCan-545), and cattle (BoBac367) based on the detection of gut bacteria (Bacteroidales). An internal positive control was also added to the assays by designing a DNA sequence that does not code or match any species. A total of four multiplexes with 3–4 targets were optimized and compared to qPCR on a variety of body fluids ( $n=50$ ). These assays are rapid, easy to perform, and produce quantitative data from as low as 5pg of DNA. The digital test gives it outstanding sensitivity for trace quantities of the target species, even in the presence of higher concentrations of a major contributor. This makes it an excellent tool for mixture deconvolution. We will show sensitivity, combination identification, and the impact of different inhibitors. Overall, this assay provides the forensic community with a novel way to use digital PCR to accurately and sensitively identify species. To sum up, this study examined the applicability of a state-of-the-art nanoplate-based digital PCR in forensic applications such as species identification, fecal contamination, and mtDNA quantification.

**Reference:**

- <sup>1</sup>. Tan, L. L., Loganathan, N., Agarwalla, S., Yang, C., Yuan, W., Zeng, J., & Duraiswamy, S. (2022). Current commercial dPCR platforms: Technology and market review. *Critical Reviews in Biotechnology*, 1-32.

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**Digital PCR; Species Identification; Fecal Contamination**

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**B143 The Social Amoeba in Forensic Science: Microbial Fingerprinting and Epigenetics**

*Andrea Alvashere, PhD\*, Western Illinois University, Macomb, IL*

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**Learning Objective:** This presentation will examine the potential utility of the social amoeba, *Dictyostelium discoideum*, in forensic science casework, research, and education. Attendees will learn how *D. discoideum* can be used in microbial soil fingerprinting and as a model organism for epigenetic, proteomic, and transcriptome analyses, using both traditional sequencing and portable nanopore sequencing devices. This overview will also feature recent case studies demonstrating the use of *D. discoideum* in each of these analysis areas.

**Impact Statement:** This presentation will impact the forensic science community by providing the basic knowledge and competence needed to begin incorporating *D. discoideum* into research or training programs. This presentation will be of special relevance to forensic scientists interested in environmental characterization and/or gene expression studies, and to forensic science educators seeking engaging, cost-effective options for student laboratory experiences.

Social amoebae (*Dictyostelium sp.*) can be useful tools in forensic biology, both for soil-based environmental characterization, and as model organisms for development of new techniques for genetic, epigenetic, transcriptomic, and proteomic studies. Dictyostelium-derived protocols for gene expression analysis can be developed at a much more rapid and affordable rate than exclusively human-based studies. Later, when optimized for human samples, these protocols may provide information about tissue/fluid type, subject age estimation, and other markers of epigenetic effects on gene expression. This information can facilitate sample classification and can provide details that help to narrow the candidate pool for subject identification when a DNA database match is not obtained.

*Dictyostelia* is a genus containing ~120 species of eukaryotic amoebae (a.k.a. slime molds) found in the soil and leaf litter of terrestrial ecosystems, worldwide. The best-studied of these, *D. discoideum*, has long been a popular model organism for genetic research due to its short life cycle, multiple developmental pathways, and numerous orthologs to human disease genes. *D. discoideum*'s six-chromosome, 34 Mb haploid genome is abundant in trinucleotide tandem repeats and encodes ~12,000 proteins that vary in expression throughout the life cycle. These single-celled, asexually reproducing amoebae can, under certain conditions, also reproduce sexually, having three different sexes that can each reproduce only with a partner of a different sex class. Additionally, when experiencing nutrient stress, the multiple genetic lineages will aggregate into a multicellular slug, cooperatively migrate to a better food patch, then produce a fruiting body that releases spores that will mature into new, unicellular offspring. Each of these stages can be induced and observed in colonies grown on simple agar plates in the laboratory.

Collection of wild-dwelling colonies for laboratory study is also quick and inexpensive, and available right in many researchers' back yards. Colonies can be frozen to pause research and will resume activity when thawed. The above qualities combine to provide an ideal organism for multiple areas of forensic biology research and, especially, for hands-on forensic student research that is extraordinarily flexible, budget-friendly, and does not typically require the time-consuming process of obtaining Institutional Review Board (IRB) approval before each study can commence.

Case studies that will be briefly summarized for illustrative purposes include: (1) the harvesting and genetic characterization of wild Dictyostelium colonies from different mock crime scene locations; (2) isolation and comparison of (reverse-transcribed) messenger RNA and select expressed proteins under varying conditions and throughout the life cycle; (3) proteins useful for taxonomic classification by amino acid sequence; and (4) assessment of highly economical, portable nanopore sequencing devices, compared to established sequencing systems, for conducting these types of studies.

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**Education; Epigenetics; Environment**

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**B144 Developing a Rapid DNA Response Plan for Mass Identification Events: An Inter-Laboratory Approach to Mobilizing a Forensic DNA Laboratory**

*Dana DeVito, BS\*, Marshall University Forensic Science Graduate Program, Huntington, WV; Amy McGuckian, MS, Palm Beach County Sheriff's Office, West Palm Beach, FL; Julie Sikorsky, MS, Palm Beach County Sheriff's Office, Forensic Biology Unit, West Palm Beach, FL; Taylor Koepfler, MS, Marshall University Forensic Science Center, Huntington, WV; Jason Chute, MFSF, Marshall University Forensic Science Cen, Huntington, WV; Kelly Beatty, MFSF, Marshall University, Huntington, WV*

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**Learning Objective:** After attending this presentation, attendees will understand the planning and outcome of two independent simulated mass identification events with mobilized Rapid DNA instrumentation conducted by Marshall University Forensic Science Center and Palm Beach County Sheriff's Office Forensic Biology Unit.

**Impact Statement:** This presentation will provide the forensic biology community with guidance for developing a plan to mobilize Rapid DNA instrumentation for a mass identification event.

Rapid DNA instruments have been around for close to a decade and are entering into human identification workflows across the nation. Both early and recent adopters can attest to the practicality and success of their use. Additionally, Rapid DNA has proven to be ideal for use at mass identification events, such as the California Camp Fire in 2018 and the Surfside condominium collapse in 2021. In these types of situations, a comprehensive plan for victim identification is critical. The identification of individuals who cannot or will not identify themselves, such as victims of mass disasters or human trafficking, when combined with the mobilization capability of Rapid DNA instruments, serves as a catalyst for forensic DNA laboratories to develop a laboratory mobilization plan.

In June of 2021, a collaboration between Marshall University Forensic Science Center and Palm Beach County Sheriff's Office Forensic Biology Unit was formed to develop a blueprint for the mobilization of Rapid DNA instruments. The purpose of this inter-laboratory approach was to build a deployment plan that could be modified for use by all forensic DNA laboratories and to test both laboratories' internally validated Rapid DNA protocols in a non-laboratory setting. A plan was developed that included the necessary supplies, logistics, role assignments, humanitarian needs, and more. To test the developed workflow, the two laboratories conducted independent simulated mass identification events in West Virginia and in Florida. As part of these events, buccal, bone, soft tissue, and blood samples were processed outside of the laboratory using the mobilized Rapid DNA instruments. Considerations for various aspects of forensic DNA laboratory mobilization as well as lessons learned were included in the materials developed throughout this collaboration. The goal is for the developed guidance to help facilitate the discussion and design of additional emergency preparedness and response plans for Rapid DNA deployment by other laboratories in the forensic biology community.

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**Rapid DNA; Mass Identification; Forensic DNA Laboratory**

## B145 Toward a Rapid DNA Tool for Integrated Forensic Epigenetic Sample Preparation

Rachelle Turiello, MS\*, University of Virginia Chemistry Department, Charlottesville, VA; Renna Nouwairi, BS, University of Virginia, Charlottesville, VA; Jordan Keller, BS, University of Virginia, Centreville, VA; Francine Garrett-Bakelman, MD, PhD, University of Virginia, Charlottesville, VA; James Landers, PhD, University of Virginia, Charlottesville, VA

**Learning Objective:** After attending this presentation, attendees will gain insight into a microfluidic technology and the integration of an automated tool for streamlined sample preparation for forensic epigenetic analyses.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with an alternative, automated technique for forensic DNA methylation sample preparation that combines enzymatic DNA extraction and sodium bisulfite conversion for use with forensically relevant amounts of input DNA.

Around the world, the toolkit for nucleic acid-based forensic analysis has exploded to include more predictive strategies that go beyond Short Tandem Repeat (STR) profiling.<sup>1</sup> Laboratories and investigators alike have been made aware of alternative strategies for identification, including genetic genealogy mapping and DNA phenotyping.<sup>2</sup> In parallel, investigation of the human epigenome has been suggested for a variety of forensic applications; in particular, multiple well-supported strategies have characterized the utility of methylation status to predict human chronological age.<sup>3</sup> However, there is an argument that the integration of epigenetic analysis into the forensic DNA workflow is not a simple task due to the high degree of DNA fragmentation and loss known to occur during sample preparation via sodium bisulfite conversion—this is a large hurdle to overcome considering the low levels of DNA typically encountered in the forensic context.<sup>4</sup> Beyond these issues, the preparation process requires both DNA extraction and cytosine deamination—two processes that are laborious, time-consuming, and prone to analyst contamination due to the series of open-tube transfer steps.

We propose a microfluidic device for the rapid, automated sample preparation of nucleic acids for downstream forensic epigenetic analysis, including age prediction. This tool integrates an enzymatic lysis strategy with silica bead-facilitated sodium bisulfite conversion to streamline the traditional sample preparation workflow. The extraction approach utilizes a one-step method that releases nucleic acids with the assistance of a neutral protease working in a PCR-compatible buffer solution.<sup>5</sup> The eluate is then subjected to the sequential unit operations associated with the conversion reaction, which has been optimized on the microdevice to enhance DNA recovery and maintain conversion efficiency when compared with the gold-standard method. Samples originating from human K-562 erythroleukemia cell lines and human blood were tested with several downstream strategies, including Real-Time Polymerase Chain Reaction (RT-PCR), High Resolution Melting (HRM), and pyrosequencing using age-associated targets FHL2 and ELOVL2 for proof-of-concept.<sup>6</sup> Results suggest the extraction strategy is compatible with the bisulfite conversion chemistry sans nucleic acid purification on the microdevice. Continued work is focused on complete automation with in-house engineered systems and characterization with forensically relevant samples.

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### Forensic Epigenetics; Centrifugal Microfluidics; Epigenetic Sample Preparation

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**B146 Discovering the Mysteries About Degradation of Extracted DNA From Trace Evidence When Stored in Different Conditions: State-of-the-Art and Future Perspectives**

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**Learning Objective:** After attending this presentation, attendees will understand the consequences that inappropriate or inaccurate sample preservation can have on judicial caseworks and will gain knowledge on the best isolation and preservation strategies for DNA extracts for forensic genetics.

**Impact Statement:** This presentation will impact the forensic science community by presenting experimental studies on DNA degradation of extracts preserved in non-ideal conditions and by providing guidelines on the best approaches to conserve them without losing any genetic information.

The storage of DNA extracts is a debated topic in the forensic arena; despite the existing guidelines on the preservation of biological evidence, there are no clear agreements on the preservation of the extracts originated from the evidence in terms of duration nor temperature. According to NIST guidelines, extracted DNA to be preserved for the long term should be kept frozen.<sup>1,2</sup> However, it is not infrequent to encounter situations in which extracts are kept at fridge or room temperature, with unknown implications for its degradation and, consequently, for the final analytical results.

In order to evaluate DNA survival and degradation rates, different samples, including blood stains, buccal swabs and blood/saliva mixtures in increasing ratios, were extracted with several methods commonly used in forensic genetics and stored at different temperatures (+20°C, +4°C, and -20°C) for up to 150 days. Aliquots were quantified with qPCR to obtain a quantitative and qualitative assessment at time "0," then every four days for the first 16 days and every 30 days after one month (from 30 to 150 days). In addition to qPCR, samples were amplified with commercial kits to evaluate their degree of efficiency and typing, on at least two instruments (Applied Biosystems® SeqStudio™ Genetic Analyzer and 3500 Genetic Analyzer) for performance comparisons. The NGS VISAGE panel for phenotype, as well as a new in-house-developed ancestry and phenotype panel, will be additionally applied to selected samples to evaluate the reliability of the NGS data on these samples.

Preliminary results indicated that the frozen samples as well as room temperature vacuum-dried samples maintain a complete genetic profile useful for identification purposes and for NGS analyses; the complete results presented at the conference will clarify to what extent mixture samples can be informative on the identity of both contributors to the trace and for how long preservation at different temperatures can provide useful information, and will propose "best practices" that should be applied by forensic genetics laboratories and police forces in order to maximize the preservation of the genetic information in DNA extracts.

**References:**

1. S.M. Ballou, M.C. Kline, M.D. Stolorow, M.K. Taylor, S.R. Williams, P.S. Bamberger, B. Yvette, L. Brown, C.E. Jones, R. Keaton, *The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers*, 2013.
2. G. Sguazzi, F. Lovisolo, S. Gino, Is Genomic DNA extracted and stored at -20° C for long time useful in forensic field? *Forensic Sci. Int. Genet.* 7 (Supplement Series) (2019) 629–631.

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**DNA Degradation; STR; SNP**



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**B147 Investigator® 24plex QS Amplification Kit Validation and Evaluation of New Formulation**

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**Learning Objective:** After attending this presentation, attendees will understand key troubleshooting that can be performed during validation to assess the suitability of an amplification kit for use with probabilistic genotyping and address what to do when critical reagent changes lead to unintuitive results.

**Impact Statement:** Validation is a key component for any forensic DNA laboratory to establish the expected limits of reagent and instrument performance. Changes in critical reagents or other components of the testing process (e.g., instrumentation) can have a substantial impact on DNA results and downstream reporting.

In December 2020, QIAGEN® published a memo to provide advance notice of their upcoming formulation change for their Investigator® 24plex QS amplification kits.<sup>1</sup> The formulation change was accompanied by a change in the final extension time, with both efforts focused on eliminating minus A at some loci in the amplified product. In June 2021, QIAGEN® published a memo on the final update regarding these changes.<sup>2</sup> This memo reported that, per the NDIS custodian that performed the review, the changes did not affect NDIS approval. In coordination with the Institute of Environmental Science and Research of New Zealand (ESR), the recommendation for laboratories that use STRmix™ was to perform a performance check of the new formulation/extension time prior to use. Signature Science assessed this recommendation and elected to perform additional validation efforts to assess the new formulation with an increase in PCR cycle as well as an additional CE injection assay.

In July 2022, QIAGEN® released a memo stating that varying levels of stutter product formation between 24plex QS amplification kits have been observed by customers due to a raw material in a component of the formulation.<sup>3</sup> Accurate modeling of expected stutter product percentages is critical to the application of the STRmix™ software for mixture deconvolution. To ensure continued data quality within the Signature Science casework laboratory, validation data from the original formulation and new formulation of the 24plex QS amplification kit were re-evaluated in conjunction with the STRmix™ software. This re-evaluation was undertaken to determine if the validations were acceptable or if supplemental validation efforts were needed to fully address the variability in stutter product formation. The evaluation involved the examination of validation mixtures, particularly mixtures where a trace contributor was detected, for the presence of elevated stutter, the confirmation that the estimation of the number of contributors conformed to scientific expectations, and to ensure the resultant likelihood ratio associated with the trace contributor(s) were intuitive.

**Reference:**

1. QIAGEN® December 2020, Notification Memo - Investigator 24plex QS Kits.
2. QIAGEN® June 2021, Notification Memo - Investigator 24plex QS Kits.
3. QIAGEN® July 2022, Product Notification Investigator 24plex QS Kit, cat nos 382415 and 382417.

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**DNA; Probabilistic Genotyping; Validation**

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**B148 The Development of a Five-Dye Insertion/Deletion (INDEL) Panel for Human Identification**

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**Learning Objective:** After attending this presentation, attendees will understand the benefits of Insertion/Deletion (INDEL) markers for human identification purposes. This presentation will focus on their application with low quantity and quality DNA. The kit can be used as a secondary or in conjunction with the routine methods when adequate recovery is not possible. This research developed an INDEL panel for human identification markers followed by a validation according to the Scientific Working Group on DNA Analysis Methods (SWGDM).

**Impact Statement:** This research will provide an alternative maker novel INDEL panel for human identification markers. The benefits of INDEL recovery can be used to assist in scenarios where low quantity or challenging samples are present and current methods are limited.

The use of Short Tandem Repeat (STR) markers to find individualizing features in the human genome in conjunction with the national Combined DNA Index System (CODIS) database has streamlined human identification. DNA evidence continues to evolve as improvements in sensitivity allow for utilization of many forensic samples. This can be in the form of various instrumentation and methods, which can be more costly and unfamiliar, or alternative techniques and markers, which can provide similar types of data and information. INDEL markers are similar to STRs in that they can provide genetic identification, but there are some necessary considerations. INDELs are generally bi-allelic, which can create some limitations, but INDELs can be genotyped using small amplicons, which allows for greater success with degraded/low quantity sample types. The markers chosen were selected for their low *F<sub>st</sub>* (Fixation Index) values to obtain optimal targets during analysis.

This research focuses on the use of INDEL polymorphisms that were selected for human identification. These Human Identification markers, known as HIDs, can distinguish between various individuals and can be measured in various allele frequencies between individuals. Another decision during the making of the kit was to use INDELs that can be designed to be differentiated by their smaller size into a Capillary Electrophoresis (CE) platform. STR kits can range from 100–400bp or more. An advantage INDELs have over STRs is the ability to amplify many markers using amplicons less than 200bp. INDELs have the added benefit of no stutter artifacts, allowing for easier interpretation from degraded samples and providing more confidence when running a lower concentration sample.

Routine practices of forensic laboratories routine practices may be useful when known and reference samples are obtainable, but aged or degraded biological samples may be difficult during STR analysis. This presentation will discuss the limitations of current testing and how the developed panel can serve as a complement to forensic cases. The INDEL panel can be used on the CE platform and read like any other STR based kit. The INDELs unlike STRs will not have stutter due to method of analysis. The panel consists of 33 markers configured across five dye channels and validated according to SWGDM guidelines. Stability, mixtures, sensitivity, and reproducibility were evaluated to ensure the effectiveness of the panel. The panel was designed with markers with high discriminatory power. The 33-marker INDEL panel was developed, and preliminary results indicate the assay functions and can obtain near 100% or full profiles down to 32pg and partial profiles at 16pg.

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**Insertion Deletion Polymorphisms (INDEL); Human Identification Markers (HID); Alternative Markers Kit Development**

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**B149 The Status of the DNA Backlog in South Africa**

*Antonel Olckers, PhD\*, DNAbiotec (Pty) Ltd, Pretoria, Gauteng, SOUTH AFRICA*

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**Learning Objective:** The presentation will provide a comprehensive overview of the DNA backlog in South Africa with proposed solutions at various levels.

**Impact Statement:** DNA backlogs exist across the globe. In sharing our experiences of how to deal with this vexing problem in the forensic DNA field, we can not only learn from each other, but work together to solve one of the biggest forensic DNA challenges of our time.

The DNA backlog in South Africa persists as a result of several complex and multi-systemic challenges. In the past, the South African Police Service (SAPS) Forensic Science Laboratory (FSL) used an automated Genetic Sample Processing System (GSPS) system to eradicate the backlog at the time. The SAPS reported that the GSPS would quadruple its processing capacity and the backlog was indeed eradicated.<sup>1</sup> The GSPS has since been decommissioned and a so-called semi-automated DNA processing system is now in use. As anticipated, the DNA backlog rapidly increased in the wake of these decisions. It was reported in 2021 that the DNA backlog was 240,000.<sup>2</sup> The devastating impact of this backlog continues to percolate through the justice system.

In 2022, a High Court judge attributed the rape and murder of an 11-year-old minor to the SAPS DNA backlog, in his judgement, stating: “I also cannot ignore the fact that after he raped the first victim, he was apprehended but the charges were provisionally withdrawn because of the delay in obtaining the DNA analysis result. . . . I am also aware . . . of the adverse impact of the DNA analyses delays on the Victims of GBV and the administration of justice. This is a clear example of how one such case was impacted with severe and tragic consequences. . . . Tragic consequences and the loss of an innocent life.”<sup>3</sup> This 2022 judgement represents the proverbial line in the sand for South Africa and the impact its persistent DNA backlog has on the justice system.

South Africa can look at other jurisdictions to find examples of best practice on how to clear DNA backlogs. Whichever solution is ultimately deployed, it must be sustainable, robust with high scientific rigor, and address the root cause(s) of the current DNA backlog—including holding professionals accountable and fostering transparency. In addition, the problem with quality in DNA testing was flagged more than two decades years ago with a permanent solution being overdue.<sup>4</sup> South Africa has a painful past where the State did not act in the best interest of its citizens. The Truth and Reconciliation Commission (TRC) hearings highlighted how the justice system failed to hold perpetrators accountable over decades.<sup>5</sup> Failing to address the DNA backlog is equally devastating to the victims of crime in South Africa today.

DNA backlogs are not unique to South Africa and are presently seen across many jurisdictions globally. The solution for this particular problem in South Africa must include a few factors such as accountability and transparency as overarching goals. The solution itself is resource-based, whether it is financial, technological, human, or from other resources. Some of these solutions are less complex and can be solved by advances in technology. If the problem lies at the human resource level, as will be discussed in this presentation, it will take a momentous effort to craft sustainable solutions, given the context in which South Africa currently finds itself.

**References:**

1. SAPS press release on 06 September 2006: <https://www.gov.za/south-african-police-service-new-dna-technology-forensic-science-laboratory>.
2. Gerber J. *News24* online, accessible via: <https://www.news24.com/news24/southafrica/news/saps-dna-backlog-grows-to-almost-240-000-cases-20210921>.
3. *State versus LMM*. Judgement by Moodley J: State versus LMM, Case no: CCD14/2022. Heard in the High Court of South Africa, KwaZulu-Natal Division, Mthunzini. Judgement delivered on 18 March 2022.
4. Sv Maqhina. *SACR* 241 (1), p 241-252, 2001.
5. Truth and Reconciliation Commission of South Africa: <https://www.justice.gov.za/trc/>.

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**DNA Backlog; South Africa; High Court Judgement**

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**B150 The Impact of Key Factors Absent in Forensic DNA Analysis in South Africa**

*Antonel Olckers, PhD\*, DNAbiotec (Pty) Ltd, Pretoria, Gauteng, SOUTH AFRICA*

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**Learning Objective:** This presentation will provide an understanding of the challenges and impact in the South African context that a lack of these critical factors brings about.

**Impact Statement:** The forensic science community needs to collaborate to shape best practice globally. Sharing challenges and successes within our various contexts and systems greatly assists in this goal and allows us to learn from each other. The context in South Africa is unique in that forensic science is not regulated nor are the main laboratories accredited. Challenges to transparency and accountability are systemic. The forensic science community can learn from our challenges and our efforts to limit the impact of this on our justice system, as well as provide assistance as appropriate from their own jurisdictions.

The forensic science landscape in South Africa has deep-rooted and multi-factorial challenges that have persisted over time. At the basis of this are factors such as the lack of effective standards at the national level in the forensic science sector, a problematic structure, lack of transparency and accountability, as well as corruption, to highlight only a few.

Accreditation serves as the minimum standards in the field globally, yet this necessary first step toward sustained quality of service delivery remains lacking in the public sector forensic DNA testing laboratories of South Africa. The impact of this is far reaching given the challenges of the massive DNA backlog and the most significant pandemic in South Africa, namely Gender-Based Violence and Femicide (GBVF).

South Africa has one of the most liberal constitutions in the world. Given its history, such a constitution is not only appropriate but a necessary step to restoring the faith in the justice system of South Africa. The Constitution of South Africa outlines standards for service delivery by the State in Chapter 10. Section 195(b) states that “Efficient, economic and effective use of resources must be promoted.”<sup>1</sup> It also directly addresses accountability (Section 195(f)) and transparency (Section 195(g)). Moreover, service delivery is expressly mentioned: “Services must be provided impartially, fairly, equitably and without bias” (Section 195(d)). If these terms are not met a service is delivered unconstitutionally.

Corruption is a strong word and should not be used lightly in the forensic science context. However, the Minister of Police stated publicly that one of the reasons for the DNA backlog is “a lot of corruption.”<sup>2</sup> The impact of this unethical practice is devastating to the forensic science profession as well as the public to whom justice is denied. South Africa has been dealing with this plague over the past few decades and key aspects of the level to which corruption has risen are now widely reported in the press. State Capture was documented in the recently released report of The Judicial Commission of Inquiry into Allegations of State Capture in South Africa.<sup>3</sup> This paints a bleak picture of how far the corruption has spread and its impact on, for example, forensic science service delivery.

Given the above reality, it will be outlined how forensic science professionals are working to not only change the status quo. but also restore forensic science to a level where it can be used as a tool to obtain justice for the victims of crime in South Africa. A proposed solution will be outlined that includes basic requirements of transparency, accountability, appropriate structures, and an enabling environment to deliver on the promise of justice made possible by the credible results generated by a competent forensic science sector.

**References:**

1. Constitution of the Republic of South Africa, 1996.
2. Gerber J., “A lot of corruption’ is why SAPS has a forensic case backlog of over 117 000 - Bheki Cele”. News24 Online, 18 November 2018, Available from: <https://www.news24.com/news24/southafrica/news/a-lot-of-corruption-is-why-saps-has-a-forensic-case-backlog-of-over-117-000-bheki-cele-20201118>.
3. The Judicial Commission of Inquiry into Allegations of State Capture in South Africa. <https://www.statecapture.org.za/#>.

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**DNA Analysis; Accountability and Transparency; South Africa**

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**B151 Gas Chromatography-Vacuum Ultraviolet (GC-VUV) Detection of JWH-18 Positional Isomers and Select Diastereomeric Synthetic Cannabinoids**

*Diana Le, BS\*, The George Washington University, Washington, DC; Ira Lurie, PhD, The George Washington University, Washington, DC*

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**Learning Objective:** After attending this presentation, attendees will understand the capabilities of GC-VUV detection to distinguish between select positional isomers and diastereomers of synthetic cannabinoids and recognize GC-VUV as a complementary analytical technique to staple forensic methods such as Gas Chromatography with Electron Ionization/Mass Spectrometry (GC/EI/MS) on structurally similar compounds.

**Impact Statement:** This presentation will impact the forensic science community by providing evidence for the plausible usage of an alternative GC method employing VUV detection in analyzing structurally similar compounds, especially isomeric synthetic cannabinoids. This methodology can prove to be especially useful in providing greater confidence in identification. In addition, this research aims to demonstrate that by utilizing spectral data from VUV and fragmentation patterns from MS in tandem with the high resolving power from a GC, it will be possible to identify compounds that would otherwise be indistinguishable with electron ionization or chromatography alone.

When analyzing seized drugs, identification and detection methods such as Gas Chromatography with flame Ionization Detection (GC/FID) and GC/MS are staples in forensic laboratories. However, as a greater number of illicit psychoactive substances are synthesized to circumvent existing drug laws, these popular and routine methods are becoming insufficient, as they are unable to be specific enough to distinguish between structurally similar compounds by chromatographic and spectral means. In particular, GC/FID lacks specificity because it is unable to identify the components under individual peaks, leading to low confidence in identification. Due to similar or identical fragmentation, GC/EI/MS is often unable to distinguish between positional isomers, especially those exhibiting substitution on the benzene ring, and is unable to differentiate between diastereomers.

VUV detection enables the analysis of electron transitions in single bonds ( $\sigma$  to  $\sigma^*$  excitation) and in double bonds ( $\pi$  to  $\pi^*$  excitation) within molecules, which results in an extended analytical wavelength range compared to liquid phase UV detectors. Thus, data taken in the VUV region (125nm–200nm) as well as the UV and visible region (200nm–430nm) can analyze changes in the electron excitation in both the aliphatic and the entire aromatic region of compounds, which can prove useful for analyzing differences in spectra between isomeric compounds. VUV detection also has the capability to deconvolute peaks with coeluting compounds, which is not possible with GC/FID or GC/MS detection.

The research presented will study the specificity of VUV detection for 16 positional isomers of JWH-018 and three pairs of synthetic cannabinoid diastereomers. Within the positional isomers, VUV spectra were able to distinguish between substituted positional isomers with the same -R groups that had otherwise produced identical mass spectra and were able to deconvolute coeluting peaks. Most of the synthetic cannabinoid isomers and diastereomers exhibited unique VUV spectra that could be identified via user-generated and reference libraries, and the repeatability of this detection method was demonstrated for both short-term run-to-run and long-term day-to-day repetition. Together with MS data collected under the same GC conditions as for VUV detection, the complementary nature of the latter technique was shown due to its ability to identify compounds in cases where MS detection could not. This study will show that utilizing VUV detection will allow for a far more enhanced identification protocol for structurally similar compounds found in seized drugs.

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**Gas Chromatography/Vacuum Ultraviolet Detection; Synthetic Cannabinoids; Isomer Identification**

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**B152 The Investigation of Analytical Methods Using Real-Time Mass Spectrometry for the Rapid Analysis of Seized Drugs**

*Madeline Bloom, BS\*, The George Washington University, Washington, DC; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Ira Lurie, PhD, The George Washington University, Washington, DC*

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**Learning Objective:** After attending this presentation, attendees will have an understanding about current methodologies that utilize real-time mass spectrometry for the rapid, presumptive analysis of seized drugs. This presentation will specifically discuss the use of rapid Gas Chromatography/Mass Spectrometry (GC/MS) and Direct Analysis in Real Time Mass Spectrometry (DART®-MS).

**Impact Statement:** This presentation will impact the forensic science community by providing a thorough method validation of a novel, rapid GC/MS method for seized drugs analysis in addition to a proposed complementary technique such as DART®-MS. This information can be used to implement new technology to controlled substance laboratories based on the validation of novel workflows using real-time mass spectrometry.

Recent increases of novel psychoactive substances and fake prescription pills have increased case backlogs in forensic laboratories across the United States, limiting timely analyses of seized drug exhibits. Innovative improvements to analytical methodology in controlled substance laboratories are needed to identify drugs rapidly and accurately in circulation for legal, intelligence, and public health purposes. Mass spectrometry is known to be one of the most discriminatory detection techniques for the purpose of seized drug analysis. This project sought to identify different ways a real-time mass spectrometry technique could be used for the rapid analysis of seized drugs.

In this study, a qualitative comparison between rapid GC/MS and DART®-MS was made, as these are two rapid analytical techniques employ mass spectrometry for seized drug analysis. A rapid GC/MS method was developed and optimized to ensure upmost selectivity for run times around one minute using a suite of drug mixtures containing opioids, synthetic cannabinoids, stimulants, hallucinogens, depressants, and substituted phenethylamines. Subsequently, a complete method validation of the rapid GC/MS method to assess retention time repeatability, analyte identification accuracy, limits of detection, selectivity, and carryover was completed. Finally, a comparison study using multiple analysts and adjudicated case samples was completed to determine the strengths and limitations of rapid GC/MS and DART®-MS for seized drug screening in a practical setting.

Overall, the optimized method for rapid GC/MS allowed for overall satisfactory chromatographic resolution for the compounds in the drug class mixtures with a total runtime of just over one minute. Retention time repeatability for same day runs showed an average of 0.66 % RSD between all compounds ( $n=7$ ) and the inter-day precision over the course of six weeks was approximately 2.02 % ( $n=12$ ). The proposed methodology using adjudicated case samples showed a high level of accuracy for analyte identification and the total analysis time of approximately 10 minutes from blank injections to sample identification, which is a decrease of about 90 % in comparison to validated traditional GC/MS methods. Preliminary results between rapid GC/MS and DART®-MS showed that Limits Of Detection (LOD) values were one order of magnitude lower for DART®-MS, but analyte identification was consistent for both techniques. In conclusion, the rapid analysis of seized drugs can be improved with a proposed workflow including rapid GC/MS and DART®-MS, as retention time differences, the specific fragmentation mass spectra from EI/MS and protonated molecular ion peak from DART®-MS can be used together to increase accuracy in positive identification of controlled substances in seized drugs cases.

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**Seized Drugs; Rapid GC/MS; DART®-MS**



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**B153 The Investigation of Cannabidiol Conversion Into Delta-9-Tetrahydrocannabinol in Electronic Cigarettes**

*Laerissa Reveil, MS\*, Virginia Commonwealth University, Richmond, VA; Justin Poklis, BS, Virginia Commonwealth University, Richmond, VA; Matthew S. Halquist, PhD, Virginia Commonwealth University, Richmond, VA; Michelle Peace, PhD, Virginia Commonwealth University, Richmond, VA*

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**Learning Objective:** Attendees will learn about the potential conversion of CBD into delta-9-THC in electronic cigarettes.

**Impact Statement:** With an increase in electronic cigarette use, understanding the potential conversion of CBD into delta-9-THC is imperative as it has an impact in drug testing programs and DUI/DUID investigations with regard to positive THC results.

**Introduction:** There are over 100 phytocannabinoids derived from *Cannabis sativa*. Delta-9-Tetrahydrocannabinol (d9-THC) is the main psychoactive compound that produces euphoria while Cannabidiol (CBD) does not. The natural precursor for the formation of THC is tetrahydrocannabinolic acid, which is formed from cannabigerolic acid. Organically and naturally, CBD is not in the *Cannabis sativa* plant's synthesis pathway for THC. The synthesis pathway for CBD remains under debate in the literature. The CBD defense postulates that CBD can convert to THC when exposed to acidic conditions, when a CBD product is adulterated with THC, or through *in vivo* synthesis. As CBD is a common pharmacologically active ingredient vaped in electronic cigarettes (e-cigarettes), understanding the conversion of these two compounds is imperative due to its overwhelming impact on substance use treatment centers, workplace and federal drug testing, drug courts, and DUI/DUID investigations. The purpose of this study was to determine the conversion rate, if any, of CBD to THC using an aerosol capture method adopted from the Cooperation Centre for the Scientific Research Relative to Tobacco (CORESTA) E-cigarette Task Force, an untargeted Gas Chromatography/Mass Spectrometry (GC/MS) method, and a validated High Pressure Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS) method.

**Methods:** A CBD e-liquid was prepared at 100mg/mL in 100% PEG 400. Aerosol capture was performed using parameters adopted from the CORESTA method. The puff profile was an inhale duration of 3 seconds, an exhale duration of 10 seconds, and a puff volume of 60mL for a total of 15 puffs. Aerosol samples were analyzed with LC/MS/MS at a 10–1,000ng/mL linear range for drug recovery and dose of and screened with GC/MS for 15 major cannabinoids: CBD, d9-THC, d8-THC, d10-THC, CBG, CBDV, CBC, CBN, THCV, THCA-A, CBDA, CBGA, CBDVA, CBCA, THCVA.

**Results:** From the GC/MS screen, CBD, CBC, and d9-THC were identified in the aerosol sample. In 15 puffs ( $n=3$ ),  $0.31 \pm 0.04$ g of e-liquid was aerosolized. In the 100mg/mL CBD e-liquid ( $n=3$ ),  $15 \pm 1$ mg of CBD,  $0.06 \pm 0.03$ mg d9-THC,  $0.07 \pm 0.03$ mg CBN, and  $0.003 \pm 0.001$ mg CBG were aerosolized, respectively. The conversion rate of CBD to d9-THC is less than 1%.

**Conclusion/discussion:** The aerosolization of CBD in e-cigarettes results in a conversion rate to d9-THC of less than 1%. This conversion rate can precipitate positive d9-THC results in drug testing programs.

*This project was supported by the National Institute of Justice (2018-75-CX-0036, 2019-MU-MU-007) and the National Institutes of Health (P30DA033934). Opinions/findings/conclusions/recommendations expressed in this publication/program/exhibition are those of the author(s) and do not necessarily reflect those of the DOJ and NIH.*

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**E-Cigarettes; Cannabidiol; Delta-9-tetrahydrocannabinol**

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**B154 The Nanogram Level Detection of Five Illicit Drugs Using the Ion Mobility Spectrometer: A Field-Testing Report**

*John Wang, PhD\*, California State University-Long Beach, Artesia, CA; Rong Zhang, PhD, Mass Detect Inc. CA, Cupertino, CA*

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**Learning Objective:** After attending this presentation, attendees will understand a nanogram level detecting method of the five common illicit drugs (heroin, cocaine, methamphetamine, ketamine, and morphine) using the Ion Mobility Spectrometer (IMS). In the field, the portable device was able to detect successfully the five illicit drugs at a nanogram level (10~100ng) and at real time (5~8 seconds). The sampling process only needs to wipe the surface of the suspicious objects without opening the container with an *in situ* and non-intrusive manner.

**Impact Statement:** This presentation will impact the forensic community by providing a cutting-edge method of detecting the five common drugs in the field. The attendees will learn the principles, the sampling method, and the testing process of the IMS.

One of the challenging issues in the field-testing situations is to examine suspicious “White Powders” either in transparent containers of small white plastic bags or in opaque boxes of mail packages. Without a search warrant by a probable cause to open the suspicious container in the field, it is commonly recommended to send the targeting drug to the lab for further examination, thus delaying the investigation efforts.

The IMS was used to detect drugs in seven real field tests as a supplementary device by law enforcement agencies. The following results are reported: (1) The Testing Circumstances: The IBM was used to detect suspicions white powders in seven cases and the device provided accurate and reliable positive results that were confirmed by other testing methods in the field and later in the lab. (2) The Sensitivity: The sample quantity requires only a nanogram level of the drug. In other words, the sensitivity level of the IMS can be as low as 1ng, which is approximately one-thousandth of a needle tip in volume, much smaller than a naked human eye can see. (3) The Sampling Preparation: The sampling process requires only a simple swipe on the suspicious surface without opening the targeting container and without any sample preparation. (4) The Testing Time: The detection and analysis needs 5 to 8 seconds to produce a detection result. (5) The Readability: The 5-inch TFT color LCD touch screen displays direct indications in sound, light, and characters results while the silent alarm mode can be selected by the user. (6) The Mixture Situation: In one case, the IMS was able to detect from a suspect’s fingernail clips a mixture of methamphetamine, cocaine, and morphine. New substances can be added into the library if needed. (7) The Calibration: The device does not require any calibration during operation, which is a unique feature and allows the device to be ready all the time even when the usage environment parameters, such as temperature, pressure, and attitude, have changed. The device also has an automated cleaning mechanism. (8) The Wi-Fi Connection: The system’s log record and alarm history information can be connected with a command center if selected. (9) The Safety: This IMS system employs the use of VUV (vacuum ultra-violet) photons to ionize the sample and provides a personally and environmentally safe usage in operation, transportation, storage, and disposal. (10) The Future Applications: The authors conclude that the ion mobility spectrometer is able to provide a practical tool at the scene, in the lab, or even at the courtroom testimony. We contend that the study results add a new effective approach for law enforcement, medical examinations, postal inspections, and public health agencies in detecting illicit drugs in the field with rapid, real time, and *in situ* manners at a nanogram quantity level. In summary, the science of the ion mobility spectrometry does work in our cases.

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**Illicit Drugs; Nanogram Detection; Ion Mobility Spectrometer**

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**B155 A Quantitative Determination of Four Designer Benzodiazepines by High-Performance Thin-Layer Chromatography (HPTLC)**

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**Learning Objective:** After attending this presentation, attendees will gain a better understanding of the quantitative determination of four commonly occurring designer benzodiazepines using High-Performance Thin-Layer Chromatography (HPTLC) with an internal standard.

**Impact Statement:** This presentation will impact the forensic community by demonstrating that HPTLC is a viable instrument for quantitation of counterfeit benzodiazepines, both controlled and uncontrolled.

The goal of this project was to develop a high-performance thin-layer chromatography method that can quantify four common designer benzodiazepines. Designer benzodiazepines are a class of Novel Psychoactive Substances (NPS) that are analogs of existing benzodiazepines prepared to elude existing laws. This illegal market has been growing rapidly, resulting in an increase of designer benzodiazepines appearing in forensic laboratories. These drugs lack any form of regulation regarding known dosage, mixtures, or potencies and the similarity between the structures makes analysis in laboratories challenging. Thin-layer chromatography is a simple chromatographic technique that has been used for the analysis of drugs for many years. However, traditional thin-layer chromatography lacks the automation, resolution, and reproducibility needed for quantitative analysis.

In this study, high-performance thin-layer chromatography was used to separate and quantify four designer benzodiazepines: etizolam, clonazepam, flualprazolam, and bromazolam. The mobile phase system used was 17:2:1 ethyl acetate/methanol/concentrated ammonium hydroxide. An internal standard, thymidine, was used at a concentration of 375 µg/mL to quantify each of the four benzodiazepines. Calibration curves were made for each designer benzodiazepine with eight calibrators ranging in concentrations of 10–250 µg/mL. Linear regression of all four calibration plots produced coefficient of determinations > 0.99, indicating the data was best explained by the regression model. Residual plots of the data for each of the designer drugs displayed random residual plots confirming the linearity of the data and confirming regression model adequacy. Assessment of Limit Of Detection (LOD) produced LODs < 7 µg/mL and assessment of the Lower Limit Of Quantitation (LLOQ) produced LLOQs ≤ 20 µg/mL with good CV% (< 10%).

Results from this study indicate high-performance thin-layer chromatography is a suitable method to quantitatively determine designer benzodiazepines with good reproducibility, sensitivity, and resolution for the analysis of solid dosage drugs that are received in crime laboratories.

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**HPTLC; Benzodiazepines; Designer Drugs**

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**B156 The Separation of Lysergic Acid Diethylamide (LSD) and its Analogs by High-Performance Thin-Layer Chromatography (HPTLC)**

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**Learning Objective:** After attending this presentation, attendees will gain a greater awareness on the evaluation of eight Thin-Layer Chromatographic (TLC) mobile phase systems to separate Lysergic Acid Diethylamide (LSD) and nine LSD analogs using High-Performance Thin-Layer Chromatography (HPTLC).

**Impact Statement:** This presentation will impact the forensic community by demonstrating that HPTLC can be a useful and viable alternative tool in the analysis of lysergamides.

As New Psychoactive Substances (NPS) emerge and are submitted as seized drugs for analysis to crime laboratories, new methods and techniques are necessary to distinguish and identify these new compounds. The lysergamides are one class of NPS. Compounds in this class are derived from Lysergic Acid Diethylamide (LSD) by altering its structure to form new compounds. Due to the similar structures, these compounds can be difficult to distinguish from one another using current techniques. Although traditional Thin-Layer Chromatography (TLC) was one of the first techniques to separate LSD from related substances, TLC does not always provide the resolution or sensitivity necessary to distinguish these similar compounds from one another. High-performance thin-layer chromatography has the advantage of overcoming the poor resolution and having better sensitivity compared to traditional TLC.

In this study, HPTLC was used to separate LSD and nine LSD analogs. The analogs analyzed in this study included the commonly analyzed N-methyl-N-propyllysergamide (LAMP) and iso-LSD along with N-methyl-N-isopropyllysergamide (MiPLA), 1-valeroyl-d-lysergic acid diethylamide (1V-LSD), 1-propionyl-d-lysergic acid diethylamide (1P-LSD), N6-allyl-6-norlysergic acid diethylamide (AL-LAD), 1-cyclopropanoyl-d-lysergic acid diethylamide (1cP-LSD), 1-butanoyl-d-lysergic acid diethylamide (1B-LSD), and 1-acetyl-LSD (ALD-52). Eight different mobile phase systems were evaluated in this study to determine which produced optimal separation between all compounds. These systems were previously reported in the literature and included 2:1:1 n-butanol/glacial acetic acid/water, 4:1:1 n-butanol/glacial acetic acid/water, 1:1 acetone/chloroform, 2:1 acetone/chloroform, 8:2 acetone/chloroform, acetone, 9:1 acetone/ammonia saturated chloroform, and 9:1 chloroform/methanol. The mobile phases were evaluated based on the acceptance criteria of the number of pairs of compounds that could be resolved from each other ( $R_s > 1$ ) and reproducibility ( $CV < 5\%$ ). Each plate was visualized under 254nm and 366nm light and derivatized with the visualization reagent p-dimethylaminobenzaldehyde (p-DMAB) to make visualization of the compounds easier for the naked eye. Half of the compounds (LSD, LAMP, MiPLA, iso-LSD, and AL-LAD) would change color upon derivatization, while the other half (1B-LSD, 1V-LSD, 1P-LSD, and 1cP-LSD) remained unchanged. Based on the acceptance criteria, 80:20 acetone/chloroform (mobile phase 4) and acetone (mobile phase 5) produced the most successful results. These mobile phases provided good resolution between each of the compounds and provided good reproducibility. Mobile phase 5 (acetone) also provided the best separation between LSD and the more common analog LAMP. The use of either of these mobile phases along with using p-DMAB shows the possibility of distinguishing between LSD and 9 other lysergamides. These results demonstrate that HPTLC is a suitable method to separate lysergamides with good resolution and sensitivity.

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**HPTLC; LSD; Hallucinogens**

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**B157 The Conversion of Cannabidiol to Delta-9-Tetrahydrocannabinol (delta-9-THC) During Gas Chromatography/Mass Spectrometry (GC/MS) Analysis**

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**Learning Objective:** Attendees will be able to describe the extent to which Cannabidiol (CBD) converts to delta-9-THC during GC/MS analysis and the factors that contribute to the conversion. In addition, attendees will learn about the steps that can be taken to address the issue caused by conversion.

**Impact Statement:** This presentation will impact the forensic science community by describing how CBD present in high quantities in many strains of marijuana, hemp plant material, and derived products may be artifactually converted to delta-9-THC during analysis, leading to false positive delta-9-THC identifications, inaccurate quantitation of delta-9-THC, and incorrect designation of legal hemp-derived materials as marijuana or marijuana derived-products.

Though hemp and marijuana are the same plant, they are distinguished by delta-9-THC content. This distinction was defined in the 2018 Farm Bill. Plant material is considered marijuana if the delta-9-THC content is greater than 0.3%. This legal limit has also been applied to cannabinoid-containing products. The change in legislation required forensic testing laboratories to shift from qualitative identification of cannabinoids to quantitation of delta-9-THC.

The isomerization of CBD to delta-9-THC occurs via a ring closure. Isomerization has been performed using acid catalysts to convert CBD oils into products containing THC. It has also been suggested that conversion can occur due to heating. As such, analysis of samples containing large amounts of CBD pose a challenge because conversion may occur when the sample is introduced to the heated injection port of a GC/MS. The number of samples submitted to laboratories that contain high concentrations of CBD has increased dramatically as the popularity of CBD products increased. To determine the impact that the artifactual formation of THC would have on a decision-point analysis, conversion was monitored under various conditions. The effects of CBD concentration, injection port temperature, injection port maintenance, and derivatization were investigated.

CBD was analyzed at injection port temperatures ranging from 150°C to 300°C. As injection port temperature increased, the amount of delta-9-THC measured in the CBD samples increased. The impact of injection port maintenance on conversion was evaluated and demonstrated that dirtier liners led to greater CBD to THC conversion. We also evaluated whether derivatization of CBD could prevent ring closure and reduce or eliminate the formation of THC. CBD samples prepared at a concentration equivalent to 100% CBD were derivatized and analyzed. The delta-9-THC response observed was analytically insignificant.

Various techniques were employed to safe-guard the results from the decision-point analysis. Because experiments showed increased CBD concentrations led to more conversion, a threshold for CBD, over which delta-9-THC results would not be reported was instituted and a reporting limit under which the identification of delta-9-THC would not be reported was adopted. Complex sample matrices can introduce non-volatile components to the liner during a run. To ensure that results were not adversely affected by previous samples, CBD controls were used to monitor conversion within runs. Ultimately, the decision-point method was redeveloped to include derivatization as this was determined to be the most effective way to mitigate the risk of conversion.

Analyzing cannabinoid samples by GC/MS is challenging. The conversion of CBD to delta-9-THC can cause inaccurate designations of hemp/hemp-derived samples as marijuana. Laboratories performing these analyses must be aware of the factors that lead to conversion and characterize the artifactual formation of delta-9-THC during validation. Countermeasures should be adopted to prevent false positive results.

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**Cannabinoids; Conversion; GC/MS**

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**B158 The Utility of Flip-Flop Chromatography Employing Silica Hydride Stationary Phases With Diode Array Ultraviolet and Single Quadrupole Mass Spectrometric Detection for the Analysis of Seized Drugs**

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**Learning Objective:** By attending this presentation, attendees will be introduced to flip-flop chromatography as a method for analyzing seized drugs. The audience will gain insight into how this methodology is advantageous over traditional dual column analysis. Further there will be an understanding of how the proposed methodology is complementary to Gas Chromatography/Mass Spectrometry (GC/MS).

**Impact Statement:** This presentation will impact the forensic community by showing the utility of flip-flop as a technique for analyzing seized drugs. Flip flop chromatography can be considered an improvement to classical LC separation methods as it utilizes a singular column and combination of mobile phases for two separate chromatographic modes.

Some common methods used for the analysis of seized drugs are Thin Layer Chromatography (TLC), Gas Chromatography (GC), and Liquid Chromatography (LC). For LC, typically High-Performance Liquid Chromatography (HPLC) and Ultra-High Performance Liquid Chromatography (UHPLC) have been used in both Reversed Phase (RPC) and Hydrophilic Interaction Chromatography (HILIC) modes. Although GC/MS is considered the gold standard of analysis for seized drugs, it still has significant limitations. Compounds can co-elute, and MS lacks specificity for diastereomers and certain positional isomers, with certain classes of seized drugs also lacking molecular ions in the mass spectra. Liquid chromatography with Photo Diode Array Ultraviolet (PDA UV) and single quadrupole MS detection is a useful alternative for screening purposes or complementary technique for analyte identification. UV spectra can not only distinguish between drug classes but also between analytes within a class, including certain positional isomers. Mass spectrometric detection further provides molecular ion information.

For greater specificity of analyte retention time identification, different classical silica columns operating either in the reverse phase or complementary hydrophilic interaction chromatographic modes can be utilized. Alternatively, a Silica Hydride (SiH) stationary phase using same solvent reservoirs for both RPC and the complementary Aqueous Normal Phase (ANP) chromatography can be utilized. The use of this alternative approach allows for a reduction in analysis time as both separations are on the same column. Additionally, using SiH stationary phases with ANP chromatography compared to traditional HILIC yields faster equilibration times. A mixture of 14 drug standards representing different drug classes were analyzed using UHPLC/PDA UV/single quadrupole MS with SiH columns (C18, UDC Cholesterol, and Diamond Hydride). The stationary phases were run under both RP and ANP conditions, then compared to classical RPC and HILIC on a C18 and NP Silica column, respectively. The effect of column temperature and flow rate on the chromatographic separations was investigated. In contrast to HILIC, it is possible to achieve retention of all classes of compounds investigated using a silica hydride stationary phase in ANP mode.

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**Silica Hydride Stationary Phases; Seized Drugs; Bimodal Separations**



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**B159 The Evaluation of Fentanyl and Fentanyl-Related Compounds on United States Paper Currency**

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**Learning Objective:** After attending this presentation, attendees will better understand the extent of fentanyl contamination on paper currency and its relevance to the forensic sciences.

**Impact Statement:** This presentation will impact the forensic science community by expanding knowledge of drug-contaminated currency and how analyzing this sample type could prove beneficial to research and testing laboratories.

Previous research has evaluated the extent to which cocaine and other drugs have been detected on paper currency in the United States. The literature was in agreement that the majority of money analyzed was contaminated with cocaine. This study set out to replicate that research with respect to fentanyl to see if a similar phenomenon was true today due to the increase in fentanyl use. Fentanyl is a potent synthetic opioid that has been linked to many overdose deaths and whose prevalence has been steadily increasing in forensic casework for the past decade. Previous studies have looked for drugs other than cocaine on paper money, but not fentanyl.

A quantitative Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC/QqQ/MS) method was developed and validated with the ability to detect six analytes: fentanyl, 4-ANPP and benzylfentanyl (two precursors), acetylfentanyl (an unintentional byproduct), and cocaine and methamphetamine. Authentic samples were tested using the validated method and consisted of 188 one-dollar bills collected from 13 cities across the United States (Northeast, South, Midwest, and West). Sample preparation included soaking the bills in methanol followed by performing a single-step liquid-liquid extraction. Optimal chromatography was achieved using a C18 analytical column with 5mM pH 3 ammonium formate in water and 0.1% formic acid in acetonitrile. Gradient elution was employed with a constant flow rate of 0.40mL/min. The total assay run time was five minutes. The quantitative working range for this method was 1ng/mL to 100ng/mL (correlating to 0.1µg to 1.0µg per bill), with the lowest calibrator serving as the limit of detection and quantitation for validation purposes. No matrix effects or interferences were observed during validation. Fentanyl was detected on a majority (63%) of the dollar bill samples, with 61% having at least 0.1µg of fentanyl and 4% having greater than 1.0µg of fentanyl. Unsurprisingly, cocaine and methamphetamine were detected in all samples, typically in amounts greater than the working range of this method and therefore also in amounts greater than those of fentanyl. The remaining three analytes were detected only a handful of times and in trace amounts, exclusively in the presence of fentanyl. As expected, areas of the country with a higher incidence of fentanyl according to the literature yielded a higher frequency of positivity and higher concentrations on contaminated bills.

The information obtained from this study is beneficial to labs and researchers who are interested in novel ways to track fentanyl use in different regions, as they do not have to rely solely on data from overdose deaths or seized drug samples, both of which could suffer from extensive processing and testing backlogs. This study could be repeated a number of years later to assess to what extent the paper currency in these locations becomes more contaminated with fentanyl over time. Additionally, it offers yet another method that can accurately detect and quantitate compounds commonly encountered in forensic casework even if the matrix is something more unconventional than blood or urine. It should be noted that the authors do not believe the amounts of fentanyl detected on these dollar bills poses any health risks to the general population.

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**Fentanyl; Currency; Drug Trends**

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**B160 The Chemical Analysis of the Surface Building Collapse Samples: Updating Decontamination Protocols for Search and Rescue Canines**

*Michelle Karpinsky, BS\*, Florida International University, Miami, FL; Lauryn DeGreeff, PhD, Florida International University, Miami, FL; Erin Perry, MS, PhD, Southern Illinois University, Carbondale, IL*

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**Learning Objective:** Attendees will learn about the different contaminants found at building collapse site as well as the specific organic contaminants found at the Surfside building collapse. Attendees will also learn about the analyses performed on the samples.

**Impact Statement:** This presentation will impact the forensic science community by providing a better understanding of the contaminants present at building collapses as well as providing evidence of why the decontamination protocols for search and rescue canines needs to be updated.

On June 24, 2021, Champlain Towers South, a 12-story apartment/condo building, in Surfside, FL, partially collapsed, resulting in many injured residents and a total of 98 deaths. Only three survived the initial collapse and three dozen escaped the portion of the building that remained standing. Fires were reported under the rubble and continued throughout the night. Multiple jurisdictions surrounding Surfside responded with firefighting, search and rescue, and debris removal operations. Along with firefighters, canine units were deployed to the site to assist with search and rescue operations. During the search and rescue, canine teams were exposed to a wide range of chemical, biological, and/or radiological hazards that could negatively impact health. These hazards include aerosolized or dust particles from crush concrete, released household chemical, biological material, and so on. The canines underwent decontamination to ensure no harmful residues from the rubble were left on the canines. However, without a full understanding of the possible contaminants present at a building collapse, current decontamination protocols may be lacking. To gain a better understanding of the potential harmful exposures to canines, samples of different media, including water, gauze, and dog toy samples, were collected from the building site and sent for analysis. This research focused on the organic portion of the chemical analysis.

Chemicals of concern that can emit from the rubble of a compromised building are benzene, toluene, ethyl benzene, and xylenes (BTEX), as well as Polyaromatic Hydrocarbons (PAHs) and Polychlorinated Biphenyls (PCBs) as well as toxic metals, such as lead and antimony. An analysis of samples obtained within the search environment was completed with the use of Headspace/ and Direct Immersion/Solid Phase Microextraction (HS/SPME and DI/SPME) and gas chromatography mass spectroscopy. Headspace analysis was also completed using the AROMA-VOC (Entanglement) for low levels of BTEX. Standing liquid samples were collected from puddles throughout the search area. Gauze samples were collected after the decontamination of one of the canine teams being observed for this study. Two dog toys were also collected to be tested, as these toys had been in direct contact with the scene and the canines. Preliminary data using the AROMA-VOC for the analysis of the water samples indicated increased levels of toluene and xylene in some of the samples compared to rainwater collected around the Miami area. It is anticipated similar increased values for PAHs and PCBs from the SPME data when rainwater sample analysis is complete. These increases may indicate the need for revision of decontamination protocols for canines.

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**Surfside Building Collapse; Organic Contaminants; Canine Decontamination Protocols**

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**B161 The Development of a Safe Canine Training Aid Mimic for Fentanyl**

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**Learning Objective:** After attending this presentation, attendees will understand how a safe canine training aid was developed and verified.

**Impact Statement:** This presentation will impact the forensic scientific community by describing a safe, reliable, and effective method for narcotic detection canines to be trained to detect fentanyl.

Fentanyl has a potency 100 times greater than that of morphine and, as a result, a very small amount can cause an overdose. According to the Center for Disease Control, since 2013 fentanyl or fentanyl analog overdoses have increased from under 5 to 20 deaths per 100,000, making it the leading cause of drug overdoses in the United States. For this reason, it is imperative that law enforcement can safely detect fentanyl in field and laboratory environments. One of the most effective ways of detecting narcotics in the field is canine olfactory detection. However, currently, there are no scientifically supported, safe, and reliable ways to train canines to detect fentanyl. After attending this presentation, attendees will understand how a canine training aid was developed and verified. This presentation will impact the forensic scientific community by describing a safe, reliable, and effective method for narcotic detection canines to be trained to detect fentanyl.

The functions of detection canines vary from human search and rescue to drug and explosive detection. Canines have been widely used at crime scenes to locate illegal drugs or contraband quickly and effectively. Before a canine can be deployed, they first need to be properly trained on the scent profiles of the target substances their handler wishes them to detect. This can be done in two different ways: training the canine on the actual controlled substance or training the canine on a mimic that contains only the odor or active odorants of the controlled substance. Mimics may be created by placing a sorbent material in the vapor phase of the actual material, allowing the sorbent to collect the full headspace profile of the target material, or by spiking compounds known to be associated with the target material onto a substrate. This type of mimic is often referred to as a pseudo scent.

Studies have shown that the headspace of fentanyl contains benzaldehyde, aniline, styrene, N-phenethyl-4-piperidone, heptane, benzyl alcohol, benzeneacetylaldehyde, N-phenylpropanamide (NPPA) and 1-phenethyl-4-propinoloxypiperidine. NPPA was the best candidate for the active odorant since it's a product of degradation and the most abundant compound in the headspace. Training aid pseudo scent mimics were prepared using a mixture of headspace components with a substrate and placed into a Controlled Odor Mimic Permeation System, to control the rate of dissipation. To verify the active odorant, canine field trials were performed. The canines used included Group 1—canines with previous fentanyl training, Group 2—pet dogs without previous fentanyl training, but with previous detection training, and Group 3—operational dogs without previous fentanyl training. Since Group 1 was previously trained to detect fentanyl, they were tested on their ability to detect mimics, prepared as vapor soaks or pseudo scents, as well as positive/negative controls. Groups 2 and 3 did not have fentanyl training so a cross-over study was performed, where half of the canines were trained using a fentanyl headspace soak, and the remaining canines were trained using the pseudo scent. After six weeks of training, canines in Groups 2 and 3 were then tested on their ability to detect the fentanyl vapor soak, the pseudo, and positive/negative controls.

The results from the canine tests discussed in this presentation show that most of the canines correctly detected the substance to which they were trained. The canines were also able to alert to one specific odor mimic, the vapor soak or the pseudo scent creating the framework for the development of a safe and accurate way to train canines to detect fentanyl. This will allow law enforcement to locate and seize more fentanyl, which will have a positive impact on fentanyl-related overdoses.

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**Fentanyl; Canine; Mimic**

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**B162 A Two-Step Method for the Identification and Highly Specific Analysis of Organic Gunshot Residue Using Raman and Laser-Induced Breakdown Spectroscopies**

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**Learning Objective:** This presentation aims to elucidate the potential for a novel two-step method using Raman spectroscopy for the identification and Laser-Induced Breakdown Spectroscopy (LIBS) for highly specific analysis of individual Organic Gunshot Residue (OGSR) particles. This presentation will provide audience members with background information regarding the overall relevance of gunshot residue as a form of trace evidence and will further elaborate the importance of OGSR in particular. Through attending this presentation, audience members will gain a better understanding regarding the importance of GSR evidence. The novel two-fold method developed for the detection and analysis of OGSR particles shall be explained in detail and future work regarding this proof-of-concept study will be discussed.

**Impact Statement:** This research demonstrates the potential for the detection and highly specific analysis of OGSR via Raman spectroscopy and LIBS.

In this study, OGSR was generated from three cartridges sharing the same manufacturer, caliber, firing conditions, and discharging firearm. These three cartridges shared many similarities overall, and only differed minimally based on the bullet type. GSR samples were generated by firing using a Glock® Model 26 semi-automatic 9mm firearm, which was discharged by our collaborators from the New York State Police Forensic Investigation Center from approximately 0.3m away into a 9x9-inch cloth substrate. Raman spectroscopy was first performed to identify and confirm the identity of the OGSR particles. Then, LIBS was used to probe individual OGSR particles. A Support Vector Machine-Discriminant Analysis (SVM-DA) statistical model with five latent variables was created based on LIBS data to differentiate between OGSR generated from three different bullet types. LIBS lines for barium, lead, copper calcium, iron, sodium, potassium titanium, silicon, strontium, and the CN molecular band were notably identified as statistically significant lines. Overall, the model allowed for excellent differentiation between the three OGSR groups.

This study demonstrates that the LIBS analysis of OGSR has the potential to point to a specific ammunition type as a source of discharge and is not limited to broad identification of manufacturers. The two-step method presented has the potential to allow forensic examiners to use Raman spectroscopy to identify OGSR particles, then utilize LIBS for their highly specific analysis. Future work on exploring this methodology is needed and is intended to be performed with an expanded spectral dataset and a greater variety of ammunition samples including different manufacturers and calibers.

*This project was supported by Award No. 15PNIJ-21-GG-04153-RESS (I.K.L.) and Award No. 2019-R2-CX-0035 (S.R.K.) awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the Department of Justice.*

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**Gunshot Residue; LIBS; Raman Spectroscopy**

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**B163 Sourcing Glass Traces on Bullets as Additional Evidence for Shooting Reconstruction**

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**Learning Objective:** After attending this presentation, attendees will understand the advantages and challenges of analyzing glass traces on bullets shot through car glass.

**Impact Statement:** This presentation will impact the forensic community by adding an analytical strategy that could support or exclude scenarios proposed during shooting reconstructions.

The reconstruction of the trajectory of a bullet is important for forensic investigations of cases involving shootings, particularly when considering the transfer of material from intermediate targets.<sup>1,2</sup> 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 recovered glass fragments from a bullet can be associated with potential sources or exclude other sources, testing the proposed trajectory of the bullet.

The aim of this study was to provide independent evidence for shooting reconstructions through comparative analysis of glass evidence using the elemental composition and optical properties of the glass. Three different techniques were used: Scanning Electron Microscopy Coupled with an Energy Dispersive X-Ray Spectroscopy (SEM-EDS) for the major and some minor elements, and the Refractive Index measurement (RI) with a phase contrast microscope for the oil immersion method. These two techniques are readily available at many forensic laboratories. The third technique was Inductively Coupled Plasma/Mass Spectrometry (ICP/MS) for determining elemental composition of major, minor, and trace concentrations. The set of samples consisted of simulated casework samples from car glass (float soda-lime) prepared by shooting through different glass panes at typical angles found in vehicles. The bullets were recovered from a loose Kevlar® receiver box. Standard reference materials including NIST SRM 1830, NIST SRM 1831, and NIST SRM 612 were analyzed in parallel with samples. The known glass intermediate targets included windshield internal layers, windshield external layers, and tempered glass from side windows. These proposed source glasses were then compared to glass fragments recovered from bullets used in the shooting experiments. The sample size and complex matrix excluded the potential use of the gold standard for float glass Laser Ablation ICP/MS. The recovery of the glass fragments from the bullet for solution ICP/MS analysis required development and validation of a method to remove the contamination from the bullet lead. The advantages and disadvantages of the chemical separation of the lead will be discussed. All the elemental profiles and RIs were compared using criteria including the ones in ASTM standard methods E2330 and E1967-19. In our limited universe situation, where the number of sources were few, known, and distinguishable by the methods mentioned above, we were able to accurately associate the recovered traces to the corresponding source.

**References:**

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**Glass; Shooting Reconstruction; Trace Evidence**

## B164 The Critical Evaluation of Various Classification Systems of Lip Prints

Mukesh Thakar, PhD\*, Punjabi University, Patiala, Patiala, Punjab, INDIA; Jagmeet Kaur, MSc, Punjabi University Patiala, Patiala, Punjab, INDIA

**Learning Objective:** After attending this presentation, the attendees will learn the current challenges related to classification/analysis in lip evidence and the various existing classification systems of lip prints. Additionally, attendees will gain a better appreciation for the need for a thoroughly revised method for classifying lip prints. Finally, attendees will understand the evidentiary value of lip prints as a crucial piece of corroboratory evidence in personal identification, by adding a few more new individual features to the existing Individual features.

**Impact Statement:** The forensic science community would realize the importance of an evolved method of lip print classification with an enhanced potential of classifying partial lip prints through various class and individual characteristics.

The wrinkles and grooves present on the sulci labiorum of human lips form a characteristic pattern called lip prints and can be used for personal identification. Decades earlier (1969–2000), various scientists working with lip evidence analysis (Suzuki and Tsuchihashi, Martin Santos, Renaud, Afchar-Bayar and Jose Maria Dominguez) had classified lip prints based on the pattern of wrinkles and forms of furrows present on the Klein's zone of lips.<sup>1,2,4-6</sup> Later, Kasprzak classified lip prints based on certain specific individual characteristics (23 in number).<sup>3</sup> Recently, Kaur and Thakar devised an alternate system to classify lip print patterns.<sup>7,8</sup> In the present study, all the classification systems mentioned above have been evaluated critically to determine the strengths and limitations of these systems to ascertain their role in personal identification based on lip evidence analysis. Furthermore, an attempt has been made to widen the scope of the Individual classification system in lip prints by adding 25 newly found individual characteristics besides the existing 23.

Lip print samples were collected from 200 healthy individuals (non-smoker/non-tobacco chewer, without any oral disease) using the standard tape lifting method. The samples were photographed, and the digital images were enhanced using Picasa software and MS® Office Paint. Every digital print was divided into ten equal-sized sections (five each on the upper and lower lip) so that every corner/fragment of the lip may become part of the analysis. A total of 2,000 sections were assessed separately according to various classification systems to analyze the inclination, orientation, numerical superiority, and co-existence of diverse lip print types along with determining the presence of Individual characteristics (Kasprzak's and new features).<sup>3</sup>

Detailed examination of the partial/fragmented lip print samples was conducted. It is evident from the results that some samples (fragmented lip prints) have only a single pattern while others have more than one pattern (classified as mixed/combination). Another important observation was related to simple linear patterns, which were classifiable with all the older and current classification systems. However, the curved/angular patterns could not be classified using the most commonly and frequently used Suzuki and Tsuchihashi's and Renaud's classification systems of lip classification.<sup>4,6</sup> The mixed/combination patterns could only be identified with the Kaur and Thakar classification system.<sup>7,8</sup> Moreover, this system was found to exclusively classify the composite curved/angular patterns (curved branches, curved intersected branches) as the system is entirely based on the structural and spatial arrangement of lip wrinkles, unlike the older systems, which was solely based on the general shapes of the patterns. It was observed that every successive classification system is a better version of its predecessor in classifying the type of patterns. The individual characteristics defined in the Kasprzak and the Kaur and Thakar systems were identified in the partial/fragmented lip prints and successfully analyzed.<sup>3,7,8</sup>

The findings concluded that the recently formulated classification system based on Individual features (like the second-level details of fingerprints) classification system presents a better way to evaluate and classify even the fragment of lip prints. Additionally, the newly implored unique features can be added to the existing Kasprzak's Individual features for successful evaluation of lip print evidence in the lab and goes a long way to help in the scientific investigation of crime.<sup>3</sup>

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### Lip Prints; Classification Systems of Lip Prints; Individual Features of Lip Prints

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## B165 The Development of Latent Fingerprints on a Cigarette Using Ruthenium Tetroxide

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**Learning Objective:** After attending this presentation, attendees will be aware of a new development for retrieving latent fingerprints on cigarettes.

**Impact Statement:** This is a new technique of the development of latent fingerprints. The technology presented in this paper fosters the fingerprint analysis ability of forensic science.

Cigarette-related garbage can be easily found anywhere, even at the crime scene. Cigarette evidence can provide DNA evidence and has been used in investigation for a long time. Although DNA analysis can be done from very small amounts of material or from a mixture with contaminants, even if DNA profiles are obtained, a DNA database is not built like a fingerprint database (especially in South Korea). In such cases, DNA evidence becomes less useful without a matching suspect in the database. There are some cases that turned into cold cases, even though DNA samples were collected from cigarette evidence. This occurred mainly because suspects refused to allow their DNA to be collected, scientists were unable to obtain control DNA profiles from suspects, or because there were no matching suspects in the database. In these cases, fingerprints can be conclusive evidence to identify suspects. However, when 1,2-Indanedione (1,2-IND) was applied to cigarettes, the background was stained, making it difficult to identify fingerprint ridges. Therefore, latent fingerprint development research is needed in such cases.

In this study, 1,2-IND and Ruthenium Tetroxide (RTX) were used to compare the development of latent fingerprints on cigarettes, which are porous surfaces. On the filter part of cigarettes, latent fingerprints were clearly developed on both 1,2-IND and RTX. On the body part of cigarettes, 1,2-IND reacted with tobacco leaves and showed a fluorescence reaction on the background, making it difficult to identify the ridges of the fingerprints. On the other hand, when RTX was applied, clear ridges appeared on the body part of cigarettes. In addition, the RTX did not leave many stains on the background. The 1,2-IND is currently the most effective latent fingerprint development technique on porous surfaces and is commonly used, but it is estimated to react with amino acid components contained in a cigarette. Therefore, when there is a need to develop latent fingerprints on cigarettes, it is confirmed that applying RTX reagent is much more effective than applying 1,2-IND reagent.<sup>1-21</sup>

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### **Latent Fingerprints; Cigarette; Ruthenium Tetroxide**

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**B166 Quantitative Measurements for Fingerprint Quality Determination**

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**Learning Objective:** After attending this presentation, the attendees will understand an alternative process to utilize the information gathered from a fingerprint image, using quantitative measurements and a scoring system.

**Impact Statement:** We believe that this presentation will help advance the efforts for creating a clearer and more objective process for fingerprint collection and evaluation.

Finding methods to improve the objectivity and reliability of the analysis of forensic evidence has been an ongoing task. Fingerprint examination is a major part of this effort due to their abundance and significance at solving a crime scene. But even though there are advances in image collection and processing, fingerprint examination is still a complex task, which can be influenced by multiple factors. Among those factors, there are some that can influence and limit the overall objectivity and reliability of the process. The process mostly depends on the analysis and judgement of trained human examiners. Furthermore, the fingerprints collected from crime scenes usually contain defects, which can limit their ability to provide clear and accurate data. Previously, there have been multiple studies on identifying the factors that influence the quality of a fingerprint image to verify the validity of a collected fingerprint.

In this study, we intend to propose a basic system to quantitatively measure the overall quality of a given fingerprint image, providing examiners with a quick and easy method to help them obtain additional information from the fingerprint. We have chosen a number of quantitative measurements that influence image quality, such as intensity, contrast, standard deviation, and signal to noise ratio, and some that affect the outcome of an examination, such as the existence of the core and delta region. To set a reference, we proposed an ideal image as a binary fingerprint image with clear differentiation between ridges, and where the ridges and background exist at a 1 to 1 ratio. We converted the measurements of these factors influencing image quality to be relative to our purposed ideal case and added a multiple for each existing core and delta region. By collecting and combining these factors, we have created a score system which rates the overall quality of a fingerprint image.

Afterward, we performed an experiment to test the validity of our system, which compared the performance of our system to a paper in which fingerprint examination experts scored latent fingerprint development methods. Our system showed similar results to the other methods, differing in the two cases with the lowest score out of six and showing the possibility of using our system for quantitatively measuring the quality of fingerprint images.

In the future, we intend to improve the reliability of our system by creating a larger database, adding additional factors for quality measurements, and implementing machine learning in the process.

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**Fingerprint; Latent Fingerprint; Image Quality**

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**B167 The Standardization Process of the Fingerprint Profession: The European Approach**

*Aldo Mattei, PhD, MSc\*, RIS Carabinieri, Messina, Sicilia, ITALY*

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**Learning Objective:** After attending this presentation, attendees will have a thorough overview of the standardization process in the fingerprint profession in Europe.

**Impact Statement:** This presentation will impact the forensic science community when forensic professional organizations are tasked to actively contribute to the transnational standardization process.

In 2004, the Fingerprint Working Group of the European Network of Forensic Science Institutes (ENFSI) started the process of developing a Best Practice Manual. The completion of the document and its review from the EFPWG members was a cumbersome process, which was incredibly boosted by a funding plan made available through a specific funding plan of the European Commission (EC) within the 2012 Annual Work Programme (AWP) for the Prevention of and Fight against Crime as part of the General Programme “Security and Safeguarding Liberties” (ISEC Programme). The so-called ENFSI Monopoly Program 2012 “Towards European Forensic Standardization through Best Practice Manuals” (TEFSBPM), established, among others, the Project no. B6 in the publication of the Best Practice Manual for Fingerprint Examination in 2015. An outline of the document published will be presented during this presentation. The approach of this document was the first attempt to establish a common ground through 71 laboratories of 38 nations of the Council of Europe.

Following this great achievement, within the Monopoly Program 2016 “Steps Towards a European Forensic Science Area (STEFA),” aimed to promote the European Union Vision for European Forensic Science 2020 with the creation of a European Forensic Science Area, the Project no. G10 consisted of the realization of the Best Practice Manual for Fingerprint Visualization at the scene of crime. This document aimed to cover the detection of fingermarks at the scene of crime, while the BPM for Fingerprint Examination covers the laboratory activities. A brief outline of this manual will be presented as well.

Finally, considering the huge standardization effort made in recent years in the fingerprint world, mainly thanks to the efforts of the United States government through NIST, with the establishment of the OSAC subcommittees and lately by the AAFS with the creation of a dedicated Standard Developing Organization (SDO), the American Standards Board (ASB), there is a consistent on-going process aimed to standardize all the aspects of the fingerprint profession. Additionally, the United Kingdom government, through the Forensic Science Regulator producing other relevant documents, aimed at normalizing the fingerprint profession in the United Kingdom. All these valuable publications need to be considered and carefully evaluated by the European countries. However, given the multitude of different nations and different legislations, it is not possible to enforce the application of rigid standards in the European context. Relevant differences exist in the various nations in the admissibility of evidence in court, as well as there are different thresholds for sufficiency for comparison decisions. All the above impose a necessary compromise. As a result, the ENFSI EFPWG, through the funding made available with the on-going CERTAIN-FORS Monopoly Project 2020, Work Package 10, considered all the recent publications in the fingerprint field, produced by ASB, OSAC, and the Forensic Regulator, proposed a reference document, the 2nd edition of the Best Practice Manual for Fingerprint Examination, which is aimed at suggesting the recommended approach to all the various aspects of the fingerprint profession. An outline of the draft manual will be presented, thus demonstrating how crucial the international cooperation for the advances of forensic sciences is and how instrumental the European Commission financial support toward the harmonization of forensic processes and procedure in the fingerprint domain across Europe also is.

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**Fingerprint; Standardization; Best Practices**

### **B168 The Effect of Smoking, Tobacco Chewing, and Various Pathological Conditions on Lip Prints**

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**WITHDRAWN**

**B169 The Discrimination of Smokers and Non-Smokers Based on the Analysis of Fingermarks Using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) Spectroscopy and Chemometrics**

*Mohamed Amin, MSc\*, Kuwait University, Khaldyia, Al Asimah, KUWAIT; Entesar Al-Hetlani, PhD, Kuwait University, Khaldyia, Al Asimah, KUWAIT; Igor Lednev, PhD, Department of Chemistry, University at Albany, SUNY, Albany, NY*

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**WITHDRAWN**



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**B170 The Effect of Elevated Temperatures on the Microscopical Identification of Vegetable Fibers**

*Walter Rowe, PhD\*, The George Washington University, Woodbridge, VA*

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**Learning Objective:** After attending this presentation, attendees will understand the effect of heating on the microscopical identification of vegetable fibers.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the effect of elevated temperatures on the microscopical features of vegetable fibers.

Vegetable fibers have been used since antiquity for textiles and cordage. These fibers derive from three sources: there are seed fibers (e.g., cotton or kapok), bast fibers from the stems of plants (e.g., flax, hemp, ramie, and jute) and leaf fibers (sisal and abaca). The vegetable fibers may occur as trace evidence in criminal cases. In the forensic science laboratory, these fibers are commonly identified microscopically using a combination of brightfield and polarized light examinations. Petraco and Kubik present a comprehensive analytical procedure for the identification of vegetable fibers.<sup>1</sup> The fibers are viewed with transmitted light (brightfield illumination), between crossed polars with the fibers oriented for maximum birefringence, between crossed polars with the fibers oriented at extinction, and, finally, between crossed polars at extinction with a first order red accessory plate inserted. The last observation is the Herzog or red plate test used to distinguish bast fibers based on the S-twist or Z-twist orientation of the cellulose molecules in the fibers. These observations permit the microscopical identification of the most common vegetable fibers. Fibers transferred during the commission of crimes and their sources (cordage and fabrics) may be damaged in a variety of ways. Fibers may be exposed to elevated temperatures in structure and vehicle fires. Criminals may attempt to destroy both trace evidence and their sources by burning. There has been virtually no research published on the effect of elevated temperatures on vegetable fibers and on the microscopical features used in their identification.

Examples of each type of the three types of vegetable fiber were selected for exposure to elevated temperatures: cotton and coir as representative of seed fibers; flax, ramie, and jute as representative of bast fibers; and sisal as representative of leaf fibers. Fiber samples were heated at temperatures ranging from 200°C to 250°C for one hour in a domestic oven. Oven temperatures were verified with a NIST-certified Digi-Sense Traceable Digital Thermometer with a type K thermocouple (Cole-Parmer, Vernon Hills, IL). The fibers were then mounted on microscope slides with Permount™ mounting medium. Two microscopes were used to examine the mounted fiber samples: a stereomicroscope capable of both reflected light and darkfield observations (AmScope™) and a transmitted light polarizing microscope (AmScope™). Photomicrographs were captured for all observations with an OMAC 18-megapixel digital eyepiece camera (AmScope™). The following observations were made for each fiber sample:

- Reflected light
- Darkfield
- Brightfield (no polarizing filters)
- Crossed polarizing filters (fibers oriented for maximum birefringence)
- Crossed polarizing filters (fibers oriented at extinction)
- Crossed polarizing filters (fibers oriented at extinction with first order red plate inserted)

The following results were observed for all the fiber samples: an increase in temperature resulted in a darkening of the fibers; and at the highest temperature, the fibers were found to be extremely friable. Fibers exposed to temperatures above 250°C are expected to be difficult to identify by light microscopy. Other identification techniques such as scanning electron microscopy would have to be employed. The fragility of vegetable fibers heated to 250°C and above will require specialized sample collection and handling techniques, both for evidence collection at crime scenes and for evidence examination in forensic laboratories. The fiber samples heated at 250°C retained significant diagnostic morphological features (e.g., nodes or dislocations in the case of bast fibers). However, the discoloration of some of the fibers altered the interference colors observed with crossed polarizing filters. The discoloration also interfered with the application of the Herzog test to the bast fibers.

**Reference:**

- <sup>1</sup>. Petraco, N., and Kubik, T. *Color Atlas and Manual of Microscopy for Criminalists, Chemists and Conservators*. CRC Press, Boca Raton, FL, 2003.

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**Vegetable Fibers; Polarized Light Microscopy; Herzog Test**

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**B171 Optimal Machine Learning Model Selection on Tire Classification**

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**Learning Objective:** After attending this presentation, attendees will see how well the popular machine learning algorithms classify the different types of tire samples

**Impact Statement:** This presentation will impact the forensic science community by introducing a high-performance combination of high-dimension reduction methods PCA or PLS-DA and machine learning models for tire classification

In forensic investigations, in particular for violent crimes or accident scenes, tire trace is considered as a crucial evidence. In this regard, the chemical profile is used to link a car and a tire based on its trace materials found in the accident scene.<sup>1</sup> In this study, we explore the optimal machine learning techniques for the classification of 34 different FDLE codes for tire rubber from Rosedent and Non-Rosent datasets in which the predictors are elemental signatures derived by Laser-Induced Breakdown Spectroscopy (LIBS). Both Resonant and Non-Resonant datasets contain 340 observations of tire samples but with 15 and 24 elemental signatures, respectively. Each class of tires contains 10 observations. Both Principal Components (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) as popular unsupervised and supervised of high-dimension reduction techniques were conducted.<sup>2,3</sup> Through some visualization techniques including loading and score plots, we could also explore the effective role of each elemental signature in the components as well as how similar the elements are one another for each type of tire regarding first two components accounting for the largest portion of variation in both datasets. The new low-dimension datasets are built by selecting some first components derived by PCA and PLS-DA that are explaining approximately 95% of variations in Resonant and Non-Resonant Datasets. These new datasets were then fed into the candidate machine learning algorithms such as Linear Discriminant Analysis (LDA), Random Forest (RF), Naïve Bayes (NB), Neural Network (NNET), K-Nearest Neighbors (KNN), and Super Vector Machine with Linear and Radial kernels.

To validate the outcomes, 90% of data for each classifier were trained by 10-folds cross-validation. Random Forest, Naïve Bayes, and Super vector with Linear kernel have higher accuracy in the classification for training dataset created by the components are from PCA and PLS-DA. Also, it was observed that the performance of these classifiers for PCA data is higher than that of PLS-DA. Random Forest was selected as the optimal model among the candidates. This is because it outperformed the Naïve Bayes and Linear Super Vector Machine for the test data sets of Non-Resonant and Resonant.

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- <sup>1.</sup> John Lucchi, Dan Gluck, Sidney Rials, Larry Tang, and Matthieu Baudalet, Tire Classification by Elemental Signatures Using Laser-Induced Breakdown Spectroscopy, *Applied Spectroscopy*. 2021, Vol 75(6), 747-752.
- <sup>2.</sup> Lukas Gal, Michal Oravec, Pavol Gemeiner, Michal Ceppan, Principal component analysis for the forensic discrimination of black inkjet inks based on the VIS-NIR fibre optics reflection spectra, *Forensic Science International*. 2015.

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**Tires; Elemental Analysis; Machine Learning**

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**B172 Wrestling With the Evaluation of Fiber Evidence: Transfer and Persistence Modeled Through Jiu-Jitsu Activities**

*Victoria Lau, MSc\*, University of Technology Sydney, Ultimo, NSW, AUSTRALIA; Claude Roux, PhD, University of Technology Sydney (UTS), Ultimo, NSW, AUSTRALIA; Xanthe Spindler, PhD, University of Technology Sydney, Broadway, NSW, AUSTRALIA*

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**Learning Objective:** After attending this presentation, attendees will have a greater understanding of the influence of physical activity and elapsed time on fiber transfer and persistence. They will be informed of current research aided by jiu-jitsu activities to improve the interpretation and evaluation of fiber evidence. Attendees will ultimately gain a greater appreciation of the value of microtraces in informing activity level evaluations and their role in investigative operations and the judicial system.

**Impact Statement:** This presentation will impact the forensic science community by improving the awareness and capacity of forensic practitioners to interpret and evaluate fiber evidence and address questions of not just source, but also activity, in casework. This in turn will actualize the value of microtraces in investigations and benefit the criminal justice system.

The purpose of this research is to develop, test, and validate a model framework for the transfer and persistence of textile fibers by conducting realistic studies with human participants performing jiu-jitsu movements.

Procedures for forensic fiber examination have been long established. However, at the end of such examinations, the analytical results must be evaluated to provide any value to the investigation or the Court. Over the years, the value of microtraces (including textile fibers) at the activity level has received much less research attention than source level discrimination. However, addressing the question of, “How did that material get there?” is of most benefit to the Court. This discrepancy has clear repercussions for the criminal justice system. This presentation will discuss how jiu-jitsu activities have aided “action-packed” research to improve our understanding of microtrace transfer and persistence.

Fiber transfer and persistence studies have until now largely been laboratory-based mechanical simulations. However, transfer events in casework are uncontrolled in nature. This study takes a novel approach with skilled jiu-jitsu practitioners performing choreographed moves mimicking “standard” assault situations for transfer experiments to gather results more directly applicable to real life events in a modern context. This was followed by a study of the effects of physical activity on persistence of the transferred fibers at intervals of up to 4 hours.

Decay curves showed rapid initial loss followed by apparent exponential decay. Typical results indicated that 42.3% of fibers were lost after 10 minutes, increasing to 71.3% by 30 minutes. Length distribution demonstrated a shift toward shorter fibers with time. Overall zonal distribution of target fibers was greatest in the frontal upper regions of t-shirts (16.7–19.9%), and sleeves of hooded sweatshirts (19.1%).

These experimental results will be added to a contemporary repository of information in the form of a Knowledge-Based System (KBS). The concept of a KBS in forensic science was proposed in the past but not developed to its full potential.<sup>1</sup> The digital landscape continues to grow yet remains unembraced by this discipline area. This project will create such KBS, integrating contemporary digital technologies with results of fiber research and casework. This could prove a valuable tool to aid practitioners and ultimately improve the reliability of forensic evidence.

**Reference:**

<sup>1</sup>. Curran, J. M. (2009). Use of knowledge-based systems in forensic science. In: *Wiley Encyclopedia of Forensic Science*.

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**Textile Fiber Transfer and Persistence; Forensic Evaluation; Activity**

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**B173 A Chemical Approach to Tire Mark Analysis**

*John Lucchi, MS\*, University of Central Florida, Orlando, FL; Matthieu Baudalet, PhD, University of Central Florida, Orlando, FL*

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**Learning Objective:** After attending this presentation, attendees will understand different spectral characterization techniques for skid mark lifts. These techniques allow discrimination of tire and asphalt particles.

**Impact Statement:** This presentation will impact the forensic science community by providing another step toward using skid mark tire particles as a piece of forensic evidence.

With close to 27,000 fatal traffic accidents involving hit and runs in the United States between 2004 and 2019 (with almost 1,000 occurring between 2018–2021 in Florida alone), all the information that can be extracted from the accident scene is crucial. While traditional tire mark analysis is full of physical information about the reconstruction of the event, more information can be extracted from the rubber left during the braking event. As rubber elemental analysis has shown that it can differentiate between tire models, it is important to show the feasibility to link the rubber left from a mark on the road to its originating tire.<sup>1</sup> Once these particles are sampled, high sensitivity elemental analysis can be done quantitatively using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS).

Nonetheless, the material must first be extracted from the road surface. This is more efficiently done using lifts, in which a lifting tape is applied to the road surface and then removed, taking the loose particles with it. While this technique will collect most of the tire particles, other particles from the road material will be on that lifting tape as well. It is beneficial to assess a lift to know how much tire material vs. other material is present on the sample in order to facilitate the analysis. This presentation focuses on RGB analysis to characterize the difference between asphalt and rubber on a microscopic level.

By looking at the distribution of RGB values within the materials can be discriminated from each other. This RGB analysis is simple and non-destructive, not altering the material at all before it is further characterized elementally. This process also requires minimal time investment while providing a photographic record of what was on the lift before the material is then separated for analysis.

**Reference:**

- <sup>1</sup>. Lucchi J, Gluck D, Rials S, Tang L, Baudalet M. Tire Classification by Elemental Signatures Using Laser-Induced Breakdown Spectroscopy. *Applied Spectroscopy*. 2021;75(6):747-752. doi:[10.1177/0003702821995585](https://doi.org/10.1177/0003702821995585).

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**Tires; Trace Evidence; Skid Mark**

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**B174 Image Analysis and Neural Networks Techniques for Hair Examinations**

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**Learning Objective:** In this presentation, attendees will learn basic techniques used in image pre-processing, neural networks, and their use in digital microscopy. Image processing techniques are particularly growing in relevance due to their wide applications. These methods show the potential to be used to analyze various types of evidence. This presentation will provide an introductory overview of image processing techniques and neural networks, and more specifically, applications for hair analysis.

**Impact Statement:** This presentation is intended to provide an introductory overview of image processing and neural networks. Attendees will learn the basics of image processing, neural network, and a novel approach to hair analysis.

Hair has a high transfer potential due to natural shed; therefore, it is commonly observed evidence in crime scenes. Microscopical techniques are practical, given that they are inexpensive but may provide limited information. Additionally, the associative capabilities of microscopical evaluation of hair may range from a low value without a comparable sample set and known sample for comparison to a high value when sufficient information is known regarding the possible source (e.g., determining the animal species). As for human hair, variation and overlap can be seen within population groups and individuals, making associations difficult. Hair cannot be used to isolate a single donor but may provide information to reduce the number of sources. Although through visual comparisons donors may be eliminated, image analysis techniques may be used as an aid and to explore additional levels of data that are not visible. Given increased imaging technology, such as high-resolution digital microscopes and computer vision algorithms, their forensic applications need to be explored. It is hypothesized that the combination of neural networks and image processing can increase hair comparison accuracy; however, it is greatly dependent on obtaining a large number of images to train a model. Combining image processing techniques with measurable features may provide comparable results to a neural network.

A neural network model was built using images of 1,500 hairs from 30 individuals. Each sample set consisted of 25 shed hairs and 25 pulled hairs from each individual of varying ancestry. A VHX 6000 Keyence digital microscope was used to obtain the images at three different locations through the midshaft of the hair at 1000x magnification (a total of 4,500 images). Images were then either used as a whole or segmented into 3, 5, or 10 equal-size images (for a total of 4,500, 13,500, 22,500, or 45,000 images). Pulled hair images were used to train a neural network, and shed hairs were used as the test set. Additionally, image processing techniques were used to streamline individual measurements for the hair's color, texture, density, thickness, and medulla presence. All image analysis and neural networks were done using MATLAB® and MATLAB® image processing toolbox.

This study will provide insight into image processing and neural networks used in hair analysis. The results will demonstrate the accuracy of using a neural network and novel methods for statistically comparing hair features. The methods presented are intended to aid the comparison of hair for forensic examinations.

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**Hair Analysis; Neural Networks; Microscopy**

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**B175 The Microscopical Recognition and Characterization of Pigmented Fibers**

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**Learning Objective:** This presentation is intended to provide attendees with an approach to recognize, prepare, characterize, and compare pigmented (i.e., solution-dyed) fibers in a practical manner intended to be integrated into the existing workflow of a forensic fiber examination.

**Impact Statement:** This research is intended to be a practical approach to recognize, prepare, characterize, and compare pigmented (i.e., solution-dyed) fibers in a manner intended to be integrated into the existing workflow of a forensic fiber examination.

Pigmented (i.e., solution-dyed) fibers, in contrast to dyed fibers, are colored by the fiber manufacturer prior to extrusion through the addition of fine, microscopic, and sub-microscopic solid pigment particles. Due to the historically lower production of pigmented fibers and the extremely fine nature of the pigments, forensic research on this topic has been limited. However, solution-dyed fibers have been steadily gaining market share, utilizing an increasingly broad range of pigments, and are being used in an increasing variety of polymers for an expanding range of applications (including trunk liners of vehicles as well as commercial and consumer carpeting). Despite their size, the visualization and identification of these fine pigment particles is accessible using robust methods that are already common (e.g., polarized light and fluorescence microscopes) or are becoming more common (e.g., Raman microspectroscopy) in trace evidence laboratories.

This research aims to expand the knowledge base surrounding pigmented fibers through a systematic, microscopical study of a population of pigmented fibers using visual, microscopical methods accessible to a trace laboratory. The fibers selected for this study have been drawn from our internally developed, curated fiber collection consisting of thousands of pigmented fibers. The fibers studied here were chosen to represent a range of manufacturers that span a variety of commercial applications, colors, and fiber types. For example, polyolefins have always been pigmented since they cannot be dyed; however, the range of commercially available pigmented fibers has expanded to include polymers such as nylon, polyester, and rayon that traditionally have been colored using dyestuffs.

This presentation will summarize results arising from a critical microscopical study of this sample set by polarized light, oil immersion, and fluorescence microscopy. This direct study of pigments within fibers is supported by concurrent research into sample preparation techniques, including longitudinal whole mounts and transverse, thin cross sections. These methods of preparation, which include considerations for both mounting and embedding media, as well as cross-section thickness and microscopical imaging conditions, have been optimized to maximize the resolution of individual pigment particles that often approach, or at times exceed (are smaller than), the resolution limits afforded by white light microscopy. For example, the imaging of longitudinal mounts of fibers (which represents the main mounting approach presently used in forensic laboratories to study fibers) colored by pigmentation can result in a mass tone color in which individual pigments may not be readily observed or characterizable due to the relative size of the pigments compared to the overall thickness of the fiber. In fact, the discrimination of a delustered (TiO<sub>2</sub> inclusions) and a pigmented fiber may be challenging in longitudinal preparations. Using thin sections ranging from a thickness of 2µm down to as thin as 200nm, higher magnification objectives, oil immersion, and fluorescence microscopy, individual pigment crystals can be visualized, enumerated, and characterized by size and morphology.

The primary result of this research is the development of a systematic technical method to: (1) recognize pigmented fibers, (2) microscopically characterize the range and properties of pigments within the fibers, and (3) offer insights into trends and significance of the pigmented encountered. Ultimately, this research is focused on providing bench-level trace evidence analysts with a supplemental, complementary approach to existing fiber examination protocols and thus advance microscopical fiber examinations utilizing equipment that is readily available.

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**Solution-Dyed Fibers; Microscopy; Pigment**



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**B176 An Analysis of Automotive Paint Layers on Plastic Substrates Using Chemical Imaging Fourier Transform Infrared (FTIR) Microscopy**

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**Learning Objective:** Following this presentation, attendees will understand the layer system present in automotive paints, their forensic relevance, the challenges involved in analyzing primer layers on plastic substrates, and a new method for doing so.

**Impact Statement:** This presentation will have a positive impact on the forensic science community by reporting on the new application of FTIR microscopy to conventional forensic paint samples as well as detailing necessary procedures for validation of the new method.

Analysis of paint chips left at the crime scene of automobile accidents can provide a multitude of leads for forensic investigators. The Royal Canadian Mounted Police Paint Data Query Program (PDQ) is comprised of two components: a database that contains the complete color, chemical composition, layer sequence, and sourcing information of more than 23,000 known paint systems, and the PDQ Spectral Libraries that contain the FTIR spectra of the samples in PDQ. Once an analyst has identified key components in each of the layers, they can input that information into PDQ, which generates a hit list of possible makes, models, and years, potentially including whether the vehicle is a car or truck; the plant where the vehicle was manufactured; and the chemical and structural analysis of the sample. After a hit list has been generated, analysts can compare FTIR spectra of their sample to those in the database to confirm identification.

FTIR microscopy is ideal for the forensic analysis of layered automotive paints due to its rapid and non-destructive nature. The main forensic focus of these paints is on the clear coat and the primer layers. While clear coats and primers applied to metal parts can be well characterized, paint applied to plastics tends to have adhesive-like primer layers that, due to their thinness, are much more difficult to physically separate and analyze as is typically done for inclusion in the PDQ database. While traditional methods may be suitable for most primers, FTIR chemical imaging can provide analysts with an alternative method for especially thin or otherwise challenging primer layers. As plastic bumpers are typically painted with primers at their manufacturing plant (not automotive assembly plants) separate from the rest of the paint system, they may provide an additional source of origination information. Forensic methods by American Standard Testing Materials (ASTM) and Scientific Working Group on Materials Analysis (SWGMA) already permit  $\mu$ -FTIR of paint cross sections, but do not describe chemical imaging with a focal plane array detector.

This presentation will cover a new method for collecting  $\mu$ -FTIR spectra of primer layers applied to plastic automotive bumpers using new chemical imaging technology. Additionally, the presentation will discuss the process of developing this new method. Method validation is critical for demonstrating the robust and trustworthy results needed for presentation in legal cases. As such, this presentation will also detail the use of samples of known provenance, comparison to data obtained from the traditional method, and the implementation of sample blinding for the researcher collecting the spectra to ensure objective and reliable results.

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**Automotive Paint; FTIR Microscopy; Chemical Imaging**

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**B177 Combatting Illegal Logging Through the Use of Direct Analysis in Real-Time High Resolution Mass Spectrometry (DART®-HRMS) and Statistical Logarithms**

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**Learning Objective:** Attendees will be introduced to the development of a method to facilitate identification of illegally traded timber for use in wildlife forensics laboratories. An understanding of multivariate statistical analysis to aid in the classification will be gained.

**Impact Statement:** This presentation will impact the forensic science community by providing a new and efficient methodology for the identification of illegally traded timber. This approach will extend the capabilities of law enforcement in the use of a stand-off approach for identification of trafficked wood species.

One of the prevailing concerns in wildlife forensics is the ability to accurately identify illegally traded endangered species. While trade in fauna, including elephant parts (such as tusks), rhinoceros horns, and pangolin scales, are well-known examples of wildlife crimes, there are a host of flora that are heavily trafficked, tree logs serving as a case in point. Illegal trade of endangered wood is common because it is highly prized for making exclusive furniture, cabinetry, musical instruments, and construction materials. This causes environmental and economic damage because it leads to forest degradation and deforestation and deprives countries of billions of dollars in revenue. Illegal logging is one of the most lucrative natural resource crimes and is valued at \$52 billion to \$157 billion per year, which is a magnet to some of the world's largest organized crime groups.

The Convention on International Trade of Endangered Species (CITES) was created to address the conservation of imperiled wildlife by controlling their trade. Regulation status is defined by appendices: CITES Appendix I species are threatened with extinction, and trade of any kind is illegal; CITES Appendix II species are threatened in the wild and international trade is controlled to aid in their survival; and CITES Appendix III species are regulated by a particular nation. Therefore, depending on the species, trade is either totally or heavily restricted. Moreover, when specimens are intercepted by law enforcement, it is extremely challenging to identify the evidence as either legal or illegal, because many of the species that are illegal to trade have an appearance that is similar to species that are not restricted.

A current technique used by law enforcement to differentiate species of wood is Direct Analysis in Real Time-High Resolution Mass Spectrometry (DART®-HRMS), coupled with multivariate statistical analysis. Here, the added dimension of wood headspace analysis featuring Solid Phase Microextraction (SPME) was used to generate data to complement that acquired using the conventional wood analysis technique. This could facilitate the development of “stand-off” approaches to the differentiation of wood species based on their volatiles profiles. Eight genera, including *Dalbergia*, *Swietenia*, *Pericopsis*, *Araucaria*, *Pterocarpus*, *Cedrela*, *Diospyros*, and *Millettia*, were provided by the United States Fish & Wildlife Forensic Lab, all of which contain species that are listed within CITES Appendices I–III. The headspace volatiles of the wood samples were concentrated on SPME fibers for 30 minutes and analyzed by DART®-HRMS. Multivariate statistical analysis processing of the DART®-HRMS data revealed intra-genus similarities and inter-genus differences that resulted in the ability to assign genus attribution to the chemical signatures. The classification model that was developed could therefore be used for rapid forensic identification of species based on simple analysis of the headspace of the wood. The results show that this approach can contribute to the enhancement of techniques that enable law enforcement to distinguish between endangered wood and species that are legal to trade.

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**Illegal Logging; DART®-HRMS; Multivariate Statistical Analysis**

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**B178 Electrochemical Characterization of Common Cutting Agents in Illicit Drugs**

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**Learning Objective:** Attendees will learn about electrochemistry and how electroanalytical methods can be used for the identification and quantification of illicit drugs and cutting agents, as well as for source tracing purposes.

**Impact Statement:** This work presents the premises of developing a database of electrochemical characterizations of common illicit drugs and cutting agents to expand the use of electrochemistry in forensic analyses.

The opioid crisis intensified over the past few decades, and we saw an increase in drug seizures and consequently an increase in the backlog of forensic drug evidence. Most seized drug samples are complex mixtures of drugs and cutting agents, which can complicate the detection and quantification of the illicit drugs in the sample. The presence of these cutting agents can, however, be beneficial for source tracing purposes as the majority of cutting agents are selected based on availability in the area the bulk drug was prepared.

Seized drug samples are commonly analyzed using Fourier Transform Infrared Spectroscopy (FTIR) or Gas Chromatography/Mass Spectrometry (GC/MS). These methods, however, can be difficult to use in-field and may require extensive sample preparation that will vary depending on the sample and its matrix. We are developing electroanalytical methods for forensic analysis. Electroanalytical techniques can be rapid and low-cost while being accurate, selective, and sensitive, as well as easily adaptable for in-field detection.

In this work, our goal is to electrochemically characterize the most common cutting agents found in illicit drugs, such as diphenhydramine, paracetamol, ascorbic acid, and caffeine. To date, we have characterized 14 common cutting agents using Cyclic Voltammetry (CV). We have also optimized their detection using Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV) as these methods are generally more sensitive than CV and better suited for quantification purposes. Some cutting agents such as calcium carbonate can alter the pH of the tested sample, then impacting the measurement of the redox activity of other cutting agents. As an example, a pH study was carried out on the pH-sensitive cutting agent ascorbic acid. Additionally, some analytes such as caffeine have high oxidation potentials that can overlap with the oxidation of common solvents or have various degrees of electron transfer efficiency depending on their solvent. As such, the choice of supporting electrolyte for the detection of caffeine detection was explored, including sulfuric acid, and different chloride salts with sulfuric and hydrochloric acids.

The next step of the project is to perform analyses of mixtures of cutting agents and test mixtures with fentanyl and other currently relevant drugs. One long-term goal is to establish an extensive database of electrochemical characterizations of cutting agents encountered in street-seized drug samples to help expand the developing field of forensic electrochemical analyses.

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**Electrochemistry; Source Tracing; Drug Identification**

## C1 A Lossy Compression Analysis of GoPro® Audios Before and After YouTube® Laundry

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the impact of uploading videos to YouTube® as relates to camera attribution, and the ability to attribute the audios from GoPro®-created videos uploaded to YouTube® to the GoPro® camera make and model.

**Impact Statement:** This presentation will impact the forensic science community by serving as a baseline study of the impact of uploading videos to YouTube® on GoPro® camera attribution from the downloaded audio streams.

Action cameras are digital cameras designed to capture immersive action shots, and GoPro® is a leading manufacturer of action cameras. When a GoPro® camera captures potential evidence of a crime or other occurrence of interest, the authenticity of the video may need to be established, especially for judicial or extra-judicial purposes. Establishing a video's authenticity is made more difficult when the video has been uploaded to the YouTube® platform because the uploading process strips out or "launders" the metadata pointing back to the source camera. Because of this laundering effect, new methods for authentication of YouTube®-downloaded GoPro® audios and videos should be explored.

An exploratory study was conducted of the Modified Discrete Cosine Transform (MDCT) coefficients of GoPro® audios before and after YouTube® laundry. To that end, 400 audios from ten GoPro® cameras—200 audios from before YouTube® laundry and 200 audios from after YouTube® laundry—were analyzed and compared to a reference database of 156 models, including samples from the same ten GoPro® cameras.

Six sets of MDCT coefficients were computed for each of the 400 audios, one set for each of the following popular verified compression algorithms: AAC-LC v1 (Sine-based window), AAC-LC v2 (Kaiser-Bessel-Derived Window [KBD]), AC3, MP3, OGG-Vorbis, and WMA. The resulting coefficients then were compared to coefficients computed for the 156 reference database models, and the results were reported as Logarithmic Likelihood Ratios (LLRs) and measurement uncertainties ("Standard Error of the Mean [SEM]"). The highest LLR indicated the most consistent reference model to the test file.

The study reported the rate at which the most consistent reference model matched the make and model of the test file. These make-and-model matches were encouraging for one of the tested GoPro® models (five models were tested, two cameras per model), but the other four models didn't perform as well. In fact, when the results from all five GoPro® models were averaged, the camera models matched only 69% of the time before YouTube® and 46% of the time after YouTube®.

This study also reported the rate at which the most consistent reference model matched the make—but not the model—of the test file. These make-only matches revealed much more encouraging results. In fact, for three of the five GoPro® models tested, the camera makes (i.e., GoPro®) matched at least 95% of the time. When all five GoPro® models were averaged, the camera makes matched 95% of the time before YouTube® and 83% of the time after YouTube®.

The results raised some questions about whether, and to what degree, the format and recording environment of the reference database models can stray from those of the GoPro® audios without affecting accuracy. In other words, at what point do we start comparing apples to oranges? The GoPro® audios for this study were AAC-LC v2/48KHz/192kbps/2-ch and were made indoors with room noise only. By contrast, the 156 reference database models represented multiple AAC versions, sample rates, bit rates, and channel configurations, and were made in a variety of indoor and outdoor recording environments capturing a variety of sounds, including traffic noise and speech. These mismatched formats and recording environments may have affected the results. To test this, two additional more-optimized reference databases were created and were compared to a limited number of test files. The results were extremely promising and indicated that future research could benefit from more optimized reference databases. Finally, some additional scientific limitations and possibilities apparent from the analysis were discussed.

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### GoPro®; YouTube®; Modified Discrete Cosine Transform (MDCT)

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## C2 Can You Accurately Determine Clothing Tones in Infrared Closed-Circuit Television (CCTV) Imagery?

*Cole Whitecotton, MSc, National Center for Media Forensics—University of Colorado Denver, Englewood, CO; Gretchel Lomboy, MS\*, Seattle Police Department, Shoreline, WA; James Zjalic, MSc, Verden Forensics, Birmingham, England*

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**Learning Objective:** After attending this presentation, attendees will better understand the need for caution when making judgements relating to the tone of clothing captured within infrared CCTV imagery and be presented with the methodology, results, and conclusions from preliminary research performed in relation to such.

**Impact Statement:** This study aims to aid examiners in making informed decisions and providing the limitations of their analysis relating to the interpretation of clothing captured by infrared CCTV imagery.

Analysis and comparisons of clothing worn by suspects within infrared CCTV imagery play a vital role in both criminal investigations and subsequent trials. Digital imagery examiners will often be asked to compare the clothing worn by the unknown individual to that of a suspect, and as the process is subjective, the reliability of conclusions is in direct correlation with the reliability of the examiner's interpretation. There is currently very little research into this area, and thus the limitations are not known. Is what appears to be a light-toned jacket captured within infrared CCTV imagery, in fact, light in tone? Ill-informed and potentially incorrect responses to a question such as this given during courtroom testimony could have a significant influence on the final verdict rendered by the trier of fact. It is therefore essential that further research into this area is conducted.

To address the above, the research presented tested the following hypotheses:

- The tones of clothing represented by infrared CCTV imagery differ based on the camera system used;
- The tones of clothing represented by infrared CCTV imagery differ based on the distance of the item from the camera lens;
- The tones of clothing represented by infrared CCTV imagery differ based on illumination by artificial lighting;
- The tones of clothing represented by infrared CCTV imagery do not correlate accurately with those represented by the Human Visual System.

To test the proposed hypotheses, different-colored shirts were captured using two different infrared CCTV systems at a number of controlled distances and under different lighting conditions. As an examiner, the interpretation of an image's tone is based on the pixel intensity, so the measurements were then taken and analyzed.

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### Infrared CCTV; Morphological Analysis; Clothing Tone Analysis

## C3 Digital Reality: Light Reflection vs. Gunfire Muzzle Flashes

Barry Dickey\*, Audio Evidence Lab, Mansfield, TX; Gil Sapir, JD, MSc, Chicago, IL

**Learning Objective:** After attending this presentation, attendees will understand the need for video analysis of critical events of an alleged gun discharge (“muzzle flash”).

**Impact Statement:** The forensic science community will be impacted through the forensic analysis of video evidence that can drastically alter the facts of a case, causing an inaccurate and confusing impression of events.

Digital evidence includes the retrieval, storage, and analysis of digital data for criminal investigations. It includes body cams, dash cams, surreptitious recordings, computers, hard drives, mobile phones, and other recording devices.

For this presentation, video from a case in which a defendant is accused of entering a victim’s Jeep® at night, shooting him twice, and then exiting the Jeep® is utilized. Although informed of the allegations, counsel did not provide reports from other experts or law enforcement, requesting a completely independent evaluation of the video.<sup>1</sup>

The video images in the case were captured from an adjacent building’s rooftop on an Advanced Technology Video (ATV) Falcon surveillance system from approximately 127 feet.<sup>2</sup> The images were encoded at a resolution of 352 x 240. The video was provided in an executable format. Initial examination of the file utilized the ATV Application Player (v3.5.5) for overview.<sup>3</sup> The files were further evaluated through various forensic software. Analysis followed Scientific Working Group on Digital Evidence (SWGDE) protocol.<sup>4</sup>

Forensic analysis employed the examination of each individual image throughout the questioned event(s). Overall continuity and specific parameters (timing, location, shape, tonality, brightness) in each sequential image was analyzed. The Jeep® arrives. A reflection travels down its passenger side. The reflection stops just behind the passenger door. This reflection/illumination maintains a constant size, brightness, and shape. An individual enters the Jeep®. The reflection changes its overall shape, tonality, and brightness. The area of reflection/illumination is generally muted in tonality/brightness and distorted from its previous state. The area of reflection momentarily changes back to the identical shape, tonality, and brightness prior to entry, then immediately returns to its muted characteristics after entry. The consistency supports that the momentary change is the result of movement or repositioning of individuals inside the Jeep®. A muzzle blast inside the vehicle would generate a light source that had not previously existed, causing a unique different shape, size, and brightness in comparison to the prior reflection/illumination. The reflection/illumination returns to the identical size, tonality, brightness, and shape after the individual exits the Jeep®. The reflection/illumination consistently maintains these characteristics as the individual walked away from the vehicle.<sup>5</sup> In conclusion, forensic analysis of the images verified a lack of credible evidence to support the existence of any “gunfire muzzle flashes” in any of the images examined.

### References:

- <sup>1.</sup> *People of Illinois v. Paul Zalewski, Jr.*, No. 18-CR-03841, Criminal Division, 3rd Dist., Cook County, IL.
- <sup>2.</sup> Digital Recoding Camera: ATV Falcon Model, #:FA-DR16500DVD, Serial #:106611088709030005, Mfg. date: March 2009).
- <sup>3.</sup> Software: ATV Player [System Embedded], InputAce, Ikena Forensic, Dhex, MediaInfo, Cognitech Investigator, Izotope RX, & Sony SF/VS Pro.
- <sup>4.</sup> Scientific Working Group on Digital Evidence. (SWGDE) *Best Practices for Data Acquisition from Digital Video Recorders*, Version: 1.0 (April 25, 2018). SWGDE are accepted guidelines established for forensic acquisition of digital video recording data.
- <sup>5.</sup> *Barry E. Dickey Laboratory Report*, April 27, 2021 (filed May 21, 2021) in *People v. Zalewski*, supra, n.1.

### Digital Analysis; Surveillance Camera; Video



### **C4      An Investigation of Closed-Circuit Television (CCTV) Footage for the Detection of Facial Features**

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**WITHDRAWN**

## C5 An Overview of Facial Identification as a Forensic Discipline

*Steven Johnson, BS\*, Ideal Innovations, Inc., Arlington, VA*

**Learning Objective:** After attending this presentation, attendees will have a better understanding and insight into the value of facial identification as a forensic practice.

**Impact Statement:** The forensic science community is experiencing intense scrutiny across the spectrum of all practices, none more so than the facial identification discipline. This presentation will impact attendees by increasing understanding of the challenges and benefits of facial identification and will provide stakeholders with the tools for proper and professional applications of facial identification practices, procedures, and methodologies.

**Objectives:** To provide a better understanding of the growing interest in the facial identification discipline, the differences between facial recognition/facial examination, how the two elements work together to provide forensics intelligence for criminal investigations, and where the discipline is heading. The presentation will cover, at a high-level:

- facial features used in comparisons
- challenges of facial analysis
- differences between facial assessment, review, and examination
- the various methodologies employed in the facial examination arena
- the physiology and morphology of the face, head, and neck
- how to articulate the results of a comparison
- current legislative efforts to support training and standards for the use of facial recognition
- emergence of a Facial Identification Certification program

This overview of facial examination is intended to bring understanding to the forensic and other stakeholder communities as to how facial analysis and examination can be utilized as a tool for investigations. Like many other forensic disciplines, facial identification is under significant public scrutiny, and educating the public and the legal community is of importance. This includes identifying the limits of the technology, the need for qualified and competent examiners to conduct analysis of less-than-quality images (both known and probe), and the efforts to professionalize the discipline. Other elements in the presentation include:

- some of the standards published or in development to regulate the application of facial identification
- the history of facial recognition and examination
- the limitations of facial recognition systems
- the impact of expression on the examination
- the effects of age, environment, and illness on the face
- the features that are accepted for examination purposes
- image quality issues
- certification requirements and testing protocols

This presentation will include some examples of feature and facial comparisons to further assist attendees in understanding the facial examination processes and procedures. It is hoped that attendees will see the value in obtaining training in facial examination in conjunction with the deployment and utilization of a facial recognition system and the benefits of a certification program.

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### Facial Identification; Facial Recognition; Certification

**C6     The Application of TPS Software in Ear Print Identification: Matching/Non-Matching of the Ear Photograph and Ear Print**

*Deepika Rani, MSc\*, Panjab University, Chandigarh, INDIA; Kewal Krishan, PhD, Panjab University, Chandigarh, INDIA*

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**WITHDRAWN**

## C7 Exploring Bias in Estimating the Age of Subadults in Images

Petra Urbanova, PhD\*, Masaryk University/Department of Anthropology, Brno, Jihomoravsky kraj, CZECH REPUBLIC

**Learning Objective:** After attending this presentation, attendees will be acquainted with the basic principles underlying age estimation in images of subadults using deep learning algorithms. They will be acquainted with the performance of algorithms that use convolutional neural networks when tested on a large biometric database of subadult faces.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into the biases in forensic image age assessment using deep learning algorithms.

Assessing the age of individuals is an important forensic task. There are numerous situations in which an individual's age needs to be estimated, such as when age has crucial legal consequences (e.g., age of consent) or when an individual is put in harm's way (e.g., child abuse cases). Currently, forensic casework requiring age assessment is mostly conducted using images (photographs, video stills) and real-time video or recordings (Closed-Circuit Television [CCTV] or webcam), and there are ongoing attempts to develop age assessment algorithms that would perform most accurately. To date, however, most studies have ignored the fact that the estimation error for subadult individuals varies significantly depending on the age, ancestry, and biological sex of the individual. Similarly, there are no data on the accuracy of age estimation by experts.

The present study examines the performance of state-of-the-art automated algorithms for image age estimation in subadults. It also explores the limitations and biases arising from the non-linearity of craniofacial growth and developmental patterns on which age estimation is based, focusing in particular on the relationship with biological sex and interpopulation variation. In addition, the study confronts the performance of automated with classification error by forensic experts.

The studied sample consisted of 1,164 individuals of predominantly European descent, aged between 6 and 19 years (mean 11.13 years). Image data were sampled from the FIDENTIS 3D Face Database. The 3D images were converted to 2D color images that varied in viewpoint and technical parameters to mimic the ever-changing acquisition conditions (image resolution, pose, distance between subject and camera, focal length). The conversion was performed in a non-commercial converter.<sup>1</sup> To train the automated systems, the dataset was divided into a training (75%) and a test (25%) dataset. Subsequently, Convolutional Neural Networks (CNNs) were tested on a pooled dataset and separately on males and females. The CNN architecture was based on pre-trained ResNet18 and VGG-16 networks. Finally, a subset of 100 images randomly selected from the converted images was scored by 20 participants trained in age estimation. The participants' task was to classify each image into one of seven age categories (spanning two years).

Results showed that the algorithms tested were challenged by known sex-specific growth patterns. For both sexes, error increased as individuals approached adulthood. For females, age less than 14 years was consistently overestimated, while for older girls it was underestimated. Conversely, for males, age 14–15 years was identified as the point at which the mean absolute error began to increase significantly. The overestimation in males was evident only at early ages. When confronted with estimates from experts, the automated algorithms produced better results, but overall the estimates were less consistent.

The deep learning algorithms have proven that they can be very efficient when trained on a large training dataset. Nevertheless, there are several other issues of computational, technical, and biological nature that need to be addressed in order to develop truly reliable automated age estimation methods. The purpose of this presentation is to present the issues to the forensic community.

### Reference:

<sup>1</sup> Goldmann T. *SyDa Generator* [online]. 2021 [accessed February 1, 2021]. Available at <http://www.fit.vutbr.cz/~igoldmann/app/sydagenerator/>

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### Age Estimation; Subadults; Deep Learning Algorithms

## C8 Multimedia Stream Hashing: A Forensic Method for Content Validation and Verification

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**Learning Objective:** After attending this presentation, attendees will have the foundational knowledge of an emergent multimedia forensic method to verify the digital image, video, and audio stream integrity when file containers may change during file handling and processing.

**Impact Statement:** This presentation will impact the forensic science community by providing an alternative to traditional file hashing for multimedia evidence integrity more precisely by verifying the digital image, video, and audio streams.

How does a forensic examiner know if they have altered a multimedia stream when converting the multimedia stream from one codec or file container to another for subsequent forensic examination? Forensic standards and best practices recommend avoiding alteration or degradation of multimedia data during a transcoding process. The American Society for Testing and Materials (ASTM) International Standard E2825-21, 2021 (a standard for forensic image processing) warns to “avoid the introduction of artifacts that add misleading information to the image” and prohibits the “loss of image details that could lead to an erroneous interpretation.”<sup>1</sup> The Scientific Working Group on Digital Evidence (SWGDE) Best Practices for Digital Forensic Video Analysis section 4.3.3 (b)(ii), (2018) cautions that “transcoding could affect the content of a video file by changing its visual appearance, however small.”<sup>2</sup> The SWGDE Best Practices for Forensic Audio, in section 7.1 (2022), advises that “transcoding could affect the audio content (aliasing, compression).”<sup>3</sup>

Because a multimedia file changes when losslessly transcoded or recontainerized to a new format, the unmodified streams cannot be verified by file hash alone. The post-collection and pre-examining process of preparing multimedia streams for further examination presents a significant challenge in multimedia forensic science related to stream integrity during transformative processes. This presentation offers a method validation study of multimedia stream hashing as a forensic technique for content integrity verification.

This presentation will offer a quick overview of the underlying stream hash process used by the universal stream hashing tool Fast Forward MPEG (FFmpeg). This presentation focuses on illustrating the practical use of multimedia stream hashing as a forensic method for multimedia content integrity verification. This presentation will provide several illustrations that the multimedia stream hashing method can accurately identify the integrity of digital images, videos, and audio following transmission, transcoding, or re-containerization. In addition, this presentation illustrates the validation of the forensic use of the multimedia stream hash method while establishing the error rate for its use.

FFmpeg use for stream hashing requires an understanding of underlying processes and various options for controlling the stream hash operation. This presentation demonstrates the existence of one option for controlling the FFmpeg stream hash operation while presenting two video transcoding demonstrations. This presentation will illustrate that the multimedia stream hashing method is invaluable in the forensic examiner’s toolbox for verifying multimedia stream integrity. A case example involving an audio recording acquired from a mobile device via different methods will be presented to demonstrate the importance of stream hashing in forensic casework.

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### Multimedia Stream Hashing; Stream Hash; Stream Integrity

## C9 Source Camera Identification on Multi-Camera Phones

Stephanie Reinders, PhD\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA; Alicia Carriquiry, PhD, CSAFE-Iowa State University, Ames, IA

**Learning Objective:** After attending this presentation, attendees will have a better understanding of how selfie, ultra-wide, and telephoto cameras can affect the similarity scores that lie at the heart of camera identification methods.

**Impact Statement:** This presentation will impact the forensic science community by explaining how traditional camera identification methods could be adapted to determine whether a questioned image was taken by a Person Of Interest's (POI's) phone if that phone has multiple cameras. This presentation will also explore how selfie, ultra-wide, and telephoto cameras can affect the similarity scores that lie at the heart of these camera identification methods.

Camera identification addresses the scenario where an investigator has a questioned digital image from an unknown camera. The investigator wants to know whether the questioned image was taken by a camera on a POI's phone. Lukas, Fridrich, and Goljan discovered that slight imperfections in a camera's sensor array can be used as an identifying feature or camera fingerprint in this scenario.<sup>1</sup> These imperfections result from the manufacturing process and cause some pixels to produce consistently brighter or darker values than their neighboring pixels. A camera leaves its fingerprint in the images that it takes. A camera fingerprint is typically estimated from 50 or more reference images known to have been taken by the POI's camera. A camera fingerprint is also estimated from the questioned image.

Most camera identification methods measure the similarity between the two camera fingerprints with a similarity score such as correlation distance or peak-to-energy correlation. The score is then compared to a reference set of similarity scores to determine whether the questioned image originated from the POI's camera and quantify the strength of the evidence in favor of that determination.<sup>1-4</sup> In particular, two types of reference sets are calculated from a reference set of cameras: matching scores between an image and a camera fingerprint from the same camera, and non-matching scores between an image and a camera fingerprint from different cameras. Reinders et al. showed that changing the cameras in the reference set can change the distribution of non-matching reference scores, which can in turn change the outcome of the camera identification method<sup>4</sup>.

Previous camera identification methods have been tested on images taken by digital still cameras and the main rear cameras of mobile phones. Major smart phone brands now include a front (selfie) camera, and many offer additional rear cameras such as ultra-wide-angle and telephoto. This presentation will focus on camera identification for multi-camera phones and explore the distributions of similarity scores within (same camera) and between (different cameras) pairs of cameras on a small set of multi-camera phones.

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- <sup>2</sup> Goljan, Miroslav; Jessica Fridrich; Tomáš Filler. Large scale test of sensor fingerprint camera identification. In *Media forensics and security*, vol. 7254, pp. 170-181. SPIE, 2009.
- <sup>3</sup> van Houten, Wiger; Ivo Alberink; Zeno Geradts. Implementation of the likelihood ratio framework for camera identification based on sensor noise patterns. *Law, Probability and Risk* 10, no. 2 (2011): 149-159.
- <sup>4</sup> Reinders, Stephanie; Yong Guan, Danica Ommen; Jennifer Newman. Source-anchored, trace-anchored, and general match score-based likelihood ratios for camera device identification. *Journal of Forensic Sciences* 67, no. 3 (2022): 975-988.

### Camera Identification; Digital Evidence; Similarity Scores



## C10 A Forensic Analysis of Android™-Popular Social Networking Apps

Chen Shi, PhD\*, Iowa State University, Ames, IA; Yong Guan, PhD, Iowa State University, Ames, IA

**Learning Objective:** The goal of this presentation is to introduce the basics, challenges, and limitations of the current Android™ popular social networking apps such as WhatsApp, WeChat, Telegram, and provide a detailed analysis and results about the forensic artifacts generated and stored by social networking apps.<sup>1-3</sup> In addition, this presentation will elaborate and compare our methodology with existing mobile forensic toolkits and present large-scale experimental evaluation of the approaches to measure the performance and effectiveness of different methodologies. Overall, application developers and researchers will learn how to take advantage of the analysis results of the Android™ social networking apps to quickly locate the forensic artifacts.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how to leverage our approach to automatically discover potential evidence created by third-party private social networking apps, such as WhatsApp, WeChat, Telegram, to help investigators expedite their investigation process and develop new analysis procedures.

This presentation performs a forensic investigation to a set of Android™ social networking applications aiming at discovering sensitive information related to the owner information of the mobile device as well as the user recent activities. These applications were chosen based on the fact that: (1) they are very popular on Google® Play™ Store within the category of social networking, (2) they access and store sensitive personal information, (3) they have been studied before by other forensic toolkits, and (4) they are free to download and install.

The chosen applications are Whatsapp, Snapchat, Telegram, KakaoTalk, Facebook Messenger, Line, WeChat, Instagram, and CoverMe.<sup>1-9</sup> The evaluation of the security of the applications was performed using two techniques: live and offline static analysis. Based on our analysis results, we were able to retrieve a significant amount of information generated by these applications.

In addition, this presentation will demonstrate a set of popular social networking apps, each of which has been download more than one billion times since its first release and has been widely used by Android™ users all over the world. Both static and dynamic program analysis approaches were applied on analyzing the app code as well as the Android™ libraries and retrieving the location of evidentiary data and its corresponding evidence types.<sup>10</sup> We have made several observations from the experimental results. First, all of social networking applications chosen for the experiment access user-sensitive information and more than 40% of the applications write sensitive information to local storage. We discovered some evidentiary data that has not been found in previous studies, such as visited URLs. Another insight is that some of the sensitive information is leaked via other venues, such as being sent over to a third-party server, without writing local storage. Second, compared to the amount of evidentiary data written to database and the local file system, forensic artifacts are more likely to be written into SharedPreferences. Moreover, our approach is more efficient than previous methodologies as it requires no manual operations. On average, it needs just 49 minutes to analyze one application. Furthermore, our approach is effective in locating where forensic artifacts are eventually stored, as we discovered 132 unique file paths and URLs.

### References:

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### Mobile Forensic; Forensic Artifact; Social Networking Apps

## C11 TikTok From an Open-Source Intelligence (OSINT) Perspective: Lessons Learned

*Marcus Rogers, PhD\*, Purdue University, West Lafayette, IN; Nina Matulis, BS, Purdue University, West Lafayette, IN*

**Learning Objective:** After attending this presentation, attendees will have information on the relevant evidence artifacts that can be collected from TikTok accounts. The focus will be on open source intelligence methods that can be used with public TikTok postings from potential targets. Topics covered include verifying the posting's date and time stamps and dealing with deleted or no longer publicly visible postings.

**Impact Statement:** TikTok is now the most popular social media platform, surpassing Facebook®/Meta®. Despite TikTok's worldwide popularity, there are only a few published papers that look at collecting digital evidence from TikTok, especially from an OSINT perspective, as opposed to focusing on the devices that had the TikTok app installed. This presentation will impact the forensic science community by adding to the knowledge base of digital forensics practitioners.

Social media is an increasingly important source of potential evidence for any investigation. Traditionally, digital investigations have focused on devices (e.g., computers, mobile phones) or the network (now including the Internet of Things); however, investigators need to expand their investigations to include evidence that may only exist on social media sites. While there are numerous popular media sites, TikTok has now become the most subscribed social media platform, surpassing Facebook®/Meta®. Despite its popularity, there have only been a handful of papers published that focus on TikTok. The published work on TikTok focuses primarily on evidence artifacts that can be recovered from the TikTok app that has been installed on a mobile device (e.g., tablet, mobile phone). Vendors of digital forensics tools have pretty much ignored processing online TikTok accounts and primarily deal with artifact analysis from the devices the app was installed on as well. However, TikTok can be accessed and accounts viewed outside of the TikTok app using a web browser. Collecting potential evidence from a TikTok account using open source intelligence techniques and open source tools can greatly assist an investigation.

In this presentation, we discuss a bit of the history of TikTok and its parent company ByteDance. We also look at the difference between public data that may be available from a TikTok account and more private data that requires the investigator to authenticate (have an account) on TikTok. This presentation will examine how to view and interpret the page source from TikTok in order to identify the exact date and time the video was posted and processed by TikTok's servers. This presentation will also provide lessons learned from real cases involving TikTok and the use of some open-source OSINT tools that can assist with the identification and processing of forensic evidence from TikTok. We will also discuss some of the issues that an investigator is likely to face when trying to collect and process the evidence obtained from TikTok.

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**TikTok; Open Source Intelligence (OSINT); Digital Evidence**

## C12 The Analysis of Sysdiagnose in iOS® 15 to Identify the Sending Phone Number of AirDrop® Data

Brandon Epstein, MS\*, Middlesex County Prosecutor's Office, New Brunswick, NJ; Benjamin Klein, BA\*, Middlesex County Prosecutor's Office, New Brunswick, NJ; Derek Feuerstein, BS\*, Middlesex County Prosecutor's Office, New Brunswick, NJ

**Learning Objective:** After attending this presentation, attendees will better understand a novel technique to identify the sending phone number of an otherwise anonymous AirDrop® file.

**Impact Statement:** Instances of unwelcome illicit images (cyberflashing) as well general threats shared via AirDrop® are becoming increasingly prevalent in modern society, particularly in mass transit and schools. Due to this method of transmission, the sender can seemingly appear to be anonymous. This presentation will impact the forensic science community by demonstrating a method to uncover the sending phone number of AirDrop® files, which has been previously unavailable to digital forensic examiners.

The Apple® AirDrop® feature is designed to allow users to quickly and easily share content with people nearby in an encrypted manner. AirDrop® does not rely on cellular network or internet connectivity, instead using Apple® Wireless Direct Link (AWDL) to leverage an Apple® device's WiFi chip and a proprietary protocol to communicate directly with other devices.<sup>1</sup> Photos, videos, documents, websites, maps, and virtually any file on an Apple® device can be shared among Apple® devices, including other iPhones®, iPads®, Macs®, or iPod® touches.<sup>2</sup>

Since AirDrop® does not require any network connectivity, it is a fast and convenient way for two (or more) nearby Apple® products to communicate. Furthermore, AirDrop® does not require the user to know the recipient's (or sender's) mobile number or Apple® ID; any available AirDrop® connections will be displayed to all devices in the area. To facilitate this easy transfer method, yet promote privacy, there are device settings that allow for the receipt of AirDrop® files from everyone, contacts only, or receiving off. It should be noted that the setting of "contacts only" requires the receiver to have the sender's Apple® ID or mobile number in order to be available for AirDrop®.<sup>3</sup>

While AirDrop® gives users the ability to easily share files across devices, it has also been used for criminal activity. The nature of allowing any user to transmit files without a network connection has resulted in unwanted images anonymously being sent to devices that allow sharing from "everyone." Most often, this sharing is of unwelcomed sexual imagery, dubbed "Cyber Flashing" but AirDrop® has also recently been used to threaten mass violence, resulting in great public alarm.<sup>4-6</sup> Since AirDrop® does not rely on network connectivity, there is no record of the transmitted data retained by the service provider. Furthermore, if the sending device's contact information is not present in the receiver's device, only a sender's user-defined name is displayed (i.e., "Jane's iPhone" or "Luke Skywalker"). If no name is set on the device, the default identifier to the receiver will simply be "iPhone®."

In this project, we developed a method to gain insights into the device used to send a file via Apple® AirDrop®. Our method brute-forces the partial SHA256 hash entries found in the receiving Apple® device's sysdiagnose logs to reveal the sender's phone number, even if that phone number was not known by the receiving device. This research provides for a method to generate permutations of the partial hash values using potential United States area codes to identify the complete phone number of the sending device. As a result, it was found that the sender of an unknown AirDrop® file's phone number can be identified from the receiving device's sysdiagnose log files.

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### Digital Forensics; Cell Phone Forensics; iOS® Forensics

## C13 Cyber Forensic Investigations of Web3 Cryptocurrency Wallets on iOS® and Android™

*Mohammad Meraj Mirza, MS\*, Purdue University, W Lafayette, IN; Akif Ozer\*, Purdue University, West Lafayette, IN; Umit Karabiyik, PhD, Computer and Information Technology, West Lafayette, IN*

**Learning Objective:** After attending this presentation, attendees will have learned where to find forensically relevant artifacts, have common knowledge about how blockchain works, and how public ledgers can be used to validate and verify recovered digital forensics artifacts. In addition, attendees will learn how Open-Source Intelligence (OSINT) practices can impact Web3 cryptocurrency-related criminal investigations.

**Impact Statement:** This presentation will impact the forensic science community by drawing attention to how blockchain works and how Web3 artifacts found in mobile devices can help digital forensics investigators with their investigations. Furthermore, this presentation will highlight how to conduct an effective cyber forensics investigation of Web3 cryptocurrency wallets installed on Android™ and iOS® operating systems. Finally, this presentation will demonstrate a tool developed as an outcome of the research to help conduct cyber forensics analysis of Web3 cryptocurrency wallets.

The growth of cutting-edge technologies affects our way of life. These technologies include many Blockchain projects vastly implemented into various blockchain-based cryptocurrencies like Bitcoin and Ethereum, as well as Non-Fungible Tokens (NFTs) that are gaining immense popularity, representing a paradigm change in digital interactions and serving as the technology's backbone.<sup>1</sup> According to the Crypto Crime Report, the overall number of cryptocurrency transactions exceeded \$15.5 trillion in 2021.<sup>2</sup> Moreover, in order to store, manage, stack, and perform Web3 transactions, Web3 wallets were created and developed as a solution. Due to the nature of the Web3 wallets, users can create as many Web3 wallets as they want without any need to confirm Personal Identifiable Information (PII). As a result, in 2021, illegal cryptocurrency-related criminal activity exceeded \$14 billion.<sup>2</sup>

As the criminal activities in this field increase, cyber forensics tools either do not have an automated way to extract forensics artifacts from Web3 wallets or check if a Web3 wallet is present in the digital device rather than extracting artifacts. The lack of such support from forensic tools leaves the cyber forensics investigators stranded with a large amount of data. Therefore, the presenters will showcase a tool developed as an outcome of the research intended to help connect and highlight activities and transactions of the studied applications.

Although there are many ways to create a Web3 wallet where users can choose between desktop, mobile, web browser, and hardware wallets, this research focuses on recovering Web3 cryptocurrency forensics artifacts from mobile devices using a combination of open-source and commonly used commercial digital forensics tools; validating and verifying this information using a blockchain public ledger (e.g., Bitcoin and Ethereum); and determine which transactions happened to be performed using the device under investigation. Moreover, cross-checking the public blockchain ledger on transactions recovered from the device can lead to valuable information on the investigated timeline of the case.

The presentation will focus on pointing out how blockchain ledger works, what these new non-traditional Web3 wallet applications are keeping, and what can be retrieved through cyber forensics and OSINT techniques, which will help researchers, tool developers, law enforcement, and the cyber forensics community as a whole. Moreover, focus will also be on demonstrating how to use the developed tool to recover artifacts (e.g., wallet addresses and transactions) automatically, which fills the gap in capabilities of current digital forensics tools. Finally, the presentation will highlight how the use of OSINT-related information helps make notable validated and verified cyber forensics findings, which can help make decisions faster with a higher degree of certainty.

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### Cyber Forensic Investigation; Open-Source Intelligence (OSINT); Web3 Cryptocurrency Wallets

## C14 Cryptographic Techniques in Digital Forensics

Taiwo Ogunseyi, PhD\*, Yibin University, Yibin, Sichuan, CHINA, Yibin, Sichuan; Oluwasola Adedayo, PhD\*, The University of Winnipeg, Winnipeg, MB, CANADA

**Learning Objective:** The goal of this presentation is to discuss some of the current applications of cryptographic techniques in digital forensics as well as the challenges of these techniques in relation to digital forensics.

**Impact Statement:** This presentation will impact the forensic science community by providing insights into advances in the deployment of cryptographic techniques and associated challenges in addressing privacy concerns during forensic investigations. It will facilitate discussion of alternative approaches to enhance their usability in tackling privacy concerns in digital forensics.

The acquisition and analysis of data in digital forensics raises different data privacy challenges. Many existing works on digital forensic readiness discuss what information should be stored and how to collect the data to facilitate investigations. Often, the cost of this readiness directly impacts the privacy of innocent third parties or even that of the suspect if the collected information is irrelevant or the suspect is found innocent. Approaches that have been suggested for privacy-aware digital forensics focus on the use of policy and cryptography-based solutions even though cryptography itself is often considered an anti-forensics technology.<sup>1</sup> Cryptographic techniques such as Homomorphic Encryption (HE), commutative encryption, secret sharing, searchable encryption, and identity-based encryption have been proposed to address issues of data privacy and the ability to analyze data.<sup>2</sup> With the standardization of some of these techniques underway, it is important to evaluate their applicability and challenges in relation to digital forensics processes.

Several studies have utilized these cryptographic techniques for data privacy protection; however, some of the techniques still have drawbacks that prevent their viability for data protection in digital forensics. HE permits computations on encrypted data. The use of HE for data privacy in digital forensics implies that evidential information can be encrypted and analyzed without first decrypting the data.<sup>3</sup> This technique was deployed for secure forensic log management that continuously captures and preserves logs for Internet of Things (IoT) devices in a cloud environment. The proposed system achieves automated log collection and privacy.<sup>4</sup> However, its shortcoming is the large ciphertext generated after encryption, which leads to more investigation costs and time as well as performance complexity. Searchable encryption for privacy-preserving digital forensics allows a case-relevant keywords search over encrypted evidential information, without gaining access to suspect personal information. An application of this technique for email forensics allows an investigator to perform keyword searches on encrypted data and later decrypt the emails matching the search.<sup>5</sup> In addition to the other limitations highlighted in the study, this approach lacks a verifiable method to determine if the keywords searched are case-related or not. Using commutative encryption in a privacy-aware digital forensics model allows both investigator and server administrator to encrypt evidential information using different encryption keys, hence ensuring that an investigator has access to case-related information only and that the relevant data comes from the server without alteration as demonstrated in a cloud forensic investigation study.<sup>6</sup> However, since both investigator and server administrator are required to use different encryption keys, this scheme is less efficient.

For many of the discussed encryption schemes to be a viable approach for privacy-aware digital forensics, we recommend that: (1) only case-related and relevant data should be collected and encrypted to reduce ciphertext size and consequently reduce investigation cost and time; and (2) verification techniques such as the bloom filter or cuckoo filter may be adopted in searchable encryption so that case-related keywords can be verified. In conclusion, the standardization of these encryption schemes will have some implications for digital forensics. This study highlights the current application of cryptographic techniques and their shortcomings from a digital forensics perspective and presents some recommendations to make them viable for data privacy in digital forensics.

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### Cryptographic Techniques; Privacy-Preserving Digital Forensics; Data Privacy



## C15 Leveraging Memory Forensics Artifacts for Android™ Malware Classification

Aisha Ali-Gombe, PhD\*, Louisiana State University, Baton Rouge, LA; Sneha Sudhakaran, PhD, Florida Institute of Technology, West Melbourne, FL; Golden Richard, PhD, Louisiana State University, Baton Rouge, LA

**Learning Objective:** After attending this presentation, attendees will better understand the drawbacks of existing mobile malware analysis techniques. They will also be introduced to memory forensics as an alternative approach for developing effective feature vectors for malware analysis. Attendees will gain an understanding of the feature extraction process for postmortem analysis, malware classification, and prediction using the extracted features. The goal of the presentation is to illustrate how these new memory forensics features can aid in analysis of complex malware.

**Impact Statement:** This presentation will impact the forensic science community by illustrating a new dimension to Android™ malware analysis that utilizes memory forensics artifacts for effective malware classification. We will demonstrate how the reconstructed data from program execution patterns can serve as input feature vectors for neural and non-neural classification algorithms.

Malware analysis, especially on the Android™ platform, is still a pressing and crucial concern to the security community. In today's world, it is still very challenging to accurately classify benign or malicious Android™ applications, despite the tremendous research effort behind developing new introspection techniques.<sup>1</sup> This issue remains mainly unresolved due to over-dependence on static features by malware classifiers. In static feature extraction, malware attributes such as Application Programming Interfaces (API) calls, instruction and instruction sequences, strings, and other metadata and semantic features are extracted to form the corresponding feature vector. Nonetheless, given that most Android™ apps, both malware and benign, heavily utilize obfuscation mechanisms such as encryption, dynamic class loading, and Java reflection, the extracted static features do not form an accurate characterization of the application. In some instances where dynamic features are utilized, the overhead during analysis, the environment setup, and configuration make it infeasible to hook and log all important points within program execution.<sup>2</sup> Thus, the overarching objective of this presentation is to bridge the gap between static and dynamic analysis systems by exploring the use of memory forensics artifacts for effective malware classification. Our proposed research contributions include: (1) data collection, (2) feature engineering, and (3) malware classification and prediction.

We developed an automated tool for Android™ app installation, execution, and memory imaging for data collection called Adbautomate, which was utilized to capture 1,404 memory images from executing 580 malware and 824 benign apps. The extracted memory images are then pre-processed using DroidScraper—a tool for post-execution object allocation recovery and reconstruction.<sup>3</sup> Object allocations are recovered for each app in our dataset to form an execution pattern. Afterward, the patterns are binarized using a sequence dictionary to form corresponding feature vectors. Finally, for classification and prediction, we used Support Vector Machine (SVM), Decision Tree (DT), K-Nearest Neighbors (KNN), and Random Forest (RF) for the non-neural network algorithms and a Convolution Neural Network (CNN) for the neural network algorithm. The selected machine learning algorithms are designed to be diverse in terms of how core features are used for creating classification criteria. Our classification accuracy results are 80%, 80%, 80%, and 68% for SVM, DT, KNN, and RF, respectively. On the other hand, the CNN-based classification achieved a classification accuracy of 92% on the same test data. These results indicate that memory forensics artifacts can provide a new dimension for Android™ malware classification. The post-execution features, which are not impeded by any obfuscation and hooking constraints, provide a more accurate characterization of an app and, thus, are more suitable for classification.

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### Memory Forensics; Android™; Malware



## C16 Going Beyond Forensics With Virtual Private Network (VPN) Application Fingerprinting

*Shinelle Hutchinson\*, Cisco Systems, West Lafayette, IN; Blake Anderson, PhD, Cisco, Chapel Hill, NC; Umit Karabiyik, PhD, Computer and Information Technology, West Lafayette, IN; David McGrew, PhD, Cisco Systems, Poolesville, MD*

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the types and locations of forensically relevant artifacts that can be recovered from Android™-based, Virtual Private Network (VPN) applications. Attendees will also be introduced to a novel method of fingerprinting those VPN applications, which incorporates multiple data sources, including artifacts recovered during the forensic investigation.

**Impact Statement:** This presentation will impact the forensic science community by providing extensive details on forensically investigating VPN applications on Android™ devices with particular focus on users' usage history/behavior, Internet Protocol (IP) tracing, and server details. Due to the increased use of VPN applications in recent years, it has become pertinent to investigate how these applications can help forensic investigators in the field. This presentation will also benefit the forensic science community by demonstrating how security analysts and network security practitioners can leverage artifacts collected from a forensic investigation to improve the detection of cybercriminals using these or similar evasive tools.

VPNs allow users to circumvent online censorship and monitoring by an Internet Service Provider (ISP). In recent years, VPN use has been increasing due primarily to changes in work culture during the COVID-19 pandemic and users' heightened concerns about surveillance and censorship. Millions of persons worldwide rely on VPNs to safeguard their data and activity online. However, cybercriminals also employ VPNs to facilitate their criminal undertakings, including for ransomware campaigns and malware distribution. Despite this continued misuse, there is still a lack of practical research into how law enforcement officers can circumvent or benefit from the traces left behind on devices when a VPN is used.

Despite VPN technology being widely used for over two decades, VPN forensic research is still understudied, especially over the past five years. Alternatively, VPN fingerprinting research has garnered more attention and has been focused on various tasks, including developing methods to help detect malware and to facilitate service disruption (i.e., blocking VPN use). However, there is no previous work that incorporates the forensic analysis findings of these applications to improve the efficacy of VPN fingerprinting.

The focus of this presentation is to highlight the effectiveness and importance of utilizing digital forensics in service of both public and private organizations. To this end, the first half of this presentation provides a walkthrough of a forensic investigation of ten Android™ VPN applications that covers usage behaviors, server identifiers, location services, and other important artifacts. Namely, the VPN apps we investigate are Cyberghost, Express VPN, Nord VPN, Psiphon Pro, Super VPN, Surfshark, Turbo VPN, VyprVPN, X-VPN, and Zenmate. With the ever-increasing use of mobile VPN applications, the forensic findings presented will give digital forensics investigators another source of evidence during an investigation involving these or similar applications. For instance, many of the investigated applications retain logs of all the servers the user would have connected to, along with server details, assigned IP addresses, duration of use, and the user's real physical location at the time. Additionally, insights learned from the forensic investigation, such as details pertaining to all available servers provided by a specific app, are converted to actionable intelligence to aid the development of VPN fingerprinting.

This leads to the focus of the second half of this presentation, which introduces a novel framework for fingerprinting VPN applications. Such a framework would allow network security vendors to identify the tools cybercriminals use to evade detection. The fundamental techniques extract byte sequences from the network traffic to act as an initial fingerprint string that is an index into a database. The destination context, including the destination IP address and port, is then used to identify the most probable process out of all processes associated with the fingerprint string.<sup>1,2</sup> These works are the current state-of-the-art in identifying VPN applications based on their network traffic.<sup>1,2</sup> The limitation of these techniques with respect to more evasive VPNs is that it is difficult to get an accurate picture of all destinations that these applications go to with even large-scale data collection systems. By extracting and incorporating the forensic artifacts from the VPNs, we have developed a method to collect a significantly more detailed view into these applications' behaviors in an efficient way. By augmenting the training data with the ground truth from the forensic artifacts, we can improve the performance of the classifier on new data from the application.

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### VPN Forensics; VPN Fingerprinting; Digital Forensics

## C17 Unlocking the Audit Trail of an Unsupported Programmable Deadbolt Lock

Nicole Odom, MSFS\*, Virginia Department of Forensic Science - DME, Richmond, VA

**Learning Objective:** After attending this presentation, attendees will understand how to recover, interpret, and verify audit logs obtained from a programmable electronic deadbolt lock when the associated native interfacing equipment is no longer available and how this data was able to aid investigators in establishing a timeline of events and associated parties of interest in an arson and homicide investigation.

**Impact Statement:** This presentation will impact the forensic science community by providing techniques for approaching the acquisition and analysis of unknown data sets through the use of the scientific method. Specifically, this presentation will outline the steps taken from initial intake of an “unsupported” device through verification of the data produced.

In early 2022, a Schlage® BE367 iButton® Electronic Deadbolt Lock, which was removed from an arson site, was received by the Virginia Department of Forensic Science in reference to a homicide investigation. This specific offline digital device only operates when a recognized electronic signature is encountered, storing date and time information in the form of an audit trail with up to 1,000 events. Additionally, it is capable of adding up to 1,000 iButton®-credentialed users for controlled access.<sup>1</sup>

Typically, any data programmed for or stored within the BE367 is able to be easily accessed and downloaded by the property manager through use of the Schlage® Utility/Express™ Software and native interfacing hand-held device.<sup>2</sup> However, in this instance, the property manager reported to investigators that they had recently changed the hardware/software utilized for their residences and were, therefore, unable to interface with the device to natively obtain the audit trail. Additionally, upon speaking with support from both the security company and product manufacturer, it was determined that the device is only accessible with the original interfacing equipment/license that was utilized to program the device, rendering the possibility of accessing these logs through either a trial license or loaned hardware specific to the device an unlikely success.

Through in-depth research of the device, a good understanding of how the native logs should be displayed was able to be obtained. Multiple techniques were attempted to acquire data from the device, resulting in a raw data extraction that was initially difficult to interpret. However, by using open-source digital forensic tools to visualize and deconstruct the raw data, the underlying structure was able to be identified and the requested audit trail was able to be produced. Furthermore, the produced data was able to be verified based upon device documentation and known events relayed by the submitting agency.

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### Digital Evidence; Open-Source; Data Verification

### **C18 Digital Evidence From the Dark Net: Technology Gaps and Research Opportunities**

*Martin Novak, MPA\*, National Institute of Justice, Washington, DC*

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**WITHDRAWN**

## C19 Facial Recognition Searches Using Postmortem Images

*Ella Uren, BS\*, Northern Michigan University, Negaunee, MI; Kelcy McArthur, MS\*, Michigan State Police, Dimondale, MI; Krystal Howard, MSW, Michigan State Police, Dimondale, MI; Jennifer Coulson, BA, Michigan State Police, Dimondale, MI; Jane Harris, PhD, Northern Michigan University, Marquette, MI*

**Learning Objective:** After attending this presentation, attendees will better understand how facial recognition software is used to aid in the identification of unknown decedents, including some of the limitations of this type of investigative process. Attendees will also learn about success rates for postmortem image searching.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how far into the postmortem interval investigative leads can be generated and the point at which algorithms employed by the Michigan State Police are no longer useable. The algorithms used by the Michigan State Police are used by many other law enforcement agencies, so even though this particular study is limited in scope, it serves as a case study that is directly applicable to other agencies across the United States.

Investigative leads for aiding in the identification of unidentified human remains are generated by practitioners in the automated facial recognition community. The performance of facial recognition algorithms is highly dependent on the quality of submitted images. The quality of facial images can be affected by numerous factors, including but not limited to: focus, lighting, camera-to-subject distance, subject pose, and the level of detail visible in the facial image. Postmortem changes to the face can have a detrimental effect on the performance of algorithms intended to search the faces of living persons in the various proprietary databases to which law enforcement agencies have access. Even early in the postmortem interval (time since death), the eyes of decedents sink in, pupils may not be visible, muscle tone is lost, and color changes of the skin may mimic facial marks. Later postmortem changes, such as the breakdown of soft tissues or the presence of bloating, can render an individual visually unrecognizable.

Neutral position facial photos were taken throughout the decomposition process of donors at the Northern Michigan University Forensic Research Outdoor Station. These facial images were run through facial recognition software used by the Michigan State Police Statewide Network of Agency Photos (SNAP) Unit. Facial recognition practitioners at the Michigan State Police performed searches of each postmortem photo, ascertained whether or not the software returned any known antemortem images of the subjects, and how far into the gallery of returned candidates the returned images were located. Each image was searched using two different algorithms, which run simultaneously. Individual algorithm performance is not a primary focus of this research; however, there may be evidence to suggest the algorithms perform slightly differently on postmortem facial images.

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### Facial Recognition; Postmortem; Algorithms

## C20 A Likelihood Ratio Approach for Detecting Behavioral Changes in Device Usage Over Time

Rachel Longjohn, MS\*, University of CA, Irvine, Irvine, CA; Padhraic Smyth, PhD, University of California, Irvine, Irvine, CA

**Learning Objective:** After attending this presentation, attendees will better understand a statistical approach for quantifying, from user-generated data, how likely it is that a device (such as a cell phone) was used by a single individual over a period of time or whether the device was used by two individuals, where the time of change between the two individuals is unknown.

**Impact Statement:** This presentation will introduce a statistical methodology for analyzing behavioral event data from digital devices. Quantitative methods for analyzing such data in a forensics context are relatively unexplored, so this presentation will impact the forensic science community by exploring how statistical methods may be applied in digital forensics scenarios in which this kind of data is collected as evidence.

This work focuses on the situation in which investigators have obtained as evidence logs of user-generated activities on a device, such as sending text messages or emails, opening or interacting with mobile apps, or making calls from particular locations. Quantitative methodologies for analyzing this kind of behavioral data from devices could be useful to investigators in a number of situations. For example, if a device is suspected to have not been with the owner during a time period of forensic interest, one could analyze the pattern of events on the device to try to determine if they are consistent with the device owner's behavior, or if there is evidence of a change in behavior.<sup>1</sup> Inconsistency could, for example, indicate that another person was using the device during this time. A time at which there was a change in the patterns of events on the device is referred to as a changepoint.

For this analysis, two different source hypotheses are considered for a given set of user-generated event data: the same-source hypothesis and the different-source hypothesis. The same-source hypothesis assumes that all of the events in the evidence were generated by a single source. Alternatively, the different-source hypothesis posits that the data was generated by two different sources (i.e., a changepoint occurred at some point during the time period over which the device's event data was obtained). The strength of the evidence in support of these hypotheses is reported through a likelihood ratio, which is a statistical method for quantifying the weight of the evidence and has been used in a variety of forensic applications.<sup>2,3</sup>

To arrive at a likelihood ratio, the data are modeled using a Bayesian statistical framework, in which the sequence of events generated on the device is the observed data and the underlying model parameters and the potential time of the changepoint are considered unobserved. It is shown that the proposed model leads to a straightforward formula for the likelihood ratio. This formula is flexible in that it can incorporate pre-existing knowledge about where a changepoint may have taken place (e.g., investigators may suspect a changepoint in a particular time window or feel that a changepoint is more probable within a particular time window compared to another). This formulation generalizes prior work to the practical situation in which the time of change (for the different-source hypothesis) is unknown.<sup>4</sup> The potential usefulness of the proposed method is evaluated through experiments across a combination of simulated data and real-world datasets that are relevant to digital forensics.

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### Likelihood Ratio; Digital Evidence; Changepoint Detection

## C21 Artificial Intelligence Technologies in Age Estimation: The State-of-the-Art

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**Learning Objective:** After attending this presentation, attendees will understand some principles of application of Artificial Intelligence (AI) technologies in human age estimation.

**Impact Statement:** This presentation will impact the forensic science community by highlighting AI systems for human age estimation reported in the literature and their suitability for forensic purposes.

In recent years, AI systems have widely spread in several scientific fields and are now regularly used in medical settings.<sup>1</sup> Besides diagnostic and curative purposes, the application of this technology in the field of medicine has medicolegal and criminalistic implications. In this context, age estimation and, therefore, the determination of certain age limits is a crucial parameter in asylum applications, criminal proceedings, and professional youth sports, and it is required whenever there is a lack of documentation or doubt about the reported age.<sup>2</sup> Moreover, besides its application to living people, age estimation can play a key role in the evaluation of cadavers and human remains. Traditionally, age estimation—both in cadavers and living people—is predominantly performed using the radiological examination of skeletal and dental morphology and development.

For this purpose, according to literature and to the international interdisciplinary Study Group on Forensic Age Diagnostics (AGFAD) and the Forensic Anthropology Society of Europe (FASE) recommendations, human age estimation should require a physical examination of the subject, a radiological examination with the assessment of the morphology and the ossification stage of the non-dominant hand-wrist and of the medial clavicular epiphysis, and a radiological dental examination regarding morphology and development of all tooth types and, in particular, of the third molars with the measurement of their open apices.<sup>3-6</sup>

However, these methods are not exempt of errors due to intrinsic limitations and intra- and inter-observer variability.<sup>7</sup> For example, studies described that the Greulich & Pyle method, which together with the Tanner-Whitehouse is one the most employed methods for the analysis of the radiological examination of the morphology and development of the non-dominant hand-wrist for human age estimation, suffers significantly from intra- and inter-observer variability, which range, respectively, from 0.11 to 0.89 year and from 0.07 to 1.25 years.<sup>8</sup> Recently, in order to reduce these limitations to reliable age assessment, several research groups have studied and developed AI systems dedicated to carrying out this task.<sup>9-11</sup> While these techniques show promising results in estimation accuracy, they also exhibit several shortcomings, including data requirements, limited explainability and sensitivity to biases.

The goal of this presentation is to review and analyze AI systems reported in the literature in order to evaluate their suitability as complementary approaches to traditional methods, towards a more accurate age estimation for forensic medicine purpose.

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### Age Estimation; Artificial Intelligence Technologies; Forensic Investigation

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## C22 Computer Forensics Reference Data Sets (CFReDS) for Digital Evidence

*Rick Ayers, MS, National Institute of Standards and Technology, Frederick, MD; Mehdi Shahid, MS, National Institute of Standards and Technology, Gaithersburg, MD; Barbara Guttman, BA\*, National Institute of Standards and Technology, Gaithersburg, MD*

**Learning Objective:** After attending this presentation, attendees will gain an understanding of the improvements to the CFReDS for digital forensics website.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview on the functionality of CFReDS and the importance of documented sets of simulated digital evidence.

The resources include data sets, tools and test reports that aim to provide the forensic community with documented sets of simulated digital evidence for examination. CFReDS is based upon a taxonomy that provides users with a search functionality narrowing hits specific to a resource of interest. The CFReDS project at the National Institute of Standards and Technology (NIST) will provide users with a portal of resources produced by the Computer Forensic Tool Testing (CFTT) program and other organizations.

Documented sets of digital evidence allow forensic practitioners, researchers, the general public, and others with the ability to validate software tools, insight into a forensic tool's capabilities and limitations as well as training on a specific forensic task. Numerous types of resources exist within the CFReDS repository, for example, scenario, technology, and archived-based data sets that provide users with the ability to gain insight into a digital forensic tool's functionality and perform proficiency testing for various types of digital forensic tools.

The new and improved CFReDS leverages a dynamic and ever-evolving taxonomy that includes nodes (or "tags") that can be used to quickly filter out relevant resources that are usually very information-dense. Using the taxonomy as a building pillar of the application moving forward, CFReDS performs as a full-fledged search engine in the world of digital forensics and also includes test reports and tools, allowing a three-way data correlation and completing the forensics lifecycle.

The goal of CFReDS is aimed at providing the forensic community with a centralized repository portal of digital resources created by numerous worldwide organizations. This centralized portal will provide the forensic community with the ability to quickly find resources of interests within a centralized website.

The presentation gives an overview of CFReDS and the importance of centralized portal sharing resources from numerous organizations across the country.

Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement by the author or the author's employer, nor does it imply that the products are necessarily the best available for the purpose.

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**Computer Forensics Reference Data Sets (CFReDS); Digital Data Sets; Digital Forensics**

## C23 The Discovery of Digital Forensic Dataset Characteristics With CASE-Corpora

*Alex Nelson, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Eoghan Casey, PhD, University of Lausanne, Lausanne, Vaud, SWITZERLAND*

**Learning Objective:** After attending this presentation, attendees will better understand a metadata collection that enumerates publicly available data sets. Attendees will learn of graph-based ontologies that define the data schema used to enumerate the datasets as well as describe why the datasets are potentially relevant to research subjects.

**Impact Statement:** This presentation will impact the forensic science community by providing an aggregation of publicly available data that focuses on a knowledge representation that can be applied to investigations. Patterns of describing and testing ground truth results, using dataset authors' documentation, will raise educational potential for existing datasets and inform the community's abilities in reasoning about digital evidence and chain of custody.

The digital forensics community has generated training and reference data over the course of decades. However, significant challenges persist today in the usage pipeline for that data, from research problem formulation, through discovery of applicable shared data, through local processing and analysis. The problems include classic afflictions of internet resources such as link rot and maintainer departure. Greater challenges remain in covering the gap between research questions in natural language and the structured metadata in available dataset annotations. A dataset that describes a picture sent between two accounts in a chat app entails many levels of pattern abstraction and should be discoverable whether a user is searching datasets for, for example, that app, or for any instance of picture transmission. Yet, disparate datasets today lack a unifying language that can be aggregated and queried in one location.

We present CASE-Corpora, a community index of available forensic reference and training datasets.<sup>1</sup> CASE-Corpora aggregates dataset descriptions into a single ontological knowledge graph. Several ontologies are exercised to enable representation of a dataset, its downloadable resources, and how those resources may have migrated between hosts over time. These are represented in the Data Catalog (DCAT) and Provenance (PROV) Ontologies. However, these ontologies intentionally abstain from rich representation in focused domains such as digital forensics. They can express metadata of where to find data, but not why a forensic analyst would want to find it.

CASE-Corpora describes forensically relevant qualities of datasets by way of the Cyber-investigation Analysis and Standard Expression (CASE) Ontology and the Unified Cyber Ontology (UCO). CASE-Corpora uses all of the above ontologies to describe not only what one would download, but what devices, actions, and environmental captures went into the download; the hashes of both downloadable resources and what would be extracted for analysis, verifying chain of custody; and, where available, ground truth for analytic results' cross-verification. CASE and UCO exercise ontology-level interoperability to expertise and practice with domain-agnostic dataset discovery and provenance review to apply to finely specified forensic detail.

This presentation introduces CASE-Corpora and its support for curation and growth by the community. Data review mechanisms assist with ensuring conformant usage of the employed ontologies. Queries written for the corpora show the richness of what the community has already developed for research, such as what devices have been involved in any dataset. Our experience with developing CASE has repeatedly and consistently shown immense value in defining questions and encoding them as queries. For CASE, this has grown the ontology. For the community, CASE-Corpora can grow our collective knowledge on discoverable and testable patterns in data that many in the community have put effort into making available. This presentation will improve ontological competency, analysis interoperability, and data discovery for the community.

This presentation will cover experiences growing CASE-Corpora since an earlier edition presented at the Digital Forensics Research Workshop in July of 2022. Experiences will have been shaped by encountering potential errors in dataset chain of custody, as well as the CASE version 1.0.0 release that initialized backwards compatibility guarantees until the version 2.0.0 release.

### Reference:

<sup>1</sup>. <https://github.com/casework/CASE-Corpora/>

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### Digital Forensics; Ontology; Dataset

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## C24 Manipulating the National Institute of Standards and Technology (NIST) National Software Reference Library SQLite Metadata Sets

*Douglas White, MS\*, National Institute of Standards and Technology, Gaithersburg, MD; Austin Snelick, BS\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Objective:** After attending this presentation, attendees will have learned new capabilities that are available to investigators who use the new Version 3 SQLite NIST National Software Reference Library (NSRL) metadata sets.

**Impact Statement:** This presentation will impact the forensic science community by calling attention to procedures that may increase speed and efficiency during digital investigations that use the NSRL metadata sets.

The NSRL is transitioning from the current Reference Data Set (RDS) format, RDSv2, to an updated format, known as RDSv3, which will be the only NIST-distributed format beginning March 2023. The RDSv3 format contains a number of major changes, which include publication as an SQLite3 database, the inclusion of SHA-256 hashes for published files, and incremental releases, along with other upgrades.

As a SQLite database publication, the RDSv3 format allows users to more easily manipulate the data published in the RDS, while also including more product and file metadata. The RDSv3 publication also includes a more modern set of hashes, with the publication of SHA-256 file hashes, and the removal of CRC-32 file hashes. The SQLite database schema for the RDSv3 publication is publicly available, along with being included in the distributions published on the NSRL website.

With the RDSv3 publication, it is also possible for users to construct an RDSv2-like publication from data included in the RDSv3 format. The NSRL provides a method for users to convert an RDSv3 publication into an RDSv2-like publication, for those who must use that format in tools that will not support RDSv3.

Other customizations are possible, such as conversion to data sets more amenable to ingest in Project VIC, the Child Abuse Image Database (CAID), Hashkeeper, etc. It is also possible to extend the metadata set with additional tables to accommodate other metadata collection processes. While some familiarity with SQLite will be beneficial, it should not be necessary for attendees or investigators. The NSRL attempts to make the scripts and interfaces to the metadata as straightforward as possible. There are a number of SQLite Graphical User Interface (GUI) tools that can be used to interact with the RDSv3 SQLite database. These tools can work on any Windows®, Mac®, or Linux® operating systems.

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### Software; Metadata; Investigations

## C25 Computer Forensics Tool Testing (CFTT) Cloud Artifact Extraction Tools

*James Lyle, PhD\*, Mount Airy, MD; Richard Ayers, MS, National Institute of Standards and Technology, Gaithersburg, MD; Barbara Guttman, BA, National Institute of Standards and Technology, Gaithersburg, MD*

**Learning Objective:** After attending this presentation, attendees will be aware of what can be learned from testing digital forensic tools for extracting remotely stored artifacts.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of tool test strategies for tools that extract remotely stored application artifacts using cloud account credentials. This presentation will aid the forensic practitioner in recognizing the limitations of testing such forensic tools and being aware of the implications of choices of what to test or not test. The goal of testing forensic tools by a forensic laboratory is not to prove the software is always correct, but to show evidence that the software is appropriate for the task at hand.

The CFTT project at the National Institute of Standards and Technology (NIST) develops methodologies for testing computer forensic tools. Currently there are CFTT methodologies for testing the following:

- Disk imaging
- Write blocking
- Deleted File Recovery
- File Carving
- Forensic Media Preparation
- Mobile Devices
- SQLite Deleted Record Recovery

A variety of tools in each of these categories have been tested, and observed flaws have been reported by the National Institute of Justice (NIJ) and the Department of Homeland Security (DHS). These results can be used as a basis for identifying the types of likely failures that occur in forensic tools.

The newest CFTT digital forensic tool category to be tested is forensics tools that use account credentials to extract remotely stored artifacts. This presentation will describe some of the challenges, the approach developed, and some preliminary results.

Some of the biggest challenges are:

- Deciding on how to limit items tested. Testing extraction of all the artifacts created by all the supported applications is beyond the resources of most forensic labs.
- Creation of test data.

We decided to limit testing to these requirements:

1. Can the tool use account credentials to access the account and extract artifacts?
2. If account access fails, does the tool notify the user?
3. Does the tool render any extracted artifacts accurately and completely?
4. If the tool supports token access to an account, can a token be used to extract artifacts?

We limited testing to several common categories of applications and selected several applications from each category:

- Storage
- Email
- Location
- Productivity
- Social Media and Messaging

Creation of the test data must be done by the same applications that create the artifacts to be extracted. Several cloud accounts must be created and populated by user actions to create the artifacts to be extracted.

We have preliminary test results for three tools that show only minor anomalies.

Certain trade names and company products are mentioned in the text or identified. In no case does such identification imply recommendation or endorsement by the author or the author's employer, nor does it imply that the products are necessarily the best available for the purpose.

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### Forensic Tool Testing; Cloud Forensics; Digital Forensics

## C26 Standards Development Activities in Digital and Multimedia Forensic Sciences

*Tracy Walraven, MFS\*, Wells Fargo, Minneapolis, MN*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of digital and multimedia forensic science.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to digital and multimedia issues. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to digital and multimedia standards development will be discussed. These include: (1) recent standards that have been added to the OSAC Registry; (2) OSAC proposed standards; (3) published standards from the American Society for Testing and Materials (ASTM) and other authoritative bodies that have yet to go through the Registry approval process or are currently undergoing the approval process; (4) priorities for new documents or work products, and other highlights; and (5) projects undertaken by working groups within the digital evidence subcommittee, specifically the Quality Study Working Group's project entitled "Quality Management in Digital Evidence Examination" that interviews various members of the Digital & Multimedia Evidence (DME) and legal community to determine which elements of a quality system are most effective in digital forensics. 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.

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**Standards; Digital Evidence; Digital Forensics**

## C27 Escaping the Monolithic Architecture for Digital Forensics and Incident Response Systems Using a Microservices Approach

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**Learning Objective:** After attending this presentation, attendees will discover and understand how the use of microservices architecture can help to enhance data analytics tools and machine learning to assist with the processing of large volumes of structured, unstructured, and varied data types for Digital Forensics and Incident Response (DFIR) systems.

**Impact Statement:** This presentation will impact the forensic science community by enhancing the knowledge of how microservices and distributed systems function to aid in providing DFIR for big data, which can help provide responsive investigations. Moreover, this presentation will demonstrate how microservices architecture can effectively help DFIR developers for better and more robust system development.

Microservices architecture has gained popularity among innovative distributed systems and application designs. According to a recent survey, 85% of large firms and organizations have started to use microservices as it has become increasingly important in the operations of larger companies.<sup>1</sup> The nature of microservices architecture enables numerous services to be built independently without interfering due to the scalable design, which leads each service to be independently deployed.<sup>2</sup> This provides better failure tolerance than a traditional monolithic design. Furthermore, since the critical operations of the backend system can be isolated into their service code, the subsystems can be scaled independently. These capabilities and features of microservice architectures could be indispensable for the DFIR community by increasing the efficiency of digital forensics systems, hence decreasing the time taken for these processes during big data forensics investigations.

In this research, we aimed to ease the new features' implementation by applying the needed functionality to the back end of a newly developed forensics tool independently while being able to load balance each process efficiently. Moreover, investigators no longer need to wait while the system finishes data ingestion where the design makes the data available as soon as it finishes processing in real-time. This can help significantly speed up the investigation timeline. Furthermore, the proposed system uses event streaming and parallelization to split up the task processing and gain significant performance boosts. This presentation discusses a case study that demonstrates the abovementioned contributions, which are meant to assist digital investigators in dealing with the difficulties of big data forensics.

In addition, this presentation aims to provide the foundations of microservices architecture and design suggestions that can be carried out on different scales and are not reliant on any stack of technology from both technical and non-technical perspectives. Although employing microservices can avoid the pitfalls of monolithic design, sometimes it is not suitable and not the best option to go with. Therefore, this presentation will provide help to determine when to use or avoid the use of microservices for big data forensics by discussing the drawbacks and benefits of each design. Finally, the presenters will provide microservices incremental adoption strategies that will assist those in leadership positions in the industry, system architects, and experienced developers interested in gaining knowledge on how to establish and construct a microservice architecture for DFIR systems.

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### Big Data Forensics; Digital Forensics and Incident Response Systems; Microservices Architecture



## C28 Disambiguating “Ground Truth” for Forensic Applications in Artificial Intelligence

Cristina Aggazzotti, PhD\*, University of Quebec at Montreal, Meridian, ID; E. Allyn Smith, PhD\*, University of Quebec at Montreal, Montreal, QC, CANADA

**Learning Objective:** After attending this presentation, attendees will better understand what ground truth data is, why it is important, how its understanding differs by community, and what some of the challenges are for obtaining it within the subfield of forensic linguistics.

**Impact Statement:** This presentation will impact the forensic science community by clarifying crucial terminology and calling attention to an important, but often overlooked, challenge to conducting any kind of scientific forensic examination.

“Ground truth” refers to a fundamental truth, or in regard to data, to “the real or underlying facts; information that has been checked or facts that have been collected at source.”<sup>1</sup> We present the evolution of this concept and its more specific definition in several domains, including the following two. In the field of forensic linguistics, ground truth data is verified, accurate, and known data, often used for testing methods. Meanwhile, in Machine Learning ([ML], a leading technique in artificial intelligence), ground truth refers to any labeled data that can be used to train supervised models, but the labeled data are often an estimation of the truth and not necessarily accurate or verified.<sup>2</sup>

To better understand just how different these perceptions of ground truth can be, consider the following four scenarios of collecting deception detection data to test whether a change in pitch indicates lying.

1. There are naturally occurring recordings of people speaking, who have both lied and told the truth and can tell us which of their utterances were true and which were false. Their data are labeled accordingly.
2. We as researchers bring people into the lab and ask them to say true and false things, which we record. The speakers then tell us which of their utterances were true and which were false, with their data labeled accordingly.
3. We as researchers have a corpus of recorded conversations and we ask people who were not involved in those conversations to judge whether someone is telling the truth or not as they listen to the conversations. We then label the data based on the majority opinion of these participants, sometimes called “human annotators.” That is, if three out of four annotators think a sentence sounds false, it is labeled “false.”
4. We as researchers have a corpus of recorded conversations, and we create an algorithm to “learn” (whether via supervised or unsupervised learning) to label data as true or false, though we may or may not know what information it is using to make that decision. (Is it fact-checking via internet sources? Picking up on differences in sentence structure or pitch? Other?)

In all of these scenarios, we would then use the human-generated or automatically generated labels to train a system that will tell us whether there is a significant pitch difference between the labeled-as-true data and the labeled-as-false data. ML’s definition of “ground truth” would consider all four examples as “ground truth.” For most forensic scientists and linguists, however, only #1 and possibly #2 (if #1 is not possible, which we will discuss) would allow us to meet the ethical standards of our work, as has been argued successfully for a number of fields (e.g., for forensic linguistics).<sup>3-5</sup> We will also discuss other scenarios to help tease apart the nuances of ground truth.

In the world of big data and artificial intelligence that increasingly intersects with the forensic workflow, it is thus important to clarify and define terminology to facilitate collaboration and communication between fields. Agreeing on a definition is merely one of the challenges to obtaining and working with ground truth data; others include the costs of collecting direct measurements, the exploitation of loopholes (e.g., obtaining data from places with fewer privacy policies in place), and a lack of education and training on understanding data, its uses, and its limitations.<sup>6</sup> We will discuss these challenges and present several concrete best practices, such as writing good data usage statements, that can be immediately deployed in your work to avoid some of these pitfalls.

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### Ground Truth; Data Quality; Artificial Intelligence

## C29 Comparing Defense Attorneys' and Prosecutors' Knowledge of Digital Evidence

*Danielle Crimmins, PhD\*, University of Baltimore, Baltimore, MD; Kathryn Seigfried-Spellar, PhD, Purdue University, West Lafayette, IN; Marcus Rogers, PhD, Purdue University, West Lafayette, IN*

**Learning Objective:** After attending this presentation, attendees will understand: (1) the difference in attitudes toward and knowledge of digital evidence between defense attorneys and prosecutors; and (2) future research to advance the understanding of digital evidence in the courts.

**Impact Statement:** This presentation will impact the forensic science community by: (1) addressing the differences in attitudes toward digital evidence among defense attorneys and prosecutors; (2) comparing the difference in knowledge of digital evidence between defense attorneys and prosecutors; and (3) discussing future research to advance the understanding of digital evidence in the courts.

Researchers expect that all criminal investigations will eventually include some form of digital evidence.<sup>1-3</sup> Although digital evidence may be a vital part of a case, it is difficult to present this highly technical and ever-changing process to novice individuals. Therefore, it is important to determine how courtroom actors, which include lawyers, judges, and the jury, understand digital evidence. The goal of this mixed-method study was to investigate lawyers', both prosecutors and defense attorneys, attitudes toward and knowledge of digital evidence in criminal court cases.

The current study followed an explanatory sequential design, which included two phases. For Phase 1, snowball sampling was used to solicit participation in the online, anonymous survey, which consisted of questionnaires and open-ended questions. The final sample for Phase 1 included 11 prosecutors and 5 defense attorneys. Overall, results indicated prosecutors have a higher opinion of digital evidence. Emerging themes from the open-ended questions suggested a lack of understanding of digital evidence by lawyers and judges and prevalence of digital evidence with regard to type of data and amount of data. Driven by the emerging themes, the goal of Phase 2 was to further explore and explain the emerging themes identified in Phase 1. Phase 2 used purposeful sampling to recruit four lawyers (two prosecutors and two defense attorneys) who had experience with digital evidence. Results from the interviews confirmed findings from Phase 1 regarding the prevalence of digital evidence in court cases and a lack of understanding of digital evidence. In addition, a lack of resources was identified, which included digital evidence training for lawyers and judges. The current study also suggested that lawyers were concerned the jury would not understand and/or would put too much weight on digital evidence during trial.

Limitations of the current study, specifically with regard to the sample size, will be discussed. In addition, recent cases involving digital evidence, such as the Rittenhouse case, will be discussed as well as future research directions.

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### Digital Evidence; Lawyers; Jury

## C30 The Possibilities of Artificial Intelligence in Digital and Multimedia Sciences AI4Forensics

Zeno Geradts, PhD\*, Netherlands Forensic Institute, Den Haag, Zuid-Holland, NETHERLANDS

**Learning Objective:** Artificial Intelligence (AI) is used in digital and multimedia forensics products. With the use deep learning is important to have an expert in the loop. After attending this presentation, attendees will be aware of the limitations and the practical use of artificial intelligence.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that AI can be used in forensic science, though training and knowledge of how it is used is important.

In many software packages in digital and multimedia science, AI is included.<sup>1</sup> The use of deep learning is important since we can have new insights in data as such, which may not have been seen before. In this presentation, we will look at the different practical examples of the use of AI:

- deepfakes
- activity based on data from mobile devices and wearables
- stego analysis
- speaker recognition
- face comparison

With deepfakes, the deepfake itself is often produced with AI and deep learning.<sup>2</sup> The way to detect these deep fakes can be done with AI, in combination with video manipulation detection methods, such as the Photo Response Uniformity, Electric Network Frequency, and manual methods, as well as with facial comparison.

More research is needed to extract data from fitness trackers and mobile phones. AI is often used to analyze the data and can also be used for forensic use.

Research is also in progress on stego detection methods and unknown stego methods, so that new algorithms can be implemented in the detection software.

Also with speaker analysis software in combination with location information and other data, the use of algorithms is important, and of course here also the interaction with the expert is important.

Finally, face comparison algorithms were much improved due to deep learning algorithms, which are often used for re-identification and can also be used in combination with the human expert.<sup>3</sup> These can all be used in combination with a likelihood ratio, in combination with calibration.

New fields emerge with explainableAI, where the definition is not always the same. In court, we need to explain how the algorithm works, and since it has many layers, the generic method can be explained and visualized as such, through a complete understanding of the algorithms. Based on the examples, we provide a practical way to use AI in court, with the limitations that algorithms have, such as bias based on training sets. The Netherlands Forensic Institute and the University of Amsterdam have established a joint AI laboratory, AI4Forensics, for researching these aspects further, with five PhD students to start.

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### Deep Learning; Artificial Intelligence; Digital and Multimedia Forensic Sciences

### **C31 The Implementation and Testing of Triplet Convolutional Neural Networks for Forensic Image Matching: Phase I—Setting Up the Algorithm**

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**WITHDRAWN**

## C32 A Universal Disaster Victim Identification Digital Tool for the Management of Fragmented Cross-Jurisdictional Missing Persons' Lists and Antemortem Data in Mass Disasters With Multinational Victims

*Shada Alsalamah, PhD\*, King Saud University, Riyadh, Ar Riyad, SAUDI ARABIA; Afrah Alotaibi, MSc, King Saud University, Riyadh, Ar Riyad, SAUDI ARABIA; Sakher Al-Qahtani, PhD\*, King Saud University, Riyadh, Ar Riyad, SAUDI ARABIA*

**Learning Objective:** After attending this presentation, attendees will better understand the potential value of integrating blockchain technology into the field of forensic science by managing missing persons' lists in mass disasters with multinational victims, aggregating fragmented Antemortem (AM) data across jurisdictions in an immutable distributed ledger, and distributing it among international Disaster Victim Identification (DVI) teams from all around the world in a secure and timelier manner than traditional methods. This should allow the collection of crucial decentralized information and facilitate seamless access to it for global victims' identification.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how blockchain technology adoption has the potential of addressing key challenges international DVI teams face in mass disasters with multinational victims. This is achieved by equipping such teams with a universal, trustworthy digital tool that can be utilized to assist in the identification of global victims more effectively, securely, accurately, and in a timely manner.

Disasters are difficult to predict, and, in most cases, they cannot be prevented. Although the number of casualties involved varies depending on the disaster's type and location, such disasters leave behind thousands of victims from many nations, making the process of identifying multinational victims a challenging task for the international DVI teams. Collecting AM data records from multiple nations is the key requirement in the reconciliation process with Postmortem (PM) data. However, forensic scientists face key issues when dealing with missing persons in mass disasters involving cross-jurisdictional multinational victims, which can hamper the identification process. First, the number of missing persons' reports during mass disasters usually exceeds the actual number of victims, which makes responding to such an overwhelming volume of reports a major problem facing police organizations and contributes to the complexity of missing persons' management. Second, managing missing persons' lists containing years, and sometimes decades, of overwhelming family AM data resources' updates is significantly challenging. Finally, collected victims' AM data is traditionally limited to a specific jurisdiction where the reporter lives. Therefore, it is normally stored in a siloed police department's information system so that no one can access it without privileges. This results in fragmented lists of missing persons across multiple discrete systems making it extremely challenging for the police to have a holistic view needed to provide forensic experts with the required information in a timely manner. The above challenges hinder the DVI's reconciliation process and can result in inadvertently irreconcilable discrepancies.

This project proposes a universal DVI digital tool that, first, allows family members to remotely report missing persons through a web-based application in an effective manner for better management of missing persons' reports and AM data collection. Second, it creates an immutable distributed ledger, where a record of each victim related to a disaster and reported missing along with their collected AM data is added to the ledger with a timestamp. Finally, this tool distributes this digital ledger among international DVI teams across jurisdictions to grant them instant access to such details and allows them to add further updates related to any particular victim in a new timestamped record. This tool should grant authorized DVI teams from all around the world seamless access to up-to-date AM records at any time and from any place. The key goal is to build the right digital tool for DVI teams to identify global victims accurately and in a timely manner and, by creating this universal tool, preserve their human rights along with their families.

### Multinational Victims; Disaster Victim Identification; Blockchain Technology

## C33 Augmented Reality Applications for Craniofacial Reconstruction and Fracture Margin Assessment in Forensic Anthropology

*Katharine Kolpan, PhD\*, University of Idaho, Moscow, ID; Jeffrey Vadala, PhD, Penn VR Neurology Laboratory, Philadelphia, PA*

**Learning Objective:** After attending this presentation, attendees will have gained a better understanding of how augmented reality application technology can be utilized in craniofacial reconstruction and assessment of the fracture margin.

**Impact Statement:** This presentation will impact the forensic science community by highlighting how virtual reality and augmented reality technology can benefit forensic anthropologists in their efforts to assess and reconstruct craniofacial fracturing with minimal manipulation and damage to the fractured bones themselves.

One of the difficulties of assessing bone fractures is that overmanipulation of fragile and/or highly fragmentary skeletal elements can lead to further damage. That damage can alter the fracture margin, making it difficult, if not impossible, to accurately reconstruct or assess fracture patterning. Therefore, in many cases, it is to the forensic anthropologist's advantage to spend a minimal amount of time manually manipulating these fragmented bony elements.

This presentation examines how augmented reality application technology originally designed for cranial-facial surgical procedures also provides forensic anthropology practitioners with a means of rendering and manipulating fractured bones in three-dimensional space, while ensuring that they cause minimal damage to the fracture margin. To do this, an image, such as a radiograph, of each skeletal element is visualized by a HoloLens™, a type of wearable smart headset, using open-source software, such as 3D Slicer, that converts it into a three-dimensional mesh where it is then rendered in Unreal Engine™. In Unreal Engine™, a holographic field is produced wherein holograms can be manipulated in the same manner as if one were holding the physical bony elements in one's hands. Thus, one of the advantages of this type of computer technology is that it allows the user to use naturalistic hand gestures to manipulate the elements, which is not always the case when working with 3D technology. Another benefit is the rendered 3D collision mesh also accounts for the weight of the element by itself and in relation other bony elements, while considering the physics of the skeletal elements as well as the user's hands. Additionally, employing augmented reality allows the researcher to make side-by-side comparisons of real-life objects and holograms, meaning the practitioner can toggle back and forth between the rendered holograms and bony elements if necessary.

While this can be done with any skeletal element, the focus here is on craniofacial fracturing and how the 3D collision mesh can be used to create greater accuracy in refitting and assessment of craniofacial fracture margins themselves. This is useful if the practitioner does not wish to reconstruct the physical remains for fear of causing further damage. However, it is also possible to lock these holographic objects in space, mark the rendered holograms with tags, and draw on the holographic image, all features that would allow a practitioner to practice and/or create an advanced plan if skeletal reconstruction is necessary.

Furthermore, this technology can also be utilized on intact experiment skulls to assess how the force from certain kinds of blunt force, sharp force, and ballistic trauma impacts the skeleton. This creates the potential for certain kinds of predictive modeling about how specific types of traumatic injuries, such a hammer blows or knife wounds, will impact the bone, perhaps in ways that have not already been encountered based on forensic anthropologists' experiences assessing the remains of individuals who have already been subject to these forms of trauma.

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### Augmented Reality; Bone Fracture; Forensic Anthropology



## C34 Potentialities of the Anatomage Table in Forensic Training and Education Programs

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**Learning Objective:** After attending this presentation, attendees will have learned concepts and foundations of the Anatomage Table and its relevance not only for the human anatomy teaching but also for the virtual human dissection in forensic pathology and odontology training course.

**Impact Statement:** This presentation will impact the forensic science community by helping attendees learn the use of virtual and augmented reality to familiarize themselves with postmortem procedures and collection of autoptic parameters.

The Anatomage is a modern digital tool for studying human and animal anatomy using life-sized 3D reconstructions obtained from segmentation of multimodal images (Computed Tomography [CT] scans, Magnetic Resonance Imaging [MRI], photogrammetry).<sup>1</sup> The Anatomage Table is a life-size 3D intuitive touchscreen that allows virtual dissection, interactions, and control features, including the turning on and off of selected structures categorized on various cadaver models as well as real size measurements. Users learn human anatomy and can virtually dissect or observe selected structures by applying arbitrary clipping planes on all directions required. Cases Library contains an extensive collection of CT and MRI scans, including 4D scans showing changes over time. Forensic training programs for pathologists and odontologists include autopsy training sessions on cadavers to learn how to assess the cause of death, collect data for human identification, and interpret radiological scans.

Human anatomy is a fundamental element in the process of teaching and training programs, which requires the availability of cadavers.<sup>2,3</sup> In certain countries, this training may be challenging given the limited availability of cadaver lab programs and the cost of such training. Following the COVID-19 pandemic, it has become increasingly common to study using online tools and virtual or augmented reality.<sup>4,5</sup> The Anatomage Table can mark a turning point in the learning process of postmortem data and radiological data collection and analysis for the purpose of teaching autopsy procedures and standardized collection of autoptic parameters as well as to familiarize oneself with radiological images. The Anatomage virtual dissection should be considered an adjunct training tool as it cannot replace the *in situ* examination of cadavers of the forensic training program, but it is an effective tool in forensic pathology and odontology education and training. This tool could also be used remotely for real forensic casework or to request a second expert opinion.

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### Anatomage; Education; Virtual Dissection

## D1 The Design of a Centrifugal Microfluidic Disc for the Deamination of Cytosines for Forensic Epigenetics

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**Learning Objective:** After attending this presentation, attendees will understand a mechanism by which microfluidics can be utilized for investigative forensic DNA analysis; here, we demonstrate the design process of a centrifugal device for the deamination of cytosines using sodium bisulfite conversion in preparation for forensic epigenetic analysis.

**Impact Statement:** This presentation will impact the forensic science community by introducing an analytical tool for downstream forensic epigenetic analysis that utilizes small input sample volumes.

Within recent years, the forensic community has become more familiar with integrated rapid DNA tools, including the ANDE™ and RapidHIT® ID systems.<sup>1,2</sup> These types of microfluidic tools have entered the marketplace with the purpose of providing a fully integrated mode to streamline forensic DNA analysis through automation, decrease testing intervals, and minimize contamination. Developing microfluidic tools for investigative purposes in particular, requires further expansion into areas beyond STR profiling. In particular, the determination of human chorological age via epigenetics is one area that may benefit from microfluidic intervention. Currently, sample preparation for this type of analysis goes beyond DNA extraction to include a process called bisulfite conversion (BSC), in which unmethylated cytosines are converted to uracil to provide the potential for further genetic interpretation. Current BSC methods, however, are known to cause DNA degradation, are time consuming and labor intensive, and pose high risks to possible human contamination due to various open-tube procedures.<sup>3,4</sup>

The aim of this work is to design and optimize a centrifugal microfluidic tool to be used for epigenetic sample preparation prior to downstream analysis for forensic human investigations. Utilizing microfluidic tools for forensic analysis has many advantages. Sample volumes, and in turn the amount of input DNA required, are significantly decreased. Reagent costs required for sample preparation and incubation times are also reduced. In addition, the cost effectiveness of the disc fabrication, shortened analysis time, and lower vulnerability to human contamination make microfluidics an increasingly popular commodity within the forensics community. The integrated, on-disc features allow for bisulfite conversion chemistry and sequential unit operations to be fully automated, without requiring human interference. It also allows for the simultaneous conversion of four independent samples within a singular disc.

This presentation will take attendees through the design and optimization of microfluidic features via software and fluid studies performed with engineered systems for streamlined sample preparation. Microfluidic architecture was designed using AutoCAD software and tested with numerous aqueous dye studies in conjunction with objective image analysis software. Dye studies were conducted for the integration of sequential unit operations, including: loading dye into wash buffer chambers, opening and closing channels within the disc using a laser, and magnetically mixing silica magnetic beads using a dynamic solid phase extraction system (dSPE). A heating element enabled the testing of heated incubations on disc to prepare for long conversion parameters. Objective image analysis was conducted using Fiji ImageJ software to quantify fluid loss on the disc. Engineering software and mechatronic hardware was used in concert to interface with the disc and allow for automation. Early dye studies were integrated to show proof-of-concept for bisulfite conversion chemistry.

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### Microfluidics; Forensic Epigenetics; Forensic DNA Analysis

## D2 A Preliminary Machine Learning Study for Short Tandem Repeats (STRs) Profile Completion

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**Learning Objective:** After attending this presentation, attendees will understand the principles behind a machine learning technique known as Artificial Neural Networks (ANNs) and the necessary elements for their application in forensic investigations to support the completion of STR profiles obtained from DNA evidence collected at the crime scene. The completed DNA profiles could be used to confirm or refuse the presence of a person on a crime scene.

**Impact Statement:** This presentation will have a strong impact on the forensic science community, as it can augment traditional means of investigation by providing an automatic intelligent tool for supporting DNA analysis and the evaluation of investigation hypotheses.

ANNs are biologically inspired computer programs designed to simulate the way in which the human brain processes information. An ANN is formed from hundreds of single units, artificial neurons, connected with coefficients (weights), which constitute the neural structure and are organized in layers. The weights are the adjustable parameters of the network of neurons. During training, neurons are optimized until the error in predictions is minimized and the network reaches the specified level of accuracy. Once the network is trained and tested it can be given new input information to predict the output. Currently, ANNs are used for different goals in forensic science such as blood stain pattern analysis classification, face recognition, DNA methylation-based forensic age prediction, and so on.<sup>1-3</sup> However, no research has yet explored the potential of ANNs solving the completion of an STR profile found on a crime scene sample.

This report will present how it is possible to use ANNs to learn the relation between a partial human STR DNA profile and the corresponding reference profile. To achieve this goal, a set of partial DNA profiles obtained from the reference profile is necessary. In an entrance-controlled sterile room, a total of eight samples (6.6pg/ul) were obtained by AmpF(STR)<sup>™</sup> DNA Control 007's serial. Sample concentrations were confirmed by using Quantifiler<sup>®</sup> Trio DNA Quantification kit and PowerQuant<sup>®</sup> System kit. Quantifications were conducted, in duplicate, in an Applied Biosystems<sup>®</sup> 7500 Real-Time PCR System according to the manufacturer's instructions.<sup>4,5</sup> Samples were amplified in duplicate (N=16) by GlobalFiler<sup>™</sup> PCR Amplification Kit following manufacturer's protocol.<sup>6</sup> A partial STR profile was generated for each analyzed sample by The Applied Biosystems<sup>™</sup> 3500 Genetic Analyzer. Genotyping analysis was conducted by GeneMapper 1.4 IDX software. Since ANNs require a large number of partial DNA profiles to be trained, the 16 partial profiles were used to understand stochastic errors, necessary to produce a software's informatic rules and to generate several *in silico* partial profiles. In detail, to achieve this goal, software capable of applying the computed rules and generating a sufficient number of DNA partial profiles was designed and developed in Python language. Once an ANN is trained, by considering the computed set of partial DNA profiles, it can recognize if a partial DNA profile belongs to the given reference DNA profile. The proposed methodology has been evaluated in a preliminary experimental session involving only one reference STR DNA profile. The preliminary results showed that ANNs are a promising technique, supporting the STR profile completion by achieving high accuracy values. In the future, ANNs must be evaluated by using different reference STR DNA profiles. Moreover, a wider laboratory experimentation must be carried out to verify the rules used to generate *in silico* DNA partial profiles.

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### Investigations; Artificial Neural Networks; Partial DNA Profile

## D3 Temperature Profiling and Quantitative Analysis of Disperse Dyes in Polyester Via Direct Analysis in Real-Time Mass Spectrometry (DART®-MS)

Zoe Millbern, BS\*, North Carolina State University, Raleigh, NC; Nelson Vinueza, PhD, North Carolina State University, Raleigh, NC

**Learning Objective:** This presentation will provide attendees with more detailed information about the mass spectral analysis of mixtures of synthetic dyes in fibers. Quantitative analysis of dye concentration in potential textile trace evidence using DART®-MS will be expanded upon, as well as sample size limitations, and potential method considerations. The effect of structural composition on ionization behavior at differing temperatures will also be observed and compared with the resultant mass spectra of a mixture of dyes to provide more detailed analyses.

**Impact Statement:** Currently, very little has been done concerning the quantitation of colorants in general using DART®-MS. The determination of dye temperature profiles and ionization behavior will allow for a more accurate and detailed analysis of color mixtures in synthetic fibers and solutions. These profiles may also be applicable to other forms of fiber and colorant analysis, as temperature is a key parameter in several analytical methods.

This presentation will add detail and accuracy to the *in situ* analysis of mixtures of disperse dyes in *in situ* synthetic fibers via high-resolution mass spectrometry. To determine the effect of structural characteristics of disperse dyes on the optimum ionization temperature via DART®, the temperature profiles of several disperse dyes will be evaluated. DART®-MS is not only relatively non-destructive, but also rapid and requires less sample preparation and personnel training.<sup>1-5</sup> Following an in-lab-generated methodology for the analysis of disperse dyes in polyester fibers and spiked dye solutions, the average abundance per set concentration present in the medium will be compared across different temperature set points.

Synthetic fibers are widely prevalent, composing garments, furnishings, and other products, so the chance they will be present at or transferred to a scene and require analysis is high. Additionally, the products of these fibers that will be found are generally colored, often using a mixture of dyes to generate a target shade. The most commonly used dye classes for synthetic fibers are acid and disperse dyes, with disperse dyes used for the majority of synthetics such as polyester and other hydrophobic fibers. The DART® ionization source involves a heated stream of metastable helium gas flowing over the sample, ionizing it, and carrying ions into the mass spectrometer interface. This ionization method is particularly applicable to the evaluation of polyester and disperse dyes due to the physical entrapment of small, relatively inert dye molecules within the structure of the polymer as opposed to chemical attachment seen with acid, direct, and fiber reactive dyes to their target fibers.

During the initial method development, analysis of fibers colored with a mixture of dyes in some cases showed an abundance ratio of dye molecular ions that did not match well with the applied proportions. The temperature profiles generated could provide an explanation for inconsistencies in the relative abundance of one dye to another in a mixture when implementing the direct analysis of fibers if one dye is better ionized at a lower temperature than another. Sets of data summarizing concentration and temperature vs. abundance will also provide better limits of detection and potential quantitation for the method previously developed. Profiles for ten disperse dyes with structural differences (differing chromophores, molecular weights, and major structural components) will be compared for ionization behavior and response. An initial series of tests involving calibration series of concentrations of dye solutions with a common internal standard between dye sets will be run using DART®-MS at varying temperatures, and the average relative abundance of each will be incorporated into characterization profiles. Following the characterization of each dye in solution, a similar temperature and quantitation profile will be generated from polyester fiber samples dyed at varying concentrations. Finally, the generated profiles will be compared with the mass spectra of fabrics dyed with multiple dyes at varying concentrations to determine whether a correlation can be positively drawn between the observed and expected abundance of each.

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### Direct Analysis in Real Time (DART®); Synthetic Dyes; Fiber Analysis

## D4 A Study on the Application of a Human Model for the Analysis of a Fall From Height

*Chan-Seong Park, PhD\*, Division of Forensic Safety, Wonju-si, Kangwon-do, SOUTH KOREA*

**Learning Objective:** After attending this presentation, attendees will understand the necessity of a human model in a biomechanical analysis of falls from height.

**Impact Statement:** This presentation will impact the forensic science community by serving as a key aspect of fall from height investigation as it can augment traditional means of investigation in a systematized format via an interdisciplinary approach with communication and collaboration involving fields such as forensic medicine and forensic engineering.

In general, a fall is analyzed to obtain the initial conditions of the victim such as posture and velocity. The information of fall height, distance from the fall, and bodily injuries from an autopsy is also considered for reconstruction of the fall. However, there are difficulties in comparing bodily injuries identified through an autopsy with simulation results because the multibody models of fall simulation programs such as PC-Crash and MADYMO cannot analyze bodily injuries such as skull damages, organ damage, and fractures. This presentation introduces one case report of a fall from height where the THUMS human model was used for an analysis to study the possible explanations of body injuries from the fall. The accident and the analysis method can be briefly described as follows. The victim fell from a height of 21m, and the posture impacting the ground was recorded by CCTV. An autopsy of the victim was performed. The overall fall process was analyzed using the PC-Crash 13.0 Multibody model and compared with the ground impacting posture shown on the CCTV. The moment when the victim impacts the ground was also modeled by a human model, THUMS, and simulated thereafter by the LS-Dyna program with a comparison of the postures shown on the CCTV. The bodily injuries from the THUMS simulation results were compared with those identified in the autopsy.

From the investigation, we concluded that the overall reconstructed falling process using the PC-Crash multibody model satisfies the given information such as the image shown on CCTV when the victim fell backward. The THUMS human model successfully simulated the bodily injuries in comparison with the autopsy results. The effectiveness of a human model can be established when many cases of falls from height with comprehensive information such as autopsy results and CCTV is analyzed. The National Forensic Service in Korea has divisions of forensic medicine and forensic engineering, and thus we plan to study the effectiveness of the human model for falls from height via interdisciplinary communication and collaboration.

### Fall From Height; Human Model; THUMS



## D5 The Differential Performance of Dynamic Vapor Microextraction for Recovery of Ignitable Liquid Compounds Investigated Within a Sensitivity Analysis

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**Learning Objective:** Attendees will learn how sensitivity analysis can be used to quantify the performance of Dynamic Vapor Microextraction (DVME), an emerging fire debris extraction and concentration technique, and how the covariance mapping metric can detect the differential influence of instrument settings on the recovery of different chemical classes found within gasoline.

**Impact Statement:** This presentation will impact the forensic scientific community by describing metrics to quantitatively evaluate emerging methods for headspace concentration of Ignitable Liquids (ILs) from fire debris. Covariance mapping between recovered eluates and reference samples will be compared to peak ratios utilized in sufficiency graphs. Exploring the differential performance of DVME for different chemical classes within weathered gasoline provides information for instrument optimization.

The detection of IL from structural fires is typically investigated through headspace concentration methods. The Activated Carbon Strip (ACS) method is the most commonly used technique in the United States but requires the use of a neurotoxic elution solvent and results in vapor distortion in some circumstances.<sup>1,2</sup> In DVME, a potential alternative, a carrier gas pushes vapors from the container through a capillary vapor trap coated in adsorbent. While DVME was originally developed to identify vapors of low-volatility explosives, it has shown promise in the extraction and concentration of gasoline and diesel from fire debris.<sup>4-7</sup> DVME uses a relatively benign elution solvent (acetone) and allows analysts to monitor vapor distortion.<sup>3</sup> To determine the sensitivity of DVME performance to controllable instrument factors and factors reflecting debris characteristics, we applied covariance mapping to compare eluates recovered from simulated fire debris and corresponding reference samples.<sup>7</sup> Selected Ion Monitoring (SIM) of 17 ions typically monitored for petroleum-based fuels was used to collect sensitive and select mass spectral data of representative gasoline ions and to minimize any impact from interfering ions from the background or matrix. While this study identified optimal instrument settings and method robustness based on the overall correspondence between eluates and reference samples, a rich dataset of the chemical classes of gasoline remained unexplored. For example, collection volume was found to have a significant impact on performance, while capillary vapor trap sorbent and temperature had an insignificant impact, but only the overall impact is known. By investigating the influence of the different factors on the individual chemical classes, DVME performance could be further optimized, and the class response could be used to explain debris factor influences.

To address this gap, the sensitivity analysis was repeatedly performed on subsets of the data, focusing on responses from each of the chemical classes separately. The classes studied are the ones known to comprise gasoline: alkanes, cycloalkanes, aromatics, indanes, and polynuclear aromatics. These five classes span the full volatility range of gasoline, where alkanes, cycloalkanes, and aromatics comprise most of the higher volatility species, and indanes and polynuclear aromatics comprise the lower volatility species. Each factor's influence on the detection of chemicals from the five classes were individually explored through their main effects and interactions between factors. These results were then compared to the peak ratios utilized in the gasoline sufficiency plot, which was developed to increase transparency in the data interpretation leading to IL identification.<sup>8</sup>

As expected from the overall sensitivity analysis, volatility plays a large role in the response of each chemical class to the different factors. For example, for the higher volatility alkane class, the best instrumental setting is a low collection volume, while for all other classes, high collection volumes are preferred. The aromatic class mirrored the overall sensitivity analysis findings, which could be explained by the class having the highest abundance and moderate volatility. Potential chemical differences in responses were also found. Specifically, the response of alkanes to capillary sorbent was the opposite of cycloalkanes. These results could be used to determine the best instrumental settings for factors that were found to be insignificant in the overall design, such as capillary temperature. The results provide a better understanding of the robustness of the DVME method to realistic differences in debris composition. Furthermore, comparing the class analysis to the peak ratios utilized in sufficiency graphs leads to optimization for the specific chemical classes that are most important to IL identification.

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### Fire Debris; Headspace; Sensitivity Analysis

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## **D6 Water Modeling of River Streams as a Tool for the Delimitation of Areas of Potential Disposal Sites of Missing Persons' Bodies**

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**Learning Objective:** After attending this presentation, attendees will better understand a hydrodynamic modeling methodology for the search for missing persons thrown into rivers

**Impact Statement:** This methodology allows delimiting areas of forensic interest for the search through the prediction of places of entrapment of bodies in the rivers through the study of hydrodynamic and climatic variables.

Rivers as disposal sites are a complex search scenario due to their currents, taphonomic alterations, and specific forms of deposition. To identify search strategies for these contexts, EQUITAS and the Javeriano Water Institute from the Pontificia Javeriana University have undertaken a project since 2019 that has been focused on studying the movement of bodies by fluvial currents.

The objective is to design a methodology that facilitates the search for missing persons by using hydroinformatics tools, specifically hydrodynamic modeling and particle tracking to represent the transport of objects. This helps identify deposition zones that may be indicative of areas that are of forensic interest.

The study was focused on the La Miel river (Caldas) between the Municipality of Norcasia and the mouth of this water body with the Magdalena River. Three sections of approximately 1km were characterized using a topo-bathymetric survey, liquid gauging, and hydraulic profile measurements. Likewise, the remote sensing information of the area and historical hydroclimatic variables were considered. Experiments were carried out using a dummy to record transport trajectories and facilitate the calibration and validation of the mathematical hydrodynamic model for particle tracking.

Finally, the hydrological conditions corresponding to the dates of disappearance for missing persons were recreated, considering the information collected in terms of the time frame and the places of disposal documented in the reports of disappearance. Once the models had been run, zones with a higher probability of entrapment were identified, which correspond to areas where the speed and morphology of the river generate a pattern of movement of the particular bodies and in some cases can lead to a higher level of frequency of entrapment of the body in a particular area. These areas should be compared with contextual information, and the feasibility of an underwater or beachside archaeological survey should be considered to confirm or rule out the presence of human skeletal remains.

### **Hydrodynamic Modeling; Particle Tracking; Missing Persons**

## D7 The Perils of Using Linear Analysis for Metal Component Failures

Bart Kemper, BSME\*, Kemper Engineering Services, Baton Rouge, LA

**Learning Objective:** After attending this presentation, attendees will understand key principles in using linear and non-linear structural calculations and simulations in support of forensic investigations, the legal pitfalls of choosing a simpler or more available methods, and how Verification & Validation (V&V) embodies best practices to connect the user's skills to the simulation to enhance the likelihood given engineering simulations are admitted.<sup>1</sup> In this example of a metal component failure, it is with regard to a report of an explosion and piping failure in an active offshore natural gas collection field resulting in injury and eventual amputation.

**Impact Statement:** This presentation will impact the forensic science community by illustrating the need for non-linear analysis for non-linear events. In the previous century, a great deal of the engineering work used linear tools because that was not only what was widely available, it was the basis for the codes and standards. The majority of engineering codes employ linear methods for two reasons. The first reason is linear methods can be readily employed using classic means such as calculators or spreadsheets. The other reason is most products and equipment operate in the linear range, meaning its normal use does not result in loads above yield strength, which causes permanent deformation. Forensic engineering, however, often considers deformations as field data regarding forces or is focused on determining the root cause for some form of structural failure. In this example, using pressure vessel and piping design codes in failure analysis is often inappropriate because the failure is outside of the design envelope where the methods are valid.<sup>2,3</sup>

A fundamental difference between the scientific method and engineering is the scientific method is a method of investigation by testing a hypothesis, whereas engineering is about applying well-defined principles and standards to achieve a predictable, repeatable outcome.<sup>4,5</sup> Pressure vessels and related piping exemplify the need for predictable, repeatable outcomes due to their pressurized contents as well as potentially hazardous materials. Applied to forensic engineering, these principles are applied to mathematical models, ranging from equations to simulations, to determine whether a given hypothesis regarding failure is consistent with physics as well as whether it conforms with the applicable engineering code, such as those published by the American Society of Mechanical Engineers (ASME). In this example, a failure event theory was developed using linear methods to predict forces and deflections related to a failed pipeline fitting. The question regarding the appropriateness of the method and the underlying assumptions was not tested through V&V. A subsequent engineering analysis using non-linear techniques not only provided a more accurate accounting of the event, it raised questions as to whether the other testimony was the product of reliable principles and methods as well as whether the methods were reliably applied to the case. This indicated the report was vulnerable to a Federal Rule 702 challenge. Understanding the different methodologies will guide the investigator in assessing which methods are more appropriate for a given structural failure analysis and increase the likelihood of meeting a Rule 702 challenge.

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### Failure Analysis; Finite Element Analysis; Piping

## D8 The Effects of Land and Grooves on the Trajectory of a 9mm Bullet in a Denser Medium

*Ali Shahid Hussain, BS\*, Ministry of Defence, Lahore, Punjab, INDIA*

**Learning Objective:** After attending this presentation, attendees will be aware that: (1) the gyroscopic effect of the bullet has its pros and cons, depending upon the bullet; (2) behavior of the fired bullet is very different when it is fired by submerging the weapon in water; and (3) target training of the marines, regarding firing under water.

**Impact Statement:** All the forensic (ballistics-trajectory) cases that I have seen were based upon the assumption that the path of the bullet is straight from the muzzle. While testing some weapons in a submerged state, it was observed that the above statement is not always the case. The bullets fired in this study moved diagonally. This study will impact the forensic science community by being helpful in obtaining proper results if we are investigating the trajectory of a bullet that includes a liquid body in its path plus the role of gyroscopic motion of a bullet inside a denser medium. By “medium,” this study means the matter of the environment/space.

It is a well-established fact that whenever a bullet moves through a denser medium (for example, water), its velocity decreases drastically. This is also true whether a bullet is fired from outside into the water or it is fired inside the water by submerging the firearm. Most of these firings take place at some angle of depression. Angle of depression means the angle below the X-axis of properly held weapon. An observation was made while conducting a test with different water-submerged firearms of the same caliber (9mm pistols). The observation was that when the angle of firearm and the plane of water were parallel, the bullets did not maintain a straight path but rather showed a diagonal path. The level of the diagonal varied. Some bullets were more swayed away, while some were slightly swayed away; this means that the angle between the straight line of the weapon sight and the swayed path varied for bullets fired from the different firearms.

After observing the path behavior, and recovering the bullets from the water tank, it was found that the bullets with sharper and deeper lands and grooves tended to sway away from the straight path more, because a fired bullet has a gyroscopic motion and the medium was denser, thus they were moved away by depicting mechanical-gear or a water-turbine behavior. Here lands and grooves act as the teeth of a gear or the blades of a turbine. As we are discussing the ballistics of the bullet inside the denser medium, this can be helpful in determining the correct ballistics inside the water or any another liquid. In addition, this observation can help in designing the weapons for underwater firing, and it can also be helpful in liquid-submerged target training of marines (soldiers). All the available 9mm pistols that were used were with a right-twist barrel, so the movement of the bullet about its axis was toward the right side of the bullet recovery water tank. It can also be concluded that phenomenon of gyroscopic motion is very beneficial in a very low-density medium like air by increasing the range and stability of the bullet, while in a denser medium, or liquid medium, the gyroscopic effect affects the trajectory of the bullet and sway away from the straight path. As in mathematics, it is said that every diagonal line is a micro-part of a curve with some radius; just for the sake of imagination, if we imagine a bullet that is fired in water (the firing angle and the fluid layers of water are parallel) and the bullet is supposed to have an infinite energy and is able to move continuously without falling or stopping and its gyroscopic motion remains intact, it is possible that the bullet can travel in a circle of some radius like a planet revolving around a star. So, we can say that some of the bullet acrobatics which were shown in the movie *Wanted 2008* with Angelina Jolie may be possible.

### Gyroscopic Motion; Water-Submerged Firing; Diagonal Path

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## D9      Guessing the Twist of the Rifling of the 9mm Pistol From the Cartridge Case

*Ali Shahid Hussain, BS\*, Ministry of Defence, Lahore, Punjab, INDIA*

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**Learning Objective:** Attendees will learn: (1) how the motion of bullet contributes to the marking of the cartridge case; (2) how the markings on the cartridge case can be associated to the gun barrel; and (3) what the reaction of the gyroscopic motion of a bullet is.

**Impact Statement:** This presentation will impact the forensic science community by opening a new axis for the community and will contribute to the positive identification of firearms.

Every in-service firearm has a story to tell, and bullets and cartridge case are the main characters of this story. A firearm examiner usually focuses upon the lands and grooves of the bullet and breech-face or ejector-extractor marks on the head of the cartridge case. These are the fingerprints or the identification of a weapon upon the ammunition. This presentation has been set on scientific footings that can help identify how the rifling of the weapon affects the cartridge case. The idea arose while questioning whether it is possible to estimate the nature of the rifling twist of a weapon from the marks on the body of cartridge case and the drag marks of the extractor inside the extractor groove of the cartridge case. All endeavors were made to establish the idea. Extractor marks in extractor groove and chamber marks on the cartridge case body were deeply observed for this purpose, and these marks were found to be a type of identification mark of a weapon as well. Furthermore, this study helps us in knowing about the nature of the twist of rifling of barrel of the firearm used. Also, it was observed that the scratch marks (drag marks) on the outer cylindrical part of the cartridge case chamber are specific. It is amazing that with only the help of the cartridge case that is not in contact with the barrel, we can guess the nature of the rifling twist. We can see how the gyroscopic motion of the bullet affects the cartridge case during the fire. This study can help in determining the nature of the rifling twist of the firearm used in a crime even in the absence of a bullet. In addition, we can distinguish between a normally fired cartridge case and a blank fired cartridge case with the help of the slant of the scratch.

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**Rifling, Twist; Chamber-Marks; Extractor-Marks/Gyroscopic Motion**

## **D10 When Poor Knowledge of Basic Physics Results in Useless Trials**

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**WITHDRAWN**

## D11 A Haystack Falls and Injures a Dairy Farm Worker: Forensic Testing Used to Determine the Force Required to Topple the Stack

*Kurt Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA*

**Learning Objective:** After attending this presentation, attendees will be familiar with hay bale stacking, devices used to stack the bales, and the relative stability of each of three stacking methods. Hay bales measuring 3x4x8 feet weigh upwards of 1,400 pounds, depending on moisture content. As such, careful stacking and safety measures must be used.

**Impact Statement:** This presentation will demonstrate the test methodology applied to hay bale stack configurations, test equipment used, and the results of the average forces necessary to topple the four-bale stack.

A farmhand worked as a milker on a dairy for six years, cleaning machinery, bathing the cows, and milking the herd of nearly 2,000. The farmhand's role was changed to feeder, and he was tragically injured after only 28 days in his new position. Cow feed is comprised of a mixture of hay, almond hulls, silage, and other nutrients to maintain the health of the herd. Hay was delivered as needed; this particular load weighed 52,200 pounds and was delivered the evening before the incident. Forty-five large bales measuring 3x4x8 feet, with an average weight of 1,160 pounds, were moved by a squeeze from a flatbed trailer and stacked four bales high outdoors. A squeeze with long hydraulically controlled grasping arms is commonly used and can maneuver numerous bales at once (Figure 1).

Using a technique consistent with his training, the feeder used a bucket loader to push several bales to the ground opposite the stack. Four bales were needed. Bales bands were cut, and the segmented hay was added to a grinder used to mix the feed components. After exiting the bucket loader, the adjacent hay bale stack tipped over and struck the feeder, causing numerous fractures.

The dairy owner noticed this particular delivery had been stacked on a narrow base with the 3x8-foot bale side downward, whereas past deliveries had been stacked on the wider 4x8-foot base. Cross tying the stack is a common practice, offering additional stability with bales oriented perpendicular to the row beneath (Figure 2).

A test protocol was developed to contrast the force required to topple a four-bale haystack in each of three configurations: 1) 3x8-foot base, (2) 4x8-foot base, and (3) 4x8-foot base cross-tied. While the incident hay had long been consumed, the bales used in the test series weighed 1,152 pounds on average. Testing was conducted in the same location as the incident. A reach forklift was fitted with a 10,000-pound load cell (Figure 3); 120V AC power was delivered to an amplifier/conditioner by a vehicle-mounted DC/AC converter. Data was collected at 1,000Hz. A lightweight cable secured to the load cell was attached to a metal frame with a hooking feature placed on the top bail. As the reach forklift backed away from the stack, cable tension caused tipping of the haystack (Figure 4). Each four-bale stack tested was replaced by a fresh four-bale stack.

The first test of the 3x8-foot base configuration was conducted without incident. However, while clearing away the tested bales, the adjacent four-bale stack tipped over unexpectedly, much like the day when the feeder was injured. A second inadvertent tipping also occurred with the bales stacked on a 3x8-foot base. In all, eight tests were conducted, excluding the two instances where the stack tipped over inadvertently.

A three-test average pull force of approximately 126 pounds was required to topple the bales stacked on a 3x8-foot base. A three-test average pull force of approximately 385 pounds was required to tip the bales on a 4x8-foot base. Last, a two-test average pull force of approximately 411 pounds caused only the top bale of the cross-tied stack to fall. The test results contrast the average pull force required to topple the four-bale haystack. It was noted that only the bales stacked on the 3x8-foot base tipped unexpectedly. The average force to topple the stack on a 4x8-foot base was three times that necessary to topple the stack on the 3x8-foot base. Cross-tying the bales provides additional stability to the hay stack.



Fig. 1



Fig. 2





Fig. 3



Fig. 4

### Haystack; Hay Bale; Forensic Testing

## **D12 Satellite Laboratories at the Front Line: The Canadian Border Approach to Narcotics Detection Within Postal and Air Cargo Facilities**

*Sandra Ferreira, MS\*, Canada Border Services Agency, Ottawa, ON CANADA; Chad Maheux, MS, MPA\*, Canada Border Services Agency, Ottawa, ON CANADA*

**Learning Objective:** Attendees will learn how the Canada Border Services Agency has built three small laboratories within Ports of Entry known as Designated Safe Sampling Areas (DSSAs) to improve drug detection at the front line. The DSSAs are designed to provide Border Service Officers and Intelligence Officers with advanced real-time drug screening information, which improves their ability to seize narcotics, collect intelligence information, decrease response time of informing police partners, and support border integrity.

**Impact Statement:** The forensic science community will see a highly successful scientific approach to increase drug detection capabilities at Ports of Entry, in which drug analysis chemists, law enforcement officers, and intelligence officers work side-by-side.

The Canada Border Services Agency's (CBSA) is tackling drug detection in a new way by extending the laboratory to the front line. The CBSA has built three small laboratories within Ports of Entry (Montreal and Toronto postal facilities and Vancouver air cargo facility) THAT have high interdiction of illicit drugs. These compact chemist-led labs are known as Designated Safe Sampling Areas (DSSAs) and designed to provide Border Service Officers and Intelligence Officers with advanced real-time drug screening information, which improves their ability to seize narcotics, collect intelligence information, decrease response-time of informing police partners, and support border integrity while keeping health and safety a primary focus. Attendees will learn how a custom-made Laboratory Information Management System, a selected small suite of tools (FTIR, IMS, immunoassays), nationally networked systems (instruments), and safety measures (ducted fume hoods) are able to safely screen over 80% of exhibits on-site.

This presentation will share how a scientific approach was taken to increase drug detection at Ports of Entry, which includes drug analysis chemists, law enforcement officers and intelligence officers working side-by-side. This teamwork approach is of increasing importance as the growing number of novel drug substances and precursors is posing global analytical and field detection challenges. In addition, several drugs' substances are also considered highly toxic, such as low-dose opioids, which have only compounded the challenges for safe sampling and field testing. The creation of these DSSAs have demonstrated several advantages in their inaugural year, including the increase in free flow of legitimate goods, increased health of safety, increased viability of enforcement action due to maintenance of chain of custody and timeliness of scientific results, and real-time intelligence gathering to support active investigations. The results of this approach will be demonstrated through DSSA's seized drug trends (both import and export) and their novel concealment methods.

### **Field Drug Analysis; Satellite Laboratories; Opioids**

## D13 Identification on the Basis of Chamber Marks of .30 Caliber Firearms

*Ali Shahid Hussain, BS\*, Ministry of Defence, Lahore, Punjab, INDIA*

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**Learning Objective:** After attending this presentation, attendees will be aware that, like breech face marks, firing pin marks, ejector and extractor marks, there is another category of marks (i.e., chamber marks).

**Impact Statement:** The impact of this presentation is that it will reduce the chances of inconclusive results by a firearms expert due to the lack of clarity or absence of other marks.

Firearm and tool marks are a significant field of forensic science. Firearm examiners deal with complex cases of identification. There are several marks that can be used to identify the firearm called the variables of identification. These include breech face marks, firing pin marks, and ejector and extractor marks. These are the major marks on the basis of which a firearm can be identified. Sometimes, in crime investigation laboratories, the evidence submitted contains none of the above-mentioned marks (i.e., including breech face marks, firing pin marks, and ejector and extractor marks). The examiner may reach an inconclusive result because of the insufficient match to available data. To avoid an inconclusive result, the examiner must evaluate other marks that are present on cylindrical body of the cartridge case. These marks, known as chamber marks, are produced when the cartridge lies inside the chamber of the firearm. These marks are also unique and permanent, and their significance increases when other marks are not present. These marks are made when the bullet is sliding through the mouth of the chamber, rubbing the internal circular wall and the edge of the chamber.

A .30 caliber cartridge case was submitted to the laboratory along with a .30 caliber pistol. The primer of the cartridge case was pierced and contained the least marking on breech face. Test fires were produced, and a comparison was made under a comparison microscope to conclude the cartridge case. The cartridge case was identified based on chamber marks only. From this study, an examiner can learn how to scientifically rely on other microscopic markings to form the basis of a conclusion in the case, rather than give an inconclusive result. As these marks are generated by the chamber of the firearm, they are weapon specific. If we start considering these marks, a new category of identification, like breech face, firing pin, and extractor and ejector marks, can be introduced.

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### Chamber Marks; Outer Body of the Cartridge Case; Inconclusive Results

## D14 Testing Basic Toolkits for Forensic Text Analysis: Lemmatizers

Carole Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE; Angela Almela, PhD, Universidad de Murcia, Murcia, SPAIN; Pascual Cantos, PhD, Universidad de Murcia, Murcia, SPAIN

**Learning Objective:** After attending this presentation, attendees will better understand how lemmatizers from three different sources differ on English and Spanish text, and how lemmatization can be used to prevent sparse matrices used in quantitative text analysis.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of different, industry-grade toolkits for forensic linguistic analysis.

A fundamental idea in linguistic analysis is lemmatization.<sup>1</sup> Lemmatization is the conversion of word forms into the base or dictionary entry format. For example, the words *am*, *are*, *is*, *was*, *were*, *be*, *being*, and *been* are all lemmatized to the lemma *be*. Another fundamental idea in linguistic analysis is the corpus.<sup>1</sup> A corpus is a collection of documents (or texts). Usually, the corpus is designed for a specific purpose. For example, in forensic cases, a corpus may consist of documents of known authorship from several authors, or documents of a specific communicative type such as threat letters, suicide notes, or predatory chats. A third fundamental idea in linguistic analysis is the inter-relationship between documents, or information retrieval.<sup>2,3</sup> One very simple way of measuring the inter-relationship between documents is the number of words that different texts share, or word overlap.

If two documents are compared using the raw text, usually the only words that overlap will be function words such as *the*, *a*, *on*, etc., which are words that indicate syntactic relationships rather than semantic content.<sup>4</sup> But if the two documents undergo lemmatization, the number of overlapping words will increase because variant (different, non-overlapping) forms of a word are reduced as the dictionary-entry (same, overlapping) forms of a lemma are increased. For example, two texts will not have overlaps if the word forms *gun*, *guns* are counted since these words are variant forms, but the same two texts will have overlaps if the lemma format *gun* is counted. Note also that the frequency of *gun* and *guns* will be smaller than the frequency of the lemma]. Now consider that a third document is included in the corpus. In the third document, *gunning* occurs. Without lemmatization, the third document will have no overlap with the first two documents, but with lemmatization, all three documents are recognizable as having to do with the concept *gun*. Lemmatization thus reduces the possibility of a sparse matrix (a matrix filled with many zeros for frequency) and also makes inter-relationships between documents more readily evaluated.

In many machine-learning approaches, such as word embeddings or vectors, lemmatization is not used, but in symbolic approaches such as TF\*IDF, lemmatization is a standard technique for building algorithms for text inter-relationships.<sup>2,5</sup> All modern toolkits in natural language engineering include a component for lemmatization, but these can differ in particular features of how the lemmatizing is performed. This talk presents the results of comparing the lemmatizing of three industry-grade natural language engineering toolkits, the Stanford NLP library, spaCy, NLTK, and Basis Technology's Rosette.<sup>6-9</sup> Further, the three toolkits have been tested using both English and Spanish data, and these results are presented.

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### Forensic Text Analysis; Lemmatization; Linguistic Evidence

## D15 True and False Sexual Assault Victim Statements

Carole Chaski, PhD\*, ALIAS Technology, LLC, Georgetown, DE

**Learning Objective:** After attending this presentation, attendees will better understand how quantitative analysis of language use can identify truthfulness of texts generated during and after sexual assault examinations.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of quantitative linguistic analysis for solving sexual assault crimes and assisting forensic nurses.

This study presents two case studies of the truthfulness of victim statements in sexual assault investigations, using software for the quantitative linguistic analysis of the statements. These case studies differ in several ways: (1) how the statements were collected, and (2) the veracity of the statements. This presentation is part of a larger project investigating true and false claims of sexual assault and provides only one contrastive example.

Victim statements are generated at two points in the sexual assault investigation—at the very beginning and at the end. At the very beginning, the forensic nurse who conducts the Sexual Assault Nurse Examination (SANE) is the first line of investigation, collecting physical evidence and providing both physical and mental health care.<sup>1</sup> The collection of a written victim statement does not appear to be part of the national protocol for sexual assault nurse examination, but a victim statement can be collected as part of the SANE examination. At the very end, the prosecution often presents, sometimes by the victim, the victim impact statement. The victim impact statement is a well-known, albeit controversial, part of legal proceedings.<sup>2</sup> This talk applies text analysis software called WISER to the victim statements in one case in which the statement was collected as part of the SANE examination and in one case in which the victim delivered victim impact statements as part of the legal proceeding.

Victim statements may be either true or false. True claims of rape support felony convictions. False claims of rape are especially grievous for two reasons. First, false statements waste resources throughout the investigative workflow, from forensic nurse and law enforcement through prosecution and defense to judges and juries. Second, false statements are traumatizing for the falsely accused and eventually the accuser. This presentation applies WISER as an objective, algorithmic way to differentiate true and false statements with a statistical probability.<sup>3,4</sup>

In the false example, a woman claimed that her estranged husband kidnapped her during a meeting that they both agreed to and during which they had sexual intercourse, because he would not allow her to leave, and that he raped her. The husband claimed that he had never kidnapped her and that he had not raped her, because their sexual intercourse was consensual. The husband also said that after they had had sexual intercourse and were discussing their marriage, they argued and hit each other. The wife contacted the police; the husband was arrested and charged with kidnapping and rape. The wife was taken to a hospital, where a sexual assault examination was conducted and her statement describing the sexual assault taken as part of the SANE. This one-page statement was made available during the investigation of the authorship of text messages.<sup>5</sup> The prosecutor dropped the charges of kidnapping and rape after the wife's claims about text message authorship were proven false.

In the true example, Andrea Constand provided a victim impact statement at the trial of Bill Cosby for sexual assault of drug-incapacitated victims. This five-page statement is available online through Yahoo® and CNN.<sup>6,7</sup> The victim statement was used at the sentencing hearing. Mr. Cosby was convicted and was later released from detention after his conviction was vacated when a previous deal for non-prosecution was discovered.

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### Linguistic Evidence; Deception Detection; Sexual Assault Nurse Examination



## D16 Validation Testing of a Syntactic Authorship Identification Method Using Ground-Truth Data From 25 Authors

Angela Almela, PhD\*, Universidad de Murcia, Murcia, SPAIN

**Learning Objective:** In this presentation, attendees will learn about validation testing in forensic linguistic evidence, specifically author identification, to meet the reliability and error rate criteria for scientific evidence.

**Impact Statement:** This presentation will impact the forensic science community by providing results of validation testing using the syntactic analysis method (SynAID) to determine the reliability and error rate of computational forensic linguistic author identification.

The first validation testing of forensic authorship identification methods came quite late to the field. Svartvik coined the term “forensic linguistics” and used a syntactic and statistical analysis to determine authorship in a case.<sup>1</sup> But afterward, no testing of the method to determine its reliability or error rate was performed. In fact, for close to three decades, academic linguists were providing reports and sometimes even testimony about authorship using methods that had never been tested to determine their reliability or accuracy. Chaski began testing authorship identification techniques on “ground-truth” data (documents whose authorship was known), independent of any litigation, in order to determine error rate.<sup>2,3</sup> For most of the tests, Chaski used four authors who were matched by age, dialect, educational level, and gender, so that any differences in style could not be attributed to demographics. Chaski’s results demonstrated that most ideas about authorship identification were highly unreliable, and even a combination of these techniques would get no better than 50% accuracy. Chaski published a validation test of a technique based on syntactic analysis, using text analysis software to analyze the syntactic patterns, syntactically classified punctuation, and word lengths of texts to produce a numerical profile that is statistically analyzed using a leave-one-out, cross-validated discriminant function analysis (the SynAID method).<sup>4</sup> In this validation testing, Chaski used ground-truth data from ten authors who were matched for age, dialect, and educational level with five men and five women. Using the same dataset, Hirst and Feiguina performed validation testing of a different syntactic analysis method, the frequency of syntactic labels over two words in a row.<sup>5</sup> Chaski reported additional validation tests.<sup>6</sup> Nini and Grant performed validation testing using ground-truth data from three authors and, using non-syntactic features, could not differentiate those three authors.<sup>7</sup> Juola reports a validation test of his software using blog posts, which are probably not ground-truth data due to the nature of the internet and blog writing conventions.<sup>8</sup> Almela and Cantos presented the results of validation testing on non-native speakers using SynAID and character-level analysis.<sup>9</sup> Dar presented a validation test of SynAID using 20 male, White, middle-aged, college-educated, native English speakers with some managerial or executive experience.<sup>10</sup>

This presentation extends the validation testing of SynAID to 25 authors (Dar’s authors and five additional ones with matching demographics). Their writing samples were all spontaneously written during the course of their daily lives. Test results with error rate and accuracy rate are reported for well over 100 documents from these 25 authors. The 25 authors’ document-level numerical profiles were subjected to pair-wise statistical analysis using the protocol described in Chaski.<sup>4</sup>

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### Validation Testing; Forensic Computational Linguistics; Authorship Identification



**D17    Recreational Vehicle (RV) Crashworthiness: Enhanced Injury Caused by Unsafe Vehicle Interior Structures Combined With Predictable Seat and Belt Failures**

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Vijay Gupta, PhD, University of California, Los Angeles, Sherman Oaks, CA; Jack Bish, PhD, Safety and Forensic Laboratories, Goleta, CA*

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**WITHDRAWN**

## D18 The Mechanics of All-Terrain Vehicle (ATV) Rollovers

*David Pienkowski, PhD\*, University of Kentucky, Lexington, KY*

**Learning Objective:** Presentation attendees will gain new information regarding why ATVs cause injury or death due to sideways and backward rollovers.

**Impact Statement:** ATV rollovers often occur unwitnessed and thus details about the cause of such accidents are generally unknown. Given that ATV rollovers often cause serious injury or death, the mechanics by which such accidents occur is of interest to the forensic investigator. While ATV accidents are frequently attributed to excess speed or careless operation, this presentation shows that sideways or backwards ATV rollovers can occur due to other factors. This presentation impacts the forensic science community because it quantifies the risk of ATV rollover relative to controllable variables, including ATV size, number of riders, turning circle, and throttle input.

ATVs offer new mobility options for those who traverse rugged terrain, but these vehicles are also associated with considerable injuries and deaths, especially among young riders who use ATVs for recreation. Among the most harmful ATV mishaps are those involving sideways or backward rollovers. These mishaps frequently occur unwitnessed and are thus commonly attributed to excess speed or careless operation. Given the consequences of ATV rollovers, the mechanics by which such accidents occur is of interest to the forensic investigator. The objective of this laboratory and computer modeling study was to determine if other underappreciated variables (i.e., youth- vs. adult-sized ATVs, single vs. dual riders, varying terrain angles, and varying engine throttle inputs may be related to the risks of sideways or backward rollovers).

Two new exemplar ATVs (one youth-sized, one adult-sized) were tested in a variety of side- and rear-directed inclinations in a controlled static laboratory environment. Centers of gravity of these ATVs were measured for one adult or one adult and one child rider (located at various seating positions on the ATV) for actual angular inclinations and simulated engine throttle positions. Rollover was considered to occur when the center of gravity extended beyond the footprint of ATV tires.

The results showed that while both ATV sizes can endure an approximate 0.77g acceleration on a level road while turning, the presence of a rider behind the driver reduces the maximum safe acceleration during turning by approximately 12%. The minimum turn radius that an ATV can safely negotiate, without the risk of violent driver-ejecting tip-over, increases sharply with increasing ATV speed. Doubling ATV speed from a few miles per hour increases the safe minimum turn radius five- to six-fold. Furthermore, the likelihood of ATV tip-over with sharp radius turns is substantially increased by the presence of inclined terrain.

The risk of sideways and backward rollovers of ATVs can occur while turning at unexpectedly low speeds, especially when done on inclined terrain, when full throttle is applied, or extra riders accompany the driver on the ATV. Compared to adult-sized ATVs, youth-sized ATVs do not provide substantial additional protection from sideways or backward rollovers.

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**All-Terrain Vehicle Injury; All-Terrain Vehicle Rollover; Mechanics of All-Terrain Vehicle Injury**

**D19 Rear Impact Crashworthiness Defects Proven by Forensic Analysis and Testing: Failures of Seats, Head Restraints, and Belts**

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Paul Lewis; MS, Kenneth Saczalski, PhD, Environmental Research and Safety Technologists, Newport Beach, CA*

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**WITHDRAWN**

### **D20 Child Fatalities in Vehicular Rear Impact: Forensic Analysis and Testing to Scientifically Prove Seat and Belt Failures**

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Kenneth Saczalski, PhD, Environmental Research and Safety Technologists, Newport Beach, CA; Todd Saczalski, BSMET, TKS Consulting, Dana Point, CA*

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**WITHDRAWN**

## D21 The Effectiveness of Roadside Barriers in Reducing Accident Severity

*Darren Franck, MSME\*, Advanced Engineering Associates, Inc, Charleston, WV; Harold Franck, MSEE, Advanced Engineering Associates, St. Petersburg, FL*

**Learning Objective:** Attendees will be presented with methods for computing vehicle speeds and the interaction of vehicles with different types of roadside barriers.

**Impact Statement:** This presentation will impact the forensic community by examining the effectiveness of roadside barriers in three separate case studies.

Roadside design involves the region beyond the shoulder edge or within a median of a divided highway. It is of interest to traffic engineers to keep vehicles within the roadway to reduce the potential for injuries or death. The installation of such barriers is within the purview of a state's highway department and is determined by a cost-benefit analysis. Guidelines are available that specify the composition of barriers for roads bounding an embankment, median, or other hazardous areas.

The first case involves a woman traveling on a two-lane road. The driver was traveling in the northbound lane at night when the tires dropped off the right shoulder. She over-corrected to the left, lost control, and traveled across the southbound lane toward a guardrail. This barrier had dropped in elevation due to instability of the embankment. This instability also caused significant settlement of the roadway. The highway department repeatedly paved the sunken roadway. However, there were no efforts to raise the elevation of the guardrail.

As a result, the top of the W-beam guardrail was only a few inches higher than the pavement. The left front wheel rolled over the top of the guardrail, causing the vehicle to vault over the embankment and the adjoining railroad. The driver survived but suffered long-term brain injuries. Iterative solutions for projectile motion were devised, which indicate the vehicle was traveling below the speed limit. Testing of guardrails by other agencies indicated that the vehicle would have been kept within the roadway given the speed, angle, weight, and dimensions of the vehicle. The plaintiff recovered damages from the State due to the condition of the guardrail and the railroad for failing to maintain stability of the embankment.

Another single-vehicle crash is examined in the second case study. A man was operating a sedan up a steep, uphill access road to a four-lane highway. As the vehicle entered a sharp curve, the driver lost control. The vehicle began to yaw, and then rolled down a steep embankment. This incident occurred late at night, and there were no witnesses. The driver died, possibly due to the lack of emergency response. The reconstruction indicated that the vehicle would have been kept within the road had the guardrail been completed.

The engineering firm that designed the road specified guardrails across the crest of this embankment. However, the contractor omitted the guardrail where the vehicle departed the road. This decision was permitted by the state highway department, which remanded the case to the court of claims. Seeking a private party defendant, the attorney attempted to recover damages from the engineering firm. While unsuccessful in his efforts, the remaining section of the guardrail was subsequently constructed.

The final case involves multiple vehicles on an interstate. Two vehicles traveling west lost control and entered the median. Although a cable median barrier was present, the vehicles traveled between the cables, resulting in a collision with an eastbound tractor-trailer. Multiple fatalities resulted from the force of impact and a fire produced when one of the vehicles impacted the tractor's gas tank. The reconstruction revealed that the vehicles were not traveling at unsafe speeds. Evidence suggested that a phantom vehicle had impacted the rear end of one of the vehicles, causing the drivers to lose control. The reconstruction was requested by insurance companies seeking a determination of liability. As such, a detailed analysis of the failure of the cable barrier was not performed. However, the low-profile vehicles traversed between the cables and were not redirected back toward the westbound lanes. The evidence indicates limitations in these barriers in preventing vehicles from crossing medians.

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### Roadside Barrier; Projectile Motion; Vehicle Speed

## **D22 Elderly Female Side Impact: An Experimental Analysis of Side Impact Injuries Using Instrumented Postmortem Human Subjects (PMHS), Current Restraint Technologies, and Real-World Crash Boundary Conditions**

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**Learning Objective:** After attending this presentation, attendees will have an appreciation for interdisciplinary research in skeletal trauma analysis, specifically focused on thoracic injuries to small, frail, female vehicle occupants. 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 differences in thoracic injuries due to changes in current restraint technologies.

**Impact Statement:** This presentation will impact the forensic community by demonstrating the need for interdisciplinary biomechanical analyses of skeletal trauma to better understand thoracic injury mechanisms. Increased understanding of the mechanisms of thoracic trauma will lead to better safety restraints for all occupants.

In 2019, 7,214 occupants 65 and older were killed in car crashes in the United States, which is a 31% increase for this age group since 2010.<sup>1</sup> Database reviews of the National Automotive Sampling System Crashworthiness Data System (NASS-CDS) revealed that for small, older female occupants, thoracic-related injuries are among the leading causes of fatality. Sunnevång et al found that for near-side crashes between 1994–2008, elderly occupants had a 4x higher risk for thoracic injury than younger occupants at impact speeds between 40–80km/h.<sup>2</sup> In a second study focused between 2000–2011, it was determined that elderly female occupants in near-side impacts are the most at-risk population for thoracic injury.<sup>3</sup>

The focus of this study was to conduct a series of small, frail PMHS side impact sled tests that mimicked real-world crash boundary conditions to investigate thoracic injury mechanisms. The sled test conditions were defined by performing a side impact car-to-car crash with instrumented doors and the SID-IIIs (5<sup>th</sup> percentile) female ATD, which predicted less than a 10% chance of AIS3+ injury for a near-side occupant. Boundary conditions for the sled series included an intruding driver's side door that was controlled using the Advanced Side Impact System (ASIS) (DSD, Austria) on a HYGE sled. The sled acceleration matched the acceleration profile of the impacted vehicle, while four pneumatic cylinders of the ASIS, covered by a door liner, produced realistic door intrusion. Each subject was seated on a mass-production driver seat, equipped with a side airbag and standard three-point restraint with a pretensioner.

The PMHS were targeted to be elderly females age 65+, approximately 5<sup>th</sup>-percentile in height and weight, with osteopenic areal bone mineral density. Instrumentation on each PMHS included strain gauges on ribs 3–10 bilaterally, to help identify fracture timing. Two chest bands were used to measure chest deflection, one at the level of the axilla and one at the level of the xiphoid process.

Injuries observed were primarily rib fractures, particularly on the struck side, and in multiple cases a flail chest was observed. Eight subjects resulted in AIS3+ injuries. Subjects crossed the threshold for AIS3 injury in the range of only 1%–9% chest compression at the level of the xiphoid process. Two different airbags were used in the sled series, which led to differences in the mechanisms of thoracic injury, as some were incurred by door interactions while others came during initial airbag interactions. These findings point to the importance of understanding that current safety systems may be contributing to thoracic injuries for small, frail occupants given the systems are mainly tuned to protect 40-year-old average-size male occupants.

### **References:**

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- <sup>2</sup>. Sunnevång C, Rosén E, Boström O. Thoracic Injury Risk as a Function of Crash Severity – Car-to-car Side Impact Tests with WorldSID Compared to Real-life Crashes. *Ann Adv Automot Med*. 2010; 54:159–168.
- <sup>3</sup>. Ramachandra R, Kashikar T, Bolte J. Injury patterns of elderly occupants involved in side crashes. Paper presented at: *International Research Council on the Biomechanics of Injury Conference (IRCOBI)*; September 13-15, 2017; Antwerp, Belgium.

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### **Thoracic Injury Mechanisms; Injury Biomechanics; Side Impact Car Crash**



## D23 Event Data Recorder (EDR) Data and Restraint Use: A Case Study

*Michelle Hoffman, MS\*, Forensic Injury Analysis, LLC, Tempe, AZ*

**Learning Objective:** Attendees will learn that EDR data is important information when conducting accident reconstruction and injury analyses, but the data needs to be interpreted properly and is not a stand-alone replacement for proper analyses.

**Impact Statement:** This presentation will impact the forensic science community by highlighting, with a case example, the importance of proper interpretation of EDR data.

A 51-year-old, 6'2, 185-pound man was the driver of a 2018 Kia® Soul® that was involved in an angled frontal impact when a delivery truck turned left in front of the Kia®. An EDR report from the Kia® noted the driver's seat belt status as "OFF." A defense expert cited the EDR report to conclude that the majority of the injuries would not have occurred if he had been restrained. A biomechanical analysis by this author revealed that the man was actually wearing his restraint system at impact. This case is a clear example of why EDR reports must be carefully interpreted in arriving at accident reconstruction and injury causation conclusions. The information in EDR reports should be carefully considered in conjunction with additional information about the case and not as a sole source of information for arriving at conclusions pertaining to accident reconstruction and injury biomechanics. Accident reconstructions from both sides with respect to the Kia's® velocity change (delta V) and Primary Direction Of Force (PDOF) were similar with the Kia's® resultant delta V at approximately 34mph with a PDOF of 35 degrees. Thus, the longitudinal delta V was about 27.5mph, and the lateral delta V was about 20mph.

The injuries sustained by the male driver included left maxillary sinus fracture, bilateral C7 transverse process fractures, left and right first rib fractures, right knee soft tissue damage, right tibial plateau fractures, compartment syndrome of right lower leg (eventually amputated), and right foot fractures.

The defense expert concluded the driver was unrestrained at impact, citing the EDR report. The defense expert's final conclusion was that if the driver had been properly restrained, he would have been significantly less likely to experience the transverse process fractures at C7, the left maxillary fracture, or the right tibial plateau fracture in the subject collision. The subject Kia® was destroyed prior to this author's involvement in the case; however, photographs of the Kia® were available, which revealed vertical denting of the posterior portion of the driver's door, heavy abrasion on the driver's side roof rail, abrasion on the driver's belt webbing, and abrasion on the driver's latch plate.

Although the EDR report noted, "Safety seat belt status, driver = OFF," the report also noted that if the power supply to the ACU was lost during an event, all or part of the data may not be recorded, and it noted "Completed File Recoded = NO." A CARFAX® report was ordered and no other frontal collisions were reported for the Kia®. There was no evidence found that supported the defense expert's claim that the driver moved forward and leftward to the A-pillar. In fact, there was evidence to the contrary, in that the driver door denting and head abrasion on the driver roof rail were in expected locations for a restrained driver. This was demonstrated with an exemplar-surrogate inspection.

In conclusion, the physical evidence and injury analysis indicated that the Kia® driver was wearing his lap and shoulder seat belt at the time of impact, and this was not consistent with the EDR report showing the driver's seat belt status as "OFF."

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### EDR Data; Restraint Use; Injury Analysis

**D24 Criminal Engineering: The Ugly 50-Year History of Federal Motor Vehicle Safety Standard (FMVSS) 207 and Proposals for an Upgraded Safety Standard That Will Prevent Catastrophic Injury and Death**

*Mark Pozzi, MS\*, Sandia Safety Sciences, Edgewood, NM; Kenneth Saczalski, PhD, Environmental Research and Safety Technologists, Newport Beach, CA*

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**WITHDRAWN**

## **D25 Reverse Engineering Techniques in Forensic Investigations: A New Approach to Bitemark Analysis**

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**WITHDRAWN**

## D26 Microfluidics: Streamlining Ink Identification Using Mass Spectrometry

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**Learning Objective:** After attending this presentation, attendees will better understand how microfluidics coupled with high-resolution mass spectrometry can be used to identify and distinguish inks from different ballpoint pens, enhancing the forensic analysis of written documents quickly and easily.

**Impact Statement:** This presentation will impact the forensics community by providing a novel way to examine inks thoroughly, quickly, and easily from written documents by microfluidics coupled with high-resolution mass spectrometry.

Written documents can play a key role as evidence during a forensic investigation. Letters, bank checks, notes, and credit card receipts are among a few types of documents that may contain handwriting in pen. In addition to handwriting comparisons, ink composition investigation can give information about document forgery and/or the type of pen used. As with most evidence, a relatively non-destructive method of investigation is ideal for evidence preservation. While Raman and photo spectroscopy are non-destructive analytical techniques used with inks, the information given is limited and lacks the more diverse picture mass spectrometry can provide.<sup>1</sup> While Desorption Electrospray Ionization (DESI) mass spectrometry is a non-destructive mass spectrometry technique, it is relatively long, expensive, and requires special expertise.<sup>2</sup> Manual extractions also have these drawbacks and require larger sample sizes. Therefore, it is important to find more simple, quick, specific, minimally destructive analytical techniques that can be successfully performed on written documents.

Microfluidics is a method of microscopic sample extraction, which can be coupled to mass spectrometry. A mass spectrometer measures an ion's mass-to-charge ratio ( $m/z$ ). Commercial inks are mixtures of various basic dyes and other components. The major component ions can be seen on the mass spectrometer, which allows the user to obtain a profile of the ink's elemental composition and relative proportion of dyes. This means that even if two pens contain the same dyes, they could still be distinguished by looking at their respective spectral profiles.

Three different blue ballpoint pens (Pilot® G-2, Uniball® Signo, and Bic® Round Stic) were used to draw lines on white printer paper. A 1mm diameter hole punch was used to punch out samples from the paper. Each sample was individually loaded onto the microfluidic chip, inserted into the device, and run through an extraction cycle. Sample extractions were collected and run through a C18 reverse phase column using a High-Performance Liquid Chromatography (HPLC) system coupled to an Electrospray Ionization quadrupole Time-Of-Flight Mass Spectrometer (ESI-qTOF-MS).

Results show that the three different pens can be distinguished from one another using mass spectrometry facilitated by microfluidics, demonstrating that this could be a promising analytical technique for written documents in the field of forensics. Further work will include looking at different color pens and markers, as well as monitoring the photodegradation of pen inks over time using this microfluidics-mass spectrometry system.

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### Mass Spectrometry; Microfluidics; Inks

## D27 Deep Learning Models to Estimate Age and Sex From Images of the Pubic Symphyseal Surface and Greater Sciatic Notch

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**Learning Objective:** After attending this presentation, attendees will be able to appreciate the possibility and applicability of deep learning models (e.g., convolutional neural networks for human identification through the examples of age and sex estimation results from pubic symphyseal surface and greater sciatic notch images).

**Impact Statement:** By introducing successful age and sex estimations via deep learning models, this presentation will impact the forensic science community by promoting deep learning applications in the community.

Recently, aside from qualitative and manual age and sex estimations, 2D and 3D images are being analyzed via various computational tools for human identification. Among computation tools, deep learning is a method based on artificial neural networks. Owing to large amounts of image data, deep learning has gained popularity for its ability to perform classifications that human experts or scientists consider difficult. In particular, a convolutional neural network, a deep learning model, mimics the structure of the human optic nerve and is the most common tool used for image analysis. Deep learning using convolutional neural networks has been performed using a variety of images such as computed tomography, X-ray, magnetic resonance, and dental imaging. Many studies in forensic anthropology have used artificial intelligence, and deep learning-based methods have yielded some promising results. These results have demonstrated the potential of deep learning and encouraged further investigation of its usage in age and sex estimation. Therefore, the purpose of this study is to automatically estimate the age and sex from 2D images of the pubic symphyseal surface and greater sciatic notch using deep learning.

Eighty-two sides of pubic symphyseal surfaces and 62 sides of the greater sciatic notch of Koreans, donated from the Yonsei University Health System, were used in this deep learning study. Specifically, the number of pubic symphyseal surfaces was 17, 35, and 30 for those in their 60s, 70s, and 80s, respectively. Also, the number of greater sciatic notch equaled 21 from those in males, and 13 from those in females. Obtaining a large number of images is difficult; therefore, manual image augmentation was used to increase the amount of image data by taking one sample from multiple angles. The Twins-PCPVT model was trained to estimate age and sex based on pubic symphysis and greater sciatic notch images.<sup>1</sup>

First, with the age estimation model using pubic symphyseal surface images, the final model classified the images in the test dataset into the 60s, 70s, and 80s age groups. For the 60s, 70s and 80s age group, the model achieved accuracy, recall, precision, F1-score, and AUROC of 0.83, 0.63, 0.71, 0.67, and 0.81; 0.79, 0.80, 0.72, 0.76, and 0.84; and 0.86, 0.81, 0.84, 0.82, and 0.94, respectively. The micro- and macro-average AUROC were 0.88 and 0.87, respectively. In the heat map of the pubic symphyseal surface age estimation model, the dentated area on the pubic symphyseal surface had the most significant influence on the age prediction of the deep learning model.

Second, in the sex estimation model using greater sciatic notch images, the model achieved accuracy, recall, precision, F1-score, and AUROC of females were 0.94, 0.87, 0.87, 0.93 and 0.98, respectively. The accuracy, recall, precision, F1-score, and AUROC of males were 0.94, 1.00, 0.90, 0.95 and 1.00, respectively. The micro- and macro-average AUROCs were 0.96 and 0.99. The dentated area on the greater sciatic notch was the deepest point in the greater sciatic notch, posterior inferior iliac spine, and ischial spine. These bony landmarks were consistent with the points that many researchers use for manual sex estimation. In this study, an age estimation model for the 60s to 80s age groups, and a sex estimation model were developed using a Twins-PCPVT-based deep learning method. The new age estimation model outperformed the previously developed pubic symphyseal surface age estimation program in terms of accuracy. Moreover, the new sex estimation model could estimate sex regardless of anatomical position. This study confirmed the ability of Twins-PCPVT to estimate the age and sex from the skeleton, and this model could be improved further if trained on larger and more diverse datasets.

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### Deep Learning; Pubic Symphyseal Surface Age Estimation; Greater Sciatic Notch Sex Estimation

## D28 A Study of a Method for Estimating Mechanism of Injury to Children Caused by External Force: Collisions of Children With the Floor

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**Learning Objective:** After attending this presentation, attendees will better understand the impact of floor type on injury risk.

**Impact Statement:** This presentation will impact the forensic and forensic engineering community by providing an injury risk assessment method that considers the impact of floor type on injury risk.

In Japan, the number of cases of physical abuse of children is increasing year by year. Currently, physical abuse is judged based on the victim's own testimony and a physician's diagnosis. However, since this method of determining physical abuse is qualitative, an objective and quantitative evaluation of the applied external force is required.

In child abuse that takes place indoors, children are injured when their bodies collide with the floor. So, it is necessary to investigate how the type of floor affects the risk of head injury. Therefore, we investigated the effects of floor type on head injury risk by head impactors falling on several types of floors, such as wooden flooring and mats. In previous studies, Head Injury Criteria (HIC) has been used to assess the risk of head injury. However, depending on the posture at the time of injury, rotational velocity may be more dominant than translational acceleration, and the range of angular velocity change of the head at impact can be used to estimate the risk of acute subdural hematoma injury, according to a study by Depreitere et al.<sup>1</sup> This study measured how the HIC and the angular velocity change range vary depending on the posture and the injury situation by dropping a pediatric dummy doll onto different floors in different postures and examined the angular velocity change range as a parameter of head injury risk.

To investigate the effect of impact surface on the risk of head injury, head impactors were dropped onto floors made of different materials at different drop heights. The selected impact surfaces were wooden flooring, mats, carpets, and tatami mats. The experimental results showed that HIC=700 was exceeded at a drop height of 70cm for the wooden flooring, 80cm for the mats, and 100cm for the tatami mats. Therefore, it was shown that the material of the impact surface affects the risk of head injury.

To investigate the effect of the posture of the injured person during the fall, the dummy doll was dropped in different postures. In order to standardize the situation at the time of injury, the impact surface was wooden flooring, and the dummy was released so that its head would impact the floor between the joists. The height of the fall was measured at 30cm and in the state of falling from a grasping position. As a result, when the subject fell face down from a height of 30cm or fell backward from a grasping position, the angular velocity change range was about 40rad/s, suggesting the possibility of an acute subdural hematoma. In this case, the HIC was lower than the risk criterion, suggesting the possibility that only acute subdural hematoma could occur without concussion or fracture. Therefore, it can be considered necessary to consider not only the HIC but also the angular velocity change range when estimating the risk of head injury.

### Reference:

- <sup>1</sup>. Depreitere, Bart & Lierde, Carl & Maene, Sigrid & Plets, Christiaan & Vander Sloten, Jos & Audekercke, Remy & Perre, Georges & Goffin, Jan. (2004). Bicycle-related head injury: A study of 86 cases. *Accident; analysis and prevention*. 36. 561-7. 10.1016/S0001-4575(03)00062-9.

### Injury Risk; Child Abuse; HIC



**D29    The Magnetic Recovery and Mass Spectrometric Analysis of Fingermarks Contaminated With Cosmetics Products**

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**WITHDRAWN**

**D30    The Application of Environmental Design Principles in Preventing Crime and Reducing Fear of Crime in Cities: The Case of Kocaeli, Turkey**

*Tugba Unsal Sapan, PhD\*, Üsküdar University Institute of Forensic Science, Istanbul, TURKEY; Busra Ozen, BArch\*, Üsküdar University Institute of Forensic Science, Istanbul, TURKEY; Sevil Atasoy, PhD, Üsküdar University Institute of Forensic Science, Istanbul, TURKEY*

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**WITHDRAWN**

## D31 Generalized Cosine Similarity for Mass Spectral Comparisons

*Anthony Kearsley, PhD\*, National Institute of Standards and Technology, Gaithersburg, MD; Arun Moorthy, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Matthew Roberts, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

**Learning Objective:** After attending this presentation, attendees will learn how they can construct high-dimensional discretized consensus mass spectra from replicate measurements and compute the similarity between these objects as a more sensitive approach to sample discrimination.

**Impact Statement:** This preliminary research has the immediate impact of introducing the forensic community to a method for leveraging replicate mass spectral measurements when comparing samples and may have long-term value as an approach to quantify uncertainty in comparisons.

An infinite dimensional variant of the cosine similarity commonly used when comparing mass spectra in tasks like mass spectral library searching and pair-wise spectral comparisons is presented. This Generalized Cosine Similarity (GCS) approach utilizes replicate mass spectral measurements to characterize variability and increase sensitivity. In particular, the method constructs a data structure referred to as High-Dimensional Discretized Consensus (HDDC) mass spectra that are identified as vectors whose statistical properties (mean, variance, standard deviation) can be assembled into a measure of similarity/dissimilarity. The result is a more sensitive measure of similarity that could have several potential applications in the forensics chemistry workflow from instrument calibration and maintenance to uncertainty quantification during compound identification.

This presentation covers a preliminary exploration of the method, where the GCS between several pairs of structurally similar compounds is computed, based on replicate in-source Collision Induced Dissociation (is-CID) mass spectra collected using Direct Analysis in Real Time-Mass Spectrometry (DART<sup>®</sup>-MS). For each compound, constructed multiple HDDC using disjoint sets of the replicate mass spectra are employed and assessed as to how well the compounds were distinguished using GCS and the min-max test for spectral discrimination. A direct comparison of GCS to traditional single-measurement cosine similarity is made, and it was determined that the GCS approach worked as well or better than single-measurement comparisons for the evaluated pairs of compounds.

Preliminary results are promising, and there are several open questions that still must be answered.<sup>1</sup> Since the GCS approach accounts for measurement variability when making computing similarity/dissimilarity, it is important to understand all possible sources of measurement variability. Ideally, sources of measurement variability can be quantified and appropriately considered in GCS computations. Additionally, future work includes exploring the applicability of GCS for comparisons of mixtures measured using DART<sup>®</sup>-MS (e.g., seized drug evidence) and other types of mass spectra of pure compounds (e.g., electron ionization mass spectra).

### Reference:

- <sup>1</sup>. Roberts MJ, Moorthy AS, Sisco E, Kearsley AJ. Incorporating measurement variability when comparing sets of high-resolution mass spectra. *Analytica Chimica Acta* 1230 (2022): 340247-340247. <https://doi.org/10.1016/j.aca.2022.340247>.

### Mass Spectrometry; Cosine Similarity; Compound Identification

## D32 Rapid Isolation of Sperm From Sexual Assault Case-Type Samples Utilizing a Microfluidic Device

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**Learning Objective:** After attending this presentation, attendees will better understand a rapid alternative to differential extraction for isolation of sperm from mock sexual assault samples that is compatible with DNA typing by targeted Next Generation Sequencing (NGS).

**Impact Statement:** This presentation will impact the forensic science community by presenting studies for improved isolation of sperm from mock sexual assault samples to reduce the signal from the female fraction in mixed samples.

Targeted NGS or Massively Parallel Sequencing (MPS) has recently emerged as an efficient method to profile both Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) markers, together or independently, at a relatively high plexity for forensic DNA analysis.<sup>1,2</sup> Some clear advantages of Targeted NGS assays for forensic DNA analysis include the ability to design small amplicons as the sequence and not the size, which is used to distinguish loci, the added information of sequence variation within repeats and flanking regions to increase the discrimination power of the loci, and the ability to multiplex more loci together in one assay such as autosomal and Y markers. However, these assays are susceptible to inter- and intra-locus imbalance and higher stutter due to the nature of library preparation and clustering for sequencing. Therefore, even with the added advantage of sequence information, Targeted NGS assays have similar sensitivity for detection of minor contributors in mixtures compared to traditional CE technology for sexual assault samples. Sexual assault samples are usually composed of the combination of two or more individual body fluids or secretions. Mixed forensic DNA profiles are expected from these types of samples, and protocols, such as differential extraction for isolating sperm from sexual assault samples, have been developed to reduce the profile from the victim in these cases.<sup>3</sup> These protocols can have varying success for recovery of the sperm fraction or removal of the epithelial DNA between operators and laboratories.<sup>4</sup>

A microfluidic device has been developed for isolation of DNA from various biological samples to assist with NGS sample preparation for sequencing applications. This device successfully allows for purification of genomic DNA (gDNA) from samples using microfeatures in a chip to entangle the DNA while allowing proteins and cell debris to flow through the chip. The technology can also be used to isolate sperm cells from samples by trapping larger epithelial cells or cell-free gDNA on the microfeatures and allowing the smaller sperm to be collected. This report details the isolation of the sperm from mixed mock sexual assault samples and subsequent analysis of autosomal and Y-STR markers using NGS. This system yields an improvement in locus and allele detection in the sperm fraction for the male contributor and reduction of the profile from the epithelial cells.

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3. Renata Jankova, Zlatko Jakovski, Robert Janevski, Differential extraction method as a golden standard in analyzing semen stains in sexual-assault cases, *Forensic Sci Int Genet.* 2019, 7(1): 838-840.
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### Differential Extraction; DNA Mixture; Next-Generation Sequencing

**D33    The Detection of Saliva Stains Using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR) Spectroscopy: The Effect of Surface and Time**

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**WITHDRAWN**

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## D34 Wheelchair User Sues Pharmacy for Access as a Violation of the Americans With Disabilities Act

*Robert Lynch, BArch\*, Architectural Expert & Consultant, Haymarket, VA*

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**Learning Objective:** After attending this presentation, attendees will know that: (1) the Americans with Disabilities Act (ADA) requires access for persons with disabilities to commercial facilities throughout the United States; (2) the accessibility is required to comply with the 2010 Accessibility Standards of the ADA; (3) maintenance of the accessibility of the commercial facility is required by the ADA; and (4) the person with a disability may bring suit against a non-compliant commercial facility.

**Impact Statement:** This presentation will impact the forensic community by informing attendees that required, specified, standardized accessibility shall be provided and maintained for persons with disabilities as designated under the ADA for commercial facilities throughout the United States.

This presentation is a report in support of the claim by Plaintiff, a man with an ambulatory disability, against a pharmacy, a commercial facility as defined by the Americans with Disabilities Act. The Plaintiff was being pushed in his wheelchair from the accessible van he used to arrive at the facility when the wheel of his chair was caught in a deteriorated depression in the bituminous pavement of the access aisle. Plaintiff was pitched from his chair, fell, and was seriously injured upon landing on the concrete pavement of the ramp to the sidewalk. The author of this presentation was asked by Plaintiff's counsel to investigate, analyze, and report on the physical conditions of accessibility for persons with disabilities within and proximate to the northerly entrance to the pharmacy located in Colorado with emphasis on the accessible vehicle parking stalls and access aisle of the commercial facility approaching the entrance to the commercial facility.

The analysis and report are based on the applicable standards of design and maintenance of the 2010 ADA Standards for Accessible Design under Title III of the Americans with Disabilities Act of 1990 (ADA), 42 U.S.C. Sec. 12101 et seq., and its related amendments and implementing regulations; and various local building and facility maintenance codes. This study utilized photographic data of the site at the northerly entrance of the pharmacy. The conditions of the two accessible parking stalls and the access aisle between them that connect the accessible parking area to the northerly entrance to the pharmacy were analyzed. During the analysis, several conditions were discovered that to a reasonable degree of certainty present as non-compliant with applicable building design and building maintenance codes of the Americans with Disabilities Act (ADA), the local regional building code, and the local building maintenance standards.

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### Accessibility; ADA; Building Maintenance Code



## D35 Electrical Injury Evidence and Sequelae

*Helmut Brosz, BSc\*, Forensic Science International Group, Markham, ON, CANADA*

**Learning Objective:** After attending this presentation, attendees will understand the important role forensic electrical engineers play in reconstructing an electrical injury accident scene through documentation and observation of sequelae presented among other factors. Scene reconstructions assist forensic nurses, doctors, and pathologists in determining if the reported symptoms are consistent with electrical engineers' findings to determine if electrical injuries are present. Some electrocutions without visible markings may have a criminal aspect by use of electricity; this is either intentional (electrical homicide, electrical torture) or accidental (negligent homicide).

**Impact Statement:** This presentation will impact the forensic science community by utilizing electrical engineering methodology alongside forensic nurses in investigations concerning suspected electrical injury or electrocutions.

In this presentation, electrical injury and electrocution cases will be presented with various outcomes and sequelae. Electrocution generally means current has passed through the heart and death ensued. Through investigation, it can be determined if death was caused intentionally, by criminal negligence, or other causes.

Electrical injuries are multifaceted, the severity of electrical trauma ranges from gross loss of limb and tissue due to high voltage and high current contact to low voltage and low current contact, not leaving any marks on the body depending on contact area, duration, and other environmental factors. However, nerve damage can lead to delayed injuries. Since some electrical injuries involve marks on the body, there can be challenges in accurately assessing the pathway(s) of current, the modes and circumstances of contact. When an electrocution is being examined, investigators must inspect the scene and the body before drawing conclusions.

In some cases, it can be difficult to determine whether the incident was deliberate, accidental, or if an electrocution actually occurred due to lack of evidence. For example, if a victim were to be electrocuted by fallen electrical wires, there are numerous possibilities as to what caused the wires to fall. One possible situation is an electrical worker who installs wires in such a manner that they could foreseeably cause an electrocution; such a case could be considered negligent homicide or manslaughter. Another situation is the electrical system being sabotaged in some way; this would be an intentional electrical homicide. An incident could also be the fault of the victim, such as electrical suicide. Natural causes such as floods, winds, storms, and lightning bringing down power lines and poles can cause electrocution.

When investigators such as forensic electrical engineers examine the scene of an electrocution, they cannot afford to make any assumptions. Although some instances may appear to have a clear cause in the view of forensic nurses and doctors, all possibilities should be taken into consideration with the expertise of a forensic electrical engineer.

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### Electrical; Injury; Electrocution

## D36 Failure of Suture Materials Used for a Cesarean Section

*Sarah Hainsworth, BENG, PhD\*, Bath University, Bath, ENGLAND*

**Learning Objective:** After attending this presentation, attendees will understand the mechanical properties of suture materials, their possible failure modes, and the type of evidence that is required for determining the root cause of the failure.

**Impact Statement:** This presentation will impact the forensic science community by allowing materials scientists, engineers, and surgeons to understand how sutures fail and the techniques that can be used to investigate failures and document the necessary evidence.

Absorbable sutures are widely used for internal wounds; one common situation in which they are used is for caesarean sections after childbirth. The materials used for the sutures are usually polymer fibers made from non-toxic monomer precursors. One of the most widely used polymers is polyglactin 910, a polysaccharide made from a copolyester of lactic acid and glycolic acid. These copolyesters are harmless products that are easily hydrolyzed by body fluids over the same time scale as wound healing and are then excreted by the body.

In several cases, sutures have been found to have failed post-surgery, leading to traumatic consequences of the wound opening and the intestine being exposed. Patients in this scenario pursue claims against the surgeon, the hospital, and/or the suture manufacturer. In some cases, the stitch that failed is not retained, owing to the emergency nature of the surgery needed to repair the wound. This leaves the question for the expert witness of how the stitch failed when investigation of the evidence is impossible.

New suture materials can be tested to determine the strength of the materials. Here we report the results of tests on three types of synthetic suture materials: Monocryl (dyed), Vicryl, and PDS. Each suture type was supplied in two gauge sizes—metric 1 and metric 0—corresponding to suture diameters of 0.4mm and 0.35mm, respectively. Monocryl and PDS are monofilaments (a single strand), while Vicryl is a braided filament. All three types of sutures were obtained from Ethicon, which is a division of Johnson and Johnson® Medical Ltd. The suture materials were found to have very high tensile strengths between 1,200 to 1,400MPa.

The sutures tested are all known to lose their strength *in vivo* as a consequence of the hydrolysis of the polymer as the material is absorbed into the body. The manufacturer's data were used to calculate the retained strengths after a period I.

In addition to the quasi-static tensile tests, tests at high strain rates were conducted to see whether “coughing” by the patient might lead to conditions under which the sutures would fail. The suture materials were found to be strain-rate insensitive for the testing speeds used here. The possible loading on the sutures from the soft tissue were estimated and found to be far lower than the forces required to exceed the tensile strength of the fiber.

Possible failure modes for sutures include: failure at knots or other attachment points; incorrect technique by the surgeon leading to the knot not being properly tied; the stitch not being placed correctly in the soft tissue; failure of the suture material itself from damage during the surgery or handling in the operating theatre; a quality-control issue in the polymer precursors; or an included defect during manufacture of the suture material.

In the absence of evidence retained during the medical emergency, it is not possible to attribute fault. This work highlights the need for evidence collection and failure analysis through the retention of failed products, or even good, contemporaneous photographs of failures that would allow the expert to comment more confidently about the cause of the failure.

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### Sutures; Failure; Tensile Strength

## D37 On-Board Video Analysis Is Used to Defend a Truck Operator in the Case of a Pedestrian Killed in the Crosswalk

*Kurt Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA*

**Learning Objective:** The goal of this presentation is to illustrate how video evidence analysis was used to determine that the actions of a truck operator were not in violation of a vehicle code. Synchronized, split-view video analysis demonstrated the actions placed a pedestrian directly into the path of an approaching truck, and therefore determined to be the cause in this fatal incident.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a methodology used in video analysis and will thereby assist in correctly determining the applicable vehicle code section.

At the incident T-intersection, east-west vehicles are through traffic, and northbound traffic is controlled by a posted stop sign. A pedestrian, walking westbound on the south sidewalk, was intending to cross northbound in a nearside crosswalk. The operator of a northbound trash truck stopped at the limit line, intending to turn right eastbound. Both operator and pedestrian waited for traffic to clear. The operator initiated his turn, then the pedestrian stepped into the roadway. While in the crosswalk, the pedestrian was struck and knocked over by the truck, subsequently dragged, and tragically killed. Law enforcement investigators concluded the fatality was caused by the truck operator who violated vehicle code 21950(a), a section that specifies a drivers shall yield the right-of-way to a pedestrian crossing the roadway within a marked crosswalk. However, a detailed video analysis helped defend the operator facing potential criminal charges.

The incident truck featured an on-board video system with six cameras capturing the driver's actions and views looking forward, rearward, left, right, and into the hopper. Only the right-side camera recorded the pedestrian's approach and their movements into the roadway before impact.

Ambient sound levels of 65.3 to 74.5dBA with the truck's engine running were measured at 10-foot increments relative to the front bumper centerline and increased with decreased distance to the truck.

The truck's flashing LED turn signal indicators were found to be operational at the time of the incident. The on-board, driver-view video proves the truck operator used his turn signal by showing his left hand activating the switch with an audible "click" and intermittent clicking sounds from the indicator relay.

The intersection was inspected and digitally scanned. The incident truck was made available. The truck's location when stopped and during the right turn maneuver, as well as the pedestrian's approximate position on the sidewalk and within the crosswalk were determined by video frame analysis.

A side-by-side, synchronized, split-view video was created to explore the truck operator's actions contemporaneously with the pedestrian's relative position leading up to the impact. Syncing the driver and right-side camera videos was achieved by aligning the videos about an event independently observed in each. Watching the operator's actions simultaneously with those of the pedestrian in real time became crucial to the operator's defense.

The on-board videos demonstrated the operator had activated his turn signal and had stopped at the limit line before moving his truck forward. From his location on the sidewalk, the pedestrian could have observed the truck's flashing right turn indicators. Furthermore, the noise of the truck was above the ambient roadway conditions.

The synchronized split-view video showed the truck operator had been negotiating his right-hand turn for more than 5 seconds before the pedestrian stepped into the roadway, and therefore was not in violation of 21950(a). However, it was determined the pedestrian entered the crosswalk and walked directly in front of the advancing trash truck. Contradicting the conclusion of law enforcement investigators, the prosecution found the pedestrian had violated 21950(b), a section that does not relieve a pedestrian from the duty of using due care for safety and specifies that no pedestrian may walk into the path of a vehicle that is so close as to constitute an immediate hazard. Ultimately, the prosecution decided against filing criminal charges against the truck operator because the pedestrian was found to be the cause of this fatal traffic incident.

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### Video Analysis; Pedestrian Fatality; Crosswalk Violation

**D38 Research on the Deep Learning-Based Arc Melting Mark Detection Technique**

*Lim Kyu-Young, MS\*, National Forensic Service, Jeju-si, Cheju-do, SOUTH KOREA*

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**WITHDRAWN**

## D39 Video Analysis Helps Defend a Municipality in a Bicyclist's Impact With a Truck

*Kurt Weiss, MS\*, Case Study Collision Science, LLC, Santa Barbara, CA*

**Learning Objective:** The goal of this presentation is to illustrate video evidence used to determine a cyclist's speed on a quick descent. Video frame analysis was used to identify the path of the cyclist, thereby demonstrating rider behavior before a tragic impact that caused significant injury.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a methodology used in video analysis—cyclist's trajectory and speed determination.

A student enrolled in a school-sponsored, mountain biking team, a sports program in existence for six years. This eager cyclist demonstrated excellent bike-handling skills. Protective gear like helmets and gloves were required, and while riding beyond the campus boundaries, all students were required to ride single file. The out-and-back course was approximately 7.2 miles long on a picturesque mountainous road. The team pedaled to the summit and descended the backside, where they regrouped. Once turning around, the team climbed up and over to head back to campus.

On the return leg, and prior to descending from the summit, the incident cyclist traded helmets with his companion, because it was equipped with a video camera. Riding up front side had already provided familiarity with the road's steep grade and sharp turns. Unfortunately, while negotiating a right-hand turn near the bottom of his descent, the cyclist went wide and impacted a truck heading in the opposite direction. A complaint was filed alleging a dangerous condition of public property and motor vehicle negligence. The video recording became crucial evidence to defend the municipality in which the school was located.

Inspection of the roadway revealed that surface artifacts observed in the video were still present. A 2D geometrically correct orthomosaic photograph with perspective and lens distortion removed was created by photogrammetry software using drone images. Ground control point coordinates were added to reduce RMS error. The cyclist's path and distance from summit to impact were determined by video frame analysis and direct measurement of the location of selected artifacts. Based on a time-distance analysis, the cyclist's descent was approximately 1,950 feet long, and lasted 62.1 seconds. His average speed was 21.4mph.

The video evidence also demonstrated the cyclist's reckless behavior: excessive speed, riding without hands on the handlebars, and, most problematic, was crossing the approximate roadway centerline (established as the midpoint between the left and right pavement edges) when oncoming traffic could not be observed due to an adjacent embankment. In one instance, if opposing traffic had been present, a frontal impact with a moving vehicle would have been eminent.

The video evidence was analyzed to determine the cyclist's path, downhill speed, and proximity to centerline and pavement edge over each roadway segment. The cyclist passed a pickup pulling a cargo trailer traveling uphill. This event provided evidence of the potential for vehicular traffic. At one point, the cyclist momentarily lost control due to his excessive speed and entered the far side shoulder on a turn. Following his dramatic save, the cyclist celebrated his good fortune, and promptly regained his downhill speed.

The cyclist seems to have ignored all the previous warnings of vehicle traffic and excessive speed and entered the last right turn at approximately 24mph. The bicycle tires passed directly over a roadway artifact consistent with fragmented and/or loose asphalt, the alleged dangerous condition. However, this artifact did not appear to significantly alter the bicycle's trajectory. Instead, his speed took him wide, and directly into the oncoming truck. The impact had a closing speed of approximately 43mph. Motor vehicle negligence arose, because at impact, the truck's left side tires were approximately 1.9 feet over the roadway centerline.

Damage to the truck (fractured trim, dents, and dark-colored transfers) suggest the bicycle struck the truck's left headlight and fender. The cyclist impacted the left side exterior mirror with his face, causing significant fractures. Analysis of the relative pre-impact trajectories shows that had the truck remained to the right of center, then the cyclist would have struck the left rear corner of the truck's utility bed.

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### Video Analysis; Dangerous Conditions; Motor Vehicle Negligence

## D40 Forensic Human Identification From Facial and Cranial Images Using Deep Learning

*Mario Villar, BS, University of Granada, Granada, Andalucia, SPAIN; Andrea Valsecchi, PhD\*, Panacea Cooperative Research and DAsCI, Capoterra, Sardegna, ITALY; Pablo Mesejo, PhD, University of Granada, Panacea Cooperative Research and DaSCI, Granada, Andalucia, SPAIN*

**Learning Objective:** After attending this presentation, attendees will be familiar with a novel, original, and groundbreaking way of approaching the problem of human identification based on a skull and a photograph of a missing person. Specifically, the developed prototype makes use of some of the most recent AI techniques for image processing.

**Impact Statement:** This presentation will impact the forensic science community by presenting some preliminary results on the application of cutting-edge AI techniques to craniofacial identification, delving into what appears to be a fruitful research line.

Forensic human identification (FI) by means of craniofacial superimposition is commonly performed in a manual manner. This FI technique involves maximizing the overlap between an antemortem facial picture and the 2D projection of a postmortem skull, as well as analyzing the morphological and anatomical correlation between skull and face. There exist some AI-based (semi-) automatic approaches, but these require the annotation of reference points (landmarks) on skull and face, and the subsequent calculation of the overlap between both structures (posed as an image registration problem). On the contrary, this work tackles the direct craniofacial identification scenario, where we try to contribute to the FI process directly from frontal cranial and facial photographs of a person, without landmarking any material or calculating any skull-face overlay.

This work uses computational techniques to learn the similarity patterns between the skull and the face when both correspond to the same identity, and the dissimilarity patterns when they do not belong to the same individual. Specifically, this study proposes a novel strategy employing Deep Learning (DL) models to simplify and speed-up the identification process. The limited amount of training data, as well as the different nature of the objects under comparison (skull and face), among other reasons, make this problem a very difficult one. This study used a set of 3D cranial models from 114 different individuals and the facial images of UTKFace (<https://susanqq.github.io/UTKFace/>), the publicly available dataset with more than 20,000 images. The 2D cranial images employed for training are extracted from the aforementioned 3D models, and different DL approaches based on Convolutional Neural Networks and Siamese Neural Networks, are proposed. From the results obtained, it was concluded that DL techniques manage to reduce the candidates' sample by 88%. In other words, according to these experimental results, given a frontal image of a skull and 100 facial pictures, 88 of those facial images can be discarded, assuming that the actual positive identity is in the remaining 12 images. We consider that these results open a promising future for the application of DL methods in craniofacial identification.

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**Craniofacial Identification; Deep Learning; Computer Vision**



## E1 A Scoping Review to Define Sexual Coercion Post-2016

Patricia Speck, DNSc\*, Hoover, AL

**Learning Objective:** Sexual Coercion (SC) is a pervasive problem that includes domestic violence, Reproductive Coercion (RC), and Intimate Partner Violence (IPV). After attending this presentation, attendees will be aware that evolving evidence requires continuous updating of definitions.

**Impact Statement:** This presentation will impact the forensic science community by imparting a full understanding of the language used to describe sexual violence, specifically coercion in relationships.

**Background:** SC is an aggressive behavior. Associated with the current definition of SC is domestic violence, RC, and IPV. Grace & Anderson's systematic review identified types of sexual coercion in 2016, but no subsequent research is published.<sup>1</sup> Legislative changes and attitudes provided the impetus for studying societies' change in understanding and law since 2016.

**Aims:** Analyze literature since 2016 for sexual coercion emerging terms and definitions.

**Design:** Scoping literature review since 2016.

**Design and method:** A focused literature search used search engines: Cumulative Index to Nursing and Allied Health Literature (CINAHL), PubMed®, and Scopus®. Search terms determined from the literature included intimate partner violence *and* reproductive coercion *or* sexual coercion.

**Inclusion criteria:** English, peer-reviewed, United States only, human beings, 2016-2021.

**Exclusion criteria:** Non-English, Grey literature, Pre-2016, EndNote Capacity, International/non-United States, animals.

**Results:** Of 11,022 articles, 10,699 met the elimination criteria, resulting in an evaluation of 353 articles.<sup>2-9</sup> Of the 353, 274 were non-responsive or duplicative, leaving 79 for review. The vocabulary implies intent, including reproductive coercion, verbal coercion, sexual coercion, financial coercion, negotiation, pregnancy coercion, stealthing, sex stress/regret, sexting, sextortion, condom refusal, coerced consent, and entitlement. Examples: condom refusal, birth control sabotage, begging for sex, guilt-tripping, not taking no for an answer, and transactional sex.

**Discussion:** SC is a broad term covering behaviors that are offensive, and sometimes illegal. The literature revealed three responses: (1) GO—where both parties are sexually consensual, sexual activities are negotiated, and No means No; (2) DANGER—boundaries are not yet set, so be cautious, where there is association with lack of permission to proceed; and (3) STOP—the individual is incapable of saying yes (age, capacity, or other disability), and stop when judgement is clouded (any substance use).

**Conclusion:** The discovery of new language encourages continued research to define sexual coercion, where more research is needed to modify aggressive behaviors with educational exposure to new evidence of criminal behavior, practiced negotiation through simulation, and language modification that identifies illicit behaviors.

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### Sexual Coercion; Intimate Partner Violence; Dating Violence

## E2 Are Nursing Students Prepared to Recognize Violence Against Children? The Evaluation of an Italian Sample

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**Learning Objective:** After attending this presentation, attendees will better understand the importance of appropriate knowledge about violence against children thematic for nursing students.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the necessity for each nursing school to define its current ability to properly train students in the child abuse field to program corrective strategies.

Violence against children can be difficult to diagnose due to misleading histories and because of the absence of specific injuries. Missing or misdiagnosing violence could result in dangerous consequences for both children and families. In cases of misdiagnosis, children will not have the chance to accede to useful treatments. Moreover, in cases of misdiagnosis, erroneous interpretation of events can generate legal issues in civil, juvenile, family, divorce, and criminal courts. Since children account for nearly one-third of emergency department visits, medical and nursing staffs should be trained to understand the various forms that child abuse can take, and the warning signs seen.

In Italy, the first health care professional who triages patients in emergency departments is usually a nurse. Moreover, nurses are usually in continuous contact with patients during hospitalization. For these reasons, they should have accurate and specific knowledge about identification and management of cases suspected for child abuse. A limited number of studies have been conducted about health workers' abilities, especially for nurses, to recognize suspected child abuse cases.<sup>1-3</sup> Health care professionals may miss sentinel injuries or incorrectly attribute them to accidental trauma. Studies and research about nursing students' knowledge of violence against children thematic are extremely limited.

The present analysis aims to: investigate nursing students' objective knowledge about this topic and identify useful recommendations to increase future nurses' awareness. The study population included 175 Italian nursing students, divided into two groups: Group-A—students who attended pediatric traineeship and/or pediatric exam, and Group-B—students who did not attend pediatric traineeship and/or pediatric exam. The 175 nursing students filled out a questionnaire consisting of 18 questions (1 general, 6 about sexual-abuse, 6 about child-maltreatment, and 5 about neglect). In addition, the students were asked to self-score their knowledge about this thematic with a score from 1 to 10 points. Objective knowledge and self-rated knowledge were analyzed separately. Quantitative and statistical analysis were carried out by Excel® formulas.

In all, 70.1% demonstrated a low objective knowledge about child abuse thematic, answering less than half of the questions correctly. In this population, students' objective knowledge about signs of non-accidental traumas, sexual abuse, and neglect appeared particularly deficient. For example, 70% incorrectly answered the following question: "Which of the following findings is more frequently associated with abusive head trauma?" (Correct answer: bilateral retinal hemorrhages). In addition, 84.57% demonstrated awareness of their lack in this field, self-scoring their knowledge from 1/10 point to 5/10 points. Both objective knowledge and self-rated knowledge were non-significant with regard to students who took the training/the pediatric exam (Group A) versus those who did not (Group B).

For all questions, results of chi-square tests yielded no statistical differences between the two groups, demonstrating that the deficiencies were not related to students' different levels of progression in school training and/or preparation. This analysis points out that nursing school education can be a weak point in future nurses' knowledge on violence against children. Thus, educational programs should improve this thematic to assist future nurses in recognizing abusive suspected cases. This goal could be easily reached due to students' high awareness of their lack of knowledge in this field, as was demonstrated in our sample. The first step for nursing schools should consist of revising the pre-existing training program by a group of experienced health care professionals. Then, it should be implemented to guarantee proper skills. Moreover, extensive review of the literature—comparing nursing schools from different geographical areas—suggests common issues independent of which accreditation system is implemented. Thus, independent of which corrective strategy will be planned, it could be useful to identify a common program for all nursing schools to standardize students' knowledge.

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### Violence Against Children; Child Abuse; Nurses

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**E3      The Investigation of Competence Levels of Nurses in Collecting Biological Evidence From Forensic Cases Incoming to Emergency Services**

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**Learning Objective:** After attending this presentation, attendees will be aware that in the results of the research, it was found that more than half of the nurses received both training on forensic cases and collected evidence from forensic cases.

**Impact Statement:** This presentation will impact the forensic science community by clarifying understanding of the importance of nurses, who are the first direct contacts of forensic cases in hospitals

Health care professionals working in emergency services are generally the first to encounter a forensic case, make the first contact with the case, and reach the victim's/suspect's belongings during the examination, treatment, and follow-up. Therefore, emergency nurses have a critical role in identifying, collecting, and preserving forensic evidence. In literature, the need for emergency workers to take training on forensic cases was stressed so they can properly collect biological materials that may have evidentiary quality and prevent them from deteriorating when they encounter suspects or victims taken for treatment.

In Turkey, the country in which this work was conducted, there are no legally defined forensic nurses with job descriptions. However, a forensic nursing course is included in the curriculum of basic nursing education as an elective course for two hours a week during one semester. For this reason, the evaluation of forensic cases is mostly conducted by emergency nurses who do not have forensic nursing education or have only limited training.

This study was conducted to examine the proficiency level of nurses in collecting biological evidence from forensic cases coming into emergency services. The population of the study consisted of the nurses of a university training and research hospital that receives 5,000 forensic cases, on average, annually. The sample of the study consisted of 67 nurses from the adult emergency service and 18 nurses from the pediatric emergency service. The survey data from 85 participants were evaluated using the IBM SPSS Statistics 24.0 (SPSS 24.0) package program. For the interpretation of the findings, the correlation between two qualitative variables was examined using the "Fisher-Exact," "correction for continuity," and "Pearson- $\chi^2$ " crosstabulation.

In the results of this research, it was found that more than half of the nurses received training in both forensic cases and collecting evidence from forensic cases, with 94.5% of the participants collecting evidence on external body examination having erroneous information. While the rate of nurses who used the wrong antiseptic before sampling was 24.7%, the rate of nurses who took wrong samples from between the fingernails and nails was similar with 24.7%. The rate of those who failed to take samples from the stomach contents was 25.9%, and the rate of nurses who made a mistake in firearm injuries was 27.1%. When discussed in terms of forensic science practice, each erroneous taking of biological evidence can lead to false conclusions in terms of investigation and prosecution. Based on the results obtained from this research, it is recommended that nurses be given training on biological evidence collection both before graduation and during in-service training programs, and that forensic nursing courses should be compulsory in undergraduate education programs.

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**Forensic Case; Nursing; Biological Evidence**

## E4 The Role of Forensic Nursing in Human Trafficking Survivor Intervention

*Jaymee Kim, PhD\*, University of Findlay, Findlay, OH; Michelle Stratton, RN, Blanchard Valley Health Systems, Findlay, OH*

**Learning Objective:** After attending this presentation, attendees will better understand the design and implementation of effective and ineffective human trafficking intervention procedures in the rural United States, disciplinary concerns when trying to track human trafficking data, and techniques used by forensic nurses to intervene in human trafficking scenarios.

**Impact Statement:** In the medicolegal community, identification of human trafficking survivors, including evidence documentation and reporting, is particularly challenging. This presentation will impact the forensic science community by providing options for forensic nurses to develop response practices that can assist in survivor intervention and criminal prosecution of perpetrators.

In 2000, the United States Congress passed the Victims of Trafficking and Violence Prevention Act that defines sex trafficking under 22 USC 7102(9)(A) as a situation in “which a commercial sex act is induced by force, fraud, or coercion, or in which the person induced to perform such an act has not attained 18 years of age.” The second component addresses labor trafficking under 22 USC 7102(9)(B) as “the recruitment, harboring, transportation, provision, or obtaining of a person for labor or services, through the use of force, fraud, or coercion for the purpose of subjection to involuntary servitude, peonage, debt bondage, or slavery.” Only having passed just over 20 years ago, human trafficking charges are infrequently pursued by law enforcement and prosecutors. Instead, related charges, such as sexual assault, may be issued. Part of the challenge of pursuing human trafficking charges lies in the fact that most survivors do not self-identify as someone who is trafficked. Unlike popular media depictions of groups of people corralled, chained, and transported in cargo containers, most victims of trafficking in the United States are trafficked by someone they know through the use of manipulation and coercion. Because of the close relationship to the trafficker and the victim’s absence of self-identification as a trafficking victim, locating and providing outreach for victims is particularly challenging—as is generating data on the prevalence of trafficking in a given region.

This study relies on five years of ethnographic data collection, consisting of informal interviews, semi-structured interviews, observation, and participant observation from rural Ohio and first-hand experience developing human trafficking response policies to present how forensic nurses and medical personnel can play an integral part in human trafficking intervention. This study answers the questions of how local responder relationships are built (or are in conflict), how survivors and survivor data may be tracked, and how public outreach can be implemented. Here, findings include the use of local public and medical training programs in human trafficking identification, development of novel emergency department protocols for sexual assault nurse examiners, and tensions in the development of relationships with local response stakeholders that impact human trafficking response protocols.

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### Human Trafficking Intervention; Forensic Nursing; Human Trafficking Prevention

## E5 An Introduction to the New Organization of Scientific Area Committees (OSAC) Forensic Nursing Subcommittee

Nancy Downing, PhD\*, Texas A&M College of Nursing, College Station, TX; Katherine Scafide, PhD, RN, George Mason University, Fairfax, VA; Joyce Williams, DNP, Randallstown, MD

**Learning Objective:** After participating in this session, attendees will be able to: (1) relate the need for development of forensic nursing standards; (2) recognize priority areas for improvement in health care delivery in the response to victims; (3) describe current proposed forensic nursing standards and research gaps; and (4) discuss potential impacts on forensic nursing science and practice and forensic science more broadly.

**Impact Statement:** The OSAC Forensic Nursing Subcommittee is new. This presentation will impact the forensic science community by raising awareness among AAFS members of the subcommittee and invite discussion on how inter-professional collaboration can serve to strengthen forensic science more broadly.

Forensic nursing has become the standard in the health care response to victims of violence. To ensure quality, reliability, efficiency, and consistency among practitioners, development of forensic nursing standards is essential. The 2009 National Research Council Report, *Strengthening Forensic Science: A Path Forward*, critiqued the forensic science community and emphasized the need for enforceable standards and promotion of best practices.<sup>1</sup> As the visibility of forensic nursing has grown, the National Institute for Standards and Technology (NIST) OSAC for Forensic Science deemed it critical to create a Forensic Nursing Subcommittee (FNSC). The FNSC was established in October 2021. The purpose of the FNSC is to propose high-quality, technically sound standards that define minimum requirements, best practices, and evidence-based protocols and to provide guidance to ensure reliable and reproducible outcomes. Their initial mission is to draft standards related to the collection and storage of physical evidence from victims of sexual assault, sexual assault examinations, development and validation of new methods, and training and continuing education of practitioners. Consisting of a diverse membership of forensic practitioners, the subcommittee will establish terminology definitions and identify gaps in research to improve evidence-based practice.

This presentation will briefly review the histories of forensic science and forensic nursing, describe the FNSC composition and OSAC standards development processes, and present progress to date on FNSC standard development and identification of research gaps. The future promise is the advancement of forensic nursing science that will strengthen practice and health care delivery for victims of violence across the nation. The impact of the FNSC will be to improve the overall health care delivery responses to victims who experience sexual assault, domestic violence, stalking, human trafficking, elder abuse, and other non-accidental crimes against persons. Outcomes will align with the missions of the Violence Against Women Act, OSAC, AAFS, and the Department of Justice to support sound forensic science principles.

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### Forensic Nursing; Standards; Forensic Science

## E6 Building Quality Metrics to Authenticate Science at Work for Your Forensic Nursing Program

Jamie Ferrell, MBA, BSN\*, Memorial Hermann Health System\*, Katy, TX

**Learning Objective:** After attending this presentation, attendees will identify the value of building quality metrics to measure improvements in their forensic nursing science practice. This commitment to validating evidence-based principles achieves improved health outcomes, best quality of forensic science results, and accurate information for judicial results.

**Impact Statement:** This presentation will impact the forensic science community by providing forensic nursing programs' demonstrated value of quality metrics, which can have improved outcomes on health and higher reliability of forensic evidence results as well as factually influencing judicial consequences.

Clinical quality measures are a cornerstone of efforts to improve care delivery and patient outcomes in all health care settings across the United States.<sup>1-</sup>

<sup>3</sup> This session will explore why quality metrics should be a key component for forensic nursing programs. Understanding exactly how quality metrics work has the potential to strengthen forensic nursing program development with patient outcomes as well as the high-reliability implications for the criminalist and judicial sciences with evidence preservation and testing. Developing outcome measures that are truly meaningful can be challenging in the field of forensic nursing. These tools help measure or quantify health care processes, best evidence recovery, improved outcomes, and patient perceptions. Organizational structure associated with the ability to provide high-quality health care and/or relate to one or more quality goals for health care and forensic science results are worthy strategies. These goals include effective, safe, efficient, patient-centered, equitable, and timely care as well as best evidence recognition, collection, and preservation.<sup>4</sup> The application of quality metrics to the forensic nursing program can yield improved health outcomes, reliable forensic evidence results, reduce burden and control cost, improve nursing satisfaction, and influence external organizations in the investigative and judicial domains.<sup>5</sup>

Forensic evidence results play an increasingly important role in bringing many criminal investigations and prosecutions to a successful conclusion. The quality of the results performed in forensic science laboratories is influenced by the precision of forensic evidence collection, sample handling, and preservation by the forensic nurse.<sup>6</sup> Many factors are important contributors to quality assurance in forensic nursing that can complement the forensic evidence results presented at trial such as the detailed documentation, including the patient history, injury identification, and collected evidence detail to provide accurate court testimony. Therefore, it is essential to ensure and maintain the highest standards of evidence-based practice and accuracy in the forensic nursing science specialty.

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### Quality Metrics; Forensic Nursing; Forensic Evidence



## E7 Building Networks, Overcoming Barriers, and Bringing Sexual Assault Nurse Examiner (SANE) Services to Rural Areas

*Tami Thomas, PhD, Florida International University, Jensen Beach, FL; Juan Carlos Nobrega, MPH\*, Florida International University, Miami, FL; Sherry Britton-Suscino, MSN, Florida International University, Boca Raton, FL; Michelle Caldera, MPH, Florida International University, Miami, FL*

**Learning Objective:** The goal of this presentation is to share findings from a project that has transformed a rural region's Sexual Assault Services through co-creation with rural law enforcement, victim services, and first responders. Attendees will learn how an International Association of Forensic Nursing (IAFN) -accredited curriculum and clinical practice hours have decreased wait times in the emergency rooms and clinics. Successful approaches and cultural awareness data and training outcomes will be shared.

**Impact Statement:** This presentation will impact the forensic science community by presenting how increasing the availability of racially and culturally diverse SANEs who understand the cultural intricacies of rural issues, language, and access barriers can be addressed by an effective program of training and co-creation with rural health care leaders, law enforcement, response teams, and support from the State Attorney's office.

**Background:** SANE programs were created to meet the medical and evidentiary needs of sexual assault victims. SANEs assess patients for acute health care needs, providing for the stabilization and treatment of the patient, as well as to provide written and photographic documentation of injuries, forensic evidence collection, and courtroom testimony. The benefits of SANE utilization are well researched and widely documented in the literature. However, despite widespread recognition of the benefits SANE programs provide, there is a chronic lack of SANEs and SANE programs, particularly in rural areas. As reported by the United States Government Accountability Office, training, funding, and availability of forensic sexual assault examiners are limited in all states, especially in rural communities where unsupported victims endure the responsibility of commuting to non-local SANE services.

**Significance:** The limited access to SANE resources throughout Florida creates challenges for sexual assault victims, Emergency Room (ER) personnel, proper rape screenings, assistance from SANE nurses, mental health counseling, and community support.

Through our program, we built new networks and assisted rural communities to overcome barriers and increase the number of SANE-trained and -certified nurses throughout the state. Our priority was to assist rural communities in training SANEs and build successful networks so victims can receive SANE services, including the forensic medical exam, evidence collection, and connection to community-based victim services.

**Purpose:** To address the present and urgent need for rural SANEs, leadership at a minority-serving university developed and implemented an innovative evidence-based training from the IAFN-accredited curriculum and clinical training in collaboration with a preeminent program in forensics. This preliminary data is focused on the development and outcomes from the initial cohort of sexual assault nurse examiners for rural and underserved communities.

**Methods:** Outreach to rural communities is based on need or presence of SANEs in these underserved communities. Trainees are recruited specifically from nine rural underserved counties of Florida and go through the SANE-training process and prepare to take their SANE certification exam within 1–2 years of receiving training. During this time, process and outcome measures are recorded. Trainees have monthly support meetings and the opportunity to schedule additional training as needed. Continuous process and outcome evaluation strategies are used to ensure rapid quality improvement of the program. Quantitative and qualitative data are collected on a regular basis to ensure that data are available for program improvement as approved by the Institutional Review Board.

**Analysis/evaluation:** Using process and outcome measures framed on evidenced-based practice and IAFN criteria, we collected quantitative data. We used qualitative feedback from the trainees and community stakeholders to improve processes and ensure the best trauma-informed care for these victims in rural areas. Analysis and evaluation are ongoing and will be presented.

**Results:** Preliminary results reveal that nine rural and underserved communities have now benefitted from an increase in the number of SANEs who can now deliver high-quality, evidence-based sexual assault evaluation and treatment. Implications for forensic science and criminal justice include the availability of racially and culturally diverse SANEs who understand the cultural intricacies of rural issues, language barriers, and access to care. Existing efforts have created an expansion plan for remaining rural and underserved areas of Florida. Specifically, detailing an effective program of training and co-creation with rural health care leaders, law enforcement, forensic laboratories, response teams, and the State Attorney's office. Complete results will be presented at the conference.

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### Sexual Assault Nurse Examiners; Rural Communities; Process and Outcome Measures



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**E8 Does Age Matter? Descriptive Data and Sexual Assault Kit DNA Analysis Findings of Elderly Sexual Assault Victims**

*Julie Valentine, PhD, MSN\*, Brigham Young University, Sandy, UT; Leslie Miles, DNP, Brigham Young University, Provo, UT*

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**Learning Objective:** After attending this presentation, attendees will have developed an increased understanding of variables associated with Sexual Assault (SA) in elderly victims, including DNA analysis findings from submitted and tested SA kits.

**Impact Statement:** This presentation will impact the forensic science community by helping attendees develop a greater understanding of SA of older women and enhanced knowledge of the Short Tandem Repeat (STR) DNA analysis findings of their SA kits.

SA is a pervasive problem affecting all ages, races, and genders. Although research has illuminated our understanding of SA within mainstream populations, few studies have examined SA of older persons. Researchers exploring characteristics of suspects who committed sexual violence against elderly individuals reported that elderly SAs are more likely to be committed by strangers in victims' homes.<sup>1</sup> Lee and colleagues reported that non-frail elderly SA victims were more likely to be assaulted in their homes by a stranger, while frail elderly SA victims were more likely to be assaulted in care centers by unrelated caregivers.<sup>2</sup>

The purpose of this presentation is to share research findings from an exploratory, retrospective study of 64 patients (ages 65 or older) seen for SA Forensic Medical Examinations (SAFME) from 2010–2019 in a United States Mountain West state. The age range was 66–102 years, mean age of 72 years of primarily female patients. Statistically significant findings will be shared, including higher rates of SA by non-related individuals with authority over patients (i.e., caregivers). SAs were less likely to happen in patients' homes, with 40% occurring in care facilities. Forty-three percent of patients were unable to answer specific questions related to assault. Fifty-seven percent of patients were found to have anogenital injuries. Additionally, data will be shared on STR DNA analysis findings from submitted and tested SA kits from these elderly victims. Age was found to be a significant factor in the likelihood of developing a probative STR DNA profile. Following menopause, our findings indicate a much lower likelihood of developing probative STR DNA profiles. We theorize that anogenital changes, including decreased lubrication, related to menopause and decreased estrogen, result in less DNA capture of foreign contributors. We propose additional research on these novel findings. Multidisciplinary practice implications will be discussed to meet the needs of this unique and often underserved victim population.<sup>3</sup>

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**Sexual Assault; Elderly Victims; Sexual Assault Kits**

## E9 The Prevention of Compassion Fatigue in Forensic Nurses During the COVID-19 Pandemic

Nancy Cabelus, DNP\*, Belleair, FL

**Learning Objective:** Following this presentation, attendees will be able to: (1) analyze the differences between compassion fatigue and burnout; (2) discuss how the COVID-19 pandemic has increased risk for compassion fatigue in forensic nursing practice; and (3) identify best practices for the prevention of compassion fatigue in forensic nurses

**Impact Statement:** This presentation will impact the forensic science community by bringing awareness to the disabling symptoms of compassion fatigue brought on by forensic nursing care during the COVID-19 pandemic. Discussion will center on recognizing such symptoms that affect the health and wellbeing of the forensic nurse. If symptoms are unrecognized or left untreated, compassion fatigue may lead to burnout for the nurse and could potentially create harm to patients.

Nurses provide the majority of health care services during a pandemic, and they have the most contact with patients.<sup>1</sup> Compassion fatigue is a contributor to nurse burnout, but it is distinctly different. Compassion fatigue may occur in nurses who work excessive hours in stressful settings and may also lack the resources needed to do the job. One definition of compassion fatigue is the “sympathetic consciousness of others’ distress, together with a desire to alleviate it.”<sup>2</sup> It is the combination of physical exhaustion, mental strain, and emotional withdrawal that is caused by caring for sick and traumatized people over an extended period of time.

Nurses who work with forensic patients are subjected to stressors in their practice settings on a regular basis. Over the past two years and since the onset of the COVID-19 pandemic, on-the-job stressors have increased even more. Additional job responsibilities, precautions, and hardships have happened more frequently than ever before. As patients were dying, nurses were bearing the burdens of helping others to cope and to survive. Coping with the stressors of typical forensic nursing practice is often challenging enough. Adding the COVID-19 virus to the mix is no easy task to deal with, and the risk for compassion fatigue will increase if nothing is done to prevent it. Some signs of compassion fatigue may include problems sleeping, having difficulty concentrating, exhaustion, irritability, ruminating thoughts, and loss of pleasure in the things that one used to enjoy. Quarantining and other efforts to reduce the spread of the COVID-19 virus, such as isolating, may only exacerbate loneliness and depression.<sup>3</sup>

The best practices for prevention of compassion fatigue are those that focus on self-care. Healthy interventions that nurses would typically recommend for their patients should be practiced for themselves. Getting enough sleep, eating a healthy diet, exercising, and staying hydrated are very important, as is taking time away from work to participate in activities with family and friends. Spending time in nature, meditation, and prayer may help the nurse to connect with their higher power or spiritual practice. While peer support and mentorship may add value to work-life balance, professional counseling or psychotherapy may be beneficial as well.

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### Prevention; Compassion Fatigue; COVID-19

## E10 The Dynamic Development of Forensic Nursing in Switzerland: Flying Forensic Nurses as a Model for Future Success in the Modern Care of Victims of Violence in Switzerland

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**Learning Objective:** After attending this presentation, attendees will have gained insight into the current situation of forensic nursing in Switzerland and the innovative implementation procedures being performed with the Department of Health of the Canton of Zurich and the Institute of Forensic Medicine in Zurich.

**Impact Statement:** This presentation will impact the forensic science community and modern patient care regarding the outcomes of the survey about Swiss forensic nursing. The support of more exchange between the already-educated forensic nurses in Switzerland will help to strengthen the forensic thought and the motivation to improve the situations in the health care institutions among them. There is a strong willingness from the forensic nurses working in Switzerland to improve the situation for victims of violence. The results of this survey allow us to create new ways of implementation and development—easily accessible but highly competent.

**Background:** Forensic nursing is a relatively new specialization for nurses in Europe. In the United States and Canada, forensic nursing has been established for more than 30 years, and it is well known and implemented in the North American health care and legal system. In Switzerland, the number of certified forensic nurses is growing steadily. The Institute of Forensic Medicine at the University of Zurich is one of the leading providers of advanced courses in forensic nursing. Since 2015, 93 forensic nurses have graduated the offered “Certificate of Advanced Studies in Forensic Nursing” course at the Institute of Forensic Medicine in Zurich.

Considering the increasing need for forensic services in cases of domestic and sexual violence, there is a growing demand for solutions. The Institute of Forensic Medicine at the University of Zurich and the Swiss Association Forensic Nursing (SAFN) offer contemporary and sustainable solutions for on-time, competent, and widely used forensic services.

**Objective:** Current policy developments in Switzerland open new pathways and perspectives in forensic care for the community, especially the adoption of the National action plan by the Federal Council of Switzerland for the implementation of the Council of Europe Convention on preventing and combating violence against women and domestic violence (Istanbul Convention). This action plan focuses on three main issues: informing the public and raising public awareness, providing basic and further training for professional staff and volunteers, and preventing and combating sexual and sexist violence. Those three main points of the action plan give the opportunity to build models in forensic care, which can be provided by forensic nurses.

**Material and methods:** To expand the forensic nursing network in a sustainable and resource-oriented manner and to develop possible concepts and models for the efficient deployment of forensic nurses, all 93 graduates of the Certificate of Advanced Studies (CAS) in Forensic Nursing at the University of Zurich were systematically surveyed. The questions related to the general motivation to take on specific tasks in an internal or even external forensic nursing network. The questions also reflected the current state of implementation of forensic nursing in various institutions where the respondents are currently working.

**Results:** The survey results showed that the questioned nurses see a high potential for forensic nurses in their institutions. They already see improvements in the forensic process, but there is room for more development. Over 50% of the respondents are already taking on certain tasks related to their advanced education in Forensic Nursing. Over 85% of respondents would like to get a more active role in their own institution as a forensic nurse. Over 85% would be actively interested in an internal and/or external forensic nursing pool. The last group especially can be recruited for an outreach service, according to the Flying Forensic Nurses (on demand). This service creates a unique opportunity to provide a sustainable model of competent forensic care through Forensic Nurses on demand.

**Conclusion:** The results of the data collection show that the offer of an on-time available forensic service in various institutions is not yet efficiently developed. Nevertheless, the respondents show a high motivation to take over an active role as a forensic nurse. Forensic nurses can play an increasingly important key role in the implementation of national policies and plans in combating violence. The concept of a Forensic Nurse on demand can be an efficient and sustainable solution to provide forensic services on time, easily accessible, and independent. The high level of motivation of forensic nurses and the political developments makes it possible to explore new pathways in forensic nursing.

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### Flying Forensic Nursing; Istanbul Convention; Forensic Network

## E11 A Collaborative Artificial Intelligence Platform for Bruise Age Analysis

*Katherine Scafide, PhD, RN\*, George Mason University, Fairfax, VA; David Lattanzi, PhD, PE\*, George Mason University, Fairfax, VA; Janusz Wojtusiak, PhD, George Mason University, Fairfax, VA*

**Learning Objective:** After attending this presentation, attendees will be informed regarding an active project involving a collaboration between forensic nursing, engineering, and health informatics that is investigating the application of deep learning with image analysis in the estimation of bruise age. Preliminary findings will also be presented.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees about the benefits of interdisciplinary collaboration in addressing important forensic clinical problems.

**Background:** Law enforcement and prosecutors rely heavily on the accuracy and interpretation of injury documentation to inform their decisions. However, assessments by forensic clinicians provide little valid or reliable data about a bruise's age beyond qualitative, subjective opinion. Image analysis using Deep Learning (DL), a subdomain of machine learning, has demonstrated significant benefits in accuracy and reliability within health care, yet few studies have applied the techniques to forensic trauma analysis. The purpose of our project is to: (1) determine whether advanced, time-series approaches to DL models of images can improve our understanding of how bruise appearance changes over time on diverse skin tones; and (2) develop a bruise image platform with DL modeling to support future research and collaboration in forensic science.

**Methods:** This three-year, federally funded project involves a collaboration between forensic nursing, engineering, and health informatics. A large (~26,000) dataset of bruise images of known age and diverse skin colors will be used to prototype and analyze advanced DL models (e.g., recurrent neural networks, attention mechanisms, manifold learning). Metrics will include predictive accuracy, false positive and negative rates, Receiver-Operator-Characteristic curves, and confusion matrices. An image collaboration web platform will also be developed based on likely use cases by forensic nurses and researchers. The prototyped and evaluated neural networks will be integrated into the image collaboration platform, which will be populated with the dataset. A mixed-methods study will then be conducted with forensic clinicians and researchers to compare the model's accuracy to expert opinion and to investigate the feasibility of the platform for end-user collaboration.

**Results and implications:** During the presentation, our team will share preliminary findings, as well as our partnership experience as an interdisciplinary team. By leveraging our team's forensic nursing, engineering, and computer science expertise, we will develop new, quantitative approaches to the forensic analysis of bruises while establishing limits of reliability and accuracy for different injury conditions. Our future results will have broad implications for forensic nursing practice, criminal justice response, and future research.

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### Machine Learning; Bruise Age; Image Platform

## E12 Aligning Forensic Nurse Core Competencies and Content Using Delphi and Qualitative Methods

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**Learning Objective:** After attending this presentation, attendees will be informed regarding: (1) the scaffolding to build common core competencies, the domains, descriptions, context, and measurable performance, with the purpose of validation of the Generalist Forensic Nurse and Advanced Forensic Nurse certification, built over 20-year modified Delphi (partly due to COVID-19); (2) national documents provided by the American Association of Colleges of Nursing that guided the discussions among educators and practitioners to arrive at consensus; and (3) consistent content derived from educator and practitioner conversations about the forensic nurse role, necessary for common core curriculum in all graduate schools of nursing.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees about the scaffolding process necessary for consensus in a modified Delphi study that aligned the three pillars of forensic nursing identified by Lynch's theoretical framework with forensic nursing domains, descriptions, context, and core competencies, identifying content necessary to align academic institutions' curriculum and pedagogy for the next generation of graduate forensic nurses. This presentation alerts the forensic science community of the scaffolding necessary to influence forensic nurse education of the future.

Forensic Nursing (FN) is an American Nurses Association (ANA) -designated specialty. FN has unique knowledge about trauma and violence, and Lynch's forensic nursing theoretical framework provided a foundation for the development of forensic nursing education. Pedagogy differed and, consequently, the FN curriculum currently varies between institutions. The Forensic Nursing Certification Board (FNCB) formed to scaffold steps specifically to bring consensus among forensic nursing educators and practitioners. The Delphi Method and qualitative analysis of conversations helped arrive at the three pillars in forensic nursing, domains of practice and their descriptions, context for practice, and measurable core competencies for performance. Additionally, a qualitative analysis of educator and practitioner conversations identified key content by using NVivo™ thematic analysis.

The study results provide the structure impact for forensic nursing curriculum and pedagogy for decades to come. Purpose included standardizing curriculum globally through the identification of core elements, competencies, descriptions, context for practice, and content. Project Design used the Delphi method, modified, when necessary (in person or virtual), using Essentials documents for guidance. Settings occurred over six meetings (2002–4, 2014, and 2020–21) at institutions with FN faculty. Participants included academic FN faculty and practitioners ( $N=126$ ). Many attended all six meetings. Implementation began similarly with each meeting, building on the previous meeting documents. Anonymous responses to questions, followed by prioritization and consensus in the content areas, improved fidelity to the Delphi, even with faculty familiarity. Measurement used NVivo™ for qualitative analysis of the recorded participant conversations. Results of the thematic analysis were presented to the participants where prioritization collapsed broad areas of agreement into component parts and consensus transpired. The results were cross-walked with previous meeting results, validating the core elements (forensic nursing, legal, and forensic science) and core competencies in the context of all FN practices. The process built on the current evidence, newest knowledge and understanding, and identified necessary core content to standardize FN curricula across institutional settings at all levels of nursing practices. Limitations included an evolutionary understanding of FN that changed over time as scholars commented on theory, frameworks, and practices as “best practices.” Modification of the Delphi method was essential to account for interpersonal familiarity among FN faculty.

Conclusions reflect the standardization of FN pedagogy and practice promises to align FN education with nursing, particularly the new Essentials (2021) and The Future of Nursing: 2020-2030 (2021), provide validation of FN expertise with certification, and improve patient health through exceptional FN trauma-informed care. The offering presents the educator-led activities from 2002, 2014, and 2021. In 2021, the FNCB brought consensus among forensic nursing educators and practitioners to align forensic nursing core competencies, context, content, and practice expectations for academic institutions globally, thereby impacting forensic nursing curriculum and pedagogy for decades.<sup>1-8</sup>

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### Forensic Nursing Core Competencies; Forensic Nursing Education; Delphi Method



## E13 The Forensic Nursing Certification Board Process and Outcomes Ensuring Validation of Generalist and Advanced Forensic Nurse Certifications

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**Learning Objective:** Constructing a valid certification examination for the Generalist Forensic Nurse and Advanced Forensic Nurse was a daunting task, taken on by the Forensic Nursing Certification Board. This presentation will explain the steps necessary to create a valid test and reveal the outcomes after the first year of testing.

**Impact Statement:** This presentation will impact the forensic science community by providing a deeper understanding of the processes to construct a valid certification test in nursing, aligning forensic nursing with the profession. Another impact relates to threading national nursing organization guidance publications with the new Generalist and Advanced Forensic Nurse certifications. Last, the impact is understanding the coordination and virtual convening of over 25 forensic nurse experts to create common core scenarios for use in test construction.

Following the Delphi study results supporting Forensic Nurse Core Competencies and Content, the content validated Lynch's three pillars unique to the specialty of forensic nursing, which included a legal foundation (in United States law, and pertinent globally with country-specific law), forensic science, and forensic nursing, confirming Virginia Lynch's theoretical framework in 1991 for the specialty practice of forensic nursing. The thematic analysis of the practitioner and educator conversations gave specific information to establish common core content, necessary for the establishment of curriculum in graduate and undergraduate schools of nursing. These studies provided the foundation allowing for the next step—embarking on writing a certification examination for the Generalist Forensic Nurse and the Advanced Forensic Nurse.

Writing a certification examination is a process requiring enlistment of experts in practice and education with knowledge about forensic nurse content, test writing, and experience in forensic nurse settings. A cohort of experts from the Academy of Forensic Nursing, working with the Forensic Nursing Certification Board, embarked on identifying and aligning with guidance documents from the American Association of Colleges of Nursing, creating scenarios familiar to forensic nurses globally. A subset of question writers aligned the core competencies and content with the three pillars of forensic nursing, using nursing's NCLEX®-style questions, now aligning the American Association of Colleges of Nursing Essentials (2021), with the new Forensic Nursing Core Competencies for Generalist and Advanced Forensic Nursing Practice (2021). A bank of 250 questions, divided by knowledge and application in each of the three pillars, were generated for the generalist and the advanced forensic nurse certification candidates. The system for analysis of questions was generated automatically as to the index (identification of those performing in the top/bottom 20%). This presentation is an in-depth look at the process and outcomes as the Forensic Nursing Certification Board moves toward validation of the Generalist Forensic Nurse (GFN-C™) and Advanced Forensic Nurse (AFN-C™) certifications.<sup>1-9</sup>

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### Forensic Nursing Certification; Forensic Nursing Certification Board; Forensic Nursing

## E14 Creating a Clinical Forensic Medicine Elective for Advanced Medical Students in the United States

*Melinda Carter, MD, PhD\*, Alabama College of Osteopathic Medicine, Auburn, AL*

**Learning Objective:** After attending this presentation, attendees will be aware of a novel effort to educate future physicians in the United States about patient encounters in which an awareness of forensic science or death investigation will benefit patient welfare and public health.

**Impact Statement:** This presentation describes a four-week elective in clinical forensic medicine for advanced medical students entering primary care residencies that will prepare them for partnering with forensic nurses. The goal of this presentation is to instill an awareness of forensic science topics and methods in physicians to encourage them to become empathic communicators with first responders, nurses, law enforcement, and other professionals involved in an investigation. Course impact will be assessed by a pre- and post-course survey on forensic science literacy created for health care professionals.

Physicians are legally responsible for many aspects of patient care, such as signing death certificates, yet many practicing physicians have not received formal instruction on the logistics of caring for victims of crime or the value of mortality data to public health. Like most of the public, physicians commonly equate “forensic science” or “mortality research” with murder investigation rather than understanding how these topics relate to living patients.

This presentation describes efforts to organize a multidisciplinary team of scientists, physicians, nurses, police detectives, and mental health professionals to share their expertise and experiences with fourth-year medical students. Since most graduates of osteopathic medical schools pursue residencies in primary care fields, like family practice, pediatrics, or internal medicine, the course focuses on limited access to skilled care, issues of health equity, and social determinants of health care in rural and other underserved communities. The course was developed at one medical school and is currently being proposed to the Curriculum Oversight Committee for adoption at another osteopathic college on the campus of Auburn University. Each week of the course covers major topics, such as introduction to forensic sciences, violence and neglect, insidious chronic abuse, and death investigation, using recorded lectures, educational videos, reading assignments (news reports, book chapters, and journal articles), culminating in a virtual discussion led by students and facilitated by experts.

A new goal for this elective is to identify and set up patient-contact experiences for students to interact with diverse health care professionals and patients. Contributing faculty will create a novel survey to evaluate forensic science literacy among health care professionals to measure student awareness and understanding of key topics before and after taking the course. Initial delivery of this virtual elective during the pandemic was encouraging. Course enrollment filled up quickly, and students were actively engaged in weekly discussions. Future endeavors include pre- and post-course feedback from students to gauge impact of topics, as well as plans to follow-up with the same student physicians near the end of their residency programs to assess value to clinical experiences. The generation of clinically relevant research ideas for future medical students is anticipated. This presentation aims to solicit collaborative partnerships and innovative ideas for enhancing medical student learning and developing future interprofessional activities across different health care programs.

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**Physician Education; Clinical Practice; Forensic Medicine**



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## E15 Precepting and Mentoring the Forensic Nurse

*Kathleen Sekula, PhD\*, Duquesne University, New Kensington, PA; Virginia Lynch, MSN, University of Colorado, Divide, CO*

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**Learning Objective:** After attending this presentation, attendees will better understand the differences between precepting and mentoring, the qualities to look for in a mentor, how to advance their practices by serving as a preceptor or mentor or both, and why is this so important to forensic nursing advanced practice.

**Impact Statement:** This presentation will impact the forensic science community by encouraging all forensic nurses to embrace their roles as both preceptors and mentors and to know the difference.

As a relatively new practice area for nurses, forensic nursing is emerging as an exciting and much-needed area of practice. Precepting and mentoring are two roles that are paramount to forensic nurses supporting forensic colleagues in their clinical and academic development. All areas of forensic nursing practice should be included in the initiative to mentor forensic nurses: Sexual Assault Nurse Examiners (SANEs), death investigators, clinical advanced practice nurses, corrections nurses, risk managers, and legal nurse consultants, among others. All forensic nurses in all areas of forensic practice must be included as we move forward to support this clinical specialty area. Unless we acknowledge all forensic practice areas, we will not move forward. Each specialty area in forensic nursing is important, and we must acknowledge their needs.

The lack of qualified preceptors and mentors has been an issue in the forensic community. Even more importantly, the lack of understanding regarding the qualities and expertise needed to become both a preceptor and a mentor is lacking. Many nurses do not realize the potential of their work and what they have to share with colleagues in the forensic nursing community. This presentation proposes to delineate the roles of the preceptor and the mentor. There are many considerations as nurses evolve into the mentoring role, and this presentation focuses on clarifying those roles.

Forensic nurse preceptors must demonstrate broad knowledge, confidence, and enthusiasm about continuing their own learning. In addition, they must answer learners' questions clearly and precisely, be open to conflicting ideas and opinions, connect information to broader concepts, communicate clear goals and expectations, and capture the learner's attention. They must give the learner honest and objective feedback while instructing, modeling, coaching, and facilitating learning. The mentor is also an expert in their field and is willing to provide guidance to a willing mentee. A mentor is a role model, and while they do not evaluate the mentee's learning, they provide support and guidance to the mentee who is developing their professional role. Mentoring relationships can be formal or informal and are time-limited only as agreed upon by the mentor-mentee relationship. Lack of access to SANE mentors is frequently identified as a barrier to educating new SANEs and maintaining practice requirements.

The purpose of this presentation is to describe a formalized educational program for SANE nurses on precepting (clinical supervision for early skill acquisition) and mentoring (longer-term role development). The approach includes identifying preceptors and mentors, providing training modules for both roles, virtual mentoring opportunities, and long-term professional development. At the end of this presentation, we propose that attendees will understand and address their own potential to serve as preceptors, mentors, or both.

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### Mentoring; Forensic; Nurses

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## E16 Who Doesn't Report? Understanding How Victim-Offender and Assault Characteristics Relate to Stated Reasons for Not Reporting Sexual Assault to the Police

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**Learning Objective:** After attending this presentation, attendees will better understand why patients who participate in a medical forensic examination decline to report the assault to law enforcement at that time. Additionally, attendees will learn about the relationship between victim-offender and assault characteristics (e.g., relationship, demographics, substance use, and inability to recall the entirety of the assault) on reasons given for not reporting.

**Impact Statement:** This presentation will impact the forensic science community by informing forensic nurse scientists on the reasons people choose not to report to law enforcement and help them better anticipate the needs of patients who have experienced sexual assault. It will provide forensic nurse scientists information they can use in cross-disciplinary collaboration to further reduce barriers to reporting sexual assault.

It has been well established that the vast majority of people who experience sexual assault do not engage with any help-seeking services. These services generally include medical care, advocacy, and law enforcement. The dynamics of sexual assault are very complex; therefore, there are many reasons for this lack of engagement. Among others, these reasons often include fear of not being believed, shame and embarrassment, and previous negative experiences with responses to help seeking. Research indicates that as many as 25% of victims who have forensic evidence collected by participating in a medical forensic examination following a sexual assault decline to release that evidence to law enforcement and report the assault. Legislative efforts such as the Violence Against Women Act have significantly increased access to medical forensic examinations for those who have experienced sexual assault. This is especially true for those who choose not to report to law enforcement but participate in forensic evidence collection. While the reasons for not engaging with help seeking services are somewhat understood, little is known about the reasons people participate in these medical forensic examinations and choose not to report to law enforcement at the time of the examination.

This presentation will share the results of a qualitative analysis of reasons for not reporting to law enforcement provided by 295 sexual assault victims who participated in a medical forensic examination but did not report the assault at that time. This analysis revealed four major themes, which will be discussed. The results include exploration of variation in reasons for not reporting by victim, offender, and assault characteristics, including patient demographics, substance use, and inability to recall the events of the assault. This knowledge can help forensic nurse scientists anticipate the potential barriers patients face and identify opportunities for cross-disciplinary collaboration with the goal of reducing those barriers.

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### Reporting Decisions; Assault Characteristics; Sexual Assault

## E17 Nursing and Engineering Partnerships Lead to Important Advances

Joyce Williams, DNP\*, Randallstown, MD

**Learning Objective:** After attending this presentation, attendees will have a better understanding of how forensic nurse and forensic engineer partnerships contribute to advances in Evidence-Based Practice (EBP) and the development of technology-enhanced practice.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how joining the two disciplines, forensic nursing + engineering, leads to greater innovations to advance scientific discovery and innovation.

Nursing science is built on EBP methods to provide the best of care or the “gold standard” to help patients recover from disease and injury. EBP combines the best research evidence available, clinical expertise, and patient values. Important discoveries that have improved the quality of life for patients were invented because nurses found that they could do better with the support of the engineering community. Engineers working in health care venues emerged during the 1960s out of concern for patient safety, especially as it related to the potential electric shock hazards of medical devices.<sup>1</sup> Nursing perspective, plus the biomedical engineering lens, has enhanced opportunities for greater technology and subsequent innovation. Combining these two disciplines heightens the relationship built on Science, Technology, Engineering, and Math (STEM), the ideal setting for conducting research to improve patient care.

“Science Works” is the focus of this presentation. A health care background with requisite clinical experience provides the underpinning for a biomedical engineer’s ability to solve clinical problems. Nursing + engineering is the intentional combination of the disciplines of nursing plus engineering producing a sum that is greater than its parts, also known as “convergence.”<sup>2</sup> The National Science Foundation (NSF) defined convergence research as, “... solving vexing research problems, in particular, complex problems focusing on societal needs. It combines knowledge, methods, and expertise from different disciplines to form novel frameworks to catalyze scientific discovery and innovation.”<sup>3</sup> Historically, partnerships between providers of health care and practitioners of engineering have produced highly successful outcomes: the development of the pacemaker (1958); the Computed Tomography (CT) scanner (1970); and slow-release medicines (1980), among many other technologies.<sup>4</sup> More recently, forensic scientist students at Duke University nursing and engineering designed a first-generation telerobotic intelligent nursing assistant, after the Ebola outbreak in 2014. The telerobotic intelligent nursing assistant serves as an alternative to human contact that diminishes the risk to health care providers caring for patients with infectious diseases. Other innovations are the use of a baby carrier that attaches to an electronic wheelchair, a crib that opens sideways to accommodate a mother in a wheelchair, and a smart technology walker to help lift patients in the event of a fall.

This presentation will detail the nexus of nursing, forensic nursing, and forensic engineering and how joining these disciplines improves safety features from ineffective, potentially unsafe care to care that is safe, effective, patient-centered, timely, efficient, and equitable and other research to improve care to populations. Furthermore, alert mechanisms (sensors in bandages) to warn us when an infection starts or adding improvements to personal protective equipment (inserting side Small Arms Protective Insert [SAPI] plates to further decrease mortality in combat) all contribute to improvements in morbidity and mortality. Forensic nursing can be more actively involved in this technology-dependent future. Exemplars such as using the use of different wavelengths to document skin differences in the visibility of bruises is one example of current research. To move the needle further, joint degrees between Colleges of Engineering and Nursing are needed to leverage nurse ingenuity and create patient care innovations. In conclusion, nurses will continue to focus on the “art of caring” and “practice of health promotion,” while engineers focus upon the “art of design” and the “practice of building.”<sup>5</sup> It is by working together that greater innovations can problem solve and advance scientific discovery and innovation.

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### Forensic Nursing; Forensic Engineering; Innovation

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**E18 Identifying Information in Protein From Sexual Assault Evidence**

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**Learning Objective:** After attending this presentation, attendees will better understand different sources of identifying information in sexual assault evidence and methods that can be used to extract and use this information. In the event that no clean male signal can be obtained, other options may still be available to the investigator.

**Impact Statement:** The use of protein sources of genetic information has the potential to significantly enhance genetic information that can be extracted from sexual assault evidence, particularly in the fraction of evidence that has evidence of a Y-chromosome but no usable Combined DNA Index System (CODIS) -quality profile, currently about 5% to 15% of all kits. This additional information can expand the proportion of kits that can provide identifying information or functional leads for investigators.

Sexual Assault Evidence (SAE) presents challenges to the investigator due to post-coital degradation of male DNA and prevalence of the victim's DNA signal. A significant fraction (5% to 15%) of SAE contains evidence of male biological material, through amplification of Y-chromosome amplicons, yet fail to provide actionable DNA Short Tandem Repeat (STR) profiles that can be applied to CODIS. Protein is more stable than DNA, so there is a potential for male protein to persist in the victim after DNA has degraded. Since many proteins contain non-synonymous SNP genotypic information in the form of single amino acid polymorphisms, this information has the potential to synergistically complement, enhance, or, if necessary, supplement, genotypic information that can be used for identification or ancestral estimation.

The purpose of this study was to determine if these single amino acid polymorphism markers in genetically variant peptides could be identified and validated from male-specific proteins in human semen. Frozen semen samples from nine European males were analyzed using proteomic measures to determine their protein content. A tryptic digestion was performed on the samples and analyzed using liquid chromatography/tandem mass spectrometry. A total of 4,419 proteins were identified across all the datasets with an average of  $491 \pm 192.96$ . There were 34 genetically variant peptides identified from 14 proteins using proteomic analysis. After validation through DNA exome sequencing of matching samples, 10 remaining proteins were confirmed with DNA coverage of 22 of the genetically variant peptides. Our predictions resulted in 33 true positives, 122 true negatives, 96 false negatives, and 2 false positives, leaving a false positive rate of 2%. Proteomic processing is fully compatible with DNA methods since seminal plasma is separated from spermatozoa during isolation of the male DNA fraction. These validated markers were obtained from the population of known male-specific proteins, including: prostate specific antigen, semenogelin 1 & 2, and prostate and semen expressed protein 1. Additional semen-specific peptides are also candidate genetic markers awaiting validation by DNA sequencing. This demonstrates that the male-specific population of genetic protein markers in semen are a potential source of genetic information that can be used to statistically associate a suspect with sexual assault evidence or provide useful leads for investigators such as potential ancestry. This approach has the potential to increase the proportion of SAE that can provide forensically usable genetic information.

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**Sexual Assault Evidence; Proteomic Genotyping; Genetically Variant Peptides**

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## E19 Right Under Our Noses: Understanding Missed Opportunities for Collection of Saliva as Evidence

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**Learning Objective:** This presentation will utilize crime lab data to demonstrate the importance of the collection of saliva as potential DNA evidence on skin surfaces for days, even after patients have showered or bathed. After attending this presentation, attendees will better understand the importance of considering this type of evidence collection, that saliva is a very hardy and an important source of potential evidence, and that the forensic nurse should always give consideration to saliva as a viable source of biological evidence. It may, in fact, be their best potential, most probative, or only source.

**Impact Statement:** This presentation will impact the forensic science community by showing that sharing the results of the DNA with the forensic nurses will provide better outcomes and collection. Forensic program managers will learn if the staff is collecting evidence with positive results. This promotes forensic collaboration and reinforces positive teamwork.

Biological evidence collection time frame recommendations published in the Department of Justice's National Protocol for Sexual Assault Medical Forensic Examinations are generally accepted as the baseline standard for evidence collection time frames throughout the United States.<sup>1,2</sup> However, in practice, these collection time frames can vary widely throughout the country. Despite the development and open access to these recommendations, as well as scientific evidence that supports them, potential DNA evidence opportunities are missed. Lack of access to trained medical professionals such as forensic nurses, knowledge gaps about time frame standards among collectors, local evidence collection policies and procedures, and resource limitations all contribute to these potential missed evidence collection opportunities.<sup>1-3</sup> Even when evidence collection is less than invasive, such as the collection of touch DNA or saliva from skin surfaces, specimens can easily be overlooked if forensic nurses and practitioners think that evidence has been washed away or degraded.<sup>4,5</sup> Specifically, specimens potentially containing saliva are more likely to provide DNA evidence than some might think.<sup>5</sup> Evidence suggests that potential DNA from skin surfaces where saliva may be present be collected for up to four days.<sup>1</sup> This is even true in cases where patients have showered, bathed, or otherwise washed their bodies.<sup>5</sup> Not only does the literature support this, but real-life application in California crime labs supports this as well.

To help with the backlog, a Research Assistantships for Diverse Scholars (RADS) program was started that allowed the forensic nurse to FedEx® directly to the crime lab three probative swabs with one reference sample. The crime lab then sent monthly RADS reports to the Forensic nursing program. This resulted in a practice change. There was an increase in collection of samples for saliva after the nurses began receiving the reports from the laboratory. This increased the number of samples for saliva past 24 hours based on history. This demonstrates the importance of communication between the crime lab and the forensic nurse who is collecting the specimens.

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**Salvia; DNA; Forensic Nursing**

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**E20 Long-Term Care Facilities (LTCFs) and Nursing Homes (NH) SARS-COV-2 Infections: Inevitable Conditions or Medical Malpractice?**

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**Learning Objective:** After attending this presentation, attendees will have better insight into the complexity of recognizing medical responsibility in cases of COVID outbreaks within long-term care facilities, which is a complex problem that involves various factors with interesting medicolegal implications.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the organizational and clinical aspects to be considered in the management of SARS-CoV-2 infection within residential facilities for the elderly, with particular reference to the related medicolegal implications.

Although more than two years have passed since the outbreak of COVID-19, SARS-CoV-2 infection is still considered a worldwide health emergency responsible for interstitial pneumonia, Severe Acute Respiratory Syndrome (SARS), multiorgan dysfunction, and death. As of July 20, 2022, COVID-19 has affected more than 560 million people, leading to a progressive increase in hospitalizations worldwide, thus raising questions about risk and protective factors. An issue of particular relevance is in specific settings, such as residential facilities for the elderly. These individuals are particularly susceptible to the most serious consequences of the SARS-CoV-2 infection, and thus suffered the first wave of the pandemic with particular severity. Long-Term Care Facilities (LTCFs) and Nursing Homes (NH), which provide care to both elders and people with disabilities, have indeed faced the highest rates of SARS-CoV-2 infection among both staff and residents, accounting for 30%–50% of all COVID-19-related deaths worldwide.

In light of this data, a review of the literature has been carried out in order to better understand what factors, within the structures mentioned, may have been responsible for such a high percentage, with particular reference to cases of COVID-19 outbreaks occurring during the first wave and within residential facilities for the elderly in the Sicilian territory. Based on current evidence, risk factors for the development of COVID-19 in adults can be divided into individual factors, including advanced age, sex, and/or chronic diseases and extrinsic factors related to the structural, organizational, and managerial characteristics of the residential structures in a pandemic context, for example, the number of guests, urban or rural location, availability of Personal Protective Equipment (PPE), and staff management.<sup>1,2</sup> On such a basis, a comprehensive understanding of the risk and protective factors for COVID-19 and their interaction can be helpful not only for the implementation of preventive activities against infection, progression, and adverse outcomes in infected patients, but also for a proper evaluation of the medicolegal implications in cases of malpractice.<sup>3</sup>

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**COVID-19 Infection; Medical Malpractice; Care Homes**



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**E21 A Descriptive Study of Non-Fatal Strangulation in Sexual Assault**

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**Learning Objective:** After attending this presentation, attendees will have increased knowledge about sexual assault victims and assault characteristics associated with Non-Fatal Strangulation (NFS) during sexual assault.

**Impact Statement:** This presentation will impact the forensic science community by increasing understanding of NFS in sexual assault and associated patient and assault characteristics.

NFS is a life-threatening “hidden epidemic” in cases of sexual assault. NFS is a gendered crime in which the majority of victims are women, and most assailants are men. Women are often assaulted in their own homes, even if children are present.<sup>1</sup> Regrettably, many survivors of NFS delay seeking care and do not seek help in an emergency department for evaluation post-assault.<sup>2</sup> NFS can not only cause neurological damage from lack of blood flow to the brain, but NFS can also result in cognitive, behavioral, and psychological trauma. The victim may be brought to the “edge of homicide” by strangulation without any medical or psychological care.<sup>3</sup>

The purpose of this study was to explore the unique victim and assault characteristics of NFS sexual assault victims in a Mountain West state in the United States between the years of 2019 and 2021. This retrospective, exploratory study of 2,262 sexual assault cases found that the following variables were statistically significant in NFS cases (~15% of total cases): age, gender, prior history of sexual assault, established suspect relationship; victims reporting of pain post-assault; physically hitting and/or strangling victim; victim reports of changes in breathing, vaginal-penile penetration; higher number of penetrative acts; and victim with anogenital injuries and non-anogenital injuries. Logistic regression models were calculated to explore the predictive power of statistically significant variables. Other studies have been conducted and found variables associated with NFS include increased assault from current or former intimate partners, home location of assault, presence of children, and an increased loss of consciousness during assault indicating higher degree of lethality.<sup>2</sup> Exploring variables associated with NFS during sexual assault cases is beneficial in practice and policy decisions for Sexual Assault Nurse Examiners (SANEs), forensic examiners, and forensic scientists. Increased awareness and understanding of NFS during sexual assault could lead to improved medical management and services for NFS victims post-assault.

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**Non-Fatal Strangulation; Sexual Assault; Violence Against Women**

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**F1      The Impact of COVID-19 on Death Rates in a Rural Missouri County**

*Marie Lasater, MSN\*, Texas County Government, Licking, MO*

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**Learning Objective:** COVID-19 has profoundly impacted the normal death rate in rural Missouri. Attendees will learn the impact the pandemic had on several causes of death, many not directly related to COVID-19 infection.

**Impact Statement:** The presentation will inform the community of non COVID-19-related causes resulting in a significant increase in the normal death rate in rural Missouri.

With the onset of the COVID-19 pandemic, the death rate in a rural Missouri county rose from 8.3 cases per 1,000 residents to 12.23 cases, while the overall death rate in the United States remained fairly stable. Of note, the overage in deaths was minimally accounted for by COVID-19 infection. Over a two-year period from 2020 through 2021, significant increases were seen in not only suicides and overdoses, but also accidental deaths of all causes, strokes (mainly affecting the middle cerebral artery), and cancers of all types. Approximately 55% of the overdose deaths involved the drug fentanyl, rarely previously encountered in the county represented. Gunshot wounds to the head, previously rare, comprised the main method of suicide, followed by self-asphyxiation. New onset stroke occurred primarily in women and were mostly evidenced in the left middle cerebral artery, an unexplained finding. Accidental deaths of all causes are up 150%, including falls, motor vehicle accidents, choking, surgical errors, carbon monoxide poisoning, and temperature related—both hypo- and hyperthermia. Four juveniles (under the age of 18) suffered vehicle fatalities, all attributed to overcorrection of the vehicle. Overdose deaths have risen 150%, with the majority due to fentanyl, followed by methamphetamine and benzodiazepines. One unusual death was due to albuterol abuse. Ischemic strokes have risen 62% since 2019. Cancer deaths have risen by 29%, with lung cancer as the leading cause, followed by pancreatic and lymphatic cancers.

With COVID-19 deaths comprising only 6.5% of the overage in the death rate, other factors must be considered, including stressors experienced by those exclusively in a rural community. For example, the nearest tertiary care centers are two-and-a-half hours away, mental health counseling is very limited, and churches did not hold services for a period of time, deeply affecting this community.

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**COVID-19; Fatalities; Rural**

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**F2 Predicting Accidental Drug Overdose as the Cause and Manner of Death in Near Real-Time Using the Suspected Potential Overdose Tracker (SPOT) Tool—New York City, 2018–2020**

*Karli Hochstatter, PhD, MPH\*, Friends Research Institute, Baltimore, MD; Jason Graham, MD, Office of Chief Medical Examiner, City of New York, New York, NY*

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**Learning Objective:** Attendees will learn about a novel tool, the Suspected Potential Overdose Tracker (SPOT), that uses data collected by death investigators to predict accidental drug overdose as the cause and manner of death in near real-time. Learning objectives include: (1) the development and functionality of SPOT; (2) how SPOT is being used by the New York City Office of Chief Medical Examiner; (3) barriers and facilitators of implementing SPOT; and (4) public health implications of adopting SPOT.

**Impact Statement:** This presentation will provide information about a novel tool developed by the New York City Office of Chief Medical Examiner, named the Suspected Potential Overdose Tracker (SPOT), that identifies suspected accidental drug overdose deaths in near real-time. By applying their investigative data to this tool, death investigators can immediately predict the likelihood that the death will later be confirmed as an accidental drug overdose and distribute this information to representatives and leaders from various partner agencies, including public health and safety officials that are most likely to intersect with people at risk of overdose. The SPOT tool overcomes many barriers of the long turnaround times for issuing finalized death certificates and allows for improved surveillance activities and rapid responses to the worsening drug overdose crisis.

**Background:** The United States is experiencing a worsening crisis of drug overdose deaths. Accurate and timely drug overdose surveillance data is needed to track the evolving crisis and mount an effective response. Death certificates are the foundation of overdose mortality surveillance. However, long turnaround times for issuing finalized death certificates in suspected drug-related deaths hinders surveillance activities. In response to significant delays in finalizing death records, the New York City (NYC) Office of Chief Medical Examiner (OCME) developed and implemented SPOT. The purpose of the SPOT tool is to rapidly identify unintentional drug overdose deaths in near-real time through a simple and replicable process using data routinely collected by death investigators, allowing for timely monitoring of overdose fatalities.<sup>1</sup> The objectives of this study are to examine the performance of the SPOT tool for predicting accidental drug overdose as the cause and manner of death.

**Methods:** The SPOT tool assigns each death a ranking of 1 through 3 based on the likelihood of it being an unintentional drug overdose, with 1 representing the highest likelihood that the death will be confirmed as an unintentional drug overdose and 3 representing the lowest. We measured the performance of the SPOT tool for predicting accidental overdose deaths by comparing suspected overdose deaths in NYC from 2018–2020 that were identified using SPOT to finalized death certificates. Performance measures included the sensitivity, positive predictive value, proportion of false positives, and type II error rate. These measures were calculated overall and for each year.

**Results:** From 2018–2020, the SPOT tool identified 4,476 suspected accidental drug overdose deaths in NYC using data collected within 72 hours of fatality. During this time, there were 5,127 death certificate-confirmed accidental overdose deaths. The sensitivity of the tool ranged from 66%–77% across the three years. The positive predictive value ranged from 93–97% for cases assigned a ranking of 1, 87–91% for cases assigned a ranking of 2, and 62–73% for cases assigned a ranking of 3. Among all unintentional overdose deaths in 2018, 2019, and 2020, 21%, 28%, and 33% were missed by the SPOT tool, respectively. The proportion of type 1 errors ranged from 15%–23%.

**Conclusions:** Using data routinely collected during death investigations, the SPOT tool allows for the rapid identification of unintentional drug overdose deaths. The tool may be used by health departments, epidemiologists, public health programs, and others for rapid data-driven decision making, identifying gaps in public health and public safety overdose response, and evaluating and responding to overdose prevention interventions, programs, and policies before finalized death certificate data become available. Ongoing efforts to improve the performance of this promising tool and capture more drug overdose deaths will be discussed.

**Reference:**

- <sup>1</sup>. Hochstatter, K.R., Rastogi, S., Klein, K. et al. Predicting accidental drug overdose as the cause of fatality in near real-time using the Suspected Potential Overdose Tracker (SPOT): public health implications. *BMC Public Health* 22, 1311 (2022). <https://doi.org/10.1186/s12889-022-13700-0>

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**Accidental Drug Overdose; Overdose Surveillance; Overdose Prediction Tool**

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**F3 Research Questionnaires to Assess Law Enforcement Use of Near-Surface Geophysics**

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**Learning Objective:** Attendees will learn about the questionnaires geared toward understanding how geophysical methods are applied for law enforcement investigations. Attendees will be introduced to the goals of three distinct questionnaires on the applications of geophysical methods. The first questionnaire aims to assess the law enforcement use of metal detectors at crime scenes, the second on law enforcement implementation of advanced geophysical methods, and the third queries geophysical service providers who have conducted geophysical surveys for law enforcement. Attendees who have applied geophysical methods in support of law enforcement will be provided information to access all three questionnaires at the presentation.

**Impact Statement:** These questionnaires will impact the forensic science community by providing insight on how advanced search strategies, specifically geophysical methodologies, are applied to law enforcement investigations. These questionnaires will encourage geophysical service providers and law enforcement personnel to discuss their experience with a variety of geophysical methods such as metal detectors, ground-penetrating radar, magnetometry, and electrical resistivity. Results of this questionnaire could assist in more appropriate survey designs, deployment, and management of geophysics for law enforcement investigations. In addition, the results can give insight on why applying certain geophysical methods were deemed effective or ineffective in detecting a suspected target. Ultimately, if there are enough responses to the questionnaires, the results will be compiled and published.

Applications of geophysical methods such as ground-penetrating radar, magnetometry, electromagnetics (to include “metal detectors”) have been deployed to detect or image the subsurface for potential targets that are of interest to law enforcement. Geophysical methods can be efficient techniques in non-invasive imaging of an area for suspected targets that can save law enforcement time and money for excavation. However, when geophysical methods are deployed to seek a buried or hidden law enforcement target, there are varying levels of “success” in detection of targets. The outcome of geophysical surveys will depend on the physical and chemical composition of the suspected targets versus its surroundings, the suspected depth and the general dimensions of the suspected target, certainty of the target is present, and the methods applied to the site. The causes of “success” and “failure” in detection of law enforcement targets are often attributed to the geophysical equipment used, but other factors are more likely to affect the outcome.

To better understand circumstances of success or failure, compiling experiences from a wide range of law enforcement personnel will better document the root causes of survey outcomes. To learn more about the efficiency of geophysical methods, three questionnaires were created to gather information on the application of geophysics for law enforcement investigations. One questionnaire, “Metal Detectors use in Crime Scene Investigations,” is based on law enforcement’s application of metal detectors at crime scenes. The second questionnaire, “Law Enforcement use of Geophysical Methods,” will ask law enforcement personnel to describe geophysical surveys they or their organization contracted or deployed geophysical methods in attempts of detecting suspected targets. The third questionnaire, “Geophysical Service Providers in Support of Law Enforcement,” is designed for geophysics professionals providing geophysical services for criminal investigations to describe the surveys they have conducted. Each questionnaire will ask participants to provide information about the methods they deployed (for example, GPR, electrical resistivity, magnetometry), the suspected targets (for example, weapons, drugs, human remains), the survey environment (for example, residential home, lake, commercial building), the concealment material (for example, soil type, fresh water, concrete/basement soil) surrounding a suspected target, and the effectiveness of the methods for detection of the target in that environment.

The results of these questionnaires will give insight on those requesting and conducting geophysical surveys for law enforcement. Participants will get a chance to discuss the searched targets, survey environments, and the overall efficiency of the method for those law enforcement investigations. Compiling the collective experiences of the use of geophysical methods for law enforcement investigations will enable the development of recommended practices for when use of specific geophysical methods are more likely to successfully detect a target.

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**Ground-Penetrating Radar; Geophysics; Metal Detector**

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**F4 Determining Diets Via Blood for Forensic Applications**

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**Learning Objective:** Attendees will learn the value of information that can be provided via a bio-affinity assay that can provide criminal investigations with vital characteristics that can support the number of individuals at a crime scene and differentiate them through their different dietary lifestyles.

**Impact Statement:** This presentation will impact the forensic science community by providing a different approach to categorizing individuals who are present at a crime scene. By looking at the metabolite levels within blood, information can be gathered that collaborate or contradict with suspect/witness statements. A bio-affinity approach, in this case, is a fast and accessible approach that is efficient in the distinction of different metabolite concentrations.

An individual's diet can be due to specific reasons, such as one's culture or health, or due to personal reasonings. Regardless of this, it can provide individualized characteristics aiding investigators in building a criminal profile. In violent crimes, such as murders and aggravated assaults, blood is typically present at the crime scene due to impact on the bodies of suspect(s) or victim(s). Linking blood at a crime scene to an individual aids forensic investigator in proving a suspect(s) was at the scene of the crime when it occurred.

Previous literature has expressed the need for additional research to understand the complete value of forensic information that blood can provide. By looking at metabolite levels within blood, we can better understand and gain more information about individuals. This will benefit forensics by obtaining more information that assists by providing characteristics that assist in determining suspects.

This research examines a method of analysis that identifies dietary habits by analyzing concentrations of alanine, an amino acid, in blood, specifically serum. Alanine is a non-essential amino acid whose concentrations are impacted by food consumption, making it a quantifiable approach in differentiating an individual's dietary habits.<sup>1</sup> Specifically, alanine is affected and detected at lower concentrations when an individual consumes meat as opposed to them not.<sup>1</sup>

In this case, a bio-affinity assay is a fast and accessible approach in interpreting these concentrations. It also allows for efficiency in determining the different concentrations present across individuals. Utilizing an enzymatic assay approach allows for the analysis of individuals' diets differentiating from those who consume meat versus those who do not consume meat, such as vegans or vegetarians. By specifically looking at alanine and its relative concentration levels, it allows for individuals to be differentiated, determining who was present at the scene, and categorized by meat vs. non-meat eaters.<sup>2</sup>

Bodily fluids hold key information about their originator, such as the individual it came from, and other genetic and lifestyle details assisting in forensic investigations. By analyzing and determining different amino acids in blood, this aids in the ability to recognize and draw conclusions based on the individual from which it originated. By conducting this research in both buffer and human serum, we mimic the accuracy of a real crime scene application. This better supports the forensic field by providing information when little evidence is left at a crime scene.

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**Alanine; Enzymatic; Bodily Fluids**

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**F5      When the Killer Is Waiting for You at the Door: A Case Report of a Katana Murder**

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**Learning Objective:** After attending this presentation, attendees will better appreciate some forensic implications in postmortem diagnostics related to a brutal katana murder.

**Impact Statement:** This presentation will impact the forensic community by sharing the experience gained following a ferocious murder perpetrated using a katana sword.

A 61-year-old woman was found lifeless on the stairs of her daughter's apartment where she had gone to pick up her grandchildren to take them to school. The son-in-law, followed in the past for psychiatric problems and with whom she did not get along, opened the door of the apartment, and threw himself on her, violently throwing the woman to the ground on the steps. He then took a katana with which he repeatedly hit her. Immediately after, he called the police for an arrest. The prosecutor asked for an inspection of the crime scene and an autopsy to reconstruct the phases of the attack. External examination of the body revealed a deep lacerated scalp wound with bruising in the left frontoparietal area with a detachment of a bone fragment from the skull and a large sharp-edged stab wound in the right nape region with underlying skull discontinuity. Furthermore, multiple defensive lesions affecting the upper limbs bilaterally and the presence of two other wounds, one localized in the cervical area and one precordial, were detected. Near the body, a katana with a total length of 46cm was found and a 28cm long blade was bent about 30 degrees. Before the autopsy, a PMCT was performed that confirmed the skull fracture with a detachment of bone fragment in the left frontal region and bone wound involving the nuchal skull and which deepened, involving the transverse process of the C2 vertebra.

During the autopsy, the dissection of the nuchal region was then performed, which allowed for the reconstruction of the path of the blow. In particular, the blade hit the cranial theca causing a cut lesion on the right cerebellar hemisphere and then deepened into the cervical region where it determined the complete section of the right vertebral artery. Histopathological examinations confirmed a significant loss of blood with almost bloodless parenchymatous organs and allowed us to document the viability of the nuchal lesion.

This clinical case shows how the medicolegal investigations carried out made it possible to reconstruct the various phases of the attack. The primary impact of the body on the steps was confirmed and then the succession of several katana blows with the fatal blow, which therefore turned out to be the one that drew on the nuchal region.

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**Forensic Pathology; Katana; Murder**



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**F6      A Thousand Ways to Die: When the Suicidal Instinct Cannot be Stopped**

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**Learning Objective:** After attending this presentation, attendees will better appreciate some forensic implications of postmortem diagnostics related to cases of suicide carried out in an atypical way.

**Impact Statement:** This presentation will impact the forensic science community by sharing the experience gained following the management of a strange suicide that at first glance may be inexplicable.

A 57-year-old woman, a farmer, was found inside a tank for the collection of rainwater closed by a heavy cast iron manhole and located in the garage of a house in the Modena Apennines. The prosecutor therefore requested a forensic examination on the scene to clarify the methods of implementation and the possible involvement of third parties in the death. The first circumstantial data collected revealed that the woman lived with her family and was in drug treatment for a condition of depression (olanzapine, paroxetine, and delorazepam). There was also a previous suicide attempt carried out a few months earlier through the inhalation of propane gas in a confined space, an attempt that failed due to the timely intervention of family members. Before proceeding with the difficult extraction of the corpse, it was necessary to empty the tank, which contained about 300 liters of water at a temperature of 8°C. Once extracted, the presence of a very abundant foamy cone and the presence of an initial wrinkling of the hands was noticed. Considering the thanatological parameters detected concerning the climatic conditions, death was placed between four and eight hours before the inspection. No traumatic injuries that could lead to suspicion of the involvement of third parties in the death were detected on external examination and no suspicious elements emerged from the police investigations.

Before autopsy, postmortem CT was performed that documented fluid material throughout the respiratory tree and in the digestive tract with diffuse alterations of the pulmonary parenchyma with a ground glass appearance. Inspection of the thorax found marked emphysema of the lung with many sub-pleural hemorrhagic petechiae, important cerebral edema, and aqueous liquid material mixed with frothy small bubbles in the larynx, trachea, and bronchial branches and a bilateral pleural effusion. In the stomach and duodenum were also found brownish aqueous material mixed with food fragments, in the context of continence of the pyloric valve and petrous ridge hemorrhage. Histological examinations showed marked aqueous emphysema in the lungs where the presence of some diatoms was also found. The toxicological analysis highlighted the presence of the drugs taken by the patient at values well within the therapeutic window.

This case report shows how a subject determined to commit suicide can carry out this gesture in ways that may seem inexplicable at first sight but which, if examined in an overall context that takes into account the circumstantial, autopsy, toxicological, and histological data, can therefore be uprooted.

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**Pathology; Drowning; Suicide**

## **F7 Godfathers on the Beach—The Hidden Corpse Under the Sand: A Case Report**

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**Learning Objective:** The aim of this presentation is to highlight the importance of forensic radiology investigations in the defining of firearm headshot injuries, in corpses in advanced state of decomposition.

**Impact Statement:** This presentation will impact the forensic science community through the provision of a proper forensic radiology investigation.

Italy has historically suffered from a high rate of criminality, exercised also through political influence and economic life control from different organized criminal organizations. The criminal groups that operate, like the Mafia, are infamous for the so-called “Lupara Bianca” (“white shotgun”) murders, with a higher number of missing persons, probably dead, where no corpse has been found.<sup>1</sup> “Lupara” is the Sicilian dialectal term for shotgun, a typical firearm associated with Mafia organizations; the other term, “Bianca” (white), symbolizes the absence of blood because bodies and traces of murders are concealed. The corpses are usually buried in the countryside, in places where it is very difficult to find them; alternatively, the body is placed in building pillars under construction, covering it with a concrete cast; another brutal mode to conceal the corpse is dissolving it in acid, with the eventual remains thrown into rivers, lakes, or the sea. In several cases, the corpse may be found for different reasons such as a confession of an affiliate. In these cases, forensic radiology may be considered a mandatory tool, in particular when there is suspension of firearm lesion.<sup>2</sup> In cases of corpses in an advanced state of decomposition, the typical external examination is no longer possible, due to advanced phenomena of putrefaction; using CT-scanning imaging, it is possible to distinguish between entrance and exit wounds and to define the sequence of the wounds by studying the fracture lines on the cranial bone.<sup>4-5</sup> Indeed, Puppe’s Rule, established by the German forensic pathologist Puppe in 1903, has been largely used in glass fracture analysis but can be applied also to the examination of skull fractures, and in several cases of multiple gunshots, the application of both Puppe’s Rule and Multislice Computed Tomography (MSCT) allowed the pathologists to reconstruct the exact sequence of fire.<sup>3,6</sup>

**Case report:** The police found the body of a man buried under the sand in a wood near the beach in an advanced state of decomposition and skeletonized in some anatomical districts. During the external examination, it was possible to detect multiple head bullet wounds, difficult to identify (entrance/exit wounds) due to the advanced state of decomposition. Consequently, a CT scan was performed on the corpse to identify the entrance, exit, and direction of the bullet wounds. One bullet wound passed through the left parietal bone (entrance wound “A”) and penetrated the opposite side, the right parietal bone (exit wound “B”). Near (6.3 inches) the entrance wound “A,” two fracture lines ran away from the central hole: one fracture line had a transversal course, stopping at the left frontal bone; the second fracture line had a coronal course stopping at the right parietal bone. The second and the third bullet wounds originated from two shots fired in rapid succession close to each other and passed through the right parietal bone (entrance wounds “C” and “D”). These wounds beveled the inner table of the frontal bone, stopping at the center of the frontal-ethmoidal region, through a single exit wound (“E”). In this case, the application of Puppe’s rule to the CT scan of the skull allows for imagining of the exact sequence of fire: the first shot was the entrance wound “A”; immediately afterward “C” and “D” were shot in rapid succession. It is possible to hypothesize that the fracture lines caused by the latter two wounds (“C” and “D”) intersect at the fracture lines of the first wound (“A”) without ever crossing them.

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### **Headshot; Forensic Radiology; Puppe’s Rule**

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**F8 Is Infrared Photography Equivalent to the Use of Chromophore Testing for Muzzle-to-Target Distance Determination in Gunshot Residue?**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding if Infrared (IR) photographs of Gunshot Residue (GSR) patterning on clothing will provide the same level of information and detail as chromophoric tests. The study consisted of the examination of dark-colored shirts that were shot with a handgun, using two bullet types, at varied distances. The resulting GSR patterns were evaluated with IR photography and chromophoric chemical tests. The pattern measurements from both processes were compared using photographic measurement software.

**Impact Statement:** This presentation will impact the forensic community by showing that infield use of IR photography to document GSR patterns on dark-colored clothing is a reliable method for preserving these patterns that could otherwise be compromised.

**Introduction:** In firearms homicides, it is uncommon to recover victim clothing from the body so that it can be preserved. GSR is transitory and during transportation of the body, the GSR pattern on the clothing could be altered. IR photography at the scene can preserve these patterns for later evaluation, muzzle-to-target distance testing, and scene reconstruction.

**Methodology:** The test subjects were 24 black colored t-shirts that were struck by a .45 ACP full metal jacket and hollow point bullets from varied defined ranges. GSR patterns were photographed with an IR camera, then sodium rhodizonate was used to visualize the GSR on the clothing; 100cm<sup>2</sup> and a 49cm<sup>2</sup> perimeter templates were used to mark the area surrounding the bullet hole to establish a known examination area. The Fiji ImageJ2 open-source image analyzing software was used on the IR and chromophoric patterns to measure the area of GSR pattern, residue perimeter, and overall GSR density.

**Results:** An independent *t*-test for two samples was used to determine if IR photography of a GSR sample would produce pattern measurements that would be significantly different than pattern measurements obtained from chromophoric testing. The value to determine significance for all tests was a  $p < 0.05$ . The *p*-value for the perimeter measurements for both IR photographs and chromophoric tests was significant. The *p*-value for the area measurements for both IR photographs and chromophoric tests was not significant. This same test was used to determine if varying muzzle-to-target distance changed the relationship between chromophoric and IR measurements. Variation of muzzle-to-target distance produces no statistical significance. A Spearman's Correlation was used to determine if different bullet types would produce significantly different GSR patterns in IR photographs given set muzzle-to-target distances. The value to determine significance for this test was a  $p < 0.05$ . The testing showed that there is a statistically significant correlation between the two bullet types for both the area and perimeter measurements. Varying muzzle-to-target distance was not significant for these examinations.

**Conclusions:** Overall, IR photography of GSR was not statistically significant to chromophoric testing. Each testing method has its own merits. IR photography allows for the viewing of all the various components of GSR that absorb IR light. Chromophoric testing allows for the visualization of micro amounts of lead expelled in GSR. These two testing methods are not synonymous or mutually exclusive, but independent testing methods that produce different viewpoints of amorphous pattern. Using Fiji ImageJ2 image processing software allowed quantitative information to be obtained objectively from abstract patterns in both IR and chromophoric samples.

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**IR Photography; GSR; Muzzle-to-Target Distance**

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**F9 Forensic Sexual Assault Evidence: Moving Past Differential Extractions**

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**Learning Objective:** This study evaluated the efficacy of performing a differential extraction on p30+, sperm-negative samples by determining how many of these cases yielded a CODIS-eligible DNA profile in the sperm fraction.

**Impact Statement:** This presentation will impact the forensic sciences community by providing understanding of the value of performing differential extractions on sperm-negative, p30-positive samples, and if taking such samples through this tedious process results in a high number of probative profiles in the sperm cell fraction.

**Introduction:** A rape kit typically has swabs from intimate areas, collected by a trained SANE nurse. If a sample tests presumptively positive for the presence semen, with BPIC (5-Bromo-4-Chloro-3-Indolyl Phosphate) and p30 tests, this sample is extracted using a differential extraction protocol, even if sperm is not confirmed visually under the microscope. This is to account for the accidental missing of sperm due to random sampling rather than actual absence of semen in the sample, or potentially an aspermia individual.

**Methodology:** A review was done of all the sexual assault cases that have been worked by this lab, and cases that were reported as being presumptively positive for the presence of semen, but where no sperm was observed, were analyzed further. The resulting DNA profiles generated from the sperm cell fractions of these shortlisted samples were analyzed and compared to the Known reference DNA profiles to determine if the DNA profile matched a perpetrator; were just leftover (undigested) epithelial cells from the victim; or yielded insufficient or uninterpretable data. The cost per sample of conducting a differential extraction as compared to a direct extraction was also tallied. This data was statistically compared.

**Results:** A p value was calculated using a *t*-test, where the number of cases where the sperm cell fraction yielded a probative CODIS-eligible DNA profile were calculated against the number of cases that didn't. In this study, of the 91 cases extracted using a differential extraction, there were only 5 CODIS-eligible profiles obtained. Of the 91 samples reviewed, 52 DNA profiles yielded single-source DNA profiles but only one was CODIS eligible; 23 yielded DNA mixtures but no CODIS profile; and 16 were deemed inconclusive (i.e., a p value < .05). For comparing the total cost incurred in the two extraction processes, the costs (per sample) of all the different consumables involved were calculated using the current cost available on the vendor's website. The total number of cases that were under consideration was multiplied by the cost per sample. When plotted on a graph, the difference in the cost was essentially doubled, as expected.

**Conclusions:** The first hypothesis demonstrated that, of the scores of cases that were extracted using the differential protocol, the number that resulted in CODIS-eligible profiles was surprisingly low. The results clearly show that a majority of the DNA profiles, when present, matched the victim. Additionally, there is a significant difference in the cost of performing differential, as opposed to, direct extractions. The samples and the cost are essentially doubled when performing differential extractions. The time taken to do these differentials is very subjective, depending on analyst comfort, technique, and experience; however, the cost incurred remains constant between the different analysts. As shown, there was a marked increase in the cost of performing differential extractions.

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**DNA-Sperm; Differential Extraction; P30**

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**F10 The Shelf Life and Effectiveness of Blood Reagents Over Time**

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**Learning Objective:** After attending this presentation, attendees will be introduced to research showing that the shelf life of blood test reagents is long lasting and well beyond manufacturer recommendations. The objective of this research was to compare the effectiveness of two commonly used blood detection reagents, BlueStar® and Hemascein®. The reagents were prepared and utilized over a 21-day period. Two sets of reagents were evaluated, with one set being kept in the dark and refrigerated and the other being left out and exposed to light and ambient temperature.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the effective use of blood reagent chemicals over multi-day crime scene processing. Once prepared, the same reagents can be used over several hours, days, or even weeks without wasting expensive reagents

**Introduction:** This research compared two blood reagent chemicals over 1, 7, 14, and 21 days and rated the results under exposure to different light conditions. One group of reagents were exposed to indoor lighting and environmental conditions, while the other group was wrapped in aluminum foil and refrigerated until it was opened on testing days.

**Methodology:** The study focused on the use of BlueStar® and Hemascein® applications on two fabrics—denim, and cotton. Canine blood was used for safety concerns and was diluted at 1/10, 1/100, and 1/1,000. Two batches of each chemical were prepared on day 1 in spray bottles. One set of each mixture was then wrapped in heavy-duty aluminum foil and the tops taped with black electrical tape, ensuring no light would contact the solutions in the bottle. They were also stored in a refrigerator, which was only opened weekly for testing. The second set of each mixture was kept at room temperature and not wrapped and was therefore exposed to environmental light and temperatures.

**Results:** The overall results showed BlueStar® to be a more reliable blood reagent chemical over Hemascein® in detecting blood at dilutions of 1/10, 1/100, and 1/1,000 on both cotton and denim fabrics throughout the 21-day testing period.

**Conclusion:** The result of the research shows BlueStar® and its chemiluminescence to be a more reliable blood reagent chemical over Hemascein® and its fluorescence. It is easier to use, with only one application necessary versus two (Hemascein® and then peroxide per the instructions). The reactions noted with BlueStar® did not require any other equipment such as an alternative light source (ALS) or protection to visualize as Hemascein® does. Furthermore, this research shows that when BlueStar® is protected from light and temperature changes, it will still provide useful reactions to crime scene investigators for several days and even weeks.

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**BlueStar®; Hemascein®; Shelf Life**

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**F11 Gun Violence: Recreating Shooting Incidents**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how recreating a crime scene shooting incident can be key forensic element in identifying the shooter, the weapon, and ammunition. The objective of this research is to correlate how caliber, projectile type, and muzzle-target distance creates different-sized bullet entry holes. This study examined bullet entry holes test fired at 5 and 15 feet into glass, plywood, and drywall substrates. The handguns used in this study were 9mm and .45 caliber with full metal jacket (FMJ) and hollow point (HP) ammunition.

**Impact Statement:** This presentation will impact the forensic community by describing the effects different caliber and projectile bullets cause when there is lack of evidence, as well as identifying the weapon and linking the suspect(s) by measuring the diameter of the bullet entry hole depending on the distance of discharge and material penetrated.

**Introduction:** Crime scene reconstruction is imperative to providing the narrative of a crime. The use of crime scene investigation assists forensic examiners in piecing together the missing links that will allow for more detailed characteristics within the crime scene and provide a greater chance of solving the crime.

**Methodology:** The materials used for this study were glass (single pane), wood (plywood), and drywall. The four types of ammunition used were 9mm Blazer Brass Luger® FMJ, 9mm Federal Luger® HP, .45 caliber TulAmmo auto FMJ, and .45 caliber Hornady® auto+p HP; and two different handguns, a 9mm Glock® 19X and a .45 caliber Rock Island Armory® (ACP) 45. Each of the four types of ammunition was test fired three times into the targeted materials from the ranges 5 and 15 feet (total of 72 rounds). The data was analyzed using *t*-test and significance determined at *p*-value < 0.05.

**Results:** Comparing the mean values of each projectile type in the bullet entry hole: the 9 mm FMJ and the .45 caliber FMJ bullet in the plywood, *p*-value at 5ft was 0.0686 and 0.0132 at 15ft. The *p*-values in the glass comparing 9mm HP and .45 caliber HP at 5ft was 0.0322 and 0.5107 at 15ft; and for the 9mm FMJ and the .45 caliber FMJ at 5ft was 0.8149 and 0.2417 at 15ft. The *p*-values in the drywall when comparing the 9mm HP and 45 caliber HP at 5ft and 15ft were both 0.0022; and comparing the 9mm FMJ and the 45 caliber FMJ at 5ft was 0.0132 and 0.0022 at 15ft.

**Conclusions:** This test study provided insight on ammunition impact of certain materials at two different ranges. Overall, the caliber size and distance will impact the bullet entry holes at different ranges in wood, as the HP bullet showed significant difference in the glass at close range; however, the HP at the far range and FMJ bullet entry holes both the close and far range had no significant differences. The HP bullet showed no significant difference in the drywall regardless of range; the FMJ showed significant difference at both ranges. Thus, bullet size, projectile type, material, and distance do impact the entry holes. These details could be very important for forensic firearm examiners recreating shooting incidents.

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**Bullet; Caliber; Projectile**



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**F12 The Variation in Suicide Note Traits Between Men and Women**

*Eleanor Abreo, MD\*, University of Oklahoma Health Sciences Center, Oklahoma City, OK; Edana Stroberg, DO, Oklahoma City Office of the Chief Medical Examiner, Oklahoma City, OK*

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**Learning Objective:** After attending this presentation, attendees will understand if there is a difference between the rates at which phenotypic females and phenotypic males leave suicide notes and if there are variations in the traits of notes left by male and female decedents.

**Impact Statement:** This presentation will impact the forensic science community by providing insight into the persons in Oklahoma who die by suicide and gives future direction for investigation into those who leave suicide notes.

Certain studies demonstrate differences between those who die by suicide and leave written communication and those who do not. However, studies that examine women's and men's propensity to leave notes and studies that examine differences in note content between men and women show marked variation in results. A retrospective study using reports by medical investigators and autopsy reports from the Oklahoma City Office of the Chief Medical Examiner's (OKCME) database was conducted in order to determine if there is a difference between the number of suicide notes and note traits between phenotypic males and phenotypic females in Oklahoma in 2021. A total of 299 people died by suicide in Oklahoma in 2021. Of those people, 257 (86%) were men and 42 (14%) were women, with an average age of 45 years; 47 men (18%) and 13 women (31%) left a written suicide note. No significant difference was found between the number of men and women in who left a written suicide note ( $X^2$  (1,  $N=299$ )=3.6,  $p=.057$ ).

In terms of variations in suicide note traits among men and women, there were no significant differences between the number of men and women who left suicide notes with instructions for disposition of their remains ( $X^2$  (1,  $N=60$ )=0.25,  $p=.614$ ) or the number of men and women who wrote multi-page notes ( $X^2$  (1,  $N=60$ )=3.2,  $p=.072$ ). When a suicide note was left, there was no difference between the number of notes by men or women that referenced reason(s) for suicide ( $X^2$  (1,  $N=60$ )=0.29,  $p=.59$ ). There were no significant differences between the number of men and women who left handwritten ( $X^2$ ,  $p > .05$ ) or cell phone text message notes ( $X^2$ ,  $p > .05$ ).

In this pool of female and male note writers, no significant differences were identified across the examined parameters. Perhaps differences between note writers and non-note writers could be identified if decedents are subclassified by parameters such as socially defined race, history of previous suicide attempts, mechanism of suicide, or history of mental illness as sex does not appear to correlate with note writing or note attributes. For future research, it would be useful to have transcribed copies of handwritten notes in order to accurately gauge note content. Currently, pictures are taken of the notes that are available. In some cases, notes are unavailable as they are in the possession of law enforcement, or the decedent was hospitalized and no scene investigation was conducted.<sup>1-5</sup>

**References:**

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**Suicide; Note; Sex**

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**F13 The Role of the Ethics Committee in Research Activities During the SARS-CoV2 Pandemic**

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**Learning Objective:** After attending this presentation, attendees will understand that COVID-19 epidemic had changed Ethics Committees (Ecs) activity that should respond in a timely manner to new evaluation requests by adapting its standard operating procedures to the new reality.

**Impact Statement:** This presentation will impact the forensic science community by showing that, in emergency conditions, it is necessary to proceed as quickly as possible in the review and assessment of the research by safeguarding, at the same time, both scientific transparency and compliance with ethical requirements.

Research participants' safety is guaranteed by ethical guidelines (e.g., Declaration of Helsinki and good clinical practice), legislation to protect participants' privacy, research ethics committees, and informed consent. "Ethics Committee," according to Directive 2001/20/EC, is an independent body consisting of health care and non-medical members responsible for protecting the rights and safety of subjects involved in a trial through "expressing an opinion on the trial protocol, the suitability of the investigators and the adequacy of facilities, and on the methods and documents to be used to inform trial subjects and obtain their informed consent". In Italy, the establishment of ECs is provided by the law in public health facilities, private hospitals, and care institutions. At the beginning of pandemic, World Health Organization and the European Commission provided indications regarding the research's evaluation by adopting an "expedited" approach of ECs approval.<sup>1,2</sup>

In Italy, both the Council of Ministers and the Italian Medicines Agency (AIFA—Agenzia Italiana del Farmaco) issued some measures for a national coordination of clinical trials and therapeutic programs management in COVID-19 emergency in 2020.<sup>3</sup> The EC of the National Institute for Infectious Diseases "Lazzaro Spallanzani" in Rome, as National Unique EC for the evaluation of clinical trials of medicines for human use and medical devices for patients with COVID-19, formulates a national opinion also on the basis of the evaluation of the AIFA Technical Scientific Committee. AIFA introduced a "fast track" for the online transmission of research documentation and the submission of request for clinical trials authorization relating to the treatment of COVID-19. The applicants were allowed to defer the sending of paper documents, which in any case must be sent as soon as possible to the Clinical Trials Office. During the COVID-19 public health emergency, applications for trials in oncology, transplants, and urgent clinical conditions requiring interventions that could not be postponed were admitted besides trials to address the COVID-19 emergency. In Turin, the Inter-Company Ethics Committee (IEC) was established in 2019 and includes three hospitals (University Hospital "City of Science and Health," Hospital "Ordine Mauriziano," and "ASL Città di Torino").

From January 2020 to December 2021, the IEC evaluated 1,100 studies of which 134 referred to COVID-19. IEC evaluated: in 2020, 472 non-COVID-19 (NCS) studies and 90 COVID-19 studies (CS); in 2021, 492 NCS and 46 CS. The trend in the number of evaluations showed two peaks, in March–July 2020 and September–November 2020, just when pandemic waves occurred and before the Comirnaty vaccine authorization. The majority of studies were observational (86.5% of CS, 59.1% of NCS). CS focused on impact on other pathologies and therapies ( $n=35$ ), SARS-CoV2 characteristics ( $n=22$ ), therapy ( $n=21$ ), long-term effects ( $n=17$ ), diagnosis ( $n=14$ ), COVID-19 vaccines ( $n=9$ ), epidemiology ( $n=8$ ); 6.7% of total CS and 2.3% of NCS referred to emergency management research. The role of ECs is fundamental in promoting ethical values in research and in ensuring ethical protection and standards for all the individuals involved in COVID and non-COVID research, even in emergency situations. None the less, the need to accelerate the research for guiding public health interventions should not come at the expense of a thorough ethics review.

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3. Italian Medicines Agency (AIFA - Agenzia Italiana del Farmaco). *Clinical trials' management in Italy during the COVID-19 (coronavirus disease 19) emergency*; 2020 [https://www.aifa.gov.it/documents/20142/1123276/Comunicazione\\_gestione\\_studi\\_clinici\\_in\\_emergenza\\_COVID-19-EN\\_17.09.2020.pdf](https://www.aifa.gov.it/documents/20142/1123276/Comunicazione_gestione_studi_clinici_in_emergenza_COVID-19-EN_17.09.2020.pdf)

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**Ethics Committee; COVID-19; Research**

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**F14 An Improbable Ballistic Trajectory in a Firearm Suicide With an Expanding Ammunition**

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**Learning Objective:** This presentation regards a particular case of ballistics. Indeed, the trajectory is not linear, and the expanding projectile performed various ricochets. In this case, it was necessary to associate autopsy and CT to understand the trajectory and conclude that the manner of death is a suicide.

**Impact Statement:** Forensic ballistics is a complex science. Each situation is unique and must be confronted with forensic experience. This case illustrates the necessity for the need for forensic investigations. For the forensic science community, this case is an example to understand complex trajectories.

**Introduction:** In the past few years, gendarmerie services have been equipped with expanding ammunition, for the simple reason that the old projectiles were subject to ricochets or could pass through targets to reach others, thus creating collateral damage. Expanding projectiles are projectiles that mushroom on impact, which severely limits their penetrating power.

**Case:** We received at the Forensic Institute of Rouen, the body of a policeman who would have committed suicide with his service firearm, a SIG PRO handgun, with an expanding ammunition of 9mm. An autopsy was performed. The observations were as follows: an orifice regarding the 4th intercostal space that had the characteristics of a ballistic inlet orifice. The following lesions were then described: a dilaceration of the left ventricle of the heart, a penetrating wound in the lower lobe of the left lung, two holes in the diaphragm, a penetrating wound in the transverse colon, and finally an orifice next to the posterior arch of the 11th left rib. We found the projectile lodged behind the 9th left rib. In total, the projectile punctured the skin regarding the 4th left intercostal space with a downward trajectory, slightly from left to right and from front to back, ricocheted on the anterior arch of the 6th rib, changed trajectory to tear the left ventricle and the lower lobe of the left lung in its path, pierced the diaphragm, reached the transverse colon, passed back into the thoracic cavity, perforated the diaphragm a second time, and then struck the posterior arch of the 11th left rib to end its course behind the 9th left rib.

**Discussion:** In the case described, we can see that the ballistic trajectory is not linear or curved. With the various ricochets, we can establish that this one is rather rectangular in appearance. This would not have been possible with a jacketed projectile, which would rather have had an almost linear trajectory. This was possible thanks to the expansive nature of the projectile, which was found with a perfect mushroom-shaped deformation. This therefore demonstrates the effectiveness of this type of ammunition, which remains intracorporeal, even when fired at close range, thus avoiding its release and potential collateral damage. If the projectile had been different (for example, FMJ), the trajectory would have been quite different, and the individual could have survived.

**Conclusion:** Each ballistic shot leads to a unique situation and therefore to a very singular trajectory. Depending on the elements encountered on its trajectory, its composition, and many other parameters, a projectile will be able to be more or less deflected and will create more or less damage. In a suicide context, if some people are miraculous, others have no chance of survival, even if the shooting conditions are apparently similar, hence the complexity of this discipline and the impossibility of predicting a trajectory without having performed an autopsy.

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**Forensic Pathology; Ballistic Trajectory; Expanding Projectile**

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**F15 Kinship vs. Unidentified Human Remains (UHR)**

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**Learning Objective:** attendees will be made aware of the nuanced differences between calculations supporting Unidentified Human Remains (UHR) identification through familial DNA testing and traditional kinship calculations. The latter is often incorrectly used as a proxy UHR. The H1 hypotheses remains the same between Kinship and UHR calculations, but H2 differs significantly. Using visual H1 and H2 pedigrees, the differences can be easily recognized and understood.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that UHR at medical examiner's offices often require DNA testing and familial association for positive identification, so let's do it correctly.

Medical examiners often rely on DNA profiling for UHR and missing person's casework. It is a common misconception that traditional kinship calculations can be used as a proxy for UHR identification. However, the resulting likelihood ratios for UHR differ significantly from the kinship proxy. Larger medical examiner offices may have a DNA section or Rapid DNA instrumentation to assist them in obtaining a DNA profile from skeletal remains and then from family members for comparison. Some medical examiners will outsource this work to the local crime laboratory, university, or private DNA laboratory. The DNA profiling work may be impeccable, but the incorrect calculations are often applied. For this reason, we have developed a tool that allows the visualization of complex pedigrees that form the basis of the H1 and H2 likelihoods. The H1 hypothesis remains the same between traditional Kinship and UHR but the H2 hypothesis differs significantly between the traditional Kinship and UHR calculation. The resulting likelihood ratios differ in magnitudes between traditional kinship and UHR/Missing Person's casework. This tool is used to visually observe the H1 and H2 pedigrees and the symbolic formula is presented for further exploration and understanding. Relatedness testing is a niche discipline within the forensic biology community. There are specific proficiency testing and accreditation requirements and yet the experienced practitioners often fail to recognize the differences in the models.

This simple presentation will explain the differences using visual tools so the otherwise abstract models become easily recognizable and a deeper understanding is provided to the experienced practitioner and novice. I have performed relatedness calculation since 1998 and the advancement in the multiplex kits from 9 loci to 20+ unlinked loci is allowing for the familial DNA identification out through second-degree relatives with significant relatedness indices/likelihood ratios.

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**Kinship Calculations; Unidentified Human Remains Calculation; Pedigrees**

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**F16 True Believers: An Analysis of Trauma Associated With Modern Incidences of Human Sacrifice**

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**Learning Objective:** Attendees will learn about variations in patterns of trauma inflicted across types of ritual homicide cases and how methods, such as a machine learning cluster analysis, can help medicolegal professionals to perceive trends more objectively.

**Impact Statement:** This presentation will impact the forensic science community by revealing broad trends in trauma observed across types of ritual homicide.

When human sacrifice is discussed in modern circumstances it often evokes a Galadrielic mix of emotions—individuals are horrified but also fascinated by this rare and morbid act. These emotions are frequently accompanied by dissociation since human sacrifice is typically likened to “primitive” and “savage” peoples of the distant past. Yet, ritual violence is hardly a thing of the past. While news headlines purporting occult killings, vampire killers, and Satanic ritual abuse seem sensational, they can also be viewed conceptually as potential instances of modern human sacrifice. As outside observers, medicolegal professionals often explain ritually committed crimes with a Westernized focus on mental illness. However, analyzing the symbolic features of crime preparation, execution, and post-crime alterations from the viewpoint of the perpetrators can elucidate ideological belief systems and reasoning consistent with theoretical models that aim to explain the historical purposes of human sacrifice. Furthermore, analyzing the trauma present on decedents can help distinguish cases truly synonymous to modern sacrifice from other forms of ritual murder, which can provide insight into the crime and its likelihood of recurrence.

For this research, news articles from the past century were searched using key words (e.g., human sacrifice, ritual murder, ritual homicide, etc.). Only articles from known reputable sources were utilized, as well as cases that had fairly thorough disclosure of details ( $N=39$  cases). Collected cases spanned across 16 countries, at least 12 distinct religions, and 92 years. Based on case details, perpetrators were classified as “true criminals,” “dabblers,” or “true believers” using Perlmutter’s Symbolic Analysis criteria, and presence/absence of broad categories of trauma inflicted upon victims were recorded.<sup>1</sup> These categories include sharp force, blunt force, Gunshot Wound(s) (GSW), mutilation, strangulation, sexual assault, dismemberment, poisoning and/or drugging, burning and/or boiling, cannibalism, blood drinking and/or draining, and hanging.

When comparing trauma types across perpetrator classifications using a Fisher’s exact test, gunshot wounds and dismemberment were associated significantly with perpetrator classifications with p-values of 0.016 and 0.002, respectively. Dismemberment was most commonly seen among true believers (synonymous to modern human sacrifice), while gunshot wounds were most commonly seen among true criminals (those who use occult ideologies to justify their criminal actions, but whose crimes are not performed to fulfill any tenets of their belief system). Blood drinking and/or draining was not significant, although its p-value of 0.080 was fairly close to the cutoff for significance and may suggest an association that only becomes observable in a larger sample size. A machine learning clustering analysis also revealed differences in trauma, specifically methods utilized to kill an individual versus postmortem modifications, depending on the goal of the perpetrator. Collective, retrospective analyses of classes of crimes may help reveal broad trends unperceived initially. Specifically, the use of a machine learning cluster analysis helps us to discern groupings of crime features more objectively. When applied, this information can aid medicolegal professionals in interpreting patterns of trauma observed in cases of ritual homicide.

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**Trauma; Human Sacrifice; Ritual Homicide**

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**F17 A Case Report of Suicidal Hanging With Precipitation**

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**Learning Objective:** It is in the interest of forensic investigations to have a good diagnosis. A crime scene was diagnosed a natural death (a man discovered dead on the garage floor). The postmortem CT and the autopsy allowed the conclusion that the death was hanging with precipitation.

**Impact Statement:** This study of hanging with precipitation and biomechanics findings to explain lesions and injuries in these cases will impact the forensic science community.

**Introduction:** Suicidal hanging is one of the most common ways to commit suicide in the world. Suicide with precipitation is most often associated with defenestration. However, it is possible to encounter cases of suicidal hanging with precipitation. In this case, the findings and examination of the place of death may be modified by the precipitation and, therefore, initial survey findings may be biased. We present a case of suicidal hanging with precipitation in which the initial findings were based on a bloody inhalation death due to a maxillofacial trauma. We relied on literature reviews and the biomechanics of hanging to support our conclusions.

**Case report:** The victim's body was found on the floor, lying on the back with the legs to the side. In the garage, a ladder and a 15m power cable were found; the cable was attached to a beam. The height was 2.50m. Two farewell letters were sent to a relative by the victim the day before. The investigator's initial findings were that the victim had fallen without having had time to tie himself up. The body of this man weighed 90kg for a height of 172cm. At the external review, we found asphyxia syndrome. There was no circular abrasion or an abnormal mobility of the cervical spine. At autopsy, hemorrhagic infiltrates of the different planes of the neck were detected as well as fractures of the thyroid cartilage and the hyoid bone. Finally, a hemorrhagic infiltrate of the anterior aspect of the cervical spine with a fracture of the vertebral body of C3 was noted. The findings of the autopsy were consistent with a hanging with a precipitating gesture.

**Discussion:** In this case, the main issue was whether the victim had hanged himself or fallen from a high place. The lesions found in the cervical region were consistent with the hypothesis of a hanging. Concerning the precipitation, we based our conclusions on biomechanical analyses of hangings with precipitation having led to lesions of the cervical rachis or even decapitations. As a reminder, the force  $F$  applied to the neck is calculated by multiplying the weight (in kg) by the acceleration constant  $g$ . In this case, we obtain a force of  $F=882.9\text{ N}$  (in freefall, omitting frictional forces). These results are consistent with literature reviews on the subject. Thus, we were able to conclude from our observations and the literature that this case was a suicide by hanging with precipitation.

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**Suicidal Hanging; Precipitation; Cervical Fracture**

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**F18    How to Approach Drug Intoxication in Pediatric Ages: A Five-Year Retrospective Analysis**

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**WITHDRAWN**



## **F19 Ossified Cephalohematoma in a 27-Day-Old Infant: A Rare Finding Diagnosed by Postmortem Computed Tomography (CT) Scan**

*Michael Pagacz, MD\*, Maryland Medical Examiner, Baltimore, MD; Zabiullah Ali, MD, Maryland Medical Examiner, Baltimore, MD*

**Learning Objective:** After attending this presentation, attendees will have gained knowledge of ossified cephalohematomas in infants, methods for postmortem diagnosis, and how to prevent potential misdiagnosis of non-accidental trauma.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of ossified cephalohematomas in infants, methods for postmortem diagnosis, and how to prevent potential misdiagnosis of non-accidental trauma.

This presentation highlights the utility of postmortem CT in diagnosing ossified cephalohematomas in infants. Cephalohematomas are benign collections of subperiosteal hemorrhage that develop as uncommon complications of birth trauma in up to 0.2–3% of neonates.<sup>1</sup> Though most commonly associated with instrument-assisted vaginal delivery, cephalohematomas have been reported in neonates delivered via Cesarean section without labor and *in utero* diagnosed by ultrasound with premature rupture of membranes as an associated factor.<sup>2–4</sup> Other factors associated with an increased risk for cephalohematoma include prolonged difficult labor, cephalopelvic disproportion, primigravida, male gender, and increased fetal size.<sup>5</sup> The majority of cephalohematomas spontaneously resorb within one month of life.<sup>6</sup> On rare occasions, they may persist, begin to calcify, and form an ossified cephalohematoma through subperiosteal osteogenesis.<sup>7</sup> Ossified cephalohematomas have both an inner and outer layer of bone surrounding the subperiosteal hemorrhage, resulting in characteristic imaging features on Computed Tomography (CT). These features include a hypodense, non-enhancing, uniformly homogeneous core encased by bone.<sup>8</sup> The characteristic imaging findings may also be helpful in the postmortem setting to help distinguish between skull injuries due to birth trauma and injuries as a result of non-accidental trauma.

Here is reported a case of a 27-day-old female infant with an ossified cephalohematoma diagnosed at autopsy using a postmortem CT scan. The decedent was born at 39 weeks and 5 days gestation via spontaneous vaginal delivery to a primigravida mother. Physical exam at birth noted a normocephalic head with normal size fontanelles, and without evidence of a cephalohematoma or other skull trauma. The infant was discharged home on the second day of life and continued to develop normally until her death.

On the day of death, the infant was given a nighttime feeding in bed by her father who fell asleep with the infant on his chest. The infant was found approximately four hours later, face down on the mattress. Emergency medical services responded and initiated cardiopulmonary resuscitation attempts but were unsuccessful. Law enforcement investigation did not reveal any evidence of abuse or neglect. An autopsy revealed posterior parietal skull discoloration, with no subgaleal or scalp hemorrhage identified. The remainder of the autopsy was negative for trauma or abuse. A CT scan of the infant's skull was performed and revealed an ossified cephalohematoma. Cephalohematoma could be associated with underlying skull fracture due to instrumentation and potentially could lead to misdiagnosis. Therefore, review of pertinent medical records in conjunction with appropriate postmortem imaging is important for proper diagnosis. This case highlights the utilization of postmortem CT in distinguishing benign lesions associated with birth trauma from injuries from non-accidental trauma.

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### **Cephalohematoma; Infant; Radiology**

## **F20 The Optimization of 3D Fingermark Images for Use in the Federal Bureau of Intelligence's (FBI's) Universal Latent Workstation (ULW)**

*Nicole Stanaback\*, University of New Haven, Netcong, NJ; Josep De Alcaraz-Fossoul, PhD, University of New Haven, West Haven, CT*

**Learning Objective:** After attending this presentation, attendees will understand the protocol for optimizing 3D fingermark images to render them suitable and compatible with the FBI's Universal Latent Workstation (ULW) computer software. The development of this protocol will help to promote 3D analysis in routine forensic applications. ULW provides objective metrics on the visual quality/clarity of fingermarks which may be obtained to support an examination of a 3D fingermark.

**Impact Statement:** The impact of this presentation on the forensic science community is the promotion of the use of 3D analysis in everyday forensic applications. While 3D images of fingermarks are becoming commonly used in experiments, currently they cannot be used in routine forensic casework due to their inability to receive accurate automatic quality metrics via ULW or similar software. With the use of this created protocol more accurate quality metrics can be obtained for the fingermark allowing for the possibility of 3D images to be implemented in everyday casework.

After the release of the National Academy of Science's (NAS) Report in 2009 and its criticisms of many forensic disciplines—especially friction skin ridge pattern analysis—scientific research has skyrocketed. One of many focuses to investigate has encompassed the estimation of the age of fingermarks, also referred to as “time since deposition/.”<sup>1</sup> One technique proven viable to examine and distinguish the age of fingermarks is a 3D analysis via an optical profiler (OP) and the metrics it provides.<sup>2</sup>

Ideally, for a (latent) fingermark to be properly examined, it should be assigned both a quality score (QS) manually as well as an automatic quality metric, using the FBI's Universal Latent Workstation (ULW), or equivalents. Despite its great performance with 2D images, ULW is inconsistent when it comes to recognizing fingermarks captured in 3D. Herein lies the primary issue with 3D imagery's implementation in casework; the raw 3D images being analyzed do not always perform as expected and must undergo some degree of optimization by image editing software to become suitable for ULW. The main hypothesis as to why this inconsistency occurs is that ULW software cannot properly differentiate ridges from furrows in the default 3D image format provided. Interestingly, the software tends to consider the outline of the ridges as two separate ridges rather than one, possibly because the inner areas of the ridges are not dark enough compared to the background.

Based on preliminary experimental findings, the color contrast must be very high between ridges and the background to be workable in ULW. The goal of increasing contrast was to have a solid dark ridge structure over a uniformed background, almost white in color.<sup>3</sup> The contrast was not the only enhancement necessary since the image created by the OP was larger in size and resolution than what ULW can handle. These were adjusted along with the color, which was another factor that affected ULW's ability to optimally process the image.

Several combinations of image enhancements were created and tested with ULW. A total of 720 3D images were edited and examined from six donors, three males, and three females. These were deposited onto plastic and glass microscope slides and exposed to natural light and complete darkness for three months. QS values and metrics on visual quality, including the surface area extension of “BlueGreen” regions were collected.<sup>4</sup> “BlueGreen” was the physical region considered suitable for identification. Additionally, the images were binarized in color (pure blacks and whites) and ridge flow checked. The binarization option in ULW was able to show the accuracy of (ridge) areas that the software was recognizing. The enhancement sequence that provided a correct ridge flow and best quality metrics, once the image was binarized, was re-sizing to 3x1.5cm; inverting the colors; modifying resolution to 250ppi; editing to grayscale; re-adjusting the color contrast to positive 50 for both brightness and contrast and applying “blur.” Once the image was optimized, it was re-examined by ULW at 1,000ppi, and metrics were obtained. Data collected from ULW included quality/clarity scores and extension of “BlueGreen” areas. Microsoft Excel® was used to compile and calculate the percentage of “BlueGreen” and perform preliminary statistics. Further analyses were conducted with IBM® SPSS® Statistics software to detect any trends in the data. Initial results showed that the “BlueGreen” metric aligned well with the QS values after completing enhancements.

Further analysis will be performed to provide slight improvements in the protocol that could be useful for fingermarks collected under different conditions. In addition, as the software for ULW is updated, the optimization protocol will be adjusted to become compatible with the software's updates.

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### **3D Fingermarks; ULW; Optimization**

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**F21 Fingerprints: A Signature Through Time**

*Tracy Brisendine, BA\*, Denver Office of the Medical Examiner, Denver, CO*

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**Learning Objective:** Attendees will learn the history of fingerprints, including their introduction into the forensic science community in the 1800s. The basics of fingerprint patterns and characteristics will be addressed as well as what the requirements are for identification comparison. Current postmortem fingerprinting technology will be mentioned, and the top methods for decedent identification will be photographically demonstrated.

**Impact Statement:** This presentation will illuminate the ever-changing options of fingerprint technology and illustrate the most tried and true processes used by medicolegal investigators to identify decedents postmortem.

The science of fingerprint identification has been a staying force in forensics for over a century and remains the most used evidence out of all the forensic disciplines.<sup>2</sup> The oldest and largest forensic organization, the International Association for Identification, was established in 1915 to further the scientific identification process with fingerprints.<sup>3</sup> As of 1977, forensic professionals could become Certified Latent Print Examiners through the first professional forensic science certificate program.<sup>3</sup> The Chinese recorded friction ridge impressions during their crime scene investigations beginning in 210 B.C.<sup>1,2</sup> In 1823, nine fingerprint patterns were identified which laid the groundwork for the Henry classification system we use today.<sup>1,2</sup> In 1877, fingerprint identification was used in criminal court, prisons, and with the registration of deeds in India while also being used for the first time as evidentiary clues in criminal investigation in the United States.<sup>1</sup> In 1891 in Argentina, criminals' fingerprints were recorded for identification, becoming the first practical use of fingerprint science by law enforcement. A year later, in 1892, the first homicide was solved with fingerprint evidence.<sup>1</sup> In 1897, a report was issued to governments in India claiming that fingerprints were superior to the anthropometrics method for identification.<sup>1</sup> In 1900, England accepted the recommendation that fingerprints become logged as standard practice for criminal records.<sup>1</sup>

In the early 1900s, the first systematic use of fingerprints was adopted by the United States.<sup>1,2</sup> In 1904 in a federal prison in Kansas, inmate fingerprints started the first United States government fingerprints collection.<sup>1</sup> By 1911, fingerprints were accepted by United States courts as a reliable means of identification.<sup>1</sup>

There are three main fingerprint patterns: the arch, loop, and whorl.<sup>4</sup> Focal points of prints used for identification are the core and the delta and each pattern has its own required characteristics for a successful identification comparison. Standard postmortem fingerprint techniques include ink and paper, adhesive transference, casting material, and digital capture devices.<sup>4</sup> Law enforcement has used ink and paper since the late 1800s and even now it can be a valid method for postmortem identification.<sup>2</sup> Downsides of ink and paper include loss of detail due to paper texture and general process messiness.<sup>4</sup> Adhesive transference is the process of dusting a decedent's fingers with black powder and then lifting the print by means of a tacky surface.<sup>4</sup> It takes practice but can maintain the fine minutiae of the fingerprint; it can also be replicated many times on the same digit without loss of ridge detail.<sup>4</sup> Casting can be used with degloved epidermis or on regular ridge detail when fingers cannot be rehydrated.<sup>4</sup> More advanced postmortem fingerprint techniques are needed when there is maceration and decomposition, epidermal degloving, thermal modification, and desiccation.<sup>4</sup> Various substances can be utilized to re-plump sloughing or waterlogged epidermis. For instance, boiling the digits or hands can be used to bring ridge detail back to life.<sup>4</sup> Degloving allows for dermis and epidermis printing.<sup>4</sup> Thermal modification and desiccation have unique challenges but can be overcome with science, specific knowledge, and chemical rehydration. Best practices for ink printing, casting, and degloving for postmortem identification are easily demonstrated in step-by-step photographic detail.

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**Fingerprints; Identification; Techniques**

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**F22     The Examination of Latent Fingermark Degradation as a Function of Time and Temperature by 2D and 3D Optical Analyses**

*Riddhi Roy, BS\*, University of New Haven, West Haven, CT; Josep De Alcaraz-Foussoul, PhD, University of New Haven, West Haven, CT*

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**WITHDRAWN**

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**F23 Ridge Volume as a Metric for Fingermark Age Determination and Sex Discrimination**

*Nicole Stanaback\*, University of New Haven, Netcong, NJ; Joseph De Alcaraz-Foussoul, PhD, University of New Haven, West Haven, CT*

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**Learning Objective:** After viewing this presentation, attendees will understand whether the volume of ridges could be used as a metric in latent fingermark age estimations for both biological sexes. The presenters will provide additional knowledge on 3D analysis of the evidence as a potential basis in mathematical aging models.

**Impact Statement:** The findings of this research will contribute toward the overall goal of helping crime investigators exclude or include possible suspects in highly trafficked areas, thus reducing the backlog of forensic laboratories and easing the criminal justice process. However, further analyses should be conducted because this study is limited to the Caucasian population.

Recent 3D examinations of latent fingermarks have proven to be extremely helpful as the discipline of friction ridge pattern analysis is exploring the possibility of determining “time since deposition,” or the “age,” of this type of physical evidence.<sup>1</sup> 3D imaging has allowed meaningful topographical data to be collected for the purpose of age estimations that could not normally be available using more traditional methods such as a compound microscope, a stereomicroscope, a magnifying glass, or computer programs. This may aid examiners by being able to exclude or include evidence that has been left behind within the period that the crime was committed; and is especially useful in cases involving highly trafficked areas such as jewelry stores or banks. This new source of information may help alleviate the casework load in already overloaded crime laboratories. This project aims to explore the aging process of fingermarks based on the changes in the volume of residue deposited as a function of time and biological sex.

On 3D approaches to evidence examinations, a study empirically proved the significant loss in the height of fingermark ridges over time, demonstrating a clear relationship to and sight of “aging.”<sup>1</sup> This initial study led to exploring other possible factors and metrics that could be deemed as or more suitable for this purpose. One potentially influencing factor is the sex of the individual, as females have a higher density of ridges than males. Caucasian females have average densities of 13.32 ridges/25 mm<sup>2</sup>, compared to Caucasian males having a ridge density of 11.14 ridges/25 mm<sup>2</sup>.<sup>2</sup> Thus, it is hypothesized that female fingermarks may retain sweat residue (i.e., volume of ridges) for a longer period than their male counterparts.

For this study, five Caucasian males and females deposited two sets of fingermarks onto standard glass microscope slides. These were created by washing their hands with soap and water, drying completely, and placed into plastic bags for ten minutes to stimulate the production of sweat. The fingermarks were aged over a ten-day period, with data collection on day 0, which was approximately 4 to 6 hours after deposition, as well as at 1 day, 3 days, 7 days, and 10 days post-deposition. A 3D optical profiler was utilized to obtain images and collect topographical data metrics in 3D for one set. The other one was visualized with a titanium dioxide-based powder and photographed in 2D. This experiment allowed one set to be examined in 3D while the sister impressions were used for 2D analysis. A side-by-side visual comparison during the ten-day aging process was conducted. Statistical analysis was also conducted for the 3D data collected.

The findings of this research will contribute toward the overall goal of helping crime investigators exclude or include possible suspects in highly trafficked areas. Thus, reducing the backlog of forensic laboratories and easing the criminal justice process. However, further analyses should be conducted because this study is limited to the Caucasian population.

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**Age Determination; 3D Fingermarks; Biological Sex**

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**F24 The Development of Potential Chemical Print Database in the United Arab Emirates (UAE) for the Analysis of Fatty Acids in Latent Fingerprints as Evidentiary Value in Forensic Investigations**

*Mayssa Hachem, PhD\*, Khalifa University, Abu Dhabi, United Arab Emirates*

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**Learning Objective:** The surface of hands and feet of humans are covered with friction ridge skin, a special type of thickened skin. The contact between an area of friction ridge skin and another surface might result in the creation of a characteristic mark or impression on that surface, named fingerprints. Fingerprints have two main characteristics. The first one is that fingerprints are unique; no two fingerprints are identical, even those of identical twins. Therefore, fingerprints can be used as a means of personal identification. The second characteristic is that fingerprints of an individual remain unchanged throughout life. The friction ridge pattern of an individual is completely formed in the fetus about 24 weeks after conception and ridges' arrangement endure throughout life. Moreover, fingerprints persist for some time after death allowing the identification of individuals at early postmortem stages.

Fingerprints collected from a crime scene are considered one of the most valuable types of evidence during forensic investigations. One of the main challenges when dealing with fingerprints examination in a crime scene, dirty fingerprints comprise little to no ridge detail affecting examiners who are unable to make identifications or exclusions in the absence of DNA markers. Recently, researchers have studied the chemical composition of fingerprint residues to afford a substitute means for including or excluding individuals and to provide investigative leads.

Fingerprints are classified as patent, latent, or plastic impressions although all three types are usually associated with the term latent print. Latent fingerprints are composed of natural secretions of glands in the skin, mainly eccrine and sebaceous glands. Eccrine sweat contains mostly the water-soluble part of the print like salts and amino acids whereas the sebaceous secretions are composed of fatty acids both saturated and unsaturated triglycerides, cholesterol, squalene in addition to a variety of lipid esters. Among these lipids, fatty acids recovered from fingerprints are important for aging studies since fatty acid's composition in sebum may change intensely with the age of the donor due to the oxidation of C-C double bond in unsaturated fatty acids. The estimated percentage of fatty acids in 5-day-old newborns is only about 1.5% of the overall sebum composition. It increases to 20–23% in one month to 4-year-old children. Sebum production continues with age, peaking during the mid-30s, then begins to decline in middle age. Also, it was reported that fatty acids composition in latent fingerprint differs between males and females and among different races.

The chemical analysis of latent fingerprints is known in literature. However, extracting and quantifying extremely small amounts of fatty acids or trace fatty acids have not been broadly explored until recently and have widely varied depending on sampling protocol, extraction solvent, treatment methods and lipidomic analysis. To my knowledge, the uses of biometric fingerprints are well developed in UAE, but no chemical print database related to fatty acid distribution in fingerprints among citizens and residents is recognized. Therefore, the proposed project, with a limited number of participants, will show the research's project feasibility, which can be at a later stage suggested for government departments such as the police to investigate the chemical nature of fingerprints. This type of information could be useful for reducing the pool of potential suspects in criminal investigations when latent fingerprints are unsuitable for comparison by traditional methods.

**Impact Statement:** When applied on a larger scale, this study may be developed to special devices integrating artificial intelligence and measure fatty acids content in fingerprints helpful for law enforcements and police during forensic investigations especially when other evidence such as DNA can not be retrieved from the crime scene.

The aim of the present research project is to study the feasibility of developing a chemical print database in UAE for the analysis of fatty acids in latent fingerprints among Emirati first, then other nationalities. To conduct our study, we will:

- Collect fingerprints for a limited number of healthy volunteers from different sex and age ranges. For this initial phase, volunteers should not suffer from any metabolic disease and should be non-smokers. Ethical approval for human subjects' inclusion will be sought from the office of research services at Khalifa University.
- Collect fingerprints from all fingers, both hands and two different types of skin residues will be experienced: natural and groomed prints.
- Treat collected fingerprints using tape lifting or cotton swabbing, a common technique used for fingerprints' recovery.
- Optimize a solvent system to extract fatty acid from collected fingerprints, knowing that fatty acids content in fingerprints might be on a trace level. The efficiency of extraction of fatty components in fingerprints varies greatly when comparing the different solvent systems.
- Determine a lipidomic profile of fifteen fatty acids of interest content in collected fingerprints using several analytical techniques such as Gas Chromatography (GC) and Mass Spectroscopy (MS).

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**Fingerprints; Fatty Acids Profiling; Forensic Investigation**



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**F25 Surface-Enhanced Raman Spectroscopy Enables Highly Accurate Identification of Different Brands, Types, and Colors of Hair Dyes**

*Dmitry Kurouski, PhD\*, Texas A&M University, College Station, TX; Samantha Higgins, MSc, Texas A&M University, College Station, TX*

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**Learning Objective:** This presentation will introduce the attendees to surface-enhanced Raman spectroscopy (SERS), an innovative analytical approach that has single-molecule sensitivity in detection and identification of molecular analytes. It will show that using SERS, forensic analysis of hair colorants can be improved. Specifically, SERS can be used to: (1) detect and identify colorants on hair; (2) reveal whether permanent or semi-permanent colorants were used, and (3) determine the brand of the colorant

**Impact Statement:** This presentation will help broaden the scientific outlook and demonstrate the potential of novel methods of analyses in identification of hair colorants.

Hair is present at nearly all crime scenes. Forensic analysis of hair can be used to establish a connection between a suspect and a crime scene or demonstrate the absence of such connection. Almost half the people around the world color their hair. However, there is no robust and reliable forensic approach that can be used for a confirmatory analysis of artificial colorants present on hair. Some of the currently used techniques are laborious and costly, whereas others are invasive and destructive to the forensic evidence. A growing body of evidence suggests that surface-enhanced Raman spectroscopy (SERS), a modern analytical technique, can be used to detect and identify colorants present on hair.<sup>1,2</sup> The technique is based on a million-fold amplification of Raman scattering by noble metal nanostructures that can be applied on the surface of interest. Illumination of these nanostructures by light enables single molecule detection.

This presentation will include discussion of the potential of SERS in identification of hair colorants. We showed that SERS could accurately detect and identify more than 30 different colorants present on hair with 97% accuracy, on average.<sup>3</sup> We also investigated the extent to which SERS can be used to differentiate between different brands and types of colorants, as well as to identify hair color regardless of the type and brand of the colorant used to dye hair. Our results showed that individual colorants could be identified with on average 97% accuracy, whereas different brands can be predicted with nearly 100% accuracy using SERS. We also found that SERS offered nearly 100% accurate identification of the type of the colorant and on average 97.95% accurate prediction of the hair color. These results demonstrate that SERS can facilitate the forensic analysis of hair providing highly important information about the artificial colorants present on the analyzed specimens.

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**SERS; Hair Colorants; PLSDA**



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**F26     Sweat Analysis Leading to the On-Site Detection for Drugs and Alcohol in the Body**

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**Learning Objective:** After attending this presentation, attendees will have a deeper understanding of the practicality and applications of colorimetric, non-invasive, on-site drug detection systems.

**Impact Statement:** This presentation will impact the forensic science community by introducing a novel, non-invasive sensing system that has the ability transform the model of drug and alcohol testing in the field for law enforcement personnel.

As society advances, the advancement of analytical detection methods for illegal substances and their metabolites must advance in a parallel fashion. As such, there is a need for accurate, rapid testing methods for drugs and alcohol for medical purposes as well as for law enforcement personnel in the field.

The current technology used for testing bodily fluids for drugs and alcohol requires invasive sampling of blood or urine, as well as a laboratory with trained staff for analysis of the samples. The need for laboratory testing creates a time-related bottleneck for the return of results to the requesting parties, as well as introducing more room for error as the submitted sample ages between collection and analysis. This process can potentially lead to delaying any legal proceedings that depend on the accurate analysis of the sample.

Particularly, the detection methods for blood alcohol content (BAC) are outdated, as alcohol has been legal to consume for decades across the country and current on-site BAC testing methods can still only be used as preliminary evidence for suspected operation of machinery or driving while intoxicated (DWI) charges.<sup>1</sup> Additionally, the recent legalization of marijuana for medical and recreational use in many states has led to a push for on-site testing capabilities to determine if a person is under the influence of the psychoactive component of marijuana, tetrahydrocannabinol (THC), at the time of questioning under what is likely a zero-tolerance use policy.<sup>2</sup>

The aim of this research is to develop a non-invasive sensing concept for targeting and quantifying certain substances of interest in sweat, as it is easily collected from most individuals in a noninvasive manner. Two concepts for non-invasive sensing in sweat have been developed: one for ethanol quantification relating to BAC and one for THC metabolite detection.<sup>3,4</sup> These methods utilize enzymatic and immunoassay components, are non-invasive in nature, and provide colorimetric feedback. This method of result reporting provides an opportunity for advancing these systems to on-site detection via hand-held UV-visible spectrophotometry devices and even smart phones with specialized software for colorimetric analysis via the built-in camera.

This technology could pave the way for a simple, on-site visual test for multiple kinds of drugs and alcohol for law enforcement and medical staff to operate in that moment of need, instead of submitting the individual involved to traveling to a facility, having blood samples drawn for laboratory testing, and losing valuable time as the body continues to metabolize these compounds.

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**Non-Invasive Sensing; Sweat Analysis; Drug and Alcohol Detection**

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**F27 Let the Feet Do the Talking**

*Anita Hasert, BS\*, Charleston County Coroner's Office, North Charleston, SC*

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**Learning Objective:** After attending this presentation, attendees will be able to understand the principles of working as a medicolegal death investigator by utilizing all resources available even when there is only a little evidence left behind. The use of law enforcement, forensic anthropology, the media, retail companies, the National Missing and Unidentified Persons System (NamUs), DNA, medical records, and family are what was crucial in the case to be discussed.

**Impact Statement:** This presentation will impact the forensic science community by serving as a key aspect in explaining the various efforts needed to be taken to identify a deceased individual, no matter how much evidence was left behind. "Decomposition is the most common reason that a body is rendered unidentifiable."<sup>1</sup>

The location of where the remains were found also aided in the estimation of the length of time the remains were out in the elements. Were the remains intentionally dismembered or disarticulated from decomposition? In the field of forensic science, various specialties were utilized in the identification of the human remains that will be discussed in this case. Without forensic science, the human remains would still be unidentified and there would be another family still missing their loved one, hoping they would be found someday.

"The establishment or confirmation of the decedent's identity is paramount to the death investigation."<sup>2</sup> Identification can sometimes be immediate and other times could take days, weeks, or even months to years and unfortunately may never be determined. You need to do your due diligence as a medicolegal death investigator to put a name to the remains and not put it on the shelf and wait to see what happens. If that was your missing loved one, you would want to know that all hands are on deck.

In this case, a shoe was found that contained a sock with a human foot inside. Details will be discussed in the efforts taken from October 2020 until March 2021, when coincidentally, another shoe was found that contained a sock with a human foot inside. This presentation will discuss in detail the timeline of the multiple avenues taken to seek the identity of the individual and figure out the circumstances surrounding the death. It would take six months from the second foot being found to obtain a DNA profile which would subsequently be analyzed through various databases resulting in the positive identification and then it would take just one day to notify the family. The last whereabouts of the deceased individual may have been discovered but there are many questions remaining as to what, why, when, where, and how. To this day, only the feet have been recovered but the victim has been laid to rest. The cause and manner of death remain undetermined.

**References:**

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**Feet; DNA; Death Investigation**

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**F28 The Analysis of Feet, Footprints, and Gait—How Principles of Forensic Podiatry Assist in Criminal Investigations**

*Kewal Krishan, PhD, Panjab University, Chandigarh, Chandigarh, INDIA; Michael Nirenberg, DNP\*, Friendly Foot Care, Crown Point, IN*

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**Learning Objective:** In this presentation, attendees will understand the scientific principles of an emerging forensic science subdiscipline of forensic podiatry, which examines, interprets, and evaluates pedal evidence—in particular, footprints, footwear, and gait. This knowledge has applications in medicine, anthropology, biology, and engineering.

**Impact Statement:** The presentation will provide forensic scientists a greater understanding and insight into methodologies and tools available to assist in the analysis of footprints, footwear, gait, and human remains, and importantly, an understanding of how these principles may be applied to other scientific and forensic specialties.

Forensic podiatry may be defined as the application of sound and researched podiatric knowledge and experience in forensic investigations, to show the association of an individual with a scene of the crime, or to answer any other legal question concerned with the foot or footwear that requires knowledge of the functioning foot.<sup>1</sup> Forensic podiatrists contribute to personal identification in crime scene investigations when foot or footwear-related evidence is recovered.

Forensic podiatry principles are used to assist in establishing identification and determining some biologic aspects of individuals, such as anthropometric and biomechanical characteristics. A newer area of forensic podiatry is analysis of the gait—the manner of walking—of the criminal captured on video.<sup>2</sup> The need to establish the identity of dismembered remains may also arise, not only in the criminal context but in mass disasters or mass fatality incidents. In this regard, forensic podiatry methods can assist in identification by analyzing found pedal remains or footwear.

Beyond the criminal context, forensic podiatry principles have utility in other scientific areas, including medicine, biomechanics, engineering, biology, and anthropology. It may also involve the analysis and interpretation of footprints recovered from the scene of crime. The analysis of footprints includes class and individual characteristics. A forensic podiatrist can also assist in determining estimates of stature, sex, body weight, and other biologic attributes.

Importantly, this presentation will also give investigators and forensic scientists a solid introduction to the value of pedal evidence so they will be able to recognize when the assistance of a forensic podiatrist may be warranted or minimally when further analysis of foot or gait evidence should be considered. In closing, the presentation will also provide attendees with an understanding of the scientific methodologies of forensic podiatry so investigators will know when to access this discipline and forensic scientists will recognize when these techniques could be incorporated into their work and research.

**References:**

- <sup>1.</sup> DiMaggio JA, Vernon W. *Forensic Podiatry-Principles and Methods*, CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2017.
- <sup>2.</sup> Birch I., Nirenberg M., Vernon W., Birch M., editors, *Forensic Gait Analysis: Principles and Practice* textbook, CRC Press, Taylor and Francis Group, Boca Raton, Florida, 2020.

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**Forensic Science; Forensic Podiatry; Crime Scene Investigation**

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**F29 A Comparison of Various Single-Lens Reflex (SLR) Methods for the Quantitative Assessment of Footwear Evidence**

*Valerie Han, BA\*, CSAFE, Ames, IA; Alicia Carriquiry, PhD, CSAFE - Iowa State University, Ames, IA*

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**Learning Objective:** By attending this presentation, attendees will gain a better understanding of how combining multiple scores via random forest affects the score-based likelihood ratio (SLR) while also learning how to address the non-independence of observations.

**Impact Statement:** A shoeprint is found at a crime scene and investigators want to determine whether the shoeprint matches the shoeprint made by the shoe of a person of interest. This presentation will introduce the community to a new quantitative approach to compare images of footwear outsoles. The forensic science community will also be positively impacted via the exposure of participants to score-based likelihood ratios (SLRs) formed from a random forest and their comparison to SLRs formed from multiple scores, as well as to limitations of the methods and approaches to address them.

Score-based likelihood ratios (SLRs) are a statistical method used to quantify the weight of evidence and have been used in many forensic disciplines, including footwear analysis. Footwear examiners are often asked to explore whether a particular shoe might have made a print found at a crime scene. At present, examiners rely on subjective assessment because dependable, quantitative methods to quantify the similarity between two outsole images are not yet available and tested. Park and Carriquiry proposed a method based on the idea of a maximal clique to find the best alignment for a pair of shoeprint images.<sup>1</sup> Once two images are aligned, variables can be measured that may help quantify the closeness between the aligned images. Three variables are considered: maximal clique size, percent of overlapping features in the two images, and median Euclidean distance between overlapping features. The scores on their own are difficult to interpret, so they must then be interpreted relative to two different reference sets of scores: scores that are obtained using pairs of images from the same (matching) outsoles and pairs of images of outsoles that are known to be non-matching. Matching scores are computed from features of a pair of shoeprints known to come from the same shoe. Non-matching scores are computed from two shoeprints known to come from two different shoes.

Computing the SLR requires estimating the joint distribution of the three variables. Since the distribution is not a recognizable parametric distribution, kernel density estimation (KDE) is used. However, the three variables are computed from paired outsoles, and since the same outsoles appear in different pairs, the observations are dependent. This introduces a statistical problem, because the KDE approach for estimating the joint distribution of the three features assumes that the observations in the dataset are independent.

The three variables (or features) may be used to compute an SLR. They can also be combined into a univariate score using a method such as a random forest. Univariate scores are desirable because it is typically easier to model a single variable than to estimate a joint distribution for multiple variables. The present research explores the robustness to the lack of independence of observations of different approaches for computing SLRs. Seven different approaches are explored: three that rely on the three-dimensional distributions and four that rely on the distribution of the univariate similarity score. Each method addresses the dependence of the observations differently.

The performance of the different SLRs is assessed by computing their accuracy, true positive rate, true negative rate, and rate of weak/misleading evidence, as well as their agreement. This present research will address the importance of non-independence of observations with respect to different performance metrics and identify whether there is one approach that appears to do better than others.

**Reference:**

- <sup>1</sup>. Park, Soyoung, and Alicia Carriquiry. "An algorithm to compare two-dimensional footwear outsole images using maximum cliques and speeded-up robust feature." *Statistical Analysis and Data Mining: The ASA Data Science Journal* 13.2 (2020): 188-199.

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**Score-Based Likelihood Ratio; Random Forest; Footwear Evidence**

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**F30 Further Observations Into the Linking of Footprints With Footwear: Forensic Implications and Interpretations**

*Kewal Krishan, PhD\*, Panjab University, Chandigarh, Chandigarh, INDIA*

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**WITHDRAWN**

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**F31 Source Identification of Shoeprints in a Mock Crime Scene Using an Algorithm Based on Automatic Alignment**

*Hana Lee, BS\*, Iowa State University/Center for Statistics and Applications in Forensic Evidence, Ames, IA; Alicia Carriquiry, PhD, CSAFE - Iowa State University, Ames, IA*

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**Learning Objective:** Attendees will learn about an algorithm for source identification of shoeprints that is based on automatic alignment, which achieved high performance on unrealistically clean images. We implement it on more realistic images of shoeprints and evaluate its performance in terms of accuracy.

**Impact Statement:** It is hoped that this algorithmic approach impacts the forensic science community by facilitating the workflow of examining footwear impressions by reducing the manual processing of footwear evidence. Furthermore, the similarity statistics computed from the aligned images provide an objective means to compare two outsole images and estimate the probability that they originate from the same shoe.

Digital images of shoeprints are often useful as criminal evidence to connect a suspect to a crime scene and scenes to scenes. Comparing such images has been usually conducted by visual inspection based on manual alignment in practice, which is less objective and very labor intensive. Examiners would benefit from a semi-automated approach to align and compare images that is accurate and reliable. We aim to predict whether two similar shoeprints originated from the same shoe, utilizing an algorithm we proposed in our previous study.<sup>1</sup> One of two impressions compared is relatively clean while the quality of the other is like one recovered at a crime scene.

The algorithm can be divided into three main steps: alignment, computation of similarity statistics, and prediction. Suppose there are two images denoted by R and M, where R indicates a reference image and M indicates a moving image that will be aligned to R. First, it extracts feature points from each of R and M. It then finds the optimal rotation angle and shift between the two sets of points so that their distance is minimized. M and its feature points can be aligned to R and the feature points of R using the optimal transformation. Due to the transition from the real number of point coordinates to pixels of images, the transformation between M and R is further adjusted by estimating the translative offset within a small margin of angle. Next, several similarity statistics are computed based on the aligned output. Finally, whether R and M originated from the same shoe is predicted by comparing their similarity measurements to those of a reference collection of pairs of images for which ground truth is known.

Our previous study found that the matching performance of the algorithm we describe was highly accurate when such impressions captured extensive details of shoe outsoles. However, real crime scene images may lack in detail or lose information such as randomly acquired characteristics (RACs) due to the characteristics of impression material, data collection process, and image processing. Therefore, we now implement the algorithm on shoeprints collected in mock crime scenes to evaluate its usefulness on images similar to those that may be observed in real casework. In this presentation, we present results and discuss the algorithm's potential and limitations.

**Reference:**

- <sup>1</sup>. Lee H., Carriquiry A. A new algorithm for source identification of look-alike footwear impressions based on automatic alignment. *Proceedings of the American Academy of Forensic Sciences, 74<sup>th</sup> Annual Scientific Conference*. Seattle, WA 2022.

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**Algorithm for Source Identification; Automatic Alignment; Shoeprints in Mock Crime Scene**

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**F32 Available Now: The First Open Access and Globally Accessible Forensic Science Library**

*Jeff Teitelbaum, MD\*, Florida International University, Largo, FL*

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**Learning Objective:** Attendees will learn about the recently launched Research Forensic Library, a first-of-its-kind publicly accessible repository that offers thousands of articles and reports in every discipline of the forensic sciences. All material in the library is available open access. The presentation, along with historic and current images from the library's collection, will relate the rich history of the forensic sciences and the relevance of the library to current practitioners and researchers.

**Impact Statement:** The forensic sciences have never before had a centralized repository of research papers and reports. The Research Forensic Library provides a curated collection of material available at no cost to anyone working in the forensic science from crime lab practitioners and medical examiners to biomedical research companies and forensic science students.

The library officially launched in July 2022, providing access to material based on keyword search, discipline category (e.g., Firearms and Toolmarks, Bloodstain Pattern Analysis, Crime Scene Investigation), and content collections representing a variety of organizations (the DEA, OSAC, NHTSA, NIST, NIJ, and many more). All the material in the library is carefully selected and is available open access, making the library an extraordinarily useful resource to anyone working in the forensic sciences from crime lab practitioners and medical examiners to biomedical research companies and forensic science students.

The library will support not only the forensic science community in the United States, but also researchers and practitioners around the world; the library's daily listserv of articles (also free) has subscribers representing over 50 countries. The library will also feature tables of content for core forensic journals and newsletters and will present hundreds of vintage articles and books dating back to the early 20<sup>th</sup> century. Articles authored by firearms pioneer Calvin Goddard will be available to download, as will articles by criminologists Edmond Locard, Luke May, Paul Kirk, and many others. Additionally, literature search results on a variety of topics will be posted and updated, providing practitioners and researchers with resources not readily available.

In 1917, criminologist Edward Oscar Heinrich wrote an essay titled, "The co-operation of a library staff with the criminal investigator," in which he described how important his local public library had become to his investigations; it provided new material, new ideas and directions to research, and did so for free. It has taken some time, 105 years to be exact, but the forensic science community now has its own specialized, globally accessible library.

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**Forensic Science Library; Open Access; Literature**



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**F33 Are Internships History?**

*Max Houck, MA, PhD\*, Florida International University, Largo, FL*

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**Learning Objective:** Internships have been a mainstay for forensic students for decades. For several reasons, most notably the COVID-19 pandemic, the role of the internship has come into question. This presentation addresses the moral issues surrounding unpaid internships and possible options for connecting academic learning to career skills.

**Impact Statement:** Internships are a traditional factor in the forensic profession. The COVID-19 pandemic has altered this landscape and it is necessary for students and professionals to reconsider what internships are and if they are necessary.

The role of the internship is a Catch-22: employers look for work experience when they're hiring entry-level positions but freshly-minted graduates who want an entry-level position have no work experience. In forensic organizations, this "experience gap" is more profound because forensic interns cannot actually perform functions that relate to the mission of the organization: They can't handle evidence. Unless the forensic organization has prepared a project for an intern, the student tours the lab, sees how that one laboratory does things, and leaves with only a notion of how the forensic profession operates.

Moreover, unpaid internships—and the vast majority of forensic internships are unpaid—carry several moral hazards. One survey of thousands of undergraduates found the average internship costs a college student \$6,800. The notion that students should have to forgo pay and incur additional debt to gain work experience begs ethical concerns. College is already expensive, but now students are required to spend their summers paying for an experience with questionable outcomes. The cost of an unpaid internship, especially for many first-generation college students, minority students, and any students who don't come from wealth, keeps them from accessing valuable work experience. According to a recent study conducted on hundreds of undergraduate students, 69% of students claimed that they could not afford to take an unpaid internship. A recent study found statistically significant disproportionalities in students who could afford an unpaid internship across three main criteria: race, gender, and parent's education. Only the comparatively privileged can afford to make the "investment" of accepting an unpaid internship.

Second, internships exacerbate the "experience gap." Managers want employees who can get up to speed as soon as possible but an academic education is not a vocational process intended to slot a graduate into a specified job. Working groups and standing committees have worked to improve forensic educational programs but a recent pre-publication survey reported that laboratory directors would still prefer to hire non-forensic graduates and that FEPAC accreditation of programs means little to them. Employers are desperate for employees who can work cases on Day 1; students are desperate for just about any job, but students who had never had an internship received the same number of job offers as unpaid interns. So, what is the point of an internship?

Finally, universities often charge tuition for internship classes. On top of the living and potential travel expenses of an internship, the student now must pay tuition. Moreover, the university is profiting from tuition on a class they do not teach; this opens additional ethical quandaries. The prevalence of new approaches, like micro-internships and new geography-shrinking technologies like Zoom®, offer the chance to reinvent the transfer of academic learning to career skills.

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**Internships; Education; Profession**

**F34    Avoiding Alkynes of Hiring Issues: Bridging the Undergraduate Gap of Academics to Practice**

*Michelle Rippy, MFS, EdD\*, California State University East Bay, Hayward, CA*

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**WITHDRAWN**

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**F35    Medicolegal Death Investigation—A Call for Uniformity in Core Data Elements to Support Interoperability and Electronic Exchange of Information**

*Kelly Keyes, BS\*, RTI International, Yorba Linda, CA; Erica Fornaro, BS, RTI International, Research Triangle Park, NC; Jeri Ropero-Miller, PhD, RTI International, Creedmoor, NC*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the frequently used data elements collected and reported during a medicolegal death investigation.

**Impact Statement:** This presentation will impact the forensic science community by presenting information about forensic sciences data exchange and workflow stakeholder processes for medicolegal death investigation systems. This presentation specifically identifies and defines data elements commonly collected by ME/C, data relied on in a medicolegal death investigation, and data exchanged across forensic science disciplines and public health and safety communities.

Data collection in medicolegal death investigations (MDI) varies greatly from one jurisdiction to another. Accounting for all variables in the different types of death investigations is a significant challenge. No consistency or standardization currently exists to describe what core data should be collected and documented on every scene, every time, when available and exhausting all sources. A more standardized and automated approach to collecting and exchanging data between medical examiners, coroners, toxicologists, and other stakeholders will support the needs of the medicolegal death investigation community, as well as streamline data requests from stakeholders.<sup>1,2</sup> Although the MDI community has established guidelines on conducting medicolegal death investigations, consensus standards have not been developed on data to collect nor methods to store and exchange these data.

Standardization of data is complicated, and past efforts have been made to bring more uniformity to the information collected. Jurisdictional variations between Medical Examiner and Coroner (ME/C) offices, with differing norms, complicate this issue, as does the fact that certain elements are less clearly defined, such as race/ethnicity and sex/gender. In addition to discussing what the core elements are and their necessity, other identified commonly exchanged elements for a comprehensive death investigation will be further defined to include exemplars and nuances of specific elements. This presentation will build upon the information published by a working group of MDI professionals and stakeholders to discuss the points in a death investigation at which the information is collected, the challenges and variability in data collection, and focus on five of the most problematic elements to standardize: sex and gender, ethnicity, duration of cause of death, qualification of time of death, and how injury occurred. For example, how injury occurred is a free-form field and the level of detail that different jurisdictions provide varies widely (i.e., hanging vs. ligature hanging with belt from dorm room closet), and related to sex and gender, the death certificate currently requests only “sex”, but gender can play an important role in evaluating cases through a public health and public safety lens. This presentation will also highlight guidance and educational resources that can be utilized to help bring uniformity to data collected during death investigation regardless of ME/C jurisdiction.

This standardization of identified data elements can be viewed as necessary for modernized exchange of data with public health and public safety entities, including the multitude of forensic practitioners that MDI interact with, as well as all other interested stakeholders such as families, researchers, and organ procurement organizations. Commonality among data for all medical examiner and coroner offices will help provide information about death across the United States, as to how, why, when, and where deaths are occurring to ultimately help prevent untimely deaths.

**References:**

1. *Working Group on Data Exchange in Medicolegal Death Investigation* [Web page]. (n.d.). Retrieved from <https://forensiccoe.org/working-group-data-exchange-mdi/>.
2. *Organization for Scientific Area Committees for Forensic Science*. (2021). Medicolegal death investigation data commonly collected and exchanged. Retrieved from [https://www.nist.gov/system/files/documents/2021/07/14/MDI%20data%20commonly%20collected%20and%20exchanged\\_REFERENCE\\_07092021\\_0.pdf](https://www.nist.gov/system/files/documents/2021/07/14/MDI%20data%20commonly%20collected%20and%20exchanged_REFERENCE_07092021_0.pdf).

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**Medicolegal Death Investigation; MDI Core Data Elements; Forensic Pathologist**

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**F36 Standards Development Activities in Medicolegal Death Investigation**

*Kelly Keyes, BS\*, RTI International, Yorba Linda, CA; Elissia Conlon, BA, New York City Office of the Medical Examiner, New York, NY*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of medicolegal death investigation, including disaster victim identification.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to medicolegal death investigation. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in medicolegal death investigation will be discussed. These include:

1. Recent standards that have been added to the OSAC Registry: ANSI/ASB Best Practice Recommendation 009, Best Practice Recommendations for the Examination of Human Remains by Forensic Pathologists in the Disaster Victim Identification Context, First Edition, 2019.
2. Published standards from the Academy Standards Board (ASB) that have yet to complete the Registry approval process (ANSI/ASB Standard 125, Organizational and Foundational Standard for Medicolegal Death Investigation, First Edition, 2021. ANSI/ASB Best Practice Recommendation 108, Forensic Odontology in Disaster Victim Identification: Best Practice Recommendations for the Medicolegal Authority, First Edition, 2021.)
3. Documents currently in development at the OSAC or by the ASB (OSAC 2022-N-0026 Medicolegal Death Investigation: Terms and Definitions. OSAC 2022-N-0027 Medicolegal Death Investigation Response to Death Locations and Incident Scenes. OSAC 2022-S-0022, Standard for Disaster Victim Identification; Best Practice Recommendations for Interactions with Next of Kin. Best Practice Recommendations for Interactions with Organ Procurement Organizations. Best Practice Recommendation for Decedent Assessment. Standard for Commonly Exchanged Data in Medicolegal Death Investigations. 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 (second edition). Family Engagement Following a Mass fatality Incident. Victim Information Center Best Practice Recommendations for Medicolegal Authority. Mass Fatality Management During a Pandemic. ANSI/ASB Best Practice Recommendation 006, Best Practices Recommendations for DNA Analysis for Human Identification in Mass Fatality Incidents, 2nd edition. Postmortem Impression Submission Strategy for Comprehensive Searches of Essential Automated Fingerprint Identification System Databases, 2nd edition.)
4. Priorities for new documents or work products (Best Practice Recommendations for Managing Disaster Victim Identification with Chemical, Biological, Radiological, Nuclear, and Explosive Trauma. Ethical Considerations in Disaster Victim Identification. Best Practice Recommendations for Death in Custody. Best Practice Recommendation for Autopsy Technicians. Best Practice for Next of Kin Notification. Recommendations for the Medicolegal Death Investigation Report. Workload Recommendations for Medicolegal Death Investigators.) v) Reference documents (Principles to Promote Research in Medicolegal Death Investigation. Medicolegal Death Investigation Data Commonly Collected and Exchanged.)

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.

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**Medicolegal Death Investigation; Standards; Disaster Victim Identification**

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**F37 Standards Development Activities in Wildlife Forensic Biology**

*Kimberly Frazier, MS, Wyoming Game and Fish Department, Laramie, WY; Jason Byrd, PhD\*, University of Florida, Gainesville, FL; Christina Lindquist, MS, UC Davis Veterinary Genetics Laboratory-Forensics, Davis, CA*

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**Learning Objective:** 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.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to standards developing organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards.

During this presentation, updates related to standards development in 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 029, Report Writing in Wildlife Forensics: Morphology and Genetics, First Edition, 2019
  - ANSI/ASB Standard 028, Wildlife Forensics Morphology Standards, First Edition, 2019
  - ANSI/AASB Standard 047, Wildlife Forensics Validation Standard-Validating New Primers for Sequencing, First Edition, 2019
2. OSAC-proposed standards Wildlife Forensics Methods:
  - OSAC 2021-N-0001, Wildlife Forensics Method-Collection of Known DNA Samples from Domestic Mammals
  - OSAC 2021-S-0006, Standard for the Use of GenBank for Taxonomic Assignment of Wildlife
3. Published standards from the Academy Standards Board (ASB) pending Registry approval process:
  - ANSI/ASB Standard 046, Wildlife Forensics Validation Standards-STR Analysis, First Edition 2019
  - ANSI/ASB Standard 048, Wildlife Forensic DNA Standard Procedures, First Edition, 2019
  - ASB Standard 106, Wildlife Forensics-Protein Serology Method for Taxonomic Identification, First Edition, 2020
  - ANSI/ASB Standard 111, Standard for Training in Mitochondrial DNA (mtDNA) Analysis for Taxonomic Identification, First Edition, 2020
  - ANSI/ASB Standard 138, Standard for Collection of Known DNA Samples from Domestic Mammals, First Edition 2022
4. Documents currently in development at the OSAC or by the ASB:
  - Genetic methods to determine an individual of potential hybrid origin
  - Criteria for analyzing STR data in forensic wildlife cases when no allelic ladder is available
  - Standard for the development and use of in-house sequence databases for taxonomic assignment of wildlife
  - Method for geographic assignment of individual animals
  - Wildlife Forensics-Standards for Construction of Multilocus Databases

Other highlights:

- Developed several research needs

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**Wildlife Forensics; Standards; OSAC**

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**F38    Knowing What's Best: An Examination of Investigator Perspectives on the Relevance of "Best Practice"**

*Steff King, MA\*, Simon Fraser University, Vancouver, BC, CANADA; Sheri Fabian, PhD, Simon Fraser University, Burnaby, BC, CANADA; Gail Anderson, PhD, Simon Fraser University, Burnaby, BC, CANADA*

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**Learning Objective:** After this presentation, attendees will have an adequate understanding of the complexities of upholding investigative "best practices." The results will highlight how conflict between investigators and standard practices contributes to inadequate investigations and thus requires a larger conversation on how to move forward in a more effective way.

**Impact Statement:** This presentation will impact the forensic science community by encouraging both scientific professionals and investigative practitioners to examine how they inform and conduct "best practice" procedures in their daily work. The results of this study will emphasize the need to change how investigative bodies work with one another in casework and how scientific professionals can develop contemporary procedures that might accommodate investigator challenges.

"Best practice" connotes standard procedures most effective at achieving their intended goal. For investigations, these practices are lorded as the scientific and practical best for finding case facts and faults and are integral to conducting a thorough investigation. However, despite the existence of best practice standards, inadequate investigations—such as the deficient Indigenous death investigations in Thunder Bay, ON, and Prince Rupert, BC, Canada—still exist.<sup>1-2</sup> An underlying argument in these cases is that a variety of different investigators fail to conduct standard practices and their definitions of "best practice" vary greatly. To examine this relevant and important topic, this exploratory study employed semi-structured interviews with a variety of investigators from different professional backgrounds (e.g., law enforcement, medicolegal, and the forensic sciences). Investigator responses were examined to uncover potential variability of "best practice" definitions and their unique stances on how these standards achieve investigative goals. Through inductive coding methodology, the results suggest that investigators—those who regularly work together—disagreed on what constitutes "best" practice procedure and the necessity for establishing codified standards. Qualitative themes further emphasized the juxtaposition of: (1) the agreement that different investigative institutions must collaborate to achieve case goals with (2) the continuous struggle for authority between those professionals. Investigator participants contributed that this tense relationship plays a role in producing inadequate investigations, a part of which stems from investigators relying on "the old ways" instead of employing contemporary procedures and a distrust that the sciences can produce procedures better than those who work on the ground. This research highlights an imbalance between scientific and practical "best practice" definitions that may contribute to investigative failures. Given the severity of inadequate investigations in North America currently, the onus to address these procedural deficiencies is on both scientific professionals and investigative practitioners. As many participants in this study recommended, solving best practice disagreement or conflict is best done in collaboration between members of varying investigative institutions built on the understanding of compromise over authority.

**References:**

- <sup>1.</sup> King, S., Palys, T., Ross, A., & Anderson, G. S. (2022). "Our Hearts Are Not at Rest": A Critical Look at the Adequacy of Indigenous Death Investigations. *Proceedings of the American Academy of Forensic Sciences*, 74<sup>th</sup> Annual Scientific Conference. Seattle, WA 2022.
- <sup>2.</sup> Lee, M. (2022, March 17). Report urges more re-investigations into deaths of Indigenous people in Thunder Bay, Ont. *CTV News*. <https://www.ctvnews.ca/canada/report-urges-more-re-investigations-into-deaths-of-indigenous-people-in-thunder-bay-ont-1.5823592>.

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**Best Practice; Investigations; Standard Practice**

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**F39 A Blueprint to Building an International Organization for Standardization (ISO) Quality Management System**

*Nicole Jones, MS\*, RTI International, Research Triangle Park, NC; Kelly Keyes, BS, RTI International, Yorba Linda, CA; John Grassel, MS, RTI International, Lincoln, RI*

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**Learning Objective:** This presentation will provide an overview of the Quality Framework being developed as part of grant number 70NANB21H098 with the National Institute of Standards and Technology (NIST). After attending this presentation, attendees will understand some principles of ISO accreditation, the necessary elements of the requirements of each standard (17020 and 17025), guidance for meeting the requirements, the necessary documentation to meet the requirements, and the amplification documents.

**Impact Statement:** This presentation will impact the forensic science community by providing practical guidance and considerations when developing a roadmap to ISO accreditation.

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

The two ISO standards that apply to forensic science are ISO 17020 and ISO 17025. ISO 17020 specifies the requirements for inspection bodies, whereas ISO 17025 specifies the requirements for the competency of testing and calibration laboratories. Both ISO standards have equal status and should be applied based on the activities of the forensic unit. For a CSI Unit that is located within a forensic laboratory that is already accredited to ISO 17025 or is pursuing accreditation, it may be beneficial for that agency to expand the scope of accreditation to include crime scene investigation, but for other CSI units that are not located within a laboratory, it may be more beneficial to pursue accreditation to ISO 17020. Both standards include scope, normative references, terms and definitions, general requirements, as well as structural, resource, process, and management system requirements, but the biggest difference is in the type of work that is being conducted such that ISO 17020 is focused on competency of the agency and people performing inspection and ISO 17025 is focused on the agency's analytical testing laboratory and equipment performing the testing and analysis. Some of the requirements include general (impartiality and confidentiality), structural (administrative, organization and management), resource (personnel, facilities, equipment, traceability, and procurement), process (methods, procedures, records, measurement uncertainty, reporting of results, complaints, nonconformance, and control of data) and Management (documentation, control of records and documents, management reviews, internal audits, corrective and preventative actions).

While many offices find ISO accreditation overwhelming, they likely already meet many of the requirements and with documentation and a few small changes, agencies can be in compliance with many of the elements. This quality framework will provide a blueprint to help agencies understand the policies, procedures, records, and documentation that are needed and provide tips to help them make small changes to comply with the standards. Even if an agency isn't ready to dive fully into the ISO accreditation process, making these small changes to their procedures and documentation to reflect the ISO requirements will improve their overall quality management system.

**Reference:**

- <sup>1</sup>. *Strengthening Forensic Science in the United States: A Path Forward*, in National Research Council. 2009, National Academies Press: Washington, DC.

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**ISO Accreditation; Quality Management System; Standards**



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**F40    How Creating Infrastructure and Incentives Can Increase Quality in Forensic Science**

*Brian Gestring, MS\*, 4n6services, Guilderland, NY*

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**Learning Objective:** The fractured nature of how forensic science has evolved has created unique obstacles to improving overall quality. Attendees will learn about these obstacles and how to overcome them.

**Impact Statement:** This presentation will demonstrate a mechanism to improve the quality of forensic science across all disciplines.

Forensic science remains one of the few high-consequence industries that is poorly regulated. There are no universal requirements for laboratories to be accredited nor for the practitioners that perform the work to be certified. For the most part, short of an inconsistent patchwork of regulations, forensic science remains predominantly regulated through a system of voluntary compliance.

Recently, there has been significant progress in developing standards across all forensic disciplines. Ensuring that proper standards are developed is a necessary process that is both slow and arduous. While that process is ongoing, it makes sense to establish the foundation necessary to facilitate the adoption of those standards and implement additional measures to increase the reliability and resilience of the entire field.

Historically, a lack of infrastructure and incentives has impeded efforts to increase quality within forensic science. Over a decade ago, the National Academies of Science recommended that forensic laboratories be accredited. Seven years later the short-lived National Commission on Forensic Science made the same recommendation. Transitioning to an accredited environment is a significant undertaking. Few mechanisms were established to incentivize forensic providers to seek accreditation and very limited resources were ever made available to facilitate the transition. As a result, despite these clarion calls, little has changed since these recommendations were made.

In contrast, the most significant increase in forensic science quality occurred almost two decades before the NAS accreditation recommendation. The Federal Bureau of Investigation rolled out the national DNA databank and required laboratories to be accredited and meet additional quality requirements established by the FBI to access it. State and local governments soon realized that it wasn't worth operating a DNA laboratory that did not have databank access. Limiting databank access to accredited laboratories incentivized the largest migration to accreditation in forensic science history.

While this is a dramatic example of incentivizing compliance, the diverse backgrounds of forensic providers preclude using this approach in other areas without causing a significant system disruption. Unlike DNA, the investigative databases for fingerprints and firearms are accessed by providers with educational backgrounds that vary from high school degrees to terminal degrees and that are not always based in a laboratory environment. For these providers, more infrastructure support would be needed along with incentives to achieve the goal of universal accreditation.

Even if the appropriate infrastructure is in place, initiatives will fall short without suitable incentives. In 2009, the NAS Report identified the need to "find the evidence in a usable state and properly preserve it."<sup>1</sup> In response, a technical working group on Biological Evidence Preservation was formed. The TWG was composed of stakeholders from across the country and published best practices as well as a document on considerations for policymakers. The infrastructure was in place and accessible, but lacking any incentive to act, several states failed to take any steps to codify the preservation of this critical evidence. Ironically, one of the states that failed to enact a Biological Evidence Retention policy is New York, arguably the birthplace of the innocence movement which is based on the results of post-conviction DNA testing.

As these examples illustrate, there is no one-size-fits-all solution. Forensic providers are already under-resourced and overworked. The path to increasing quality across all disciplines without risking compromising the capacity of the overall system involves developing the correct combination of infrastructure supports and incentives.

**Reference:**

- <sup>1</sup>. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.

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**Infrastructure; Incentives; Quality**

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**F41 High Reliability Organizations: What Are They and How Do They Relate to Crime Laboratories?**

*Martin Novak, MPA\*, National Institute of Justice, Washington, DC*

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**Learning Objective:** Through this presentation, attendees will become familiar with: (1) the characteristics of complex systems, (2) the vulnerabilities of complex systems, (3) an introduction to High Reliability Organization Theory, (4) the strategies of anticipation and containment in recognizing and responding to their vulnerabilities, (5) the practices of mindfulness used to maintain the strategies of anticipation and containment, and (5) practical considerations when applying High Reliability Theory to your organization.

**Impact Statement:** The presentation will impact the forensic community by discussing the concept of complex systems, operating in open environments—like crime laboratories—that can benefit their organizations by adapting the strategies of High Reliability Organization.

This work is an overview of High Reliability Organization Theory and its applicability to crime laboratories. High Reliability Organizations have been adopted recently by many organizations where safety or reliability are of utmost concern, including hospitals, medical facilities, and fire-fighting organizations. But, are High Reliability Organizations applicable to crime laboratories? To ensure the equitable administration of justice through scientific procedures that ensure accurate and unfailing testing of forensic evidence, the National Commission on Forensic Science suggest that “crime laboratories should strive to be High Reliability Organizations.”<sup>1</sup>

High Reliability Organization Theory (HRT) began in the late 1980s, when researchers at the University of California at Berkeley noted that certain organizations operated in complex, high-risk circumstances for extended periods without serious errors or disastrous failures because society demands that failure is not an option.<sup>2</sup> These organizations were deemed to be High Reliability Organizations. Examples of High Reliability Organizations (HRO) identified by UC-Berkeley researchers included nuclear power plants, aircraft carriers, and the Federal Administration’s Air Traffic Control System.<sup>3</sup>

The organizations studied by the University of California at Berkeley researchers all exhibited similar characteristic behaviors, namely a preoccupation with failures, a reluctance to simplify interpretations of events, sensitivity to operations, a commitment to resilience, and deference to expertise. These behaviors are part of the strategies of anticipation and containment. Becoming a High Reliability Organization requires strong leadership committed to creating and maintaining the strategies of anticipation and containment through the practice of mindfulness.

**References:**

- <sup>1.</sup> U.S. Department of Commerce National Institute of Standards and Technology, *Optimizing Human Performance in Crime Laboratories through Testing and Feedback*, by National Commission on Forensic Science (Washington, DC: U.S. Department of Justice, 2016), 1.
- <sup>2.</sup> Bourrier, Mathilde, The Legacy of the Theory of High Reliability Organizations: An Ethnographic Endeavor (2011). *Sociograph* No. 9, 18.
- <sup>3.</sup> Karlene H. Roberts, "New Challenges in Organizational Research: High Reliability Organizations," *Industrial Crisis Quarterly* 3, no. 2 (June 1, 1989): 114.z.

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**High Reliability Organizations; Complex Systems; Forensic Laboratories**

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**F42 Canines Can Accurately Pinpoint the Location of Drowning Victims: Deep, Cold-Water Recoveries**

*Mary Cablk, PhD\*, Desert Research Institute, Reno, NV*

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**Learning Objective:** Attendees will learn: (1) canines can detect odor of drowned humans through water; (2) our best evidence-based understanding of how they do so; (3) how to integrate the information provided by certified water canines for recovery of drowned victims; and (4) examples from extremely deep recoveries in Lake Tahoe, NV, and surrounding water bodies.

**Impact Statement:** The use of canines to search from boats and along shorelines for drowned humans or human remains submerged in aquatic and marine environments is gaining favor with law enforcement as the training and skill of handlers and their canines increases. One peer-reviewed paper has been published that discusses aspects of canine detection of submerged humans in a water environment.<sup>1</sup> This presentation will provide recent case studies and current scientific understanding compiled over the past two decades of training and deployment of water canines in the American West.

Human remains detection (HRD) canines (also referred to as cadaver dogs) are well established for locating human remains on land. The use of canines to sniff the odor of a deceased human underwater is less well known but has also long been a resource for recovery of drowned or homicide victims. Little if any research has been conducted to investigate the decomposition process of human remains underwater. The scientific community lacks longitudinal studies in a variety of environments such as slow-moving warm water, deep cold lakes, fast moving rivers, tidal environments, and saline lakes, among others. Float tables, which provide estimates of when a human body would be expected to surface due to the buildup of gases allowing the body to become buoyant, are calculated based on factors such as water temperature and depth.

Recovering a submerged human is most commonly done by divers, hooks, or remotely operated vehicles (ROVs), also called underwater drones, with robotic arms and claws. The reality of the aquatic environment, such as submerged hazards or dense aquatic vegetation, can significantly affect when or if a deceased person will float. These factors, along with depth and flow rates, affect the ability of divers or equipment to locate the body safely and effectively. A water canine can significantly increase the likelihood of recovery and reduce the time and effort for successful recovery of the victim. Pinpointing the location of the submerged body or narrowing the area in very deep waters mitigates the risk to divers and is the primary purpose for the water canine. They are an effective component of a search and recovery operation in water environments.

Water canines are specifically trained to work from watercraft and to understand the submerged context from shore, much like avalanche canines are trained to ignore the scent of people on the snow surface and focus on scent emanating from the snowpack. These are not land HRD canines, rather the water-trained canines locate the highest concentration of human odor where it surfaces from a water body. Water canines require different training than on land to be effective and the type of water the team typically searches dictates some of the training protocols within the discipline of “canine water search.”

The volatile organic compounds (VOCs) that comprise human odor (living or deceased) have been studied for the land environment, although the specific odor signature that canines recognize as human (remains) specific are completely unstudied. How that signature changes as a function of movement and interaction with water or how it differs from what they recognize in a normal air environment is completely unknown and unstudied. Yet, canines locate submerged intact decedents and parts thereof with accuracy from watercraft and from shore.

This presentation will provide discussion with visual representation of how canines detect and respond to odor that moves through and surfaces from water, allowing them to pinpoint the location of the submerged subject. Case studies from the deepest documented recoveries of drowned persons, at depths greater than 1,000' in Lake Tahoe, NV, will be presented along with examples of multiple drownings and moving water transport of bodies in whitewater rivers.

**Reference:**

<sup>1</sup>. Osterkamp T., K9 Water Searches: Scent and Scent Transport Considerations. 2011, *JOFS* 56(4):907-912.

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**Canine; Drowning; Water Recovery**

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**F43 An Instrument-Based Approach to the Use of Human Scent as a Biometric**

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**Learning Objective:** After attending this presentation, attendees will understand the application of a combined targeted and untargeted approach to gas chromatography/mass spectrometry (GC/MS) data analysis as applied to the task of determining sample source origin.

**Impact Statement:** This presentation will impact the forensic science community by sharing newly cultivated fundamental knowledge regarding the variability of human hand odor and the influence this variation has on the task of instrumentally associating odor or similar samples. This work will equip attendees with techniques for extrapolating similarities in GC/MS profiles through a pattern recognition approach and encourage further development of human scent research for forensic identification purposes.

Human scent is commonly implemented as an individualizing feature used to associate an individual with an object, location, or path of travel. The distinctive qualities of human scent have been demonstrated through the use of human scent detection canines to carry out these actions. This presented work has built upon the concept of human scent as a detectable, individualizing feature to establish a foundational framework for the instrumental analysis and association of human hand odor samples. This study expands upon previous research in the field, demonstrating how natural variations in an individual's scent profile can affect the task of associating samples. This study evaluates the volatile organic compounds (VOCs) present in the headspace of human hand odor samples collected from 57 donors. Ten donors were sampled multiple times over variable time periods ranging up to 14 days, while 47 donors were sampled on one occasion. The collected samples reflected a mixture of samples donated by washed and unwashed hands.

This research evaluates human hand odor composition and its observed variability over the course of multiple weeks; headspace/solid phase microextraction-gas chromatography/mass spectrometry (HS/SPME-GC/MS) is implemented to capture and analyze gaseous organic compounds present in the equilibrated sample headspace. With the headspace composition serving as a representative depiction of the donor's hand odor composition, both targeted and untargeted approaches to profile interpretation and association were investigated. The applied approach incorporates Spearman's rank correlation, 3-D covariance, and linear discriminant analysis (LDA) as chemometric techniques for analyzing 3D and 2D components of the human hand odor profile. The combined use of multiple model outputs is presented as a strategy to improve true positive and reduce false positive rates when associating human hand odor samples.

This study reflects an active effort toward the creation of an approach to the formal discrimination of human scent profiles. Further application of the advances made in this study will aid in the understanding of same-donor human scent similarity and inform thresholds when discriminating between different-donor samples. Additionally, this work will provide greater context to canine-performed human scent associations while continuing to build toward a quantitative laboratory test for confirmation of in-field, canine-performed human scent associations.

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**Human Scent; Chemometrics; Solid Phase/Microextraction-Gas Chromatography/Mass Spectrometry (HS/SPME-GC/MS)**

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**F44 Large Animal Veterinary Forensics**

*Cheryl Nelson, DVM\*, Nelson Reproductive Services, Versailles, KY*

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**Learning Objective:** After attending this presentation, attendees will understand the role of veterinary forensics in investigating abuse of animals and the potential for veterinary forensic in Kentucky.

**Impact Statement:** Practical applications will show early attempts at creating a veterinary forensic business and what needs to be changed to succeed. Two cases will be presented to show the value of veterinary forensics in attempting to solve large animal cases in Kentucky.

Prior to 1932, only four states had felony laws for animal abuse and Kentucky was the 49<sup>th</sup> state to make animal abuse a felony. Rules pertaining to Kentucky veterinarians reporting suspected animal abuse of client animals were only recently changed. A brief history of the development of veterinary forensics, important animal forensic investigations, and changes in AAFS protocols and procedures will be discussed.

In 2004, a veterinary practitioner in Kentucky tried to establish a Veterinary Forensics practice but it had problems thriving. In general, veterinarians are reluctant to participate in forensic cases because of time constraints, monetary commitments, liability and confidentiality issues, plus potential loss of current clientele.

Crimes against large animals do occur in the United States, and animals may be maimed or killed for insurance mortality payouts. Criminal activity can be used to remove competitors from sporting events. Investigation of animal crime scenes requires veterinarians to be entomologists, pathologists, odontologists, toxicologists, and ballistic and DNA specialists and may require advanced forensic degrees. In 2004, veterinary forensic science was a unique specialty. At this early time, much assistance was given by Dr. Emily Craig, the Kentucky State forensic anthropologist. Human-based principles and techniques were applied and most cases in Kentucky involved undernourished canines and equines.

Two cases will be presented showing the importance of veterinary forensics in Kentucky.

Case one involved a 3-month process interviewing farm owners and employees to discover the burial place of the 1970 Kentucky Derby winner “Dust Commander” so his remains could be properly identified and reburied at the Kentucky Derby Museum in Louisville, KY. After much investigation, a bag was found with four hooves and tissue (presumably heart), which is the respectable way to bury an important thoroughbred.

Case two involved a dig for forensic evidence to try to prove what substance was used to ultimately kill the Secretariat of Show Horses “Wild Eyed and Wicked,” the winner of the 5-gaited Triple Crown. Wild Eyed and Wicked, his stable mate, and three other saddlebreds were severely injured when a substance was injected into their legs above their hooves. This substance was caustic, and three of the five horses were humanely euthanized because of pain and laminitis. Two horses recovered after months of veterinary care. The Kentucky State police and Woodford County Commonwealth attorney investigated these cases. A 10-foot ditch was dug to recover skeletal remains after an 18-month legal battle between the trainer and owners, but ultimately the forensic testing was only suggestive and not sufficient for prosecution. At the time, cobra venom was suspected.

The problems of establishing a thriving large animal forensic practice in Kentucky include the ever-decreasing numbers of large animal veterinarians for the 18% of animals involved in livestock cruelty and forensic cases. The Kentucky law changed in 2020 to allow veterinarians to report clients for possible animal abuse. Veterinarians interested in forensic work will have to establish relationships with human forensic investigators and animal diagnostic labs since Kentucky has no veterinary school. The future will allow veterinarians to perform insurance claim investigations, investigate crimes against sporting event horses, cruelty cases, proof of ownership, and conduct investigations of animals that have injured people or of animals linked to crime scenes.

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**Large; Animal; Forensics**

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**F45 Cutaneous Lesions Associated With “Skin Popping”: Case Reports**

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**Learning Objective:** After attending this presentation, attendees will be aware that there are difficult-to-identify skin lesions that, in accordance with the clinical history of the subject, are most likely signs of subcutaneous injection of drugs, a practice called “skin popping.”

**Impact Statement:** This presentation will impact the forensic science community by improving the knowledge about the practice of “skin popping” and its cutaneous manifestations in order to easily recognize and associate them to drug addiction.

“Skin popping” refers to the act of subcutaneous injection of intravenous drugs, which can be, in most cases, heroin or cocaine or a mix of both called a “speedball.”<sup>1</sup> The goal is to achieve slower absorption, decrease the risk of overdose, and ease the administration.<sup>2</sup> This practice can lead to acute and chronic skin manifestations caused by the drug itself, by the adulterants and fillers commonly added to drug substances, and/or by the bacteria located on the needles used for injection, which lack sterilization. Chronic skin manifestations include a foreign-body reaction with formation of granulomas at the site of injection and cutaneous infections that may progress to life threatening complications, such as necrotizing fasciitis, extensive cellulitis, pyomyositis, bacteremia, and sepsis.<sup>3</sup> Differential diagnoses include pyoderma gangrenosum based on Hepatitis C virus, skin lesions connected with human immunodeficiency virus infection, vasculitis, endocarditis, and amyloidosis.<sup>1</sup>

This work presents the epidemiology and the clinical and pathological aspects of lesions caused by the practice of “skin popping” by using the data of two autopsy cases that occurred in the Institute of Forensic Medicine of the University of Pavia (Italy) and in the Service of Legal Medicine in Canton Ticino (Switzerland). In the first case, a 34-year-old woman with a history of drug abuse was found dead in her bed with numerous skin lesions on the right forearm and both lower limbs. These lesions were described as nummular, depressed, with sharp margins, some of grayish-white color, probably consisting of scar material, and others of reddish color, on the bottom of which it was possible to appreciate tissue with an appearance similar to granulation tissue. Opioid and cocaine were found in her urine.

In the second situation, a 42-year-old man found dead in his home with three dystrophic areas of grayish-white color, nummular, depressed, with clear margins compatible with scarring outcomes, not better characterized due to extensive phenomena of epidermolysis.

The aim of this report is to improve the knowledge about the practice of “skin popping” and its cutaneous manifestations in order to easily recognize and associate them with drug addiction.

**References:**

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2. Grunebaum A, Skupski D. Skin popping scars - a telltale sign of past and present subcutaneous drug abuse. *Case Rep Perinat Med*. 2012, 1:37-39.
3. Danijela Dejanovic, MD, and Annemarie Gjelstrup Amtoft, MD. Extensive Subcutaneous Foreign Body Granulomas Associated With “Skin Popping” Demonstrated on 18F-FDG PET/CT; *Clin Nucl Med* 2020; 45: e241–e242.

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**Drugs; Skin Popping; Cutaneous Lesions**

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**F46 Combining Color Tests With Paper Spray-Mass Spectrometry for Drug Detection**

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**Learning Objective:** After attending this presentation, attendees will learn about the use of pressure-sensitive adhesive paper for drug sample collection and subsequent analysis by color tests and paper spray-mass spectrometry (PS-MS).

**Impact Statement:** This presentation will impact the forensic community because of its application to in-field drug identification. The combination of color testing and paper spray-mass spectrometry provides a rapid and affordable method for in-field drug detection.

Collection and identification of drugs of abuse remains a leading issue in forensic chemistry. The accepted methods for drug identification involve the use of both presumptive and confirmatory techniques. Color tests are commonly utilized as a presumptive screening method for drug samples and can be carried out both in the field and in the lab. In contrast, confirmatory techniques, such as GC/MS, require samples to be analyzed at an offsite lab. For cases in which rapid drug identification is necessary, there is a continued need to improve rapid drug identification that can be achieved in the field.

Paper spray ionization combined with mass spectrometry was utilized in this work to confirm the identity of drug samples following a positive color test. Paper spray-mass spectrometry is advantageous to in-field drug detection because it requires minimal sample preparation and provides spectral data significantly quicker than traditional methods.

This work proposes a method in which presumptive and confirmatory tests are combined on the same platform for in-field drug identification. Pressure-sensitive adhesive-lined (PSA) paper is used to recover drug samples off of a surface. Pressure-sensitive adhesives are polymers that form non-reactive bonds to a surface when pressure is applied, allowing them to be used in drug recovery as they do not alter the drug. Following sample collection, a color test is performed on the PSA paper by direct addition of the reagent(s). If a positive color test results from reagent addition, the same sample is analyzed by PS-MS on a Thermo Scientific™ LTQ-XL mass spectrometer. Both full scan and MS/MS spectra are collected to confirm drug identity. Additionally, this technique could be adapted for analysis with a portable mass spectrometer for true in-field detection.

Three different color tests were investigated in this research: acidified cobalt thiocyanate for cocaine, the Simon test for methamphetamine, and the Marquis test for phenylethylamines and opiates. These tests were selected for analysis because of their frequent use and because they identify some of the most frequently encountered drugs of abuse. Preliminary results for these tests indicate that the identity of the drug of interest can be confirmed by PS-MS following a positive presumptive color test. Further details on the sensitivity and of combining color tests and PS-MS with PSA paper sampling will be discussed.

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**Paper Spray-Mass Spectrometry; Color Tests; Drugs**



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**F47 Standardization of Dogs and Sensors: Update on Current and Planned Standard of the Dogs and Sensors ASB Consensus Body**

*Kelvin Frank, Jr., MSFS, Florida International University, Miami, FL; Howard Holness, PhD, Florida International University, Miami, FL; Suzanne Perry, Sumner County Tennessee Emergency Management Agency, Castalian Springs, TN; Paola Prada-Tiedemann, PhD, Texas Tech University, Lubbock, TX; Kenneth Furton, PhD\*, Florida International University, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will learn of the processes used and current status of standard documents developed by the Dogs and Sensors subcommittee of the Organization of Scientific Area Committees (OSAC). This presentation will outline the history and future of the group's standardization efforts and inform attendees of the current roadmap being used to implement these nationwide.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of standard development activities related to substance detection utilizing canines. This will increase awareness regarding training, kenneling, and certification of these canines used by law enforcement and the broader forensic science community as effective tools to detect a wide variety of items of forensic interest.

This presentation includes the results of the latest work in drafting detection specific standard documents and working with a standard development organization (SDO) to publish dogs and sensors standards to be used by various agencies, including law enforcement and military and homeland security. There are a variety of factors that influence the measured performance of canine teams, including the source of training materials, the containment system used, how the training materials are presented to detection teams, how the teams were trained, and how they are maintained. This initiative was undertaken as a response to concerns coming from a variety of sectors regarding the need to improve the performance, reliability, and courtroom defensibility of detector dog teams and their optimized combination with electronic detection devices. This presentation will describe the development of canine training standards over the past 18 years beginning with the establishment of the Scientific Working Group on Dogs and Orthogonal Detector Guidelines (SWGDOG) in 2004 which by 2014 had completed 24 guidelines totaling 436 pages, to the guidance materials currently being developed by the Dogs and Sensors subcommittee of OSAC, which began in 2014, through the standard development process with the ASB beginning in 2016 and options for encouraging and certifying implementation of published standards, including the formation of the International Commission on Detector Dogs (ICODD). These efforts have been federally funded over the years by the NIJ, FBI, DHS, TSWG, and currently the NIST.

Since its inception, the OSAC–Dogs and Sensors subcommittee has identified 26 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 the federal registry. These documents relate to other related forensic disciplines. One technical report and seven standards have already been successfully published by the AAFS Standards Board, including: Dogs and Sensors Terms and Definitions; General Guidelines for Training, Certification, and Documentation of Canine Detection Disciplines; Dogs and Sensors Standard for Training of Pre-scented Canines Aged Trail Search; Dogs and Sensors Standard for Training of Pre-scented Canines Location Check; Dogs and Sensors Standard for Training and Certification of Canine Detection of Humans; Patrol Canine Team; Dogs and Sensors Standard for Canine Selection, Kenneling, and Healthcare and Standard for Training and Certification of Canine Detection of Explosives.

This presentation will outline the history and 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 detection threats and needs.

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**Detector Dogs; OSAC; Standards**

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**F48 The Determination of the Breakdown Products of Black Tar and Powder Heroin Via Ultra Performance Liquid Chromatography-Photo Diode Array (UPLC-PDA)**

*Alyssa Sanchez, BS\*, Florida International University, Miami, FL; Bruce McCord, PhD, Florida International University, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will have expanded knowledge of the effects of decomposition on the profiling of heroin, how the presence of excess acid and base affects the decomposition pathways, and how decomposition can be accelerated using an environmental chamber.

**Impact Statement:** This information will give law enforcement agencies a more complete profile of heroin after it has been decomposed, therefore aiding in the appropriate prosecution of transnational criminal organizations and individuals.

Heroin (diacetylmorphine) is a semi-synthetic opioid classified as a Schedule I substance in the United States. Heroin remains the most reported drug to National Forensic Laboratory Information System (NFLIS), maintaining its significance in the United States drug market. Black tar heroin is typically the result of post-processing hydrolysis in samples containing non-bound water or an excess of acid. White powder heroin comes in the form of a hydrochloric acid (HCl) salt, making it more stable and lightweight with higher purity and solubility.<sup>1,2</sup>

This work focuses on developing a better understanding of heroin's decomposition processes. Heroin readily hydrolyzes into primarily O6-MAM and then back to morphine; therefore, the concentration levels of these compounds can provide information on the decomposition kinetics. In this project, heroin samples of different geographical origins were examined using a UPLC-PDA, and decomposition was simulated using an environmental chamber to tract the relative kinetics of decomposition to O3- and O6-monoacetylmorphine (O3- and O6-MAM) and morphine. Known samples from four regions of origin were studied: Mexico, South America, Southeast Asia, and Southwest Asia. Mexican heroin has low purity and a high O6-MAM content with respect to heroin. South American heroin typically has high acidity and comes from Colombia. Southeast Asian heroin is the purest form and is highly water soluble. Southwest Asian heroin is the only basic form of the drug and has poor water solubility.<sup>3</sup>

Previous studies have demonstrated that differences in manufacture and impurity profile affect decomposition. Therefore, we selected a trial set of original samples and spiked samples with excess acetic acid and sodium carbonate for decomposition analysis. The method optimization experiments resulted in an updated gradient method with a flow rate of 0.350mL/min, a column temperature of 30°C, and a gradient as follows: 95% solvent A for 10min, 75.5% solvent A for 2 minutes, 35% solvent A for 2 minutes, and back to 95% solvent A for 3 minutes. Solvent A is a sodium phosphate buffer at pH 1.9, and solvent B is 100% HPLC grade acetonitrile. 2D and 3D UV data were collected, a scan was done from 210–360nm, and the 2D channels chosen were 210, 215, 251, and 285nm. Reproducibility studies yielded relative standard deviations within acceptable ranges for retention time, peak height, and peak area, respectively, for a standard mixture at 10mg/mL of morphine, codeine, thebaine, and papaverine. This led us to decide that, in the future, these will be the conditions under which the instrument will be operated for optimal results. All this data combined serves to determine the ability of heroin profiling after extensive decomposition has occurred and is essential for the prosecution of the responsible parties.

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**Heroin Decomposition; Ultra Performance Liquid Chromatography (UPLC); Drug Profiling**

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**F49 Fetal Alcohol Spectrum Disorder (FASD) and Criminal Justice: An Italian Research Project**

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**Learning Objective:** Following this presentation, the forensic practitioners may comprehend the usefulness of forensic sciences in order to mitigate the adverse effects of fetal alcohol spectrum disorder (FASD), in particular in the countermeasures that could be taken to attenuate the so-called secondary disabilities related to FASD (prison risks).

**Impact Statement:** This presentation will impact the forensic community by presenting the preliminary findings about the support that forensic sciences could offer in order to mitigate the adverse effects of fetal alcohol spectrum disorder (FASD), in particular in the countermeasures to attenuate the so-called secondary disabilities related to FASD (prison risks).

Fetal alcohol spectrum disorder (FASD) is an umbrella term that includes a wide range of neurological and behavioral problems that can affect a person who was born to a woman who had abused alcohol during pregnancy.<sup>1</sup>

Prenatal alcohol exposure is considered one of the main causes of preventable birth defects; however, it represents the main form of developmental delay in the world.<sup>2</sup> An early diagnosis of FASD can improve the patient's outcome, for example, preventing the development of secondary disabilities and helping them to lead a near-normal life, in relation to the severity of the disorder. In particular, among the so-called secondary disabilities, there is a close correlation between subjects with FASD and criminal offenses.<sup>3</sup> It should be pointed out that FASD entails high public costs both for health care and the juridical system. On the one hand, there are the costs of the health measures to support the affected subjects and their relatives; on the other hand, there are the costs related both to the trial and to the social impact of the criminal act.<sup>4-6</sup> Moreover, FASD is relevant across the legal spectrum from offensive behavior and arrest through the entire adjudication process to incarceration. For example, brain damage in FASD may be relevant, reducing the individual's self-control and ability to recognize when his/her conduct is subjecting others to harm.

Due to the centrality of this thematic in a criminal trial, the University of Catania, in collaboration with the Sicily Region is conducting research (still in the testing phase, the preliminary results should be available by December 2022) to achieve the following goals:

Determining the regional epidemiological picture, verifying the percentage of subjects affected by FASD in the population subjected to screening (both in school and in prison)

- Genotyping of the enrolled population in order to determine the number of subjects affected by FASD who presents an unfavorable genotype in the management of stress factors
- Determination of the so-called "subjects at risk" (affected by FASD with or without the presence of a stressogenic genotype) in the reference school population, providing adequate supportive therapies

In conclusion, part of changing the landscape of FASD is to modify the way the community perceives and reacts to FASD. A multidisciplinary approach to address this problem is increasingly necessary.<sup>7,8</sup> Rather than a purely psychological or medical approach, the integration of FASD subjects is the most important measure that can be taken to prevent the risk of criminal behavior. This becomes a way to change the conversation around FASD from the current sense of certain failure to one of the possibilities of success.

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**Fetal Alcohol Spectrum Disorder (FASD); Criminal Justice; Brain Impairment**

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**F50    Unlocking the Mysteries of Faith: The Importance of Forensic Sciences in a Case Series**

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**Learning Objective:** Following this presentation of a Christian cold-case series, attendees will understand how a multidisciplinary forensic approach may be essential in the explanation of mysteries of faith cases.

**Impact Statement:** This presentation will impact the forensic community by providing up-to-date data about the pivotal role of forensic sciences in collecting scientific evidence to dissolve the mysteries of a Christian cold-case series.

The relationship between science and faith seems to be absolutely conflictual: indeed, it seems impossible that a scientist could be a believer and vice versa. However, in this context, a recent editorial has shown that a harmonious, complementary, and productive coexistence between faith and scientific truth is still possible today.<sup>1</sup>

In this way, while in the past some important saints and intellectuals of the Catholic Church made significant contributions to theology, philosophy, natural sciences, medicine, and bioethics, today, the Church approaches apparently inexplicable phenomena with the “eyes of science.” Today, the new Church viewpoint is to create a new Christian model giving an answer to the next generation of believers.

In this innovative scenario, through a case series related to the Catholic faith, we demonstrate how a multidisciplinary medicolegal approach can be crucial in unlocking the mysteries of Christian cold cases.

In the first case, we describe the methodological approach applied to the case of a statue of Mary, mother of Jesus, that suddenly started to weep a red substance. The repeated episodes were also filmed by the faithful, generating media uproar. However, by applying a multidisciplinary forensic approach (CSI investigations combined with radiology, toxicology, and genetics findings), it has been demonstrated that there were no supernatural actions, while there was a technical artifice.

In the second case, we describe the investigations carried out on traces found in the tear duct of a statue of a saint of the Catholic Church. The traces, after careful investigation, have been demonstrated to be blood traces. Further investigations are underway to establish the possible subject who laid them.

In the last case, the investigations carried out on the “scabbard” and “corporal” (two cloths that are used in rituals in the Catholic Church) are described. In particular, red traces were found on these garments after a religious ceremony, suddenly and without any apparent explanation. The subsequent forensic investigations proved the absence of biological material.

This case series demonstrated how the Catholic Church has a new approach to apparently inexplicable events. Whereas in the past they would immediately define them as miracles, today the Church’s approach is cautious, seeking scientific ways and not faith truths. Moreover, the changes in the Church’s viewpoint and growing public skepticism seem to promote more interest in the application of forensic science than the lack of sound theory or data.

In this context, forensic sciences are fundamental in order to ascertain the truth, combining a multidisciplinary approach. This study demonstrates that a forensic approach can only help to reinforce the dogmas of the Catholic religion and not feed myths and legends.

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**Christian Cold Case; Multidisciplinary Approach; Crime Scene Investigation**

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**F51 Croatian Forensic DNA Database: Past, Present, and Future**

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**Learning Objective:** Attendees will learn about the role of DNA databases in criminal investigation, both on a national and transnational level. They will gain knowledge about the Croatian path in establishing and maintaining the DNA database and future challenges.

**Impact Statement:** This presentation will illustrate the impact of the Croatian DNA database on combating national and cross-border crime.

A rise in cross-border crime has shown an increasing need to develop forensic intelligence, which includes the collection, integration, and analysis of data obtained through forensic investigations, thus making the results of forensic investigations an additional source of information. Data, within the context of criminal investigations, relies heavily on DNA databases. Therefore, a DNA database was one of the first forensic intelligence databases. The development of DNA technology and the establishment of a corresponding DNA database on national and transnational levels is one of the most efficient ways to detect and prevent crime. The initial DNA database we established consisted of family members' DNA profiles of missing individuals after Serbian aggression on Croatia and Bosnia and Herzegovina. However, the official Croatian DNA database, to support criminal justice, was legally formed at the beginning of the 2000s with new police regulations. In 2006, the database migrated to the CODIS platform. Since the migration to CODIS, the Croatian DNA database recorded more than 2,500 matches of unsolved crime stains with suspect DNA profiles. The International Criminal Police Organization (INTERPOL) has been our indispensable partner since the beginning of the international DNA data exchange. The INTERPOL DNA database was created in 2002, and it currently contains more than 247,000 profiles contributed by 84 countries worldwide.

Today, the Croatian DNA database counts approximately 15,000 DNA profiles, of which 11,000 are exchanged daily with other European Union member states. The benefits of Croatian forensic DNA databases are enormous, particularly in improving the results of criminal investigations. Nonetheless, with such a success, new challenges are emerging. In response to such challenges, two different directions have been considered for enhancing search capabilities. The first was adding more autosomal short tandem repeat (STR) loci to the current core set of loci (United States Core loci, ESS, ISS). The second direction was the potential establishment of a national Y-STR database. Additionally, the inclusion of the single nucleotide polymorphism (SNP) and mitochondrial DNA (mtDNA) analysis within the forensic DNA analysis routine in Croatia could be the future developmental path for this powerful investigative tool in Croatia and in Europe. In further development of the Croatian Forensic DNA Database, we will work closely with the American Academy of Forensic Sciences Academy Standards Board to ensure the highest quality standards in every aspect of our work.

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**Croatian DNA Database; CODIS; Criminal Investigations**

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**F52 Can Stab Injuries Determine the Position of the Aggressor? An Exercise With a Real Case**

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**Learning Objective:** After attending this presentation, attendees will recognize the importance of identifying, locating, and describing the direction and trajectory of injuries during medical-legal autopsic examination, either to determine the cause and manner of death or, in some cases, to clarify additional objectives, such as the position of the aggressor.

**Impact Statement:** This presentation will impact the forensic science community by emphasizing the potentialities of the autopsy, overlapping its main objectives, like cause and manner of death. In a few cases, the characteristic and location of injuries may also help in assessing other questions like the position of the aggressor, keeping in mind the caution that a medicolegal examination always implies.

Knife-like weapons are the most commonly used in stabbings and may cause incised, penetrating, and mixed injuries. The characteristics of the wounds, namely the location, direction, and depth, are often a matter of discussion, especially in homicides. Victims can present injuries in any part of the body, but the chest and abdomen are the most common regions. Despite the rarity of cervical wounds, damage to the neck vessels may easily lead to death. Moreover, it may be difficult to make the distinction between homicide and suicide and to respond to many other questions often made by the police and the courts.

We report the case of a 49-year-old man found dead outside his home, with bloody clothes and head and neck cut wounds. He was seen the day before drinking in a bar near his house. At the scene, no firearms and/or bladed weapons were found. He was depressed because he divorced recently, had several promiscuous relationships, and used to participate in sexual parties, as well as numerous financial debts probably related to gambling. He also had alcoholic habits.

The medicolegal autopsy revealed nine superficial cut injuries that penetrated only the subcutaneous cellular tissue and three abrasions, scattered throughout the right auricular, mastoid, and lateral cervical regions. It also showed two penetrating wounds: one fusiform, on the right parotid region (8mm x 2mm) that perforated the subcutaneous cellular tissue and platysma muscle; the other one in the lower third of the right latero-cervical region, oblique down and forward (1.2cm), with the upper and posterior extremity V-shaped, surrounded by a purple ecchymosis. The latter reached the subcutaneous tissue and perforated successively the platysma muscle, the right sternocleidomastoid, partially the homolateral omohyoid muscle until the posterolateral lower third of the right internal carotid artery. This one, from back to front, right to left, and sensibly horizontal, with an estimated depth of 3–4cm, was lethal. No defense injuries were observed. Toxicological examination revealed a blood alcohol level of  $2\pm0.26\text{g/L}$ .

First, the authors easily established the cause and manner of death. Then, they discussed, based on the disposition of the injuries and its characteristics, the possible position of the aggressor. This position, according to their interpretation, may have determined an “accidental” homicide if the victim, to release himself, had suddenly and unexpectedly moved his head against the knife that threatened him.

This presentation highlights the importance of identifying and locating injuries as well as describing their direction and trajectory during the medical-legal autopsic examination, first to answer to the main objectives of a medicolegal autopsy like the cause and the manner of death. The location and disposition of the injuries allows, in some cases, clarification of additional objectives, such as the position of the aggressor. This, combined with a thorough examination of the death scene and the police investigation, contributes to the correct approach to the homicide analyses.

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**Accidental Homicide; Stabbing; Position of the Aggressor**



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**F53 When Book Fiction Becomes Reality: A Case Report of a Brutal Murder With a Hammer and a Related Flubromazepam Intoxication**

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**Learning Objective:** Attendees will better appreciate some forensic implications of postmortem diagnostics related to a planned brutal hammerhead murder and concomitant attempted murder from flubromazepam intoxication.

**Impact Statement:** This presentation will impact the forensic community by sharing experience gained from handling brutal homicide with concomitant attempted homicide by administering a high dose of flubromazepam with the possible staging of a murder-suicide.

A 58-year-old man was found lifeless in a pool of blood inside the house with numerous lacerated/bruised scalp wounds and a hammer placed near his body. The external examination also revealed a cut injury to the right wrist with poor vitality characteristics. The unconscious wife was also found in the same house with a stab wound on her left wrist and some bilateral marks compatible with a ligature. She was immediately rescued and taken to the Emergency Room where toxicological tests revealed severe benzodiazepine intoxication with flubromazepam (value equal to 1,350ng/ml on peripheral blood and 330ng /ml on urine samples with negative results at the hair test).

The public prosecutor arranged an autopsy and toxicological tests on the husband to clarify the dynamics of the attack and evaluate a possible administration of poisonous substances or medicines. Before the autopsy, a PMCT was performed that revealed five mold fractures of the skull and the breakthrough of the left orbit with no other traumatic skeletal injury.

The autopsy on the husband showed repeated blunt injuries to the head (at least five blows) compatible with the hammer found in the vicinity of the body. Histological examinations confirmed the poor vitality of the wrist cut injury. Toxicological analysis instead was negative.

These data, corroborated by what was found on his wife, namely the presence of cuts and abrasions compatible with signs of ligation as well as severe benzodiazepine intoxication, led to authorities to hypothesize a possible staging by the murderer with the attempt to simulate a homicide-suicide.

Police investigations confirmed this reconstruction, which led them to suspect the involvement in the crime of the couple's son, a thriller book writer. In fact, the son had bought flubromazepam on the black market several weeks before and some days before had carried out online research on how to fall asleep with benzodiazepines and how to kill with a hammer.

This case report shows how the integration of necropsy, histological and toxicological analysis with circumstantial data can allow for the reconstruction of a complex murderous dynamic worthy of a book plot.

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**Hammer Blow; Flubromazepam; Intoxication**



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**F54 Drug Addiction and Pregnancy: A “Crack” Baby Case**

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**Learning Objective:** After attending this presentation, attendees will understand the importance of the death scene as the starting point to retrospectively investigate the social and clinic history of the mother, according to the results of the autopsy. Attendees will be encouraged to practice fetal autopsies to be able to recognize slight differences in normal fetal morphology.

**Impact Statement:** Crack cocaine use has become a severe public health issue in many countries over the past decades, increasing the number of drug-addicted women of child-bearing age and thus increasing the rate of infants exposed prenatally to these substances. This presentation will impact the forensic community by stressing the role of a systematic investigation review of the circumstances surrounding death followed by a complete autopsy and collecting the correct histopathological and toxicological samples to fully understand the fetoplacental outcomes.

**Background and case report:** Cocaine abuse has harmful effects on its users, specially in pregnancy. Infectious diseases, psychiatric and respiratory pathologies, as well as gastrointestinal complications and acute cardiovascular diseases may be observed. In a high number of cases, other risk factors may contribute to gestational complications such as low socioeconomic status, inadequate prenatal follow up, history of domestic abuse and infections during pregnancy.

We report the case of a female fetus who died *in utero* 3 days before the partum. The 37-year-old mother was homeless, malnourished, addicted to injectable crack, and occasionally engaged in prostitution. The pregnancy was poorly monitored, so the gestation age was unknown. She went into labor on the street, witnessed by a passer-by, rapidly sent to the emergency department of the nearest area, where the placenta was extracted, and fetal death was verified.

At necropsy, the age of the fetus was estimated to be between 27 and 29 weeks. She had flat ears with low insertion, an increased distance between eyes, and bilateral bi-palpebral edema as well as abdominal distention. The placenta was oval, with a paracentral insertion of the cord. The maternal surface was spongy with hemorrhagic areas and the fetal surface had vascular engorgement. Internally, no macroscopic findings were visible.

Histopathological examination revealed an ischemic placental infarction and acute purulent funisitis. Toxicological analyses performed on the stomach, liver, and kidneys were positive for phenacetin, cocaine and its metabolites, benzoylecgonine, and ecgonine.

**Discussion and conclusion:** The necropsic findings will be related with the deleterious effects of cocaine in pregnancy, like the placental syndromes cause by vasoconstriction, possible teratogenic effects associated with the adulterant phenacetin, and others such as reduced head circumference, small for the gestational age, and congenital heart malformations. Risk factors (social and clinical) presented by the mother will also be taken into consideration.

The authors concluded the death of the fetus was *in utero* and caused by placental infarction, probably related to the mother’s cocaine consumption, associated with acute purulent funisitis infection.

This case aims to alert to the deleterious effects of the crack cocaine use on the mother’s and the fetus’s health by the increasing the risk of placental displacement, preterm delivery, and reducing the placental blood flow leading to the infarction. Moreover, the authors emphasize the importance of the steps taken during an autopsy when cocaine is known to be a significant issue in the death. In this context, they finally remember the crucial role of the classic samples for toxicologic analyses, beyond the blood—stomach, liver, and kidneys among others—that are ultimately forgotten in many forensic sets.

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**Fetus; Cocaine; Crack Babies**

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**F55 The Migratory Phenomenon in Italy During the COVID-19 Pandemic: A Technical and Methodological Approach of Hotspot Inspection in the Medicolegal Perspective for Public Health and Human Rights**

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**Learning Objective:** After attending this presentation, attendees will comprehend the usefulness of forensic science in support of public health and human rights in the site inspection of migrant Italian structures.

**Impact Statement:** This presentation will impact the forensic community by presenting the application of forensic science to the emergency related to the migratory phenomenon, demonstrating the importance of a medicolegal perspective as a guarantee for public health and human rights.

According to the statistical dashboard of the Italian Ministry of Interior, the migration phenomenon showed a regular increase starting from 2019 and there was no evidence of a decrease in migration during the COVID-19 epidemic. From August 1, 2020, to August 31, 2020, 5,328 migrants arrived in Italy by sea, with an average number of migrants/days of 171. The Italian system for the reception and integration of asylum seekers and refugees has always represented a hot topic and the relative law system has been changed frequently. When migrants arrive in Italy, they should be sent to hotspots, where they should receive early health screening, first aid, and pre-identification and asylum procedures should be initiated (D. Lgs n. 220/2017, n. 113/2018, n. 130/2020). The rule of law and universal concepts of human rights are essential foundations for a democratic society and social peace. It has been reported that human rights violations of migrants are a widespread and distinctive feature of this phenomenon. According to the Lancet Migration and the WHO Regional Office for Europe, migrants in reception facilities are the most at risk of contracting infectious diseases, including COVID-19, due to several factors, including overcrowding, poor measures to prevent the spread of infection (e.g., social distancing, hand hygiene, isolation), failure to use personal protective equipment, and poor health care. The aim of this presentation is to show the situation of the hotspots of Lampedusa and Pozzallo (two of the main Italian hotspots for the migrant's reception) during the COVID-19 pandemic.

**Results:** The site inspection occurred on September 1, 2020, for Lampedusa hotspot and August 25, 2020, for the Pozzallo hotspot. These facilities can receive 187 and 234 migrants, respectively. Based on data obtained through the site inspection, the Pozzallo and Lampedusa hotspots did not offer receptivity adequate to contain the spread of the SARS-CoV-2 infection. The following critical issues were identified: precarity conditions of order and hygiene; inadequate toilet services; overcrowding; lack of social distance (common places and dormitories); an absence of distribution of soap and sanitizing liquids; the prolonged stay of migrants in hotspots (> 72h); incorrect sorting of migrants on the basis of the antigenic test; and inadequate ventilation. We suggest different actions that should be applied in order to contain the spread of the SARS-CoV-2 infection in the hotspots. These activities are also important to prevent the spread of other widespread infectious diseases. To ensure the health of migrants and respect for human rights, it is considered important to conceive hotspots as the first place of assistance for migrants, where an early diagnosis of violence or torture can be made through a methodological medicolegal approach.

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**COVID-19; Migration; Human Rights**

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**F56 A 3D Reconstruction of a Complex Road Accident**

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**Learning Objective:** The aim of this study is to sensitize attendees about the importance of both a multidisciplinary forensic approach and a forensic pathologist in order to solve cases of complex road accidents.

**Impact Statement:** The following presentation will impact the forensic community by stressing the importance of CSI and the usefulness of a multidisciplinary forensic approach to solve road accidents with complex dynamics.

Over the past two decades, the increase in road accident fatalities has generated growing interest in the study of injuries in the forensic community. From a methodological point of view, forensic investigations in cases of road accidents are integrated with increasingly meticulous multidisciplinary investigations (biomechanical engineering, forensic radiology, etc.). These investigations include the examination of the vehicles involved, the biological traces (blood, flaps of skin, fragments of organic material), the inorganic traces (tire prints, braking, traces of paint, fragments attributable to the cars and/or vehicles involved) found at the scene of the accident or possibly found on the corpses.

In August 2017, at about 5:30 a.m., a road accident occurred; the vehicles involved were a scooter with a young couple on board (TV and LV) and a car with only the driver on board (GF). The accident occurred in a carriageway without a centerline and in the absence of witnesses. As a result of the collision, the passengers of the motorcycle reported several lesions and were aided by the emergency service. After being transferred to the hospital, TV died a few hours later. The driver of the car, who was unharmed, declared that he suddenly found himself in front of the scooter and, despite an evasive maneuver attempt, collided head-on with the scooter. After the frontal impact, the car made a rotational motion and collided with a parked car, ending up in the center of the carriageway with the front of the car in the opposite direction to the original one. He also declared that he was unable to tell who was driving the motorcycle. The crime scene investigation was conducted by the judicial police officers and subsequently by a technical expert, whose reconstruction determined that the scooter, driven by TV, occupied the opposite lane at the moment of the collision.

Two years after the accident, the judge disposed of further investigation. We were instructed to re-examine the context in order to determine the probable dynamics of the event. In the first phase of investigation, no forensic pathologist was appointed, nor was any external cadaveric examination ever carried out. The exact 3D reconstruction of the scene of the accident was created using the laser scanner. Furthermore, the study of the photographic documentation allowed us to highlight the presence on the scene of numerous fragments of the two vehicles involved in the accident and dislocated after the collision that had not been previously considered. The analysis of the position of these fragments on the scene permitted the determination of the exact position of the vehicles. We compared the explosion of the fragments that occurred after the collision to the spread of a shotgun with broken ammunition, applying the laws of parabolic motion. We studied the injuries of TV through the examination of the total body CT performed before death, clarifying the dynamics of the event before and after the collision. Our study, therefore, confirmed that the motorcycle was driven by TV and that, contrary to what was previously believed, the impact occurred in the lane belonging to the scooter. A 3D video reconstruction of the road accident dynamic was also performed.

Based on the findings of the discussed case, we demonstrated that a multidisciplinary approach is mandatory, particularly in complex car accidents. In the late phase of the investigation, thanks to modern technologies such as the laser scanner, several pieces of information could be recovered, even though it is desirable that CSI should be performed in an optimal manner in the first phase.

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**Road Accident; Crime Scene Investigation; Multidisciplinary Approach**

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**F57 Strangulation Asphyxia: Homicide or Suicide? A Practical Approach**

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**Learning Objective:** The present study aims to suggest a multidisciplinary approach valuable in deaths from strangulation to make the complex differential diagnosis between homicidal and suicidal occurrence.

**Impact Statement:** This presentation will impact the forensic scientific community by demonstrating the validity of a multidisciplinary forensic investigation protocol useful for distinguishing self-induced strangulation from a third-party killing.

**Background:** Strangulation asphyxia is quite a common event in forensic practice; most strangulation cases are homicides or accidental events, while suicidal ones are very rare, as the required strength to collapse the airway is progressively lost when the victim begins to become unconscious, and the attempt fails.<sup>1</sup> Suicidal strangulation can be defined as neck compression resulting exclusively from the self-application of a ligature without the contribution of the corpse's weight.<sup>2</sup> The distinction between homicide and suicide events in these cases can be a challenge for forensic pathologists; for this reason, it is mandatory to conduct a systematic crime scene investigation and a careful postmortem multidisciplinary examination. Three self-strangulation case reports are reported below in which methodological forensic approaches allowed the diagnoses of suicides.

**Case reports:** (1) A 60-year-old woman, suffering from depression and inoperable carcinoma of the anus, was found lying on the floor in her bedroom with a curtain cord around her neck. The corpse was found in the morning by her husband, who was sleeping in a different bedroom to avoid being awakened due to the woman's repeated trips to the bathroom due to her neoplastic pathology. The man alerted the police, and the public prosecutor ordered an autopsy examination. (2) A 75-year-old woman was found lifeless in her room in the nursing home where she was resident, supine on the bed, with a nylon stocking wrapped around her neck, the free ends of which were fastened to her fingers. She had a history of surgery for rectum cancer and anxiety disorder. (3) The body of a 50-year-old man was found inside a van parked on his property. The coroner on duty went onsite and found a wire around the neck of the corpse. Inside the house there was a box containing some letters written by the man in which he pointed out he no longer had any reason to live.

In all three cases, the external examination of the corpse revealed no signs of struggle and the presence of a continuous, horizontal, and uniform ligature mark around the neck and conjunctival petechiae. The autopsy examination showed the presence of hemorrhagic infarctions of the infrahyoid muscles of the neck, as well as the fracture of the small left horn of the hyoid bone in the first case and the right posterior horn and the corniculate cartilages of the hyoid bone in the third case. Histological investigations revealed pulmonary edema with emphysema and poly visceral congestion. In all cases, toxicological investigations excluded drugs, psychotropic substances, or alcohol intake. The toxicological analysis is strongly recommended to exclude a blunting of the subject's senses. Literature studies show a correlation between cerebral oxidative stress and suicidal behavior, suggesting the usefulness of immunohistochemical analyzes to corroborate suicidal or homicidal dynamic in a forensic scenario that may be suspicious.<sup>3</sup> In the reported cases, immunohistochemical analysis was performed on brain samples to verify the correlation between oxidative stress and suicide. A careful crime scene investigation and postmortem examination, including histological, histopathological studies, and toxicological analysis, integrated with a detailed collection of anamnestic data, is mandatory to establish suicidal death and exclude other possible causes in ligature strangulation deaths.

**References:**

1. Rabl, W., Markwalder, C., & Sigrist, T. (1992). "Self-asphyxia"--a forensic medicine-criminal challenge. *Archiv fur Kriminologie*, 189(1-2), 1-8.
2. Cordner, S., Clay, F. J., Bassed, R., & Thomsen, A. H. (2020). Suicidal ligature strangulation: a systematic review of the published literature. *Forensic Science, Medicine and Pathology*, 16(1), 123-133.
3. Schiavone, S., Neri, M., Mhillaj, E., Morgese, M. G., Cantatore, S., Bove, M., Riezzo, I., Tucci, P., Pomara, C., Turillazzi, E., Cuomo, V., & Trabace, L. (2016). The NADPH oxidase NOX2 as a novel biomarker for suicidality: evidence from human post mortem brain samples. *Translational psychiatry*, 6(5), e813. <https://doi.org/10.1038/tp.2016.76>.

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**Suicide; Strangulation; Asphyxia**

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**F58 To Die in Pursuit of Beauty: A Case of Systemic Silicone Oil Embolism After a Breast Beauty Treatment**

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**Learning Objective:** Attendees will better appreciate how the application of a rigorous methodology can aid in the diagnosis of a silicone oil embolism.

**Impact Statement:** This presentation will impact the forensic community by sharing the experience gained following the management of a case of silicone oil systemic embolism that occurred following an injective cosmetic treatment to the breast.

A request for help was made for a 37-year-old woman who had felt ill immediately after an unauthorized person had been given injections for aesthetic purposes in the breast. Upon the arrival of the rescuers, the patient was mydriatic, poorly reactive with jugular turgor, sinus tachycardia with right bundle branch block, and deviation of the electrical axis to the right at ECG. During ambulance transport, she first developed bradycardia and then PEA (pulseless electric activity). Once in the emergency room, an echocardiographic examination highlighted a hyperechoic material in the heart chambers for thrombolytic therapy. Despite the continuation of the resuscitation maneuvers for over one hour, the woman did not revive.

An autopsy was ordered by the public prosecutor to clarify the cause of the woman's death and to identify the injected substance. Preliminarily to the autopsy, a postmortem CT examination showed a large collection of hyperdense material under the right breast, in ventricular chambers, and in the pulmonary vessels as well as the presence of diffuse nodular formations filled with hyperdense material at the level of the thorax bilaterally.

External body examination found a semilunar wound in the right submammary sulcus from which a transparent oily-like substance seeped abundantly. Dissection of the soft tissues of the thorax showed the presence of marked structural subversion with multiple nodular formations containing oily material, a marked fibrosclerotic alteration of soft tissue, and a widespread presence of transparent oily material. There was also a large collection containing about 300cs of similar material immediately below the pectoralis major muscle that was in communication with the wound in the right submammary sulcus. *In situ* opening of the pulmonary artery highlighted the leakage of fluid blood mixed with abundant oily material, material that rises above the blood level. The same oil was also found in the heart chambers.

The histological investigations carried out (staining with HE) subsequently confirmed the diagnosis of systemic embolization by showing the presence of multiple optically empty vacuoles in cerebral, cerebellar, cardiac, pulmonary, and renal vessels. Chemical investigations carried out on the material found in the autopsy and on that found inside a syringe found at the crime scene confirmed the presence of polydimethylsiloxane silicone oil. This case report shows how the use of a rigorous methodology with an autopsy, histopathological examinations, and integration with radiological and chemical-toxicological analysis can allow the diagnosis of an uncommon condition such as systemic silicone oil embolization after an illegal aesthetic treatment.

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**Forensic Pathology; Aesthetic Treatment; Silicone Oil**

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**F59 Self-Immolation: A Case Series Investigating the Motivations and Risk Factors of a Rare Method of Suicide**

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**Learning Objective:** This presentation aims to deliver an insight into the circumstances, motivations, and risk factors of a rare method of suicide, self-immolation, by presenting two interesting cases and comparing them with the findings of 14 other cases of self-immolation.

**Impact Statement:** Self-immolation is a form of suicide that is not commonly seen in developed countries. It is a method of suicide more commonly seen in Iran, India, and China. This case series will examine a total of 16 cases of self-immolation from 2013 to 2022 that were accepted to the District of Columbia Office of the Chief Medical Examiner (OCME). There are many different methods of suicide with many different associations and risk factors; however, self-immolation is a particularly painful, unpleasant, and drawn-out method of committing suicide. Two case examples of self-immolation and a review of 14 other cases that have come through the District of Columbia OCME give us a glimpse into the different motivations for self-immolation.

The first example is the case of a 50-year-old male who was admitted to the hospital with severe burns involving approximately 70% of his total body surface area. He succumbed to his injuries one day after sustaining his burns. The decedent was seen in front of the Supreme Court dousing himself in an unknown fluid and setting himself on fire in an act of protest concerning climate change inaction. This example demonstrates a politically motivated suicide with the suicide method acting as a message to spark discussion in a community.

The second example is that of a 75-year-old male who reportedly threw gas on his wife at her place of work. After his unsuccessful attempts at lighting her on fire, and subsequently attempting to stab her, the decedent fled the scene. Not long after the incident, a call was received that the decedent was at a gas station where he had lit himself on fire. He passed away at a hospital shortly thereafter. This example demonstrates a more social motivation for suicide that was domestically motivated.

Reviewing all 16 cases of self-immolation revealed many differences and many similarities. This method of suicide was most likely to be a first attempt, with only one of the cases having a confirmed previous suicide attempt. Many of the cases involved individuals with mental health issues, with 12 out of 16 cases having diagnosed mental illness or symptoms of a mental illness, including schizoaffective disorder, schizophrenia, depression, bipolar disorder, post-traumatic stress disorder, and paranoia. The majority of the cases took place outside, with the exception of 5 cases where the self-immolation took place inside their home, vehicle, or in the shed outside their house. Most of the cases occurred during the winter months of the year. The majority of cases were male. The age ranged from 19 to 75 years of age, and there was no predominant race in the examined cases. Some of these findings align with what is seen internationally, wherein most cases of self-immolation were first attempts at suicide. However, there are differences as well, where most of the cases of self-immolation in Iran were committed by females.

Self-immolation is a method of suicide with risk factors and motivations that vary greatly across the globe with big differences between developed and developing countries. The purpose of this presentation is to attempt to understand the population that is most likely to attempt this violent method of suicide and what these people have in common.

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**Self-Immolation; Suicide; Thermal Injuries**



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**F60 Trainspotting: Sudden Unexpected Death After Cannabis Consumption**

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**Learning Objective:** This study aims to investigate the possible relationship between cardiac adverse effects of cannabinoids consumption, promoting caution when approaching these issues.

**Impact Statement:** This presentation will impact forensic science by strengthening the scientific evidence that correlates the use of cannabinoids and sudden cardiac death, sustained in the cases under examination by a histopathological picture not dependent on another cause.

**Background:** There are several species of cannabis, each with a different amount of the two main active ingredients, delta 9-tetrahydrocannabinol (THC), responsible for the euphoric effects, and cannabidiol (CBD), marketed for its anti-inflammatory actions. Synthetic THC-like dronabinol and nabilone are used as treatments for nausea and vomiting as well as for the treatment of neuropathic pain associated with tumors and neurological disorders. In 2018, the United States Food and Drug Administration approved a CBD oral solution for the treatment of two forms of epilepsy, Lennox-Gastaut syndrome, and Dravet syndrome. However, it is the most widely used illicit drug in the United States and the most widely used in the young adult population of Western countries. Therefore, the acute toxicity of cannabinoids has always been held to be low, so there is little public awareness of the potentially hazardous cardiovascular effects of cannabis. Thus, the present report will show cases of young subjects found dead for whom a forensic autopsy was requested.

**Case series:** Three young Black men were found in their houses in November 2019, March 2020, and April 2022, aged 37, 37, and 28 years old, respectively. In all three cases, the investigation of the scene of the death did not suggest the involvement of third parties, nor did the circumstantial data and testimonial evidence. The pathological anamnesis collected was not worthy of note. The autopsy examination conducted subsequently allowed the exclusion of traumatic causes as the origin of the death. However, organ and biological fluids were taken for subsequent histological and toxicological investigations. Heart and brain were fixed *in toto* for the detailed study, which was carried out at 28 days of formalin fixation allowing the exclusion of macroscopic alterations causally relevant in death. The samples taken and fixed were then embedded in paraffin and the sections were stained with the hematoxylin-eosin technique, revealing a cardiac finding of a polymorphic pathological picture, characterized by areas of edema and fiber segmentation. The death was consequently attributable to sudden cardiac death on an arrhythmic basis, with no signs of other factors supporting it (infectious, coronary artery disease, or anatomic abnormalities). In all these cases, the toxicological tests were positive for THC in the urinary and blood matrices.

**Literature review:** To better understand the link between the intake of cannabinoids and sudden cardiac death, a systematic review of the literature was carried out that showed that smoking marijuana causes tachycardia and an increase in blood pressure with effects on the heart rate within ten minutes of inhalation of marijuana for up to two to three hours, mediated by activation of the CB1 receptor, supporting the idea that cannabis-induced tachycardia is attributable to activation of the sympathetic nervous system. Furthermore, a parasympathetic mechanism with bradycardia and vasodilation has also been hypothesized, inducing reflex tachycardia, which is likely to be an additional mechanism for cannabis-induced tachycardia. This biphasic effect of cannabinoids appears to be dose-dependent, whereby lower doses caused sympathetic stimulation and norepinephrine release while higher doses caused parasympathetic stimulation. On the other hand, some studies have shown that marijuana users have a double incidence as the abstinent population and heavy users have a greater risk than occasional users.

**Conclusion:** From the integration of autopsy data in the cases in question and the review of the literature, it is possible to deduce, although the present study lacks genetic investigations (having only investigated channelopathies through immunohistochemical techniques), an at least concurrent causal role of the assumption of marijuana in the mechanism pathophysiological that led to the death that appears to be suggestive. Therefore, further trials will be done to better clarify the scientific validity of this connection.

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**Sudden Unexpected Death; Cannabinoids; Cardiac Arrhythmias**



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**F61 Measuring the Environmental Degradation of Traditional Drug and Novel Psychoactive Substance (NPS) Residues**

*Elizabeth Robinson, MS\*, National Institute of Standards and Technology, Gaithersburg, MD; Edward Sisco, PhD, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Objective:** After attending this presentation, attendees will understand how exposure to different environmental conditions affects the stability of traditional drug and novel psychoactive substance residues.

**Impact Statement:** This presentation will impact the forensic science community by providing key information on the stability of drug residues after exposure to six different environmental conditions to help determine the likelihood of detection after exposure.

A crucial aspect of the detection of trace drug residues that is often overlooked is understanding the behavior of these compounds in real-world environments. The ability to predict both the lifetime of drug residues as well as their degradation products after exposure to different environmental conditions can provide critical information to aid in: determining if collection of a residue is possible, refining alarm algorithms of screening tools, providing guidance on proper storage of samples containing suspected residues, and possibly give insight into potential decontamination protocols. This information is crucial for law enforcement, first responders, and forensic practitioners.

This work investigated the stability of trace (tens of nanograms) deposits of drugs when exposed to six environments (-4°C, 23°C, 30°C, 47°C, 30°C, and 90% relative humidity, UV exposure). Quantitative data was obtained by direct injection electrospray ionization mass spectrometry (ESI-MS). The mass loss measurements were then used to establish fitted degradation curves for all drugs and all conditions. Pairwise comparison analysis between different environments for individual drugs was also completed to understand both the similarity between environments and the effect different environmental conditions had on mass loss. In addition, full mass spectral data was collected using thermal desorption direct analysis in real time mass spectrometry (TD-DART®-MS) in both positive and negative ionization modes to identify and understand spectral abnormalities that could be attributed to degradation or decomposition products formed after different environmental exposures.

Representative drugs from major classes were examined. Mass loss was found to depend on both the environmental exposure as well as the compound analyzed. Exposure to UV light as well as elevated humidity most often had the greatest impact. Major degradation products and pathways for some compounds have also been established. This data may ultimately provide law enforcement, first responders, and forensic practitioners with a better understanding of how trace drug residues will behave under real-world condition.

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**Seized Drug Analysis; Mass Spectrometry; Environmental Degradation**

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**F62    Unidentified Child Remains: Analysis of Identifications**

*Amanda Bixler, MA\*, National Center for Missing & Exploited Child, Alexandria, VA*

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**Learning Objective:** Attendees will learn of specific investigative steps and resources that appear to have the most impact resolving cases of unidentified children. Attendees will also observe the benefits of developing a multifaceted approach that incorporates multiple layers of resources to their cases.

**Impact Statement:** This presentation will show the forensic community the importance of forensic resource application to unidentified child cases and the significance they play in making identifications.

There are approximately 45,000 sets of unidentified remains in the United States, and it is estimated that more than 1,000 of these remains are those of children. The National Center for Missing & Exploited Children (NCMEC) currently assists law enforcement agencies, medical examiners, and coroners with just over 650 unidentified John and Jane Does believed to be children.<sup>1</sup> NCMEC stands as a national clearinghouse to these agencies to provide resources, analytical services, and forensic assistance in the hopes of identifying these victims and providing information to investigators about their death. NCMEC has the unique opportunity to observe recovery and identification methods at a national level and provide insight of resolved cases.

Two hundred thirty-six identifications of unidentified child cases intaked with NCMEC were reviewed, dating from May 2000 to December 2020. The results of the analysis recommend investigators tasked with resolving an unidentified child case should develop a multifaceted approach that incorporates multiple layers of resources. The goal of this analysis was to review how remains of deceased children have been successfully identified and to develop data-supported recommendations for law enforcement agencies, medical examiners, and coroners working similar cases.

The findings indicate that proactive investigated methods play a critical role in identifying these victims. Additionally, proactive and early collection of biometrics and entry into biometric databases played a significant role in making identifications. Conducting parallel investigations into the victim's and suspect's identities could also increase the chances of finding a resolution.

Finally, there were four findings of particular interest for investigators working unidentified child cases that emerged from the study: most of the children's bodies were recovered within the same city or state from which they went missing; most of the children were deceased within hours or days of going missing; identifications primarily stemmed from a tip submitted to law enforcement; and if a suspect was identified, the perpetrator was not known to the child in most cases, unless the child was under the age of ten. These are challenging investigations, and the majority took over one year to resolve. By focusing investigative efforts on the steps that have proven successful and taking advantage of emerging technologies such as forensic genealogy, more and more cases could be resolved in a shorter timeframe.

**Reference:**

<sup>1</sup>. Ritter, Nancy. Missing Persons and Unidentified Remains: The Nation's Silent Mass Disaster. *NIJ Journal* (256), 2007.

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**Unidentified Child Cases; Forensics; Investigations**

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**F63 Eros and Thanatos: When Autoerotic Stimulation Leads to Death: A Presentation of Three Cases**

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**Learning Objective:** after attending this presentation, attendees will better understand the role of a thorough analysis of the crime scene in three cases of death from autoerotic stimulation.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of the relevance of crime scene analysis as well as the importance of additional circumstantial information to face death from autoerotic stimulation.

Autoerotic deaths can be defined as accidental deaths occurring during solitary sexual activity. These cases are rare but well-known occurrences in forensic practice, including inserting rectal and genital foreign bodies, cross-dressing in conditions of extreme heat, and fatal accidents due to asphyxiation. Three cases of fatal accidents related to autoerotic activities are reported and compared with forensic literature.

In the first case, a 71-year-old man was found dead inside the bathtub with the shower hose close to the anal orifice and tightly fixed by a rubber band all around his legs. The water was still flowing out of the shower hose with a water temperature of 38.4 °C (101.1°F) and a rectal temperature of 42.5 °C (108.5°F). The police reported that the victim lived alone, and he kept sexual paraphernalia in his house. The body did not reveal any signs of traumatic injuries, but it showed extensive burns and skin maceration. Autopsy examination documented diffuse atheromatic disease, mitral and aortic valve sclerosis, nephroangiosclerosis, and fatty liver. Toxicological analyses were negative. The cause of death was due to a heat-induced vasovagal syncope THAT occurred during autoerotic anal stimulation.

In the second case, a 29-year-old man was found dead on his knees inside the shower stall with a cable fastened around his neck. Specifically, the cable was fixed on the shower holder, and there was a mobile phone on the shower tray between the victim's legs. On a closer examination, a shower cap containing seminal fluid was tightly fastened around the penis; in the bathroom, there was also an opened Vaseline® tube. Autopsy examination revealed the hanging mark (over the larynx) with little muscle hemorrhages beneath the skin, visceral congestion, subconjunctival, and pleural petechiae. There were no signs of traumatic injuries on the body. Toxicological analyses were positive for cocaine and benzoylecgonine. The cause of death was due to hanging, which occurred during autoerotic asphyxiation.

In the third case, a 72-year-old man was found by his wife hanged in the bathroom. The body was nude and entangled with three ropes, creating a sort of self-pulley. Specifically, the first rope was fixed with a ring-like shape at the window's bathroom, whereas the second was fastened all around the torso. The lower end of the third rope was formed into a fixed loop and placed around the neck, while the upper end passed through the ring of the first rope, then fixed behind the second rope, and finally it was wrapped around the left hand. Furthermore, there was a tripod with a camera in front of the body, and a knife and a ketchup bottle on a locker nearby. On a closer examination, the body showed the hanging mark on the neck, seminal fluid on the penis, and ketchup on the groin. The police examined the camera and documented two photographs and four videos of similar autoerotic performances of the man. The public prosecutor did not authorize autopsy examination.

In all our cases, the deceased was male, between 29 and 72 years old, and there was clear evidence of accidental autoerotic fatalities during the on-site inspections. Therefore, autoerotic asphyxial deaths typically occur when a rescue mechanism fails (case no. 3) or is not in place at all (case no. 2) and the victim cannot free her-/himself, becoming unconscious. Other unusual autoerotic incidents include fatal bleedings, extreme heat temperatures, the use of water (i.e., aqua-eroticum), or electrocution where the victim tends to misjudge the situation (case no. 1). However, the on-site inspection and autopsy are always crucial in such cases, providing very important medicolegal elements to help preliminary investigations.

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**Suicide Pact; Homicide-Suicide; Crime Scene Investigation**

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**F64 Youth Suicide Trends in Georgia: A Retrospective Study on the Effects of Pop Culture, Social Media, and the COVID-19 Pandemic**

*Samantha Mattox, DO\*, Georgia Bureau of Investigation, Suwanee, GA; Cody Archer, BS, Georgia Bureau of Investigation, Marietta, GA; Elizabeth Andrews, BS, Georgia Bureau of Investigation, Decatur, GA; John Carter, PhD, MPH, Emory University, RSPH, Decatur, GA; Natasha Grandhi, MD, Georgia Bureau of Investigation, Decatur, GA*

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**Learning Objective:** The goal of this presentation is to review, analyze, and compare data on the national, regional, and state level concerning youth suicide. With the advent of the internet and an ever-growing number of social media platforms, information is rapidly both uploaded and downloaded. Social media trends, pop culture news, and the COVID-19 pandemic are plausible causes for a perceived recent increase in suicides.

**Impact Statement:** It is crucial for members serving in the public health sector to recognize dangerous trends and assist in implementing changes to policy, legislation, and prevention whenever possible.

Suicide is the second-leading cause of death within the United States in the 10–24-year-old youth population. Moreover, younger generations are exposed to the internet and electronic devices at an earlier age. Consequently, there is a constant flow of information which they are processing while also trying to understand and clarify who they are as a member of society. Herein, we aim to assess potential influences on suicides within the 17-and-under population within the Georgia Bureau of Investigation's (GBI) jurisdiction and examine possible trends regarding suicide contagion and the perceived increase during this five-year time frame.

One hundred ninety-four youth suicide cases were identified in the GBI's jurisdiction (155 of 159 counties) from 2016–2020. Multiple data points were collected to include age, gender, mechanism of death, the use of social media during the terminal event, the presence of a note or other electronic posting expressing intent (if known), and a history of maltreatment. To assess for potential suicide contagion, various pop culture events were noted and compared to the cases obtained. Pop culture events of interest included the release of Netflix's® *13 Reasons Why* (2017), the passing of popular livestreaming service users (2019–2020), and the passing of prominent musicians and celebrities based in the United States during this time frame. In addition, participation in dangerous social media challenges like the Blue Whale Challenge and the Pass-Out Challenge (also called the Choking Game and Blackout Challenge) were evaluated. Last, the nationwide shut-down caused by the spread of COVID-19 in March of 2020 was examined for potential effects on youth suicide rates.

Although our data did not reveal a significant increase in suicide rates in correlation with the above stated events, the public health sector must stay aware and keep abreast of trends and potential dangers to youth populations. Due to the fear of suicide contagion after the Netflix's® hit series' most controversial scene depicting a teen female committing a suicidal act, both the public and health professionals expressed enough concern to enact changes, including making additional resources like the "13 Reasons Why Toolkit" available. The World Health Organization (WHO) also released new guidelines for media professionals.

This report emphasizes the medical examiner office's role in documenting potential harm to the public as well as provides additional analytical data as it pertains to certain events and corresponding trends in youth suicide. Causality has been an extensively studied and debated topic in this field as a combination of variables tend to contribute to the ultimate decision as opposed to a single event.

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**Suicide; Youth; Social Media**

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**F65 Solvent Effects in the Analysis of Explosives**

*Clarissandra Braun\*, The University of Tampa, Geneva, IL; Kenyon Evans-Nguyen, PhD, University of Tampa, Tampa, FL*

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**Learning Objective:** Attendees will have a better understanding of how solvent conditions impact the results of explosives when tested using direct infusion into an MS or an LC/MS.

**Impact Statement:** The detection of explosives in forensic science is relevant in many scenarios such as terrorist attacks and civilian safety. While there are many approaches to detecting explosives, one of the most common of them is through mass spectrometry. The research presented will give attendees the opportunity to understand how various solvents and instrument parameters in mass spectrometry play a role in the detection of TNT, Tetryl, RDX, HMX, and PETN.

Mass spectrometry is a critical technique in the forensic analysis of explosive devices. Liquid chromatography/mass spectrometry using electrospray (ESI) or atmospheric pressure chemical ionization (APCI) is especially useful in detection of organic high explosives such as RDX, TNT, and HMX. However, the specific ions observed in mass spectrometry of explosives vary significantly and are highly dependent on the conditions in the ion source. There is little consensus on what specific ions should be observed in a mass spectrum obtained with ESI or APCI for a given organic explosive. In this work, we are conducting a methodical study of organic explosive ions formed under varying ion source parameters, with particular attention paid to solvent conditions. TNT, Tetryl, RDX, HMX, and PETN were used as model explosives and tested as standard solutions using direct infusion into the MS (a Thermo™ LTQ XL and a Thermo™ Exploris 120) ion source. In preliminary experiments, solvent conditions were seen to have a profound influence on the ions observed in the mass spectrum.

These model explosives were purchased as stock solutions that were further diluted in both methanol and acetonitrile at concentrations of 10µg/mL, 25µg/mL, and 50µg/mL. The deprotonated molecule peaks and/or adducts observed in these studies were highly dependent on solvent conditions. The peaks observed were observed in other studies in the literature, but such studies are inconsistent with each other regarding observed peaks.

Current work is focused on direct infusion of model explosives while running a typical LC solvent gradient using an LC system without a column. These experiments should provide insights into the variance of peaks observed for a given explosive, depending on the specific solvent conditions present when it elutes from the LC system into the ion source in an LC/MS experiment. These studies should inform analysts using LC/MS for explosive analysis.

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**Explosives; Solvent; Mass Spectrometry**

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**F66 The Architecture of a Face: Craniofacial Forensic Reconstruction on Paper**

*Sharon Moses, PhD\*, Northern Arizona University, Flagstaff, AZ*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding about the processes involved in making two-dimensional forensic facial reconstruction drawings when the human remains are no longer available to create three-dimensional reconstructions (clay-over-skull cast). This presentation discusses making reconstruction sketches based upon skull measurements and autopsy photographs. Reconstructions are a least-expensive option to aid law enforcement in the identification of unidentified human remains.

**Impact Statement:** This presentation will impact the forensic community by demonstrating and promoting an understanding of how forensic facial reconstructions are done on paper, particularly when the skull is no longer available to do a clay-over-skull cast reconstruction. Drawing reconstructions are a feasible alternative after remains have been interred. The medical examiner's office releases unidentified remains that have not been claimed within a prescribed time limit as storage is often limited. Storage timelines vary from state to state (or county). In lieu of physical skeletal references, a reconstruction can be rendered from autopsy photos.

A facial reconstruction requires an understanding of craniofacial musculature and bone structures when unidentified individuals are in a state of advanced decomposition when they are found. It is the forensic artist who can reconstruct a face that can be published in hope that someone may recognize the individual. Secondly, an often-neglected aspect of reconstructions released to the public is the lack of incorporating clothing and other artifacts associated with the decedent that aid in identification. This presentation will demonstrate how clothing and other worn materials as visuals should also be utilized in a reconstruction.

This presentation will demonstrate that forensic facial reconstruction drawings are a viable option for identifying human remains when only photographs of the remains are available or when decomposition is prohibitive and an image for public release is needed. DNA is helpful if it is feasible to apply it; however, many individuals have no DNA on record in a database. Missing persons will sometimes have a family member leave a DNA sample, but unless the coroner or medical examiner is relatively sure and is seeking a confirmation, DNA tests can be expensive, time-consuming, and funds are usually limited. Matching possible missing persons reports consistent with location and timeline are the most frequently successful applications. Meanwhile, a visual rendering is inexpensive and can be very helpful to facilitate public input and/or recognition.

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**Forensic Art; Forensic Facial Reconstruction; Facial Anatomy**

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**F67 Cadaver Dogs and Handlers: Detection of Ancient Bones**

*Hannah Cawley\*, Duquesne University, Pittsburgh, PA; Lisa Ludvico, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Objective:** This presentation will share the potential capabilities of cadaver dogs to detect the scent of ancient bones.

**Impact Statement:** This research will be another source supporting the work and competency of cadaver dogs and their handlers. Unlike previous research, it explores the detectable scent of ancient bones that have been removed from their original burial site. It is a step toward filling the gap in research that exists in this area.

Not only are dogs our best companions, they are also one of the best detectors of human remains. Cadaver dogs are often brought along on search and rescue missions or to the scenes of potential crimes to help locate missing and deceased persons. They have also been recently introduced in an archeological setting to locate ancient burial grounds.<sup>1</sup> This is because the olfactory of a canine is powerful enough to allow them to detect traces of scent that humans cannot, as well as recognize familiar ones. In this research, the capabilities of five cadaver dogs to locate ancient human remains were examined through a series of five trials. Two of these trials were conducted indoors and three took place outside. The type of remains used in these trials were three ancient human bones of different sizes. They originated from Greece and are dated around 580 A.D.

The first of the trials was the least challenging and they became progressively more difficult. It was hypothesized that the dogs would be able to successfully detect the bones during each trial. Five handler-dog teams from the search and rescue group RSAR (Resources in Search and Rescue) of Connecticut participated. Their searches and the environment were recorded through photographs, videos, and written notes. Other factors affecting the success of the dogs, such as training history, experience, and potential error, were documented.

To date, no research trials have been conducted and published to assess the precision, accuracy, and reproducibility of cadaver dog detections on ancient bones. This research was conducted for its potential to demonstrate how precise the capabilities of cadaver dogs are on ancient bones and to indicate if the previously reported success at locating burial sites was heavily influenced by scents that may have been trapped by surrounding soil or if human bones still give off detectable scent after so many years.

**Reference:**

- <sup>1.</sup> Glavaš, V.; Pintar, A. Human Remains Detection Dogs as a New Prospecting Method in Archaeology. *J. Archaeol. Method Theory* 2019, 26 (3), 1106–1124. <https://doi.org/10.1007/s10816-018-9406-y>.

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**Cadaver Dogs; Ancient Bones; Search and Rescue**



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**F68 A Cruel Case of Homicide: Execution by Carbonization After an Assault With a Knife**

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**Learning Objective:** The peculiarity of the case we are presenting is mainly related to the methods of execution of the crime; considerable brutality has been applied. In this presentation, we also explain how to identify and analyze injuries in a charred subject.

**Impact Statement:** The importance of defining the viability of the lesions in charred subjects and the difficulties that may exist in identifying these corpses are considerable. In addition, it is crucial to reconstruct events and understand whether the victim was burned alive or after death. In our presentation, we clarify these elements.

Fire-related deaths are not uncommon and the forensic pathologist, in his profession, can easily run into these kinds of situations. On the contrary, fire-related homicides are not very frequent. When the forensic pathologist has to analyze a charred body, he must solve several dilemmas: first, it is crucial to distinguish between murder and suicide; then it is necessary to understand if the victim was alive or dead when he/she was charred; in addition, in the case of complex lesions present on the corpse, it will be essential to check whether these lesions have potentially played a role in the determinism of death. Normally it is not easy to precisely reconstruct the homicidal dynamics and, often, it is difficult to identify the specific cause of death of these subjects (inhalation-related deaths, thermal injury, and traumatic death with immolation).

We present a particular case of homicide by carbonization following an assault with a knife. In this case, the victim belonged to a criminal gang and, due to problems with other gang members, he was attacked by one of them with a knife. In particular, the subject had two neck injuries: one superficial and another deep that, however, as later demonstrated by the autopsy, did not affect large vessels (jugular and/or carotid). Also, the body was almost completely carbonized. The defendants' defensive strategy was to deny that they had charred the subject while he was still alive; they claimed to have burned the body when the victim had already died from knife injuries. In this way the defendants wanted to avoid the aggravating circumstance of cruelty. Through on-site forensic and medicolegal scene and corpse investigation, we were able to reconstruct the dynamics of aggression and death. In addition, analysis of signs of viability (COHb concentration, mucosal lesions, etc.) led to the conclusion that the subject was still alive and conscious when he was charred and that stab wounds were not fatal. Thus, the aggressors used fire not to hide the body but to kill the victim with cruelty.

Our case shows that in cases of fire-related homicide with concomitant lesions of another nature, it is essential to analyze all the useful elements found thanks to on-site investigation and autopsy. Only by combining all the evidence is it possible to reconstruct with reasonable certainty the precise dynamics of this rare type of murder.

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**Cruelty; Murder; Carbonization**

## **F69 Toxocological Effects of Amnesic Shellfish Poison (ASP) Poisoning: From Cleopatra's Controversial Death to Modern-Day Forensic Considerations**

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**Learning Objective:** After attending this presentation, attendees will delve into the toxic effects of snake venom and snakebite-related death.

**Impact Statement:** This presentation will have an impact on the forensic scientific community by demonstrating a new key in the role of current forensic techniques in the study of snake poisoning starting from the analysis of Queen Cleopatra's death.

Cleopatra VII was queen of the Ptolemaic kingdom of Egypt from 51 to 30 B.C. as its last active ruler. Cleopatra had a great inclination toward medicine and science, being attracted to the knowledge of poisons. She began testing these substances on condemned prisoners to see the different reactions produced in the body and to find the different limits of toxicity and lethality. Many attributed Cleopatra's death to the bite of an asp, but according to some Greek and Roman historians, she killed herself by drinking a poisonous beverage, getting bites into which she would insert poison, or even employing more sophisticated methods. According to some historians, the snake would have been nothing more than symbolic, meaning, therefore, that the queen's death was a real cold case, as the sovereign's body has never been found.

**Materials and methods:** In this work, we carried out a historical forensic search of all the studies found exclusively in the specialized biomedical literature that have analyzed Cleopatra's death and attempted to reconstruct it years later. It represents the first step in a much broader forensic humanities work currently being defined. Using the NCBI PubMed® search engine by entering the keywords, "Cleopatra death," "death of Cleopatra & Egypt," "death of Cleopatra in Egypt," and "Cleopatra death & forensic."<sup>1-3</sup> In addition, a search was conducted on the state-of-the-art in the forensic literature of snake deaths by entering the keywords "snakebites & death & forensic," "snakebites & death & toxicological investigations."<sup>4,5</sup>

**Results:** Only three papers emerged in which Cleopatra's death was forensically analyzed.<sup>1-3</sup> Specifically, in one an analysis is made of the common venomous vipers found in Ancient Egypt and the action of the toxins produced. Typical symptoms of viper bites included: cardiac pain, water retention in processed food in the stomach, vomiting and diarrhea, pus-filled teeth, and blood and bile discharge from the nostrils. In addition, species belonging to the Cobra family were studied, and among them the best known was the cobra *Naja haje* L., also known as the asp or banded cobra (one of the largest in Africa at five meters in length). In addition, other venomous animals include spiders and scorpions. In forensic analysis, there are a few studies in the literature describing death by snake venom but none of them refer to the case of aspid venom used by Cleopatra. Regarding modern investigations for the isolation of toxins to date, the most advanced method is sandwich-ELISA also through the use of polyclonal antibodies. The toxins to be analyzed are usually chosen according to the bite characteristics and geographic epideiomy of the snake.<sup>5,6</sup>

**Discussion:** With these studies it became clear that the snake that supposedly killed Cleopatra was an aspid (i.e., an Egyptian cobra, not an aspid viper). The viper bite generally produces violent local pain with inflammation, edema, and hemorrhage, as opposed to the cobra bite that causes rapid death from respiratory failure due to general paralysis without leaving obvious lesions on the skin. Cleopatra knew well that poisons ingested orally would cause such ailments as painful spasms, nausea, and a slow end. Therefore, while it is not possible in this context to determine the causa mortis and the exact manner of it in Cleopatra, by proposing as a theoretical case the hypothetical death by viper venom, it can be pointed out that certainly with modern technologies, to this day, it would have been easier to solve this conundrum. Even today it is still difficult for the forensic pathologist to determine the presence of a venom in the blood, especially in identifying the species and the lethal toxin.

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### **Forensic Sciences; Cleopatra; Toxicological Effects of Asp Bite**

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**F70 The 1921 Tulsa Race Massacre and the Demographics of Death**

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**Learning Objective:** After viewing this presentation, attendees will be able to describe the documentary resources in use to identify victims of the 1921 Tulsa Race Massacre. Viewers will be able to contrast the level of record keeping and retention relevant to recovering victims of this human rights violation.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the role of vital statistics in an investigation of a human rights conflict in the United States. It will describe resources available to many states for acquiring death records that may be relevant to a death investigation.

In 2018, the City of Tulsa, OK, with the support of Mayor G.T. Bynum, selected anthropologists, archaeologists, and historians to initiate investigations into the recovery of the victims of the 1921 Tulsa Race Massacre (TRM), also known as the 1921 Tulsa Disaster. From May 31 through June 1, 1921, a White mob committed homicide, looting, and systematic arson in the African American-owned, unincorporated area of Greenwood, adjacent to Tulsa's downtown. Thirty-nine blocks were incinerated, and the death toll was estimated to be in the hundreds. Although victims from all populations are sought, the TRM Physical Investigation Committee quickly focused on the African American male victims who were reportedly buried within Oaklawn Cemetery, the Tulsa city cemetery at that time, according to historical documentation. This intense scrutiny is warranted in part by the loss of the Sexton's records for Section 20, the Colored Potter's Field, the area of the cemetery in which these victims were interred. However, records for the other cemetery sections were maintained.

Here, we present our initial synthesis of Tulsa County death certificate data for the month of June 1921 with the City of Tulsa burial records for Oaklawn. The Oaklawn Cemetery records, maintained by Sexton Thomas S. Feely, provided some data and served as a basis of comparison with other data sets. In addition, [Ok2explore.health.gov](https://ok2explore.health.gov), the online Oklahoma vital statistics portal, provided access to names and death dates by county. Death events older than 50 years are frequently open records and accessible to non-related researchers. These death certificates were ordered through [VitalChek.com](https://vitalchek.com).

This is a first step in reconstructing the demographics of the June 1921 mortuary population for Oaklawn Cemetery. We hypothesize that death certificate numeration of Colored burials in Oaklawn provides an indirect census of the Colored Potter's Field population, assuming that burials in the paid plots are in the city record.

The June 1921 city records contain seven burials in Oaklawn Cemetery, for four juveniles and three adults, none in the Potter's Fields (Colored and White). These seven individuals have Tulsa County death certificates, although one is misfiled in [Ok2explore](https://ok2explore.health.gov) as a January death. [Ok2explore](https://ok2explore.health.gov) indicates 140 death certificates are available for June 1921 Tulsa County. Our review has indicated approximately 134 deaths in June 1921 with a misfile rate of about 5%.

Our analysis of the June data indicates that the null hypothesis, that death certificate data will not provide a census of the Colored Potter's Field, should be accepted. The number of June death certificates indicating Oaklawn burials well exceeded the seven documented in city records, even when excluding race massacre-related deaths. Burials from all populations are inconsistent compared with the City Sexton's records. Proportions of populations represented generally resemble 1920 census data, with Whites having the majority of interments, followed by Colored, Mexican, and Indian. Burial locations in the county vary by racial classification. Juvenile mortality was almost equal to adult mortality for Whites when the race massacre-related deaths were excluded. Loss of the Sexton's records is a handicap to the recovery of the race massacre victims. Considering how few June burials were documented by the Sexton, it is telling that the Section 20 records alone are lost. Our analyses of death certificate data are ongoing and include examination of death certificates for the surrounding counties and through the year for other race massacre-related deaths.

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**Human Rights; Race; Death Certificate**

**F71 A 1966 Unsolved Homicide, Conjecture, and Forensic Inadequacy**

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**Learning Objective:** Attendees will learn the limitations of forensics in the early 1960s and how the unsolved status of this case is likely a product of the inadequacy of forensic and scientific knowledge of that time. Progressive knowledge and scientific advancements in crime scene and postmortem processing have opened the door for cold cases such as this to be re-opened and investigated. There will be an overview of the creation of the Colorado Cold Case initiatives at the Colorado Bureau of Investigation as well as the Cold Case Task Force and Colorado State's Cold Case Database.

**Impact Statement:** This presentation will impact the forensic community by bringing up the importance of remembering the past to appreciate the present and to always be striving to learn and grow the field of forensics to support the future MDI's, scientists, and families impacted through forensic investigation.<sup>3,4</sup> It is a glimpse into history clouded by bias and ignorance, and although bias is something our society is well aware of, it hasn't fully vanished from the forensic science community.

On February 3, 1966, a 52-year-old female was found murdered in Denver, CO. Her naked body had been dumped in an alley with a pair of women's boots and a single cigarette pack. She was stabbed 24 times. By the end of the week, her autopsy was completed, and police had interviewed and subsequently released the two persons of interest. One of the incised wounds of her chest had sliced through her heart and was listed as the cause of death. The murder was reported to have been sexually motivated but only a few dark nuclei were noted on the microscopic examination of the vaginal smear and were suggestive of spermatozoa heads but were too indistinct to be of any value.<sup>7</sup> What few pieces of evidence there were from the crime scene were lost years ago and no scene photos or transcribed police interviews remain. Her case went cold weeks after her death and has had no confirmed leads since. The deceased's criminal history was minimal with a single count of prostitution and public drunkenness.

In the broadest sense, forensic science is the application of science to law. Forensic science is, and will always be, a crucial element of the criminal justice system.<sup>2</sup> Evidence from crime scenes must be collected, examined, and analyzed to develop objective truths for the prosecution of criminals or the absolution of innocents.<sup>1,2,5</sup> The high-tech reality of forensic science has come to not only be expected but also captivates the attention of the public at large.<sup>2,5</sup>

Unfortunately, the knowledge of science as it relates to forensics has not been around forever and neither has the technology.<sup>5,7</sup> The unique structure of DNA was not discovered until 1953 and DNA fingerprinting was not used in forensics until 1986.<sup>1,2</sup> Only recently has DNA from decades-old crime scenes been used to link murders to killers both dead and alive.<sup>2,6</sup> Trace evidence remained in its infancy at the time of this murder. If law enforcement knew what we know now back in 1966, maybe this homicide would be solved.<sup>1,5,6</sup> Or perhaps not, the narratives of this case are fraught with comments that implicate bias during the investigation process. The human condition may not have come as far as technology and science, but change can only happen through awareness.<sup>3,4</sup>

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**Homicide; Unsolved; Science**

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**F72 A Case Study: Was This a Homicide or Did the Decedent Get His Wish to Fly High and Be Fed to the Birds?**

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**Learning Objective:** After attending this presentation, attendees will gain an insight into the various scientific methods used in the death investigation of a decomposed and mummified individual with animal and insect activity. Also discussed will be the emphasis of the importance of utilizing partnering agencies to legally and scientifically conduct a medicolegal death investigation. In this case study, a combination of forensic odontology, forensic anthropology, forensic toxicology, forensic entomology, medical implants, and pathology was utilized.

**Impact Statement:** This presentation will impact the forensic science community by discussing the importance of obtaining independent scientific testing from multiple different agencies during this death investigation. Traditional methods of investigation were not possible in this case due to the amount of time this person was deceased, as well as being unable to obtain a reliable witness.

We will present a case study that will provide insight into the various scientific methods used in a death investigation. The decedent was located in an unlocked residence, under a set of blankets and a tarp, and in an advanced state of mummification.

When questioned, the decedent's wife wanted investigators to believe the decedent was still alive and provided multiple explanations as to where he might be. It was not until almost a full year later that law enforcement was notified of the death. When the decedent's wife was interviewed about her husband's death, she did explain she was aware of her husband's body being in the house and how she was attempting to grant her husband's wishes of having a quasi-traditional bird feeding burial with Hindu-Indian elements. The evidence did not match up with her claims, which led to her seeking out news outlets in an attempt to build support.

Forensic anthropology and forensic entomology helped to disprove the wife's claims that the decedent was alive days before his mummified body was found by investigators, but one very important question remained; was this a homicide? An autopsy was performed and despite all forensic science methodologies that were utilized throughout the medicolegal death investigation, the cause and manner of death are currently listed as undetermined.

With the use of forensic science, the wife's statements were scientifically proven to be impossible and ultimately led to her being convicted of welfare fraud, theft, and failure to report a dead body. The wife of the decedent still claims to this day that she last saw him alive only days before he was found dead, even though forensic examination of the body determined that there was no truth to those words. Although the pathologist was restricted in his examination due to the mummified skeletal remains, the additional forensic tests were able to answer at least a few questions that would have been left unanswered. Was this a homicide, or did the decedent get his wish to fly high and be fed to the birds?

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**Homicide; Forensic Anthropology; Forensic Entomology**

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**F73 A Case of Filicide: Combined Homicide by Asphyxiation and Stabbing**

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**Learning Objective:** Our presentation is particularly interesting because it describes an unusual case of a filicide (by 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.

**Impact Statement:** This presentation will impact the forensic science community by describing how to analyze the circumstantial data and the results of on site inspection. In addition, an in-depth description of the particular lesions found will also help in future cases of combined homicide.

This study presents the case of a child found dead inside a chest of drawers of the bed at his home. At the time of the discovery, the child presented with his nose and mouth wrapped in masking tape, multiple weapon wounds, and a kitchen knife penetrating the anterior cervical region. At the same house, the child's father was found, unconscious and with half-empty bottles of alcohol next to him. Transported to the emergency room, the father of the child had very high levels of alcohol; the external inspection carried out on the man did not detect defensive injuries.

The forensic examination, the external examination, and the autopsy showed the cause of death of the child to be murder by a combination of two different modes, that is, attributable to the combined action of an asphyxiation (by adhesive tape) and a cutting injury (by kitchen knife).

The purpose of this work is to present a case of heinous filicide characterized by the absence of defensive injuries on the victim and on the aggressor, motivated by the physical disproportion and the relationship of kinship between the aggressor and the victim. In addition, we aim to emphasize the importance of forensic examinations in clarifying the dynamics of death in the case of combined homicide.

In fact, in homicides, the discovery of more than one potentially lethal instrument (and therefore of as many potentially fatal injuries) can make the reconstruction of the dynamics and the ultimate cause of death by the medical examiner difficult. This difficulty can be accentuated even more if, as in the case presented, we are faced with the scarcity of postmortem pathological signs of violent mechanical asphyxiation.

In these circumstances, it is therefore essential to carry out a meticulous forensic inspection, the first unrepeatable investigation that allows us to analyze the crime scene, 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. This is necessary, first, to understand if the victim, at the time of each injury, was still alive or had already died, but also to exclude aggravating factors such as overkill. Finally, it is essential to search for macroscopic and microscopic signs suggestive of asphyxia to determine whether and when the asphyxia has acted in the determinism of death.

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**Child; Murder; Combined Homicide**



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**F74 A Successful Multidisciplinary Medicolegal Investigation in the Absence of a Body**

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**Learning Objective:** After attending this presentation, attendees will be able to recognize that aspects of the medicolegal investigation may have a role in homicide investigations despite the absence of a body.

**Impact Statement:** Attendees will also understand how this type of homicide investigation necessitates the utilization of a multidisciplinary approach to not only proceed with a prosecution but to provide evidence that a death, in fact, occurred.

From the perspective of investigators, postmortem changes to a decedent can manufacture and leave behind telltale clues that a death has occurred, even after the removal of a decedent.

Scenes of death have many facets that are observed and evaluated as part of standard medicolegal and criminal death investigations. The evaluation and examination of the body at the scene of death significantly impacts the direction and composition of the investigation as a whole. Injuries identified to the body can often be preliminarily evaluated on scene to determine if they are consistent with initially reported circumstances of a death. Suicides, homicides, accidents, and natural deaths typically have differing scene presentations that can guide the investigative professional in the appropriate direction to focus their efforts.

This study presents an unusual case of a medicolegal investigation conducted in the absence of a body. The atypical involvement of medicolegal investigators at this death scene contributed to the determination that a death indeed had occurred and resulted in aiding the successful prosecution of the offending individuals.

An 84-year-old woman disappeared from her long-term private residence where she had lived alone for more than a decade. A previous welfare check by sheriff's deputies, months before her disappearance, found several convicted felons reportedly working for the elderly woman in a handyman-type capacity. At that time, the elderly woman stated she was fine and that no one was taking advantage of her.

Upon her disappearance, a missing person's report was filed, and law enforcement responded to the home to conduct a welfare check inside the residence. Law enforcement entered the home through an unlocked door and found the house to be in disarray. No decedent was found, but what appeared to be dried blood stains were identified on the carpet and floor. These stains were streaked in the direction of a door leading to the exterior of the residence. Additionally, there was an air of decomposition that led investigators to a couch where what appeared to be decompositional fluid and maggots were identified.

Despite the absence of a body, law enforcement contacted the medical examiner's office for consultation. A forensic pathologist and two medicolegal death investigators responded to the scene. The scene was investigated for potential medicolegal evidence and photographically documented. The maggots found on scene were collected and retained, both in a preserved state and live for entomological evaluation.

This unusual case required a multidisciplinary medicolegal investigation that subsequently revealed the scene to be consistent with a death despite the atypical presentation of no decedent. This case conclusively illustrates the importance of utilizing a multidisciplinary medicolegal approach in death investigations.

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**Multidisciplinary; Medicolegal; Homicide**



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**F75 A Case of Death From Pneumonia Due to Medical Malpractice in a Young Man Suffering From Psychiatric Disorders**

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**Learning Objective:** After attending this presentation, attendees will remember the importance of an autopsy approach in cases of negligence and malpractice, especially when dealing with extremely deficient clinical records.

**Impact Statement:** This presentation will impact the forensic science community by illustrating a case of death by an undiagnosed bacterial pneumonia in a young man hosted in a therapeutic community that could have been avoided if the correct diagnostic assessment were adopted.

A 17-year-old man previously diagnosed with psychiatric disorders (low IQ, developmental disorder, and ADHD) was hosted in a therapeutic community where a complex pharmacological treatment scheme was set up. During the stay in the community, the young man showed an oppositional behavior, a severe incapacity of withholding impulses, and frequent episodes of sudden behavioral changes difficultly managed by health workers. In some cases, the man took self-injurious actions following a need for hospitalization and frequent, significant changes of the treatment scheme. During the last period of his stay, according to the clinical diary annotations, his health conditions worsened, with the arising of muscle stiffness, enuresis, and dysphagia. One day, a nurse found the man unexpectedly dead in his bed. When first aid arrived, rescuers noticed the presence of abundant purulent drainage from the man's airways.

Upon suspicion of medical malpractice, considering that the clinical diary showed a lack of sufficient documentation regarding the health conditions of the patient, notably during the last month of his stay, a judicial autopsy was ordered.

The autopsy showed hematic congestion of the viscera, heavy lungs with abundant pleural serosanguineous effusion. The young man also presented extensive necrotizing lesions on the heels, possible expression of infectious complications of traumatic wounds.

The histological investigations were fundamental and revealed, in the lungs, a microscopical pattern of diffuse alveolar damage characterized by inflammatory exudate extended to all lung lobes and a significant red hepatization with blood cells, fibrinosuppurative exudate, and neutrophils, identifying the cause of death as a bacterial pneumonia. Neutrophilic inflammatory infiltrates were present also in the heel wounds, suggesting a possible correlation between the two infection sites.

Toxicological analysis revealed the presence of antipsychotic clozapine and benzodiazepines within the therapeutic range; this allowed the exclusion of acute toxicity effects on the body. However, investigations revealed a mismatch between the pharmacological treatment scheme prescription reported in the clinical diary and the drugs detected in the blood samples.

With such severe and widespread pneumonia, although it is not possible to establish its etiology, the young man must have presented some symptoms (such as temperature, cough, dyspnea, and oxygen desaturation). Any of these symptom patterns are described in clinical documentation compiled by the caregivers, raising suspicion about inadequate and superficial evaluation of the patient by health workers.

The forensic examination showed that the health professionals who treated the young boy acted negligently. Unfortunately, since there was not a correct microbiological analysis (antibiogram), it is not reasonably possible to understand if the boy could have been saved by applying a targeted therapy. A juridical process is now in progress to assess the amount of reward that the Health Structure owes to the relatives of the boy.

The hope of this descriptive report is to improve health quality of the facilities for patients suffering from psychiatric illness, underlying the importance of individuating clinical manifestations of organic pathologies affecting people with mental health disorders.

The goal of the present case report is to underline the importance of a thorough medicolegal investigation in cases of unexpected deaths of subjects with mental health disorders. In this case, a proper integration of data resulting from a careful examination of the clinical records, autopsy data, and microscopic investigation allowed the identification of possible malpractice. Even though it was not possible to identify each individual responsibility, a civil lawsuit allowed adequate compensation, taking into account the young age of the subject and that the death could have been avoided in the case of a correct and timely diagnosis.

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**Medical Malpractice; Psychiatric Disorders; Professional Liability**

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**F76     Examining Trends in Juvenile Deaths in Harris County, Texas: A Seven-Year Retrospective Review (2015–2021)**

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**Learning Objective:** After attending this presentation, attendees will be knowledgeable regarding death trends of the juvenile population (ages 6–17) in Harris County, TX, and how the data may provide guidance on where resources may be directed to mitigate juvenile deaths.

**Impact Statement:** This presentation will impact the forensic science community by increasing understanding of trends in juvenile deaths and demonstrating the prevalence of traumatic deaths in juveniles. It is important to spread awareness on some of the major issues facing the juvenile population in the hopes that more intervention can be established.

Using death records from the Harris County Institute of Forensic Sciences (HCIFS) located in Houston, TX, a retrospective review was performed of juvenile deaths (ages 6–17) from 2015–2021 in which an examination was performed. HCIFS performs death investigations in accordance with the Texas Code of Criminal Procedures 49.25 that stipulates the types of deaths that are reportable to HCIFS, including sudden, traumatic, or unexplained deaths of any age. Harris County is the third-largest county in the United States and provides a large and diverse population from which to analyze data.

From 2015 to 2021, there were 796 juvenile deaths that received examinations at HCIFS. From this population, 25.5% of these deaths were classified as Homicide and with the manner of death, Suicide, making up 24.1%. General accidental deaths made up 7.3% of the population, motor vehicle accident (MVA) -related deaths made up 19.5%, and natural deaths comprised 15.8% of the population. Ages 15–17 made up 55.8% of the total population. Of note, 89.2% of the homicides have a cause of death relating to a gunshot wound(s).

The percentage of deaths for each manner of classification remained consistent from 2015 through March 2020 and after the start of the COVID-19 pandemic from March 2020 through 2021. Firearm-related deaths contributed to the majority of juvenile deaths from 2015–2021. Evaluation of this data can potentially provide insight on types of education and awareness that could be provided to the juvenile population of Harris County and what geographic areas of the county may benefit from increased education.

We will be analyzing geographic trends in the juvenile deaths by zip code and school district in an effort to identify areas that could benefit from increased educational awareness. The education can be targeted based on the types of deaths that are predominantly occurring in specific areas. MVA-related deaths will be examined to look at whether the decedents were a driver versus passenger and if they were of age to have a driver's license. This information is another way that educational awareness can be targeted if specific areas of concern are identified.

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**Juvenile Deaths; Harris County; Retrospective Review**

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**F77 Knowledge, Awareness, and Attitudes Toward Child Abuse and Pediatric Patient Management During the COVID-19 Pandemic Among Students of Medicine and Surgery: A Key to Improving Forensic Medicine Courses**

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**Learning Objective:** After attending this presentation, attendees will understand the knowledge about, awareness of, and attitudes toward the medicolegal relevance of child abuse and management of pediatric patients during the COVID-19 pandemic in a cohort of medicine and surgery students.

**Impact Statement:** This presentation will have an impact on the forensic science community as it could serve as a key to understanding what the strengths and weaknesses in forensic science courses during undergraduate courses are and to implement the knowledge of future doctors on these sensitive topics.

**Background:** Following the outbreak of the COVID-19 pandemic in China, the World Health Organization declared the state of pandemic.<sup>1</sup> For this reason, many countries, such as Italy, have adopted measures aimed at limiting the spread of the virus.<sup>2-4</sup> Many concerns have been raised about how these measures, particularly the lockdown, may have affected the health, especially the mental health, of the most vulnerable individuals, including children.

In fact, governments have taken steps to lessen the economic effects of the COVID-19 pandemic, but there has not been an adequate response to reduce domestic violence, child abuse, and mental health deterioration.<sup>5</sup> Added to this is the impact on the mental health of health care professionals who have been on the frontlines during the pandemic and the constant risk of professional liability.<sup>6</sup>

**Purpose:** The aim of this study was to assess how a cohort of medical students perceived, were aware of, and felt about the medicolegal relevance of these themes in the context of the COVID-19 pandemic, with a focus on child abuse and the management of pediatric patients.

**Methods:** A cross-sectional, web-based survey was conducted through an anonymous questionnaire on the personal websites of Università Cattolica del Sacro Cuore medical students. The questionnaire consisted of various sections covering the following aspects: demographic data (gender, age, course year, and region of origin), knowledge and awareness of child abuse, and, finally, the aspects of patient management during the COVID-19 pandemic. In total, the questionnaire comprised 23 questions. Students were asked to answer the questions using both binary answers and a 5-point Likert scale.

**Results:** The study included 1,166 participants, the majority of whom were experienced on the topics of child abuse and defensive medicine; only a small percentage were aware of the government's efforts to prevent child abuse and safeguard vaccination physicians. Moreover, there was no agreement on the use of telemedicine for non-serious pediatric patients or on the consequences it may have on their health. Finally, the detrimental impacts of lockdown on children's mental health have been a major worry.

**Conclusion:** Knowledge of these themes is mainly implemented by deepening these concepts during the undergraduate studies since a high level of knowledge on child maltreatment and on the management of COVID-19 pandemic was significantly associated with clinical years of course.

Specific multidisciplinary seminars held by forensic scientists, pediatricians, and social workers analyzing legislative protections concerning minors and those concerning vaccination doctors and telemedicine should be included in the study plan to raise awareness of these concepts among future doctors.

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**Child Abuse; Medical Liability; Medical Education**

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**F78 The Italian Missing Children Association Realizing Age Progression for Missing Children Organization:  
The Collaboration With Missing Child Kenya**

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**Learning Objective:** Through this presentation, it will be possible to communicate the importance of applying the age progression technique for the creation of updated identikit of missing persons.

**Impact Statement:** Thanks to the practical application of the age progression technique, it will be possible to show how this technique could have been of support to the Missing Child Kenya organization.

Age progression is a technique that has been supporting the search for missing children around the world for many years and is used by many international organizations. The age progression technique makes it possible to update the identification photos of individuals who have been missing for years. This method is based on the study of the variation of facial morphology and of the dynamics of growth and change of facial features over time. The changes in the distribution of facial fat and the tone of the cartilage are elements that allow us to make realistic predictions of the appearance of a face after a long time. Age progression is supported not only by these elements. Similar features between close family members, such as parents and siblings, can be of great help. Therefore the photos of the missing persons and family members are used to create an age progression, making the new identikit very accurate, albeit considering a margin of error.

The Italian Missing Children association provides an age progression service, created thanks to the support of forensic anthropology. Its collaboration with the Missing Child Kenya organization made it possible to disseminate many photos updated according to the current appearance of missing children. With the activation of a protocol consisting of guidelines and a form for the request for age progression by family members, the Italian Missing Children has made it possible to start a collaboration with Missing Child Kenya, which has supported many families in search of their loved missing ones. This presentation describes the results obtained so far.

Social media, such as Facebook®, Twitter®, Instagram®, and YouTube® have been exploited, thanks to video documentaries made by Missing Child Kenya. Thanks to these means, it was possible to continue the spread of age progression during the COVID-19 quarantines, thus keeping the population active in the search for the missing. The collaboration with Missing Child Kenya represents a practical example of the application of age progression. This technique is able to catalyze the search process for missing children and potentially favor their finding. For this reason, it must be known and spread internationally.

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**Forensic Anthropology; Age Progression; Missing Children**

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**F79 Sexual Violence Among Female Youth Gang Members in Our Communities**

*Cliff Akiyama, MPH, MA\*, Akiyama and Associates, LLC, Philadelphia, PA*

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**Learning Objective:** The purpose of this presentation is to present timely data on the dramatic increase nationwide of sexual violence and female youth gangs, an often-difficult population to assess. This presentation is critical for death investigators, forensic nurses, law enforcement, behavioral health, and those in charge of the direct care of these children.

**Impact Statement:** This presentation will impact the forensic science community by presenting timely data that is so often overlooked and, in many cities, where there are female gangs present, a total devoid of the data. This presentation will help solve that dilemma, so that we may open our eyes and hearts to this growing problem in our communities.

Youth gangs throughout the United States continue to rise as well as 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. There are over 28,500 gangs in the United States with a total gang membership of 850,000, according to the Office of Juvenile Justice and Delinquency Prevention of the United States Department of Justice. Often overlooked in gangs is the pervasive bondage of sexual violence among female youth gang members. In Los Angeles County, CA, alone, there are currently 12,000 female gang members, while in Philadelphia County, PA, there are 960 female gang members. Demographics show across both counties a female gang member average age of 15 with a range of 8–22 years. The author interviewed over 500 female gang members in the streets, jails, and juvenile halls, using a target questionnaire in Los Angeles and Philadelphia.

The author found that over 93% of all female youth gang members are “sexed in,” as an initiation tactic of getting into a youth gang. Getting “sexed in” the gang means that the prospective female gang member has to have sexual intercourse with all of the male gang members in that particular gang set or clique.

Moreover, the study found that 56% of the female gang members interviewed have a history of child sexual abuse. Unfortunately, female gang members are least likely to report rape victimization due to fear of death by the male gang members. The study also found that of the female gang members that are physically and/or sexually abused by the male gang members within the gang, 97% have never utilized any rape or physical abuse services such as a rape crisis center or shelter within their communities, while 65% of the female gang members do not even know about the services available to them.

Furthermore, with the technical assistance of the Anti-Violence Partnership of Philadelphia (AVP), an anti-violence and trauma therapy non-profit organization, this study identified 12 distinct barriers to disclosure and service utilization of female youth gang members who experience sexual and physical violence across their lifespan (i.e., immigration issues, racism, depression, anxiety, fear of death, shame, isolation, age, lack of health insurance, lack of child care, lack of affordable housing, lack of independent income, lack of support from the family/community, cultural fluency, and just not knowing the available resources surrounding intimate partner violence).

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**Youth Gangs; Sexual Violence; Female Violence**

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## **F80     Are Women Always Right?**

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**WITHDRAWN**

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**F81 The Multidisciplinary Role for the Multiform Issues of Medicolegal Assessment of Physical Abuses in Asylum Seekers**

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**Learning Objective:** After attending this presentation, attendees will better understand the importance of the role of a multidisciplinary approach in the medicolegal assessment of asylum seekers who claim to be victims of alleged torture.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of the relevance of a multidisciplinary approach in cases involving the assessment of alleged victims of torture according to the Istanbul Protocol.

In the increase of migratory flows, forensic assessment of physical violence plays an essential part for the proper functioning of humanitarian procedures of recognition of asylum. For this reason, many professional figures dealing with vulnerable migrants must be prepared to respond to the specific medical and legal needs, particularly for assessment of asylum seekers' condition. Among professionals dealing with vulnerable migrants, clinical forensic practitioners help in identifying injuries related to physical abuses and relate them to the victims' narrative.

The Istanbul Protocol has delineated the guidelines for the correct management of physical examination and methods of assigning the degrees of consistency in cases of torture. In ordinary practice, the assessment of the compatibility of physical evidence with alleged torture is very challenging for the clinical forensic practitioner. Most cases exhibit highly heterogeneous lesions in terms of morphology (margins, shape, size, color, texture, etc.) with no specific features.

The present study focuses on the potential role of a multidisciplinary approach on the final assessment of scars and disability and what effects were exerted on the medicolegal evaluation of asylum seekers who claim to suffer from physical abuse. The University Institute of Legal Medicine together with the Municipality of Milan and other institutions and organizations, actively collaborates by performing clinical forensic evaluations of scars and/or lesions in those asylum applicants who have declared to be a victim of physical violence, in order to determine the compatibility between allegations of physical violence and evidence of healed wounds.

A retrospective study was conducted on all the cases of asylum seekers evaluated at the University Institute of Legal Medicine from 2008 to 2020 to investigate in which cases further investigations were needed and what impacts this decision has. Of all the examinations, 92 asylum seekers were subjected to further forensic instrumental examinations (80.4%) and specialized medical consultations (33.7%). The most investigated type of injuries was due to blunt force (72.8%). Radiography is the most widely used instrumental exam to investigate injuries (90%) and the most frequent consultation was odontological (17.4%), followed by urological/gynecological (6.5%). In 62 cases (47.7%), the presence of scars was confirmed by the identification of further skeletal and visceral lesions.

The present research highlights how the adoption of a multidisciplinary approach, in such delicate cases, facilitated and improved clinical forensic evaluation, thereby reducing errors when assessing the presence of confounding factors as the ones regarding healing processes. Therefore, the systematic introduction of diagnostic analyses and the increase of consultations necessarily lead to higher costs and take longer to complete the final report.

In light of the recent new version of Istanbul Protocol, in which it has been made explicit for the clinicians to provide an interpretation as to the probable relationship of the physical and psychological findings to possible torture or ill-treatment, the need for an extensive approach becomes extremely obvious. Training and knowledge of the Istanbul Protocol by as many clinical forensic medicine practitioners as possible is just the tip of the iceberg. Different professionals ought to work together to correctly assess every single case and need to be properly prepared.

For the future, an increasing number of these specialized supports is expected in clinical forensic medicine in order to ensure relevant tools to practitioners for the assessment of physical violence. In consideration of the extreme sensitivity of the area of application of the Istanbul Protocol, the time is ripe for an attempt to standardize the evaluation and to make the assessment as reproducible as possible.

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**Clinical Forensic Medicine; Physical Assessment; Istanbul Protocol**



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**F82 The Management of Dead Migrants: Standardization of Data Collected and Centralization of Databases  
Toward Helping Families Find Their Loved Ones**

*Sherry Fox, PhD\*, International Committee of the Red Cross, Ypsilanti, MI; Admir Jugo, PhD, International Committee of the Red Cross, Tunis, TUNISIA*

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**Learning Objective:** After attending this presentation, attendees will have gained an appreciation for the magnitude of migrant deaths and how the identification process could be enhanced through both standardization of data collected by medicolegal systems and centralized databases.

**Impact Statement:** This presentation will impact the forensic sciences by showing how they can contribute to helping solve humanitarian problems by: ensuring that the dignity of the deceased and the dignity of the bereaved are preserved; restoring identities to the unidentified; providing families of migrants with information on their loved ones; and returning those identified to their families. It is hoped that the forensic science community will take steps to help work toward recovering and identifying migrant deaths in a dignified manner.

Humanity has “survived and thrived because we have never stopped moving.”<sup>1</sup> The largest number of people in history, 281 million globally, is migrating today.<sup>2</sup> Deaths occur during these journeys and are often underreported. Since 2014, around 50,000 migrants have died around the world, frequently with bodies neither being recovered nor identified.<sup>3</sup> Families of missing migrants may thus be dealing with the ambiguous loss of their loved ones as they search for answers to their fates and the whereabouts of their loved ones.

Drowning is the leading cause of recorded deaths of migrants, exceeding the combined causes of deaths across the globe.<sup>4</sup> The Mediterranean Sea is the deadliest place on the planet for migrants as they seek access to Europe from North Africa and West Asia. Migrants also take precarious and dangerous routes through forests, deserts, and across rivers, leading to deaths in less-traveled and poorly accessed areas. Bodies are therefore often not recovered for extended periods, if at all, or only partial remains are recovered. There are many issues surrounding migrant deaths that complicate the identification of their remains. The methods for recovery, documentation, examination, and identification of remains vary by jurisdictions around the globe.

Difficulties arise within the practice of different medicolegal systems within and between different countries. Lack of diplomatic relations between countries of origin and death is, unfortunately, not an uncommon fact, again complicating the identification process. There are contexts in which little-to-no attempt is made to autopsy or identify dead migrants, and differences in language, religion, and culture, including burial practice, can additionally negatively impact the identification process and handling of the remains in a dignified manner.

In the absence of regional mechanisms in much of the world, Red Cross/Red Crescent national society chapters may collect key information about the missing, but there is no systematic approach to linking this with the data on unidentified deceased migrants that various authorities may be responsible for managing. A global approach to the collection of standardized data through the ICRC’s Family Links Network, along with centralization of databases could help families find their loved ones.

This presentation sheds light on the often-overlooked deaths of migrants and aims toward the reduction of ambiguous loss for families by recommending that data collected by medicolegal systems in migrant deaths is standardized and that databases of dead migrants are centralized, at least within countries. It is hoped that attendees will gain an appreciation for the magnitude of migrant deaths and take steps to help work toward recovering and identifying migrant deaths in a dignified manner.

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**Migrants; Management of the Dead; Regional Mechanisms**

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**F83 A Novel Surveillance Method for Suspected Drug-Related Deaths**

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**Learning Objective:** The objective of this presentation is to highlight the quality assurance process and data precision pertaining to how New Jersey accounts for Drug-Related Mortality statistics by way of the New Jersey Office of the Chief State Medical Examiner.

**Impact Statement:** As daily mortality totals rapidly increase, and new lethal substances emerge in the drug environment, there will be imminent public health implications for society. It is crucial to broadcast suspected Drug-Related Death Data in real-time for public knowledge to combat illicit opioid usage in hot spots and susceptible areas. Utilizing a multi-step verification process to identify drug toxicity in decedents not only ensures accurate data, but allows for interprofessional dynamics to strengthen between investigators, medical professionals, and data/IT personnel.

Opioids provide therapeutic relief to combat physical discomfort, but they have been abused for decades as the human mind is made to believe the substance is necessary for survival.<sup>1</sup> The opioid epidemic has severely taken over the United States notably for the past 20 years.<sup>1</sup> In turn, this has fueled a demand for faster and more detailed drug-related death statistics while for various reasons Medical Examiner (ME) and coroner's offices struggle to meet these demands. At best, it can take 4–6 weeks to complete autopsy reports, which may still be too late to address critical situations requiring a more immediate response. New Jersey is a county-based ME system with the Office of the Chief State Medical Examiner (OCSME) overseeing all ten offices in the state. The OCSME maintains a statewide database and mandates that all ME cases must be recorded as soon as they are reported. Effective January 2018, all offices were notified of changes to the database wherein a check box was added for suspected drug-related deaths. After reviewing the history, scene examination, and autopsy findings, the pathologist would determine whether the death was suspected to be drug-related, and the case classified accordingly. This allows the OCSME to monitor such deaths on a weekly basis in near real-time. Analysis of confirmed cases revealed a noteworthy positive predictive value (PPV) between the numbers reported as suspected cases.

The OCSME database (CMS) tracks cases from the time they are reported to the stage of completion, with modules and tabs for each section within the office that handles the cases. In the Investigations Module, a check box was added which identifies Suspected Drug Toxicity deaths. For accepted cases, investigators are required to check off “Yes” or “No,” indicating whether the death is a Suspected Drug Toxicity case. Indicators of a Suspected Drug Toxicity death include:

- History of illicit/ prescription drug abuse
- Drug paraphernalia on scene
- Presence of prescription or over-the-counter medication
- Foam-type substance at the mouth or nose
- Presence of needle track marks on the body

This field is only meant as an indicator of suspected drug-related death and can be changed by the pathologist upon completion of the autopsy.

A Drug Toxicity field was also added in the Pathology Module, next to the Cause/Manner of Death. The information in the “Yes” or “No” check box will be copied from the Investigations module, allowing the doctor to view what the investigator had checked off. Once the ME is ready to amend the cause of death and the toxicology is available, the Pending checkbox needs to be unchecked, and the Cause of Death entered. At this point, the ME must indicate whether the death is in fact a suspected Drug Toxicity death by checking “Yes” or “No,” then checking “Confirmed.”

The purpose of this new field is to identify potential drug-related deaths in a timely fashion, understanding that the final number of confirmed drug-related deaths will be different from the initial number.

An examination of data comprising suspected/confirmed cases for 2018–2020 showed a close correlation. The probability of a case being finalized as a drug-related death if the decedent was initially flagged in CMS as a suspected drug overdose is significantly predictive, yielding about a 93–96% PPV yearly. A limitation of this surveillance method is the time at which toxicology reports are made available. Limited information on specific drugs involved are provided, so initial information is based on Suspected Drug Toxicity. Further evaluation of the checkboxes will allow for refinement of prediction at the case level highlighting predictive value differences among various substances.

**Reference:**

- <sup>1</sup>. Centers for Disease Control and Prevention, *Understanding the Epidemic*, 17 March 2021. [Online]. Available: <https://www.cdc.gov/opioids/basics/epidemic.html>.

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**Drug-Related; Surveillance; Deaths**

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**F84 The Harassment and Bullying of Forensic Scientists: The Impact of Gender, Organizational Hierarchy, and Civil Servant Status**

*Rana DellaRocco, BS, MS, MPA\*, Baltimore Police Department, Reisterstown, MD; Ryan Krone, PhD, Elite Research LLC, Dallas, TX; Nancy Wayne, PhD, University of California-Los Angeles, Ann Arbor, MI*

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**Learning Objective:** In general, women report higher levels of harassment at work than their male counterparts. In forensic science, women are typically the majority. This study investigates, for the first time, the extent and nature of harassment and bullying in forensic science laboratories associated with law enforcement. The results show that civil servant status (sworn vs. non-sworn) did not have an impact, that typically harassment comes from those higher in authority except in physical harassment, and that harassment had an impact on the intention to leave an agency. Mitigation of the intent to leave was seen by employees who reported high work performance despite harassment. Employees who were categorized as student, administrative, or "other" report much higher levels of personal harassment from sworn officers than lab techs or managers while levels of physical harassment were higher coming from subordinates among non-sworn scientists. The goal of this presentation is to discuss how this knowledge can help managers and employers of forensic scientists identify those most likely to experience harassment, prevent decreases in job satisfaction, and help address employee retention.

**Impact Statement:** The investment in hiring, training, and retaining high-quality forensic scientists is costly to law enforcement agencies, many of whom are struggling with resource issues. The costs of training programs, especially those conducted by law enforcement agencies from college to competency, can be in the thousands or hundreds of thousands of dollars. In addition to these costs, lost productivity due to the movement of fully competent case workers to train new hires can set back workflows and create unwanted backlogs. There is a direct link between the harassment and an employee's intention to leave an agency. This presentation will impact the forensic science community by knowing who in a forensic service may be most likely to experience harassment and how one way to mitigate the resultant intention to leave can be beneficial in employee satisfaction and retention, potentially saving an agency thousands of dollars and lost casework.

Uniquely among STEM fields, women forensic scientists are usually the majority in their workplaces. Although women report higher levels of harassment in general than their male counterparts, we are unaware of published studies investigating harassment and bullying in the field of forensic sciences. The purpose of this proposed study is to investigate, for the first time, the extent and nature of harassment and bullying in forensic science laboratories associated with law enforcement. We hypothesized that the most common harassment will be that of male sworn civil servants working in forensics targeting women non-sworn forensic scientists, regardless of their authority in the organization. A secondary aim of this study was to assess whether levels of harassment were associated with workplace performance, absenteeism, and intention to leave. We hypothesized that harassment is positively associated with intention to leave and absenteeism and negatively associated with workplace performance.

This work presents an analysis of self-reported harassment and bullying by forensic scientists across the United States. There were three types of harassment investigated: work performance-related, interpersonal, and physical intimidation. There were three categories of respondents: lab technician, manager/supervisor, and "Other" that included students, administrative support personnel, assistants, and consultants. Survey responses were used to construct harassment subscales and to measure intention to leave, work performance, and days absent, which were evaluated using generalized linear regression analysis. We found that levels and types of harassment varied depending upon the inter-relationship between the harassed and the harasser. There was no significant impact of gender on any of the three types of harassment investigated. Additional findings indicated that higher levels of harassment were reported by participants coming from harassers above them in authority, except in cases of physical harassment where, surprisingly, levels of harassment were higher coming from subordinates compared to peers or higher authorities. Participants that were classified as "Other" experienced higher levels of personal harassment from sworn harassers as compared to lab technicians and managers/supervisors. Conversely, respondents from the "Other" category experienced higher levels of physical harassment from non-sworn harassers compared to lab technicians and manager/supervisors.

The results also showed that work-related harassment and bullying is associated with a decrease in work performance and increased intention of forensic scientists to leave their agencies. Mediation analysis found that work-related harassment was associated with intention to leave by causing lower work performance. This intention to leave can be mitigated by the employee maintaining high work performance despite the harassment. Work-related harassment also had a positive and significant direct effect on intention to leave. According to the Equal Opportunity Employment Commissions' Task Force on Harassment in the Workplace, the least common responses to harassment are to report it or to file a formal complaint to management. The information in this study can be valuable to forensic scientist employers seeking to identify those most likely to experience harassment and bullying so that agencies may intervene, prevent decreases in productivity and job satisfaction, and to address employee retention.

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**Harassment; Workplace Bullying; Employee Retention**

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**F85 Offender Motivation, Victim Vulnerability, and Systemic Bias: Understanding Patterns in Transgender Homicide Victimization Data**

*Robert Eckstein, PsyD\*, University of New Hampshire, Durham, NH; Amy Michael, PhD\*, University of New Hampshire, Durham, NH*

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**Learning Objective:** After attending this presentation, attendees will better understand the sociocultural dynamics that underlie homicide victimization of transgender decedents, the implications for investigating such cases, and the potential for public health-related preventative measures.

**Impact Statement:** This presentation proposes that individual risk factors related to offenders and victims conspire with more systemic sociocultural factors, adjacent to the offense, that make transgender people especially vulnerable to homicide.

Transgender people are disproportionately victims of violent crime, with The American Medical Association and the Department of Justice declaring homicides against transgender people an epidemic and crisis, respectively.<sup>1</sup> Transgender people experience several risk factors for homicide vulnerability at higher rates than the general population including polyvictimization, housing insecurity, rejection from family, and intimate partner violence.<sup>2</sup> The current study examines instances of transgender homicide victimization ( $n=205$ ) to identify patterns and factors commonly found across cases. This includes victim-related (i.e., race, age, gender identity), offender-related (i.e., stated motivation, relationship with victim), case related (i.e., evidence of overkill, manner of death), and sociocultural (i.e., proximity to sex work, homelessness) variables. Data includes cases from 1988–2021 culled from news articles, court documents, Department of Corrections documents, and where possible, primary case records. Each case was coded and analyzed. A series of correlational analyses were conducted for the quantitative data. For the qualitative data, a grounded theory approach was used to identify major themes that emerged across cases whose results indicate that, in general, these cases are not homogeneous. Instead, there is significant variance in terms of relationship to the offender, offender motivation, and manner of death, among other variables.

It is argued that transgender people are disproportionately murdered at higher rates than cisgender people because they are vulnerable in multiple, often intersecting, ways: hate crime victimization, intimate partner violence, sexual violence, and situational violence (i.e., robbery) that arises from being oppressed, marginalized, and excluded from essential community resources. Consistent with models of structural violence, these risk factors are compounded when other evidence of bias is present. This confirms earlier studies that have found that transgender women of color are significantly over-represented in homicide rates when compared to transgender men and white transgender people.<sup>3</sup>

This presentation will provide value to professionals who investigate homicides (e.g., law enforcement, death investigators, forensic anthropologists) and behavioral scientists who research homicide patterns. We provide a framework outlining how public health advocacy can be used to mitigate many of the risk factors present in these cases.

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**Transgender; Homicide; Structural Violence**

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**F86 An Ounce of Prevention: A Simple and Practical Tool for Mitigating Cognitive Bias in Forensic Decisions**

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**Learning Objective:** The free information management toolkit described in this presentation will be introduced and attendees will learn to use this toolkit as a training tool and as a practical solution for analysts and laboratories interested in implementing the Linear Sequential Unmasking-Expanded.<sup>1</sup>

**Impact Statement:** This freely available tool can guide analysts in their evaluation of case materials, encourage transparent and reliable decision-making, and serve as a training tool for forensic analysts and other legal professionals seeking guidance on judgment and decision-making processes in forensic analysis. In addition, by providing a framework for documenting and reporting their use of case information, this toolkit can protect analysts who are inadvertently exposed to task irrelevant information during their evaluation of evidence and who might face scrutiny while giving testimony.

Forensic analysts receive information from a multitude of sources, and every piece of information available to an analyst can influence their perception, interpretation, and evaluation of the evidence. Some of the information analysts receive is directly relevant to their task (e.g., trace evidence from the crime scene), some information available to them might be only tangentially related (e.g., where the trace was found at the scene), and other information might be totally irrelevant and potentially biasing (e.g., the suspect's race or criminal history).<sup>2</sup> Cognitive bias—the effect that suggestive information has on decisions—occurs when preexisting beliefs, expectations, motives, or the situational context influences how people collect, perceive, or interpret information.<sup>3,4</sup> For example, two competent examiners that are given different case information might form different, or even contradictory, opinions about the same evidence.<sup>5</sup>

Information that can influence forensic analysts can take on many forms, but standardized information management frameworks can help analysts manage the effect of that information.<sup>1</sup> Linear Sequential Unmasking–Expanded (LSU-E) is an example of a research-based information management framework designed specifically for use in forensic analyses.<sup>1</sup> Linear Sequential Unmasking–Expanded emphasizes the need to consider the relevance, objectivity, and biasing power of information available to analysts and identify situations where additional precautions are necessary.<sup>1</sup> LSU was first proposed almost a decade ago, however, and laboratories have been slow to adopt this approach, perhaps due to a lack of practical, concrete guidance from researchers.

A free toolkit was recently published that bridges the gap between this theoretical framework and forensic practice.<sup>6</sup> The toolkit centers around a simple worksheet that steps the analyst through the LSU-E approach but also includes training materials and other supporting documents. Thus, the toolkit can be used as a cognitive bias training exercise or can be incorporated into analysts' workflow to help prevent cognitive bias. The worksheet also allows analysts to document access to information and mitigation strategies in real time. Documenting this process can protect analysts who testify about their findings in court as they can show when and why they used cognitive bias mitigation strategies and when task-irrelevant information was available to them. As a result, this tool could also be used to help factfinders evaluate the reliability of forensic evidence in criminal trials.

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**Cognitive Bias; Bias Mitigation; Toolkit**



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**F87 Criminology Skills—Between Ethical Questions And Responsibilities**

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**Learning Objective:** After attending the presentation, attendees will understand as the ethical competency is substantial in the profession of criminologist and how the thought on this competency can represent an increasingly authoritative means to give meaning to the experience AND verify the validity of what has been learned with respect to the congruence and effectiveness of the actions

**Impact Statement:** This report presents a reflection on how the possession of ethical competency is fundamental for the exercise of the profession of criminologist and how the possession of ethical skills can condition the responsibility of the professional and the decision making of the criminologist himself.

Who is the criminologist and what is the image that he detects of himself? The incipit can certainly be traced back to the enucleation of responsibility in the broad sense, which fills with contents, every single action, first thought out, then acted out, which identifies a “reflective professional” and who bases his work on the elaboration of both a theoretical reflection, both on the theory of moral obligation.<sup>1,2</sup> Responsibility can be defined as an ability to answer for one’s work or commitment.<sup>3</sup> It can also be interpreted and experienced as a form of attention that manifests itself toward oneself and toward others; attention also paid to the mistakes made, which must be recognized and analyzed, with the aim of allowing more responsible action. Being responsible not only means responding to oneself, but also responding to the other, in a progressive interweaving within specific dimensions: that of subjectivity, otherness, and sociability.<sup>3</sup>

There are elements of sectoriality, which perimeter the specific professional of the criminologist and, consequently the sense of responsibility corroborated by it, which take the form of a *modus operandi*, the concrete realization of a professional ethics, or an ethics of responsibility.

The interpretation of the concept of responsibility is intrinsically connected to that of ethics. Ethics, in a general sense, mutates models of practical philosophy, which ascribe to the vision of the human being, different interpretations, based on specific paradigms and metaparadigms, which guide the conduct of both the individual and the specific professional family.<sup>4</sup> The possession of ethical skills and, therefore, ethical reflection for the criminologist should represent not only a discourse of legitimation and reasoning on ideal values, but favor a constant critical weighting on the current meanings attributed to the professional practices put in place.

Furthermore, ethics can constitute for the criminologist the device connected to the normative dimension of individual and collective action.<sup>4</sup> In fact, through ethical reflection it is possible to analyze the different orientations that allow to formulate conscious choices, which can be declined positively or negatively, through the assumption of specific responsibilities of the behaviors acted both towards oneself and towards the community, favoring excessively the acquisition of ethical skills also towards the guilty subjects, for example with respect to the reasons and reasons inherent in the life choices made by the latter.<sup>3</sup> The space of ethics and the development of this competency cannot be exhausted within the limiting field of professional ethics or the legal moment, but can find application conditions if the subject actively intervenes in the aforementioned areas, putting in place motivations, *modus operandi* specific, which allow a specific professional intervention to be carried out in practice.

The ethical competency of the criminologist also refers to the scientific ethics of criminal phenomenology.<sup>5</sup> What concerns the criminologist is the study, analysis, and understanding of the problem of crime, of the individual, personal and subjective problem of crime, for which the consideration of the epistemological approach becomes fundamental, claimed both as knowledge functional to each discipline, and as a technical-specialist competency, also closely connected to research and application areas of practical life.<sup>2</sup> This report presents a reflection on how the possession of ethical competency is fundamental for the exercise of the profession of criminologist.<sup>6</sup>

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**Ethical Competency; Responsibility; Criminology**

**F88    An Update on Ethics Committee Approval Reporting in Cadaveric-Based Research Publication: A Five-Year Study**

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**WITHDRAWN**



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**Reporting of Ethics Committee Approval; Research on Dead; Research Ethics**

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**F89 Using the Science Teachers' Assessment Literacy Model as a Framework for Implementing Specifications Grading in a Senior-Level Forensic Laboratory Quality-Assurance and Professional Practices Course**

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**Learning Objective:** After this presentation, attendees will understand how the science teachers' assessment literacy model can be used as a framework for course design and course redesign. A case study will also be presented, whereby the model was used to redesign a course and adopt the specifications grading assessment model.

**Impact Statement:** This presentation will impact the forensic science community by informing forensic science educators in how to use the science teachers' assessment literacy model to design assessment-centered learning environments.

The science teachers' assessment literacy model (STALM) outlines the types of knowledge and skills that teachers need to create an assessment-centered learning environment.<sup>1</sup> It connects a teacher's views of learning and the assessment values and principles with the knowledge of assessment purposes, the knowledge of what to assess, the knowledge of assessment strategies, and the knowledge of assessment interpretation and action taking. The science teachers' assessment literacy model is a particularly useful model for designing assessment-centered learning environments.

This presentation will give an overview of the model and describe how it can be used as a framework for course design. A case study will be presented, whereby the STALM was used as a framework to redesign a senior-level online forensic laboratory quality assurance and professional practices course. The course was redesigned to use the specifications grading assessment model. Specifications grading is an assessment model that eliminates partial credit, is focused on mastery of learning outcomes, and gives students the agency to make decisions about the level of mastery (and grades) they want to achieve.<sup>2</sup> Course redesign will be described regarding how specifications grading was implemented in an existing course curriculum without substantial changes to established assessments or learning outcomes. Even though the instructional content, learning outcomes, and assessments strategies remained the same as in previous offerings of the course, the evaluation of the assessments and the grading scheme were modified to align with the specifications grading model. In particular, since partial credit for assignments was eliminated, the grading criteria and rubrics for each assignment was modified to include more elaboration regarding the requirements to earn master of the assessment. To reduce grading time and streamline feedback, detailed grading rubrics using the learning management system were used. The course grading scheme was modified so that assessments were grouped into key course concepts (referred to as "big ideas") that highlighted the central themes of the curriculum: quality control processes, statistical methods for quality control, quality assurance processes and accreditation, professional communication, and resume writing and job searching.

This presentation will also include reflections from the instructor regarding the perceptions of grading time, challenges for implementation, and plans for future course delivery. Student perception of the learning environment and grading system will also be shared, as well as a comparison of student performance in the redesigned course using specifications grading and prior course offerings without specifications grading.

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**Forensic Science Education; Assessment Literacy; Specifications Grading**

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**F90 The National Institute of Justice's Forensic Science Strategic Research Plan**

*Lucas Zarwell, MFS\*, National Institute of Justice, Washington, DC*

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**Learning Objective:** This presentation will introduce and discuss NIJ's Forensic Science Strategic Research Plan, including the plan's origins and formation and how the plan influences NIJ's actions.<sup>1</sup> Furthermore, the presentation will discuss how crime laboratory leaders, researchers, and policymakers can use NIJ's Forensic Science Strategic Research Plan to communicate the importance of forensic science research in addressing challenges and needs faced by the community.

**Impact Statement:** This presentation will impact the forensic science community by describing how the goal is to promote research from within the community, leading to stronger technologies, better efficiencies, and solving forensic sciences' most difficult challenges.

The National Institute of Justice's (NIJ) Forensic Science Strategic Research Plan is the research, development, and evaluation agency of the United States Department of Justice, dedicated to improving knowledge and understanding of crime and justice issues through science. As the lead federal agency for forensic science research and development, NIJ, through the Office of Investigative and Forensic Sciences (OIFS), sponsors extramural research projects across the spectrum of forensic science disciplines, coordinates closely with the stakeholder community to identify needs and develop resources, and facilitates technology transfer and implementation of emerging technologies into forensic practice.

NIJ's Forensic Science Strategic Research includes a top-level summary, shared goals, and five main strategic priorities. Each priority area will be discussed and include brief examples of current NIJ research aligned with the objectives under each focus. The presentation aims to familiarize practitioners and researchers with the strategic plan and encourage the development of projects associated with the plan's goals and objectives. Finally, NIJ will briefly present program updates, including an overview of recent research awards. This brief update will include awards made in 2022 and an overview of current portfolios. In addition, this update includes a breakdown of awards by discipline and award amount.

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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**Strategic; NIJ; Grant Funding**

**F91 A Cinematic Death Investigation**

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**WITHDRAWN**

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**F92 Improving Crime Scene Investigator (CSI) Response: An Early Roadmap for the Increased Quality and Effectiveness of Crime Scene Investigations**

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**Learning Objective:** Attendees will be introduced to the results of a completed study that presents: (1) common or frequent factors that affect crime scene staffing or response, (2) how crime scene response affects the quality and effectiveness of a crime scene investigation, (3) where in an investigation an investigator feels more assistance may be needed, (4) what that assistance in an investigation may look like, and (5) how that assistance reduces the burden on the quality and effectiveness of a crime scene investigation. This presentation also contextualizes the landscape of crime scene investigation within the greater law enforcement framework and highlights how an incomplete picture of employment numbers reduces our ability as a discipline to make crime scene staffing and response recommendations.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with data on crime scene investigation issues that were largely anecdotal. The data now give us a point of reference in conversations about increased overall staffing, improved crime scene response, and how to improve investigative conditions for crime scene investigators.

The Crime Scene Investigation Subcommittee within the Organization of Scientific Area Committees (OSAC) for Forensic Science has highlighted the need for empirical research on adequate crime scene investigator (CSI) staffing levels via crime scene response. OSAC, policymakers, and fellow professionals cannot make appropriate recommendations to increase the quality and effectiveness of an investigation with staffing or crime scene response because of this data gap.

Other related professions benefit from full demographic studies and censuses that detail the tasks and services performed, which in turn make things like policymaking, budgeting, and improvements easier to do. Crime scene investigation is unique in that it exists inside and outside of the sphere or law enforcement. It is a mixture of civilian and sworn personnel, hybridized in some areas, and at all levels of government; and can be performed by part-time and full-time personnel, who may or may not be crime laboratory personnel who were cross-trained or are responsible for all the crime scene response of their employer. No matter the level or layer of employment, CSIs face the same tasks at a scene and only get one chance to do things correctly. That is compounded by the unique pressures they face from their employer or agency. That again is also compounded by work factors outside their control.

A study was designed to investigate what CSIs considered adequate response via a survey with questions that targeted: the factors affecting the quality and effectiveness of an investigation, what is feasible to handle alone without sacrificing quality and effectiveness, when extra staffing is needed, the barriers to hiring more personnel, and what the amount of extra staffing needed is within the focus established by the OSAC CSI Subcommittee. The survey was taken by both investigators and managers across many levels of government and in different areas of the United States.

With few exceptions, there was consensus between investigator and manager responses throughout the study. The study established: which investigations are more resource intensive; which types of scenes are more resource intensive; which tasks increase the complexity and, in turn, increase the need for improved response; how increased task loads affect investigations; where the stresses of an investigation affect the quality and effectiveness of an investigation; and early answers to what may be preventing increased staffing to alleviate inadequate crime scene response.

The data can be used for quick pitches for extra personnel to supervisors or command staff or for more meaningful conversations about staffing and CSI response throughout our discipline. Forensic science providers can use these numbers and address staffing or response according to their agency-specific needs, thereby increasing the quality and effectiveness of crime scene investigations at a customizable scale.

It is recommended that future research focus on more data and responses to these types of questions as well as address the lack of specificity of employment numbers. Full-scale studies on either of the two topics requires large amounts of time and resources, and one large study might be too unwieldy to administer. A pragmatic approach would be to collect a more robust data set on quality and effectiveness and then extrapolate on a complete employment survey of the discipline.

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**Crime Scene Investigation; Staffing and Employment; OSAC**

## **F93 An Investigation of Best Practices for Fatal and Non-Fatal Bathing-Environment Cases: Guidelines and Tools for Scene Investigators, Law Enforcement, Medical Personnel, Death Investigators, and Jurisprudence**

*Andrea Zaferes, BS\*, Forensic Aquatic Consulting & Training, Shokan, NY; Robert Bready, MS, Dutchess County Medical Examiner, Poughkeepsie, NY*

**Learning Objective:** Attendees will learn that practitioners need evidence-based resources to investigate and make determinations for bathing-environment fatal and non-fatal incidents. Elements of these resources will be provided.

**Impact Statement:** This presentation will impact the forensic science community by providing novel investigation solutions, including foundation questions, checklists, and protocols, designed to identify and collect often-missed evidence and information, that are critical for: (1) accurate cause and manner of death diagnoses in fatal cases, (2) determinations of accidental, suicidal, or criminal acts in non-fatal cases, and (3) fair jurisprudence outcomes.

Forty people drown hourly worldwide. There are 110,000 annual bathtub and shower-related accidents in the United States that may or may not involve drowning. Proper diagnoses of morbidity and accidental, suicidal, or criminal determinations are important for prevention strategies, treatments, and jurisprudence. These cases can be equivocal in nature, circumstance dependent, can involve diagnoses of exclusion, may present with few injuries, and therefore, require thorough, informed scene and circumstance investigations.

Practitioners need investigative resources to effectively manage issues that are unique and specific to bathing environment incidents. This is especially true when the incidents might involve abuse, assault, or homicide. One of the reasons is the large number of different types of incidents. Bathing environments are varied (e.g., bathtubs, hot tubs, showers, sinks, buckets, rivers), as are behaviors in those environments (e.g., bathing infants, cold showering, sex in bath clubs, suicide, aquatic child maltreatment, sexual abuse in care facilities, aquatic domestic violence, female offender homicidal drowning, aqua-eroticism).

An important investigative question to help determine natural deaths is “was the decedent’s airway exposed to fluid?,” which requires informed interviewing questions, scene documentation, and possible reconstructions, to determine if water levels or body positions changed prior to arrival of investigators. Does the drain leak, and if yes, at what depth change rate? Did anyone move the body in a failed attempt to remove it from the tub? Was the water running and then turned off by a bystander? If the victim was allegedly showering, what caused the water to fill in the tub?

In addition to training, there are two solutions to help overcome these challenges, which are the focus of this presentation.

1. Three foundation questions.
2. Proven investigation protocols, forms, and checklists.

The three foundation questions are:

1. Does it make sense that the subject is in that water at that time?
2. Do the locations, positions, postures, physical presentations, and surrounding scenes of the subject and reporting parties make sense?
3. Does it make sense that the subject is dead for fatal cases or incapacitated for non-fatal cases.

The bathing environment investigation checklist includes questions on the victim’s bathing behaviors, transient scene, and body evidence to document as soon as possible, bathtub tests, first responder observations, Archimedes principal issues, body buoyancy, documentation of reporting parties, and scene processing. Protocols include how to visually document if objects are dry, damp, or wet; why processing the bathing environment for latent prints can be important even if the suspect lives in the residence; and how specific injuries on victims can help determine where to swab for biological evidence in the bathing environment. Interview questions of survivors include sensory changes during the aquatic attack, were other forms of asphyxiation also used, and what did they think was going to happen.<sup>1-11</sup>

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### Homicide Investigations; Drowning; Bathing



## F94 Assessing an Innovative Response to Intimate Partner Violence-Related Strangulation

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**Learning Objective:** After attending this presentation, attendees will gain competency in understanding the dynamics of incidents involving intimate partner violence-related strangulation (IPVRS) and the signs and symptoms of non-fatal strangulation (NFS) as reported by victims to police and medical first responders. In addition, attendees will gain a better understanding of the practical implications for forensic science evidence collection and investigation of NFS incidents.

**Impact Statement:** This information will add to the body of knowledge of non-fatal strangulation (NFS) incidents relating to forensic evidence and investigation.

Non-fatal strangulation (NFS) is the compression of the neck and/or upper torso in a manner that impedes the airway or circulation.<sup>1,2</sup> NSF victims frequently have minor or non-visible external injuries, though often suffer from more serious, internal injuries that can be long-lasting and increase mortality.<sup>3-5</sup> The prevalence and rate of injury from NFS is largely unknown because intimate partner violence (IPV) victims routinely underreport their victimization and few obtain medical intervention following an incident of NFS (approximately 29%).<sup>3,5,6</sup> Early detection of IPVRS can provide critical information for prioritizing service decisions, improving medical outcomes, and enhancing evidence collection.<sup>2,4,7</sup>

Victims of IPVRS have an increased risk of homicide and are almost seven-and-a-half times more likely to die at a later time from the NFS perpetrator when compared to victims who have not experienced NSF.<sup>8-11</sup> Although precise IPVRS counts have varied, estimates have suggested that as many as 68% of victims have experienced NFS by intimate partner perpetrators.<sup>10,12-14</sup> Providing law enforcement with the knowledge and skills to detect the signs and symptoms of NFS has been challenging, given the lack of visible physical evidence when NSF victims present to police. Only approximately 12% of IPV investigations have contained explicit references to NFS in the police report, further reiterating the challenges that first responders have faced in identifying NSF.<sup>2,15,16</sup>

In 2018, the City of Burleson, TX, enacted the “Effective Response to Strangulation” Ordinance (that mandated specific first responder protocols in cases of alleged or suspected strangulation). The new ordinance includes: (1) a defined protocol for addressing strangulation, (2) training for first responders (police, fire, and EMS/paramedics), (3) newly designed assessment instruments to improve the identification of IPV asphyxiation, and (4) specific intervention strategies for strangulation across multiple agencies. Tarleton State University’s Institute on Violence Against Women and Human Trafficking (VAWHT) Intimate Partner Violence-Related Strangulation Study is engaged in a program evaluation of the Strangulation Ordinance. The study, titled “Assessing an Innovative Response to Intimate Partner Violence,” is funded by the National Institute of Justice, Office of Justice Programs, United States Department of Justice (award 2018-VA-CA-0005) and uses a non-equivalent-groups longitudinal quasi-experimental research design to study of the development, implementation, and success of the initiative.

This presentation involves one component of the larger program evaluation and examines the prevalence of NFS in the population of IPV incidents ( $N=587$ ) reported to the Burleson Police Department during the post-Strangulation Ordinance period (March 6, 2018, to December 31, 2020). Findings will address the nature and extent of physical strangulation signs and symptoms (e.g., bruising, petechiae) and other factors (e.g., injuries, witnesses) that provide important forensic science and investigative implications. Multivariate logistic regression and other inferential analytic techniques are used to identify correlates of NFS among the population of post-Ordinance Burleson IPV incidents. Additionally, victim willingness to engage with first responders will be explored through descriptive statistics. Finally, practical implications for forensic science evidence collection and investigation in IPVRS incidents will be presented.

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**Non-Fatal Strangulation; Intimate Partner Violence; Investigations**

**F95 Forensic Archeology and Crime Scene Management: Challenges and Shortcomings in the Colombian Context**

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**Forensic Archaeology; Forensic Anthropology; Forced Disappearance**

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**F96 The Rural-Urban Differences in Access to Medicolegal Death Investigation Resources and Services: Findings From the 2018 Census of Medical Examiner and Coroner Offices (CMEC)**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the disparities in medicolegal death investigation (MDI) resources that exist between rural and urban medical examiner and coroner offices (MECs) based upon the Bureau of Justice Statistics' (BJS) 2018 Census of Medical Examiners and Coroners Offices (CMEC).

**Impact Statement:** This presentation will impact the forensic community by showing what key resource differences there are between urban and rural MECs in the United States based on an 80.9% response rate of the 2018 CMEC administration. These disparities will be contextualized against overall death rates and rates by specific types (suicide, drug/alcohol deaths, non-injury/no intent deaths, and accidental deaths) based on the 2018 Centers for Disease Control and Prevention's (CDC) WONDER mortality data.<sup>1</sup>

The 2018 CMEC was conducted by RTI on behalf of BJS (2017-MU-CX-K052) from June 2019 through March 2020. Surveys were collected by mail, online, and email. The 2018 CMEC questionnaire was developed based on review and update of the 2004 CMEC, expert panel review, and pilot testing. In this analysis, we use the 2018 CMEC public data file to partition the 1,648 responding offices into four groups based on the population size of the jurisdiction served. United States Census Bureau categories were used, including large/metropolitan offices, small metropolitan offices, micropolitan offices, and rural/non-core offices.

Results from this study include information on the caseload, staffing, budget, and access to key resources across these four categories of MEC offices. Data show consistent downward patterns across the urban to rural spectrum, with rural/non-core MECs having the lowest proportions of access to key resources (e.g., information technology, MDI personnel) and several different types of forensic testing (e.g., toxicology, anthropology, histology, neuropathology, and odontology). Salary data are provided for autopsy pathologists, coroners/non-physicians, and death investigators across these four rural-urban MEC classification groups, which is critical given the national forensic staffing pipeline shortages.

Finally, the 2018 CMEC data were integrated with mortality data from CDC's 2018 WONDER mortality data to show that rural MECs handled higher rates of suicide, drug/alcohol deaths, non-injury/no intent deaths, and accidental deaths compared to their urban counterparts. In essence, although MECs are under-resourced as a whole, there was a consistent downward pattern among the smaller rural MECs suggesting that they must do even more with less resources in terms of staffing, caseload, budget, and access to death investigation tools (e.g., fingerprint, criminal history, and prescription drug monitoring program databases). Ratios of population served to cases will be provided as further context. These data are important to understand at a national level as federal agencies seek to mobilize resources to buttress existing forensic infrastructure to improve the United States MDI system overall, as it can show ways in which these funding streams and resources can be better targeted. These data will also help inform the BJS as it plans the next administration of the CMEC.

This analysis builds on the BJS final report summarizing the 2018 CMEC findings overall.<sup>2</sup> MECs and the agencies that govern them and work with them can use the information from this presentation to understand the challenges unique to these rural communities and guide how to optimize resource allocations to support medicolegal death investigations in these jurisdictions.

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**Medical Examiner and Coroner Offices; Resource Disparities; Mortality**

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**F97 The Trends and Demographic Differences in Overdose Deaths Before and During the COVID-19 Pandemic in Jefferson County, Alabama**

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**Learning Objective:** After attending this presentation, attendees will be familiar with overdose trends that are becoming more apparent as the COVID-19 pandemic continues to affect daily life.

**Impact Statement:** This presentation will impact the forensic science community by illustrating the changing demographics in the opioid epidemic.

Unintentional drug overdose deaths have been increasing exponentially over the past few decades with the most recent estimations topping 100,000 deaths annually and contributing to a decline in overall life expectancy.<sup>1</sup> An official health advisory from the Centers for Disease Control and Prevention (CDC) indicated substantial increases in drug overdose deaths following the onset of the COVID-19 pandemic, at least partly driven by synthetic opioids such as fentanyl.<sup>2</sup> The CDC's analysis of provisional death data across several states, including Alabama, revealed a 28.22% increase in overdose deaths in October 2021 compared to October 2020. The largest increase in overdose deaths occurred among Black individuals in 2020, surpassing the rate among White people.<sup>3</sup> To understand trends in drug overdose mortality over the course of the COVID-19 pandemic, in Alabama specifically, we present data on overdose death trends and demographic differences in these shifts from 2019–2021 in Jefferson County (the largest county in Alabama).

While there was no significant change in the age ( $p=0.14$ ) or sex ( $p=0.45$ ) distribution of overdose deaths during the study period, the proportion of Black deaths increased dramatically over time ( $p=0.0002$ ). Most deaths over the three-year period analyzed occurred among males in the 30–50 age range. However, the stark increase in the overall proportion of deaths among the Black population is concerning. This subset of the population comprised 24.4% (57/234) of deaths in 2019 which rose to 42.4% (168/396) in 2021.

The proportion of overdose deaths wherein fentanyl was detected doubled (40.6% to 80.6%) between 2019 to 2021 for all groups except White females. Methamphetamine-involved deaths decreased among Black females but remained constant for all other groups. With respect to heroin, the proportion of overdose deaths declined dramatically (38.0% vs. 18.4%) from 2019 to 2021, particularly for Black females and White males. Cocaine-related deaths increased slightly but not significantly across all race-sex groups. Finally, both overall and for all race-sex groups, there was no change in the proportion of overdose deaths wherein ethanol was detected.

The present study mirrors national trends that show an increase in overdose deaths involving fentanyl, particularly among Black individuals, during the COVID-19 pandemic. The increase in overdose deaths is likely caused by many different factors in combination, such as overcrowded hospitals, quarantine with no social interaction, the uncertainty of contracting COVID-19, financial challenges, and many more social implications. Many of these factors are derived from the sudden and drastic instability of everyday life caused by the onset of the pandemic. Social determinants of health, particularly economic stability and health care access, have been severely impacted during the pandemic due to extended quarantines and the overloading of the healthcare system with patients who contracted severe cases of COVID-19—creating more risk among vulnerable populations. Taken together with other studies, these data aid in the description of “sub-epidemics,” which are often geographically restricted and comprise the broader exponential growth of drug overdose deaths over time.<sup>4</sup> The COVID-19 pandemic has vastly overshadowed the opioid epidemic in both local and national media leading to a dangerous lack of public knowledge and awareness about the severity of this subepidemic. Knowledge of this information is vital for public health initiatives focused on surveillance among at-risk groups, preventative measures, and intervention programs.

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**COVID-19 Pandemic; Overdose Trends; Opioid Epidemic**

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**F98 A Multiagency Investigation of Multiple Murders in the United States With the Trial in Indonesia**

*Lakshmanan Sathyavagiswaran, MD\*, Arcadia, CA*

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**Learning Objective:** The goal of this presentation is: (1) to inform attendees how the justice system functions, and trial proceedings are conducted in the highest court in Jakarta, Indonesia; (2) to demonstrate that crimes are solved by the dedication, perseverance, and ingenuity of involved law enforcement/forensic professionals; and (3) emphasize the importance of cooperatively working with law enforcement and criminal justice system professionals in another country (Indonesia) by acknowledging their expertise and thus building relationships and trust. This relationship facilitated detectives from the United States and Indonesia to share information on the criminal investigation, international travel to interview witnesses, and evidence presentation /exhibit preparation during the trial in Jakarta.

**Impact Statement:** This presentation will impact the forensic science community by: (1) reviewing a high-profile trial in Jakarta, Indonesia, as an illustration of the importance of scientific evidence-based investigation, and multi-agency coordination; (2) aiding attendees in understanding the importance of forensic scientists, medical examiner, and law enforcement from the United States presenting valid, reliable testimony as the media in Indonesia regaled on the shocking, morbid details of this crime.

Three steamer-type trunks were auctioned at a U-Haul® storage center in Los Angeles County in 1994. The purchaser went to the facility and recovered the boxes. He and the U-Haul® storage site owner detected a foul odor coming from one of the trunks. Law enforcement was called. All three trunks were wrapped in duct tape. When one trunk was unwrapped, a decomposing body was found. The body was left in the trunk and transported to the LA county Department of Coroner. The other two trunks also contained decomposing bodies. The bodies were two males and one female. The trunks had been there for over a year. The three victims died from trauma caused by various weapons, including a gun, a hammer, and a wrench.

A 31-year-old Indonesian businessman who ran a small business in Los Angeles was arrested in Jakarta in January 1995 on charges of passport forgery by Indonesian Authorities. He was later charged with the triple slayings in Los Angeles County based on the forensic evidence ascertained by the diligent medicolegal death investigation, the coroner, and law enforcement. Because there was no extradition treaty between the two countries, the Indonesian Government conducted a criminal trial in Indonesia for three murders that took place in Los Angeles, CA.

The victims were the businessman's associate, girlfriend, and brother. They were all killed at different time periods. Indonesian law enforcement indicated that the suspect confessed to killing of two of the three victims (his girlfriend and brother) and admitted being present when his brother killed the businessman's associate.

Details of the forensic and medicolegal death investigation, including establishment of identification of the victims by the staff LA county Department of Coroner, LAPD–RHD, Crime laboratory, Indonesian authorities, the Prosecution, and trial details wherein the presenter testified as an expert witness will be presented.

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**Murder; International; Trial**



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**F99 Alternate Light Source (ALS) Detection of Subclinical Bruising: Lessons Learned While Assessing a Forensic Technique**

*Leonard Reinart, JD, MS\*, Hopkins, MI*

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**Learning Objective:** Attendees will gain insight into the historical background associated with both latent trauma and efforts to photograph these injuries. This presentation will also investigate technical aspects of this technique and will discuss best practices as referenced in literature. Finally, the legal practicality of the technique will be explored within the context of the Federal Rules of Evidence, the PCAST Report, and the NAS Report to consider if this technique is valid, if more research is necessary, or if a change in methodology would result in meaningful improvements. More broadly, attendees will additionally learn how legal professionals approach the validation of forensic techniques and how researchers should consider both scientific and legal audiences when presenting their findings.

**Impact Statement:** This presentation will support researchers interested in exploring various aspects of latent trauma, alternate light sources, and photography. It will also provide insight into the steps that legal professionals take in gauging the validity of a forensic technique, touching on topics ranging from study design to statistical interpretation.

The use of alternative light sources (ALS) to visualize wounds often classified as either latent trauma, subclinical bruises, or subtle injuries has been commonplace for decades.<sup>1</sup> This technique has applications in a variety of legal and clinical settings and the results of which can be offered as evidence in both criminal and civil proceedings. While research is ongoing in many aspects of this technique, some believe that the use of ALS in this manner is not a valid forensic practice, particularly when employed to diagnose an injury.<sup>2,3</sup>

Thus, this project approaches the technique from a legal perspective and describes the process used to investigate the validity of the technique, primarily focused on the scientific and legal literature research conducted to assess this application of ALS. In regard to this specific technique, factors that were present at the inception of the technique that impacted its perceived validity include the close relationship this technique had throughout its development alongside forensic odontology and controversial figures in the discipline. However, as the technique evolved, other forensic disciplines played a more prominent role in research and publications, culminating in more rigorous modern research conducted by those within the field of forensic nursing. Nevertheless, the history of this technique has resulted in a lack of standardization in terms of both methods and terminology, made even more complex by advancements in photography over the course of the technique's history.

As the technique evolved and progressed, lingering assumptions or misstatements persisted or otherwise manifested themselves in ways that called into question the foundation upon which the modern research is built. What resulted was the difficult task of gauging the validity of a forensic technique as it is currently implemented, while being mindful of its origins, in order to determine if the technique should be utilized in forensic settings. To that end, modern research appears to present findings that do not support the continued use of this technique as it currently stands, for reasons ranging from inconsistent results to a high rate of false positive results. However, the current number of published empirical studies is low. This highlighted several needs within the forensic science community. First, more research is needed in the area of using alternate light sources to visualize latent trauma. Second, research published by the forensic science community should be periodically reviewed from a modern lens to identify studies and other publications that should not be used as a basis for future research due to its lack of scientific rigor or questions pertaining to the integrity of the researchers. Finally, forensic scientists and legal professionals should have a stronger relationship and a means through which to collaborate on issues pertaining to the validity of forensic disciplines; importantly, however, this must be outside of the context of the adversarial judicial system.

**References:**

- <sup>1</sup>. J.M. Cameron et al., Ultra Violet Photography in Forensic Medicine, 2 *Forensic Photography* 9,9 (1973)
- <sup>2</sup>. President's Council of Advisors on Science and Technology, *Forensic Science In Criminal Courts: Ensuring Scientific Validity Of Feature-Comparison Methods* (Sep. 2016)
- <sup>3</sup>. Leonard F. Reinart, *Alternate Light Source Visualization of Latent Trauma: Exploring The Validity Of A Forensic Technique*, Center for Integrity in Forensic Sciences, Loyola University Chicago School of Law (2022).

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**Bruise; ALS; Validation**

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**F100 Forensic Autopsies: The Contribution of the Systematic Practice of Subcutaneous Dissection for Detecting Contusions or Deep Hematoma Not Visible on External Visual Examination**

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**Learning Objective:** The goal of this presentation is to inform attendees of the practice of subcutaneous dissection protocol in Montpellier, France, and the contribution of this practice for the detection of blood infiltration not visible on external visual examination.

**Impact Statement:** This presentation will impact the forensic science community through discussion (advantage, inconvenience) of the practice of subcutaneous dissection in all autopsy cases, all types of modes of death and not only in certain contexts.

**Introduction:** The practice of subcutaneous dissection in forensic medicine is not part of the standard protocol when performing an autopsy, not only in France, but in many other countries. Some forensic physicians performed it systematically while others carry it out only in certain contexts (homicide or suspicion, child, death in custody with an extent that varies depending on the teams and doctors). Some physicians limit it because of its “mutilating” aspect and/or the extent of its contribution (manner of death). This study aims to evaluate the contribution of the systematic practice of subcutaneous dissection in the detection of collections of extravasated blood not visible on external examination.

**Material and methods:** From November 23, 2020, to April 19, 2021, subcutaneous dissections were routinely performed on the back and four limbs in 62 autopsy cases (randomly selected, all types of modes of death). Data were collected prospectively, noting whether collections of extravasated blood (defined as not detectable by careful external examination) were detected and their location (back, upper/lower limbs), their depth (subcutaneous/fatty tissue, muscular), and their dimension.

**Results:** Unexpected collections of extravasated blood were found in 37 autopsies; all localizations included (i.e., 59.6% of cases). More specifically, we found collections of extravasated blood in the subcutaneous dissections of the back of the torso in 27 cases (43.5%), of the upper limbs in 16 cases (25.8%), and of the lower limbs in 15 cases (24.2%). They were superficial (subcutaneous/fatty tissue) in 13 cases, deeper (muscular) in 9 cases, and present superficially and deeply in 14 cases. Muscular extravasated blood was found mainly in the back (22 cases), then the upper limbs (5 cases), while the more superficial infiltrations were globally located in an equivalent way within the back (13 cases), lower limbs (12 cases), and higher limbs (10 cases). They measured on average 7.4cm in the back (1 to 40cm), 3.2cm in the upper limbs (0.5 to 10cm), and 4.5cm in the lower limbs (1 to 15cm).

**Conclusion:** In approximately 6 cases out of 10, the subcutaneous dissection revealed collections of extravasated blood that were not seen on external examination, with a predominance of the collections found in the subcutaneous dissection of the back of the torso. In view of these results, we discuss the relevance of the systematic practice of subcutaneous dissection, taking into consideration some of the obstacles (deterrent factors) confronted.

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**Subcutaneous Dissection; Contribution; Blood Infiltration**

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**F101 The Effect of Common Drugs on Human Body Decomposition and the Soil Environment**

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**Learning Objective:** Attendees will gain an understanding of the effect of commonly used and abused drugs on the rate of decomposition of the human body, and processes associated with human decomposition, such as color changes, insect activity, scavenger activity, temperature, and environmental factors such as soil composition and the surrounding ecosystem. Attendees will learn that these relationships are not linear and operating in a trilaterally reciprocal manner—wherein drugs, body, and deposition environment all influence each other. This will be achieved by introducing anthrotoxicological research, with a focus on ascertaining the dependence of toxicological variables (i.e., postmortem drug redistribution, stimulating properties of a substance) on taphonomical and osteological factors (i.e., rate of decomposition, absorption of substance into bone), and vice-versa. It also discusses the relevance of soil as a matrix for drug distribution in burial cases.

**Impact Statement:** The presented research will impact the wider scientific community by reflecting on the importance of an interdisciplinary approach when assessing interlocking taphonomical, osteological, and toxicological evidence within forensic cases. It is believed that broadening the perspective on potential mistakes and knowledge gaps involving drug-related death cases will actively influence the accuracy, precision, and time-management of forensic investigations. It is important to apply toxicological knowledge to anthropological evidence, to inform the understanding of how, and to what extent, drugs can visibly affect body and/or bone. The possible visual effects are within the realms of color, shape, and wear (usage of the teeth), which can be correlated to drug use or abuse, including the method of administration (such as orally, rubbing substance into gums, placing under the tongue). This would facilitate early identification of indicators within investigations, narrowing the possibilities of unknown victim identification. Additionally, spotting these signs of drug use on compromised human remains and bone will inform how toxicological evidence should be analyzed.

This project focusses on the interaction between decomposition, drugs, and the soil environment. An interdisciplinary approach can positively influence the process of investigation and prevent misconceptions within forensic examination, contributing to greater accuracy.

To assess the effect of drugs consumed by the individual, and of drugs in the soil, on decomposition, this project was divided into two sections: a case-study-based “thought experiment,” and a laboratory-based experiment. The first study involved a systematic review of decomposition cases reported in peer-reviewed and media articles. Three drug-related forensic cases involving decomposition were chosen to examine if correlations occur between body decomposition, substance usage, and burial environment. Total Body Score was used to assess the decomposition from images, and the results were compared with any postmortem interval estimation provided.<sup>1</sup> Information about the victim’s antemortem drug consumption was used to assess the scope for inaccuracy; factors such as body temperature, discoloration, and insect activity could be influenced by properties of the drug, including: insect growth retarded by substances taken by victim; physical discoloration of the victim’s skin, imitating a more advanced stage of decomposition; and changes observable in the surrounding ecosystem, such as the leaking of substances from the body into ground water, affecting aquatic populations. In conclusion, the relationships demonstrated by the first experiment were not straightforward.

Secondly, five mice with 5g of codeine injected into their esophagus postmortem were buried in soil for five weeks and excavated at weekly intervals to test the hypothesis that codeine can leak from a decomposing body and be detected in the surrounding soil. Codeine was chosen as a representative substance of forensic importance; it is widely used in a medical capacity, and it is commonly used as a probe. Moreover, other opioids can be modeled against codeine, and synthetic opioids, such as hydrocodone, can be based on its structure. Soil samples were taken at regular horizontal and vertical distances around the carcasses to determine the distance that codeine can travel in the soil and the likely timescales for detection of codeine in the soil. This was then extracted from the soil using solid phase extraction (SPE) and analyzed using liquid chromatography/tandem mass spectrometry (LC/MS/MS).

The laboratory experiment demonstrated the dynamics and interactions of drugs from a cadaver and the surrounding soil. The forensic importance of this research lies in establishing if drugs leak or travel across soil, the time frames necessary for a drug to become detectable in the soil, and the circumference of leakage as a function of time.

These experiments highlight the importance of a multidisciplinary approach, that victim drug-consumption can affect decomposition, and that drugs can leak from the body into the soil, potentially detectable after burial, and potentially affecting adjacent buried bodies.

This anthrotoxicological approach has potential implications for estimating the post-burial interval, determining the blood-drug level after death, decision-making about distances between graves, and impacting evidence collection in investigations, including mass graves.

**Reference:**

- <sup>1</sup>. Megyesi, Nawrocki, S., & Haskell, N. (2005). Using Accumulated Degree-Days to Estimate the Postmortem Interval from Decomposed Human Remains. *Journal of Forensic Sciences*, 50(3), 1–9. <https://doi.org/10.1520/JFS2004017>.

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**Drugs; Decomposition; Soil**

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**F102 Traceability of Human Remains From Discovery to Burial: DIVIDOC and DIVIMAP**

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**Learning Objective:** Attendees will learn about a simple way to ensure traceability of human remains from recovery to burial in technologically challenged contexts.

**Impact Statement:** The forensic community will be impacted by becoming acquainted with two free Android® mobile applications responding to the needs of first responders in technologically trying contexts.

The dignified management of the dead in disaster settings is key to preserve and/or restore their identity.<sup>1</sup> In mass disasters and other trying situations, such as the current COVID-19 pandemic, resources and capacity of first responders primarily in developing countries, may be pushed to the limit.

Two photo applications, Disaster Victim Documentation (DIVIDOC) and Disaster Victim Mapping (DIVIMAP), were created to guarantee the traceability of human remains from discovery to burial and following recommendations of best practice. The applications operate in Android® and would be used by two separate groups of people, those responsible for recovery (DIVIDOC) and those responsible for the burial/disposal of human remains (DIVIMAP).

DIVIDOC allows taking up to five pictures of the body (face and specific features); each picture is automatically labeled. Each code is unique and is made up of three fields: (1) the ISO code of the country, (2) a three-digit code provided by the system administrator in the country. (i.e., 001 and 002 for DIVIDOC and DIVIMAP, respectively), and (3) a number produced automatically by the system. An optional fourth field allows for a customary code of up to 20 characters to be entered. A short form including basic biographical data such as sex, broad age categories, and a free field for notes (i.e., name or other information) is also included. DIVIMAP allows taking up to three pictures of the body bag or coffin, including the tag with the three-field code assigned to the case (with DIVIDOC), as well as the location where the body was buried.

All photos and associated metadata in both applications (i.e., geolocation, time, etc.) are automatically arranged in a folder, zipped, and encrypted. Upon transfer, a password is needed to decrypt each file. Folders not transferred from a phone to a computer after a set of hours (usually 24) get locked and yet again a special password is needed to retrieve them.

A new super-user mode allows any transmitted files to be retrieved without security codes. The new feature also includes a server setup to receive all images from both applications visualizing their geolocation in a map and according to source (place of discovery: DIVIDOC or place of burial: DIVIMAP).

Installation and user manuals are provided in a single zip folder and can be transferred for distribution in USB drives or via email. The application has been translated to English, French, and Spanish. Data protection considerations should be assessed by the users according to existing legal provisions in the country where used.

**Reference:**

- <sup>1</sup>. International Committee of the Red Cross (ICRC). *Management of Dead Bodies after Disasters: A Field Manual for First Responders* (2020). Available at: <https://www.icrc.org/en/publication/0880-management-dead-bodies-after-disasters-field-manual-first-responders>.

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**Traceability; Android® Applications; Human Remains**

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**F103 Pesticides and Blow Flies: How Do Clothianidin and Bifenthrin Affect Calliphoridae (Diptera) Oviposition?**

*Teomie Rivera-Miranda, PhD\*, Purdue University\*, West Lafayette, IN; Krystal Hans, PhD, Purdue University, West Lafayette, IN*

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**Learning Objective:** After this presentation, attendees will have a better understanding of how exposure to two commonly used insecticides affect blow fly oviposition under laboratory conditions (species: *Lucilia sericata* (Meigen) and *Phormia regina* (Meigen)). The information shared during the presentation could be applied in real-life death investigations where pesticide use is suspected.

**Impact Statement:** Having accurate TOC and PMImin estimates is crucial to every death investigation because it can help to uncover significant evidence on death circumstances, including possible connections between the suspect and the victim. These experiments were conducted in Indiana, where croplands are extensive, pesticide residue is present in the environment, and commercial pesticides are readily available in stores. Therefore, results obtained from this study will fill the current gap on the effects of two commonly used and readily available pesticides under laboratory conditions when using pig carcasses to study factors that alter TOC and PMImin estimates. Pesticides can accelerate or slow down the TOC and determining how this happens will be useful when pesticide use is suspected in a death investigation.

Blow flies arrive to remains shortly after death and females can oviposit soon after, allowing forensic entomologists to estimate the minimum time of colonization (TOC). The TOC and larval development contribute to an estimate of the minimum postmortem interval (PMImin), or time between death and discovery of the remains. However, many factors, including chemicals such as pesticides, can be used to mask the odors of decomposition, which can lead to changes in blow fly arrival and oviposition behavior, among others. With this study, we want to determine how oviposition substrate treated with pesticide affect oviposition of blow flies under laboratory conditions. We hypothesize that spraying the oviposition substrates with pesticide will conceal the odors of decomposition, resulting in a delay of blow fly oviposition events when compared to substrates treated with water (control).

To test this, we selected two common insecticides, bifenthrin (pyrethroid) and clothianidin (neonicotinoid), and applied them to two different types of oviposition substrate: liver or fetal pigs. (Pigs were obtained from the Purdue University Animal Sciences Research and Education Center (ASREC): Swine unit (PACUC number: FOR-058)). In the laboratory, we focused on testing oviposition behavior of two common blow fly species in the United States: *Phormia regina* (Meigen) and *Lucilia sericata* (Meigen) (Diptera: Calliphoridae). A total of 40 females and 20 males were placed in 12 cm<sup>3</sup> aluminum cages. Females were then provided with one of two choices of an oviposition substrate (e.g., 25g of fresh liver or a fetal pig). Each oviposition substrate was treated with either distilled water for the control or an insecticide. Bifenthrin concentrations tested here were 1.00oz/gal (high concentration and equivalent to label maximum application rate), 1.00x10<sup>-2</sup>oz/gal (medium concentration), 1.00x10<sup>-5</sup>oz/gal (low concentration). For clothianidin, we used 1.14g/gal (high concentration and equivalent to label maximum application rate), 1.14x10<sup>-2</sup>(medium concentration), and 1.14x10<sup>-3</sup> (low concentration). All concentrations had three replicates.

After all oviposition substrates were treated, we started recording time and making observations every 15 minutes for the first two hours and every hour after that until the first oviposition event occurred. We recorded time to first oviposition event and counted number of eggs laid (when possible) and determined the oviposition site preference on the fetal pigs between the treatments and the control to determine whether there was a preference between head parts, torso, and limbs. Statistical analyses, including a two-way ANOVA, were performed using RStudio v3.4.3 (2017).

Results obtained from laboratory experiments testing bifenthrin on both blow fly species using liver as the oviposition substrate suggest that bifenthrin had a significant impact on the *L. sericata* first oviposition event ( $p=0.0376$ ,  $F=4.596$ ). However, bifenthrin concentration appears to have no significant impact on the number of eggs laid ( $p=0.234$ ,  $F=1.753$ ) by *L. sericata* females. As for *Phormia regina*, bifenthrin seems to have no significant impact on the first oviposition event ( $p=0.742$ ,  $F=0.422$ ) or number of eggs laid ( $p=0.185$ ,  $F=2.055$ ). The time it took females to oviposit varied between the replicates of each treatment. In the control, first oviposition events ranged from 75 minutes to 450 minutes with an average equal to 315 minutes. In the high concentration, time range=60-420 minutes with an avg=220 minutes; in the medium concentration, time range=240-300 minutes with an avg=260 minutes, and in the low concentration, time range=105-300 minutes with an avg=185 minutes. These results will be crucial during death investigations where pesticides use is suspected or when remains are concealed in an agricultural setting, since this could lead to incorrect TOC/PMImin estimates.

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**Blow Fly; Pesticide; Oviposition**

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**F104 The Influence of the Victim-Offender Relationship on Body Disposal in Solved United Kingdom Homicides**

*Anna Williams, PhD\*, University of Central Lancashire, Preston, England; Kirsty Bennett, MSc, Leeds Beckett University, Leeds, England, UNITED KINGDOM*

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**Learning Objective:** By attending this presentation, attendees will learn about the pattern of body disposal in United Kingdom solved, non-serial homicides, and discover how body disposal methods vary with the relationship between the victim and the murderer.

**Impact Statement:** Knowledge of how body disposal methods vary with the victim-offender relationship could aid the search strategies of police and forensic investigators by providing an indication of likely disposal sites, methods, and locations that non-serial offenders use when disposing of a victim's body. Equally, when remains are found, the disposal methods used may aid the prediction of the victim-offender relationship and narrow the list of suspects. The principles identified in the United Kingdom can be applied all over the world, potentially reducing the time taken to find victim remains and increasing the chances of a successful conviction.

There are approximately 700 homicides in the United Kingdom each year, and about 20% of these go unsolved. One reason for this is that the victims' remains are never found. This study used data from solved United Kingdom homicides to predict the pattern of body disposal methods and to determine the presence of a correlation between body disposal method and the victim-offender relationship. The intention was to assist homicide investigations in two ways: predicting the victim-offender relationship from the disposal method once a body is found, or to predict disposal type and location of the body if the body has not been found but the victim-offender relationship is known.

Factors already known to affect the method and location of body disposal include practical considerations such as the physical size of victim's body as well as the alcohol or drugs consumed by the offender prior to the crime or the risk of apprehension. Other studies have found that the offender's disposal method and location reflect his/her conscious choices or active decision-making. Also, the familiarity of an offender with the location, their physical build, and access to a car can influence their disposal of a victim's body. However, the influence of the familiarity between the offender and victim on the effort made by the offender to conceal or travel to dispose of victim remains has not yet been explored.

The hypothesis here was that the closer the relationship between the victim and the murderer, the more effort the offender will spend in disposing of the victim's body. In a pre-meditated homicide, an offender familiar to the victim may want to distance him/herself from the crime to avoid detection and try to make the remains more difficult to discover, through concealment or distant disposal.

To test this hypothesis, the victim-offender relationship, cause of death, body disposal method, distance travelled from homicide location and offender residence to dispose of victims' remains, and the nature and location of disposal sites were examined in 201 solved, non-serial homicides in the United Kingdom between 1944 and 2020. Such information was obtained from news outlets ( $n=167$ ) and Freedom of Information requests to United Kingdom police forces ( $n=34$ ). Only cases mentioning an attempt at body disposal were included.

Relationships were categorized as: intimate partner; family member; acquaintance; stranger or unknown. Disposal was categorized and coded according to the effort involved, from (1) leaving the body where it was, (2) concealment or hiding, (3) concealment in suitcase or car boot, (4) burning or water disposal, (5) burial, or (6) dismemberment.

Using the victim-offender relationship as the independent variable in a Chi-Square analysis, location of death, disposal method, and distances travelled by the offender were strongly statistically significant ( $p < 0.001$ ). Intimate partners are most often murdered in the home they shared with the victim, whereas strangers and acquaintances are more often killed in their own homes ( $p < 0.001$ ). Disposal method varied significantly ( $p < 0.001$ ) with the victim-offender relationship, as family members of the victim expended the least effort in body disposal, and acquaintances expended the most (frequency of high-effort disposal methods). Strangers tended to expend less effort in body disposal than partners.

These findings broadly support the hypothesis, and provide information that could be useful to police and forensic investigators searching for the remains of homicide victims, in situations where the victim-offender relationship is known, or when remains are found, predicting the identity of the murderer from the disposal method used. The key data is presented in the style of the United Kingdom Missing Persons Bureau iFIND operational tool, in order to facilitate quicker recovery of (non-serial) homicide victim remains in challenging circumstances.

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**Homicide; Body Disposal; Victim-Offender Relationship**



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**F105 The Positive Impact of Coordinated Implementation of the Pre-Conception and Pre-Natal Diagnostic Techniques (PC-PNDT) Act in Improving the Skewed Child Sex Ratio in Haryana, India**

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**WITHDRAWN**



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**F106 Information Analysis on Disappearance Cases for the Construction of Missing Persons Lists and the Identification of Disposed Places in River Environments: Experiences in La Miel River, Caldas, Colombia**

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**Learning Objective:** This methodology allows the automated query and organization of information associated with judicial files and data bases for dealing with cases of body disposal in rivers.

**Impact Statement:** This methodology is helpful for identifying information on people thrown into aquatic environments and places from which they were disposed. This information allows the development of transport hydrodynamic modeling in rivers and the proposal of archeological strategies for approaching entrapment sites.

Dumping bodies in rivers for the purpose of disappearance represents a challenge for searches due to the hydro-geographical and environmental characteristics of this type of environment. Adding to the complexity is the effect of criminal practices in terms of the alteration of the bodies prior to dumping and the time that elapses between the occurrence of the events. The approach to this type of complex environment requires thorough information collection and analysis about the actions of the alleged perpetrators in terms of *modus operandi*, time frame, and places of operation, as well as the characteristics of the disposal sites and information on the victims dumped in these rivers.

As part of the interdisciplinary methodology developed by EQUITAS for searches in river environments, we present the results of the process of searching, reviewing, purging, and consolidating information on victims and presumed disposal sites in rivers that have been reported in cases of disappearance in the Magdalena Caldense region of Colombia. To carry out this process, testimonial sources were documented through antemortem interviews and participatory mapping workshops were held. The data from these actions were combined with reports of disappearances contained in judicial records and official databases. To conduct the textual analysis of disappearance reports, a programming code was created to guide the processing of more than 30,000 disappearance records. A matrix was designed for the recording, organization, and analysis of geographic and contextual information on events involving river disposal.

The analysis of this information facilitated the construction of a list of 332 persons who were presumably dumped in rivers in Colombia. It was possible to extract information from this list associated with the context of each disappearance that can inform the search and identification of the bodies. In addition, the disappearance routes for these cases were reconstructed, making it possible to identify and characterize presumed sites used for the disposal of bodies in relation to 13 cases associated with La Miel river in Colombia. This information was considered during the subsequent research stages that involved the hydrodynamic modeling of the tributary and interventions using underwater archaeology methodologies as part of the search and recovery of bodies that were disposed of in the river.

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**Enforced Disappearance; Aquatic Environments; Text and Geographic Analysis**

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**F107 Domicile Unknown: Scientific Challenges of Identifying Homeless Decedents**

*Jessica Crowson, MSc\*, Multnomah County Medical Examiner's Office & the University of New Haven, Portland, OR*

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**Learning Objective:** After attending this presentation, attendees will recognize obstacles in confirming the identification of homeless decedents, including a discussion on presumptive versus confirmatory methods, investigation for potential leads, and available resources to assist in these circumstances. This dialogue will demonstrate these challenges by discussing appropriate terminology, presenting relevant statistics from one metropolitan agency (from Multnomah County Medical Examiners Office in Portland, OR), and highlighting a few case studies.

**Impact Statement:** By presenting both successes and failures in identifying homeless decedents, this can stimulate more open discussion and sharing of resources between forensic agencies to put forth sound, scientific practice in identifying decedents.

While there are varying structures to agencies charged with medicolegal investigation, their core responsibilities are consistent. Every medicolegal agency conducts scientific investigations to establish cause and manner of death, and every decedent must be properly identified.<sup>1</sup> When a decedent is with their family, or in their secured and established residence or vehicle, identifications may not be complicated to determine when proper documentation and data is readily available on scene. Even within these situations, identification could be challenged when influenced by factors such as catastrophic injuries, advanced decomposition, animal scavenging, mass fatalities with numerous decedents, or improper investigation.<sup>2</sup>

Unidentified decedents may have personal effects on them to assist and/or lead to determining who they are; however, challenging cases, including those above, may be even further complicated by homelessness. An added challenge of many metropolitan jurisdictions is determining the identity of the homeless.

Homeless decedents (among other individuals) can live particularly unattached or transient lifestyles, which may limit more common pursuits of medicolegal authorities attempting to find a person's name. The longer a decedent is unidentified, the longer it will be for a family to be given proper notification.

Presumptive information can be relevant, but as science-based investigators, it is essential that confirmatory methods are applied for identification purposes. Findings at scenes can be scrutinized for valuable leads and submission of fingerprints may result in positive identifications even without presumptive information. Making assumptions based on scene evidence alone is poor scientific practice and may result in misidentifications with significant legal ramifications.<sup>3</sup> Presumptive data can be valuable when used appropriately and it can help to lead to proper confirmation and/or exclusions. Additional resources such as local agencies, NamUs, or other tools can also be useful in these matters.

With a review of some of the successes and failures in identifying homeless decedents from one metropolitan office, other medicolegal agencies should be encouraged to more openly discuss, critique, and share resources to put forth sound, scientific practice in identifying decedents, and in turn, properly notifying their families.

**References:**

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2. Spitz, W. U. (2005). *Spitz and Fisher's Medicolegal Investigation of Death: Guidelines for the Application of Pathology to Crime Investigation.* 4th ed. Charles C Thomas.
3. Van Ryn, D. (2009). *Mistaken Identity: Two Families, One survivor, Unwavering Hope.* New York City, NY: Simon and Schuster.

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**Medicolegal; Homelessness; Identification**

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**F108 Decedent Identification: A Look at Forensic Genealogy When All Other Methods Failed**

*David Kintz, BS\*, Park County Coroners Office, Fairplay, CO*

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**Learning Objective:** This presentation will outline the example of two cases where all traditional methods of decedent identification did not succeed. These cases will outline the need for full range thinking when it comes to determining the identification of a decedent. Forensic science is a growing and expanding field and this presentation will speak to that.

**Impact Statement:** The impact of this presentation will be to educate those with the responsibility of identifying the deceased. The goal is to expand the knowledge of all methods of scientific identification. This presentation will present all traditional methods and the process of forensic genealogy that led to the successful identifications.

The presentation will begin by outlining each accepted scientific method of identification. This will include fingerprints, odontology, medical radiographical, and DNA. A brief description of the benefits and pitfalls of each will be discussed. Then two unidentified cases will be discussed with an explanation of why each method failed.

Case 1 is from 1974 when remains were found in the Colorado wilderness and were buried in a county cemetery as “unknown.” The decedent was found in the winter in a severe state of decomposition. No identifiable items were found with the subject. At the autopsy, the fingertips were removed to be sent to a lab for printing. The jaw was removed for a forensic odontology exam. No other samples were saved that could be used for DNA. The subject was exhumed in 2017 for reexamination and DNA collection. The subject was found to still be missing the fingertips and jaw. A femur was collected and submitted for DNA. DNA submission in CODIS was unsuccessful. In 2020, a forensic genealogy firm was enlisted to help. DNA was processed for proper processing through genealogy databases. The genealogist began with a 12<sup>th</sup> cousin and worked from there. Eventually, they identified a 3<sup>rd</sup> cousin on the mother’s side and a 1<sup>st</sup> cousin on the father’s side. The DNA from both was used to confirm that this individual was related to both families and further research confirmed he was the only child from a union of these families. An adopted daughter was found and notified; she had been missing her father since she was 14.

Case 2 is from a scattered skeletal case found in 2020 with no identifiable items. This individual went to great lengths, including pinging the serial number on the shotgun he used to commit suicide as well as destroying his phone beyond the ability to gather information, including removing all serial numbers. Due to the amount of injury and the backcountry conditions, no dental exam was possible. DNA was submitted into CODIS, and tips were gathered to produce possible family reference samples. All were unsuccessful. In the spring of 2022, forensic genealogy began, and the profiles were processed for database submission. Close family members, including the subject’s sister, were linked. The subject was positively identified by DNA comparison with the sister. The family was notified and revealed they had been missing him since 2019 but had not reported him missing because he told them he was going on a multiple-year trip to Guam and China.

This presentation will push the attendees to always keep thinking forward and use the latest science and technology available to solve their cases.

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**Forensic Genealogy; Identification; Decedent identification**

**F109 I-Familia: Identifying Missing Persons Globally Through Family DNA Kinship Matching**

*Francois-Xavier Laurent, PhD\*, INTERPOL, Lyon, Rhone-Alpes, FRANCE; Susan Hitchin, PhD, INTERPOL, Lyon, Rhone-Alpes, FRANCE*

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**WITHDRAWN**

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**F110    Medicolegal Issues in Cremation: A Comparative Analysis of International Legislation and a Proposal of a Unified Operating Methodology**

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**Learning Objective:** The goal of this study is to inform the attendees about similarities and differences between various States' legislations regarding the cremation process and to propose a new unified operational methodology that could significantly mitigate the medicolegal issues arising from this practice.

**Impact Statement:** These new operational guidelines could impact the medicolegal issues determined by cremation

Cremation is a well-established mode of disposal that has seen a constant increase in popularity around the world. Nations with the highest cremation rates (80% or higher) include Japan (over 99%), Hong Kong, South Korea, Switzerland, Sweden, the Czech Republic, Singapore, and Thailand.

Because of its extensively destructive nature, this practice creates a number of medicolegal issues, especially concerning the opportunity to perform a forensic autopsy or external examination, identification, histological, toxicological, and genetic exams. The study and comparison of cremation laws on government websites that show the latest official text of Italy, Germany, Spain, Portugal, France, Norway, United Kingdom, and the United States show that there are a lot of similarities but also some differences.

Similarities include the need for a certificate by a medical examiner that excludes any medicolegal issues and non-natural causes of death and the requirements in terms of consent to make the decision to cremate a body, which relies on whether deceased person had expressed the will to be cremated or on family members who, after the death, declare this wish.

Significant differences were found in Germany's law establishing the need for a second medical examination prior to cremation and in Italy's obligation, introduced by law No. 130 of 2001, "Cremation and ashes dispersal provisions," for the medical examiner to collect samples of biological fluids and skin annexations from the body and store them for a minimum period of ten years for any future purpose of justice.

Trying to tackle these issues, the Italian region of Campania proposed an operating methodology that establishes that the medical examiner (forensic medicine specialist or, in his absence, another doctor belonging to the Forensic Medicine Operative Units and properly trained), after excluding causes of death other than natural, following the request for cremation proceeds to the precise identification of the body, fills out a specific consent form and, in the presence of witnesses, also identifies and proceeds to take the buccal mucosa, skin annexations (hair, axillary or pubic hair, including bulbs) and, in the event of a negative result, a blood sample and/or vitreous humor.

These guidelines, although effectively satisfying requirements regarding sample collection, resulted in lacking a well-defined sample storing chain and in an adequate informed consent for sample collection and processing of personal data.

A new operating methodology was elaborated, improving the Campania's proposal, to try to overcome these shortcomings. This new operating methodology, together with a scrupulous external examination performed by a well-trained medical examiner, could significantly reduce the medicolegal issues caused by cremation, allowing, for a period of 10 years, a valid substrate for at least genetic and toxicological exams.

However, there still is a lack of a solid national cost-benefits study to assess the actual value and applicability of this methodology on a global scale.

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**Cremation; Legislation; Guidelines**

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**F111 Firearms Deactivation and Deactivation Process Control in Croatia/European Union**

*Damir Tomasek, MS\*, Forensic Science Center Ivan Vucetic, Zagreb, Grad Zagreb, CROATIA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of one of the ways of solving problems with illegal and unregistered firearms within Croatia and the European Union. It will show legal and technical aspects of firearms deactivation and deactivation process control in Croatia and European Union member states.

**Impact Statement:** This presentation will impact the forensic science community by introducing a unique way of firearms deactivation and deactivation process control in Croatia and European Union member states. It will also give an overview of legal and technical aspects of firearms deactivation and deactivation process control as well as a description of marks and stamps that are applied on deactivated firearms.

In this presentation, the authors are seeking to clarify problems with firearms deactivation through the valid national and international regulations (Directive of the European Parliament and of the Council on control of the acquisition and possession of weapons, Commission Implementing Regulation establishing common guidelines on deactivation standards and techniques for ensuring that deactivated firearms are rendered irreversibly inoperable) in addition to the regulations in the Republic of Croatia (the Law on Procurement and Possession of Weapons of Citizens, the Law on Explosive Substances Production and Trafficking of Weapons, the Rulebook on the procedure and techniques of disabling firearms and the appearance of the common unique mark) that regulate the above-mentioned problems and providing a practical review.<sup>1</sup>

The aim of this presentation is to acquaint the professional public with the procedures, methods, standards, and other specifics of firearm deactivation (i.e., the process of transforming a firearm from an extremely dangerous object that is suitable to become an object in a criminal act or a means of injury and self-harm, into a harmless thing).

A deactivated firearm is defined as one that is permanently deactivated in accordance with Implementing Regulation (EU) 2015/2403 (hereinafter: the Implementing Regulation), ensuring that all essential parts of such a firearm become permanently unusable and cannot be removed, replaced, or modified in order to refit the firearm in any way.

Furthermore, Directive 2021/555 of the European Parliament and the Council of March 24, 2021 on the supervision of the acquisition and possession of weapons, the concept of a deactivated firearm is defined as permanently deactivated, ensuring that all essential parts of such firearms become permanently unusable and that they cannot be removed, replaced, or modified in order to retrain the firearm in any way.

In this presentation, discussion will include the deactivation of firearms, the Directive, as an umbrella act of the European Union that regulates the field of legal weapons, provides guidelines and defines terminology, and describes in detail the norms and techniques that is the procedure and method of disabling firearms.

**Reference:**

- <sup>1.</sup> Directive (EU) 2021/555 of the European Parliament and of the Council of 24 March 2021 on control of the acquisition and possession of weapons Commission Implementing Regulation (EU) 2018/337 of 5 March 2018 amending Implementing Regulation (EU) 2015/2403 establishing common guidelines on deactivation standards and techniques for ensuring that deactivated firearms are rendered irreversibly inoperable.

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**Firearms; Deactivation; EU Regulations**

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**F112 The Direct Detection of Invisible Gun Powder Residue on Fabric Surfaces: A Nano Tape and a Biological Microscope Method**

*John Wang, PhD\*, California State University-Long Beach, Artesia, CA*

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**Learning Objective:** After attending this presentation, attendees will learn a direct detecting method of gun powder residue using a nano-tape and a biological microscope at 800X. In the field, the novel method was able to successfully detect tiny gun powder residue (GPR of nitrocellulose or nitroglycerin) that is invisible to the naked eye. The detecting process takes about 15 minutes from pressing the GPR to getting images (JPEG) with a resolution of 3664 x 2748.

**Impact Statement:** This presentation will impact the forensic community by providing a novel method of detecting invisible GPR on fabric surfaces in the field. The attendees will learn the principles, the sampling steps, and the detecting process of the novel method.

One of the challenging issues at shooting-related scenes is to detect the gun powder residue on or around a suspicious gunshot hole where the gun powder particles from a longer muzzle-to-target distance are not visible to the naked eye. However, the proof of a gunshot hole via the gun powder residue is the first step in determining the occurrence of a shooting incident, the muzzle-to-target estimation, and finally the shooter's position. While the traditional procedure is to collect the GPR by stubs and bring it back to the lab for a Scanning Electron Microscope (SEM) detection, the consequence is time-consuming and costly (most crime labs cannot afford a SEM), thus delaying investigation efforts.

This study employed a quasi-experimental design with a purposive sampling. A white T-shirt was shot by a 9mm pistol (124-gr.) from 15 feet as the muzzle-to-target distance. The longer shooting distance makes the gun powder residue invisible to the naked eye since there is no visible black soot particles on or around the bullet hole.

Instead of using studs or cotton swabs, a new type of tape called the nano-tape was used to press on the gunshot hole, then placed under a biological microscope (1,600X). The nano-tape is a crystal-clear transparency with a glue on both sides; one side is used to press on the gunshot hole to get the GPR and the other side to stick onto a regular microscopic slide. Magnification was selected with the ocular lens at 20X and the objective lens at 40X. A total of six images were taken on each side of the gunshot hole along the central axis (the upper and lower portions) for comparison purposes. Under the total magnification power at 800X, the gun powder particles can be clearly observed and taken of images via a mounted digital camera (10 MP Color CMOS).

Based on the field results as a ground truth testing, the author argues that the combination of the nano-taping and the biological microscope can provide a practical tool at the scene, in the lab, or even in courtroom testimony to prove the existence of gun powder residue on a gunshot hole. The result, which is usually invisible to the naked eye, can be further used to estimate the muzzle-to-target distance, the shooting angle, and the shooter's position for a crime scene reconstruction.

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**Gun Power Residue; Muzzle-to-Target Distance; Shooter's Position**



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**F113 A Comparison Between Normal Test Firing and Submerged Test Firing of the Same Firearm and Ammo**

*Ali Shahid Hussain, BME\*, Ministry of Defense, PAKISTAN*

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**Learning Objective:** The attendees will learn: (1) how the marks characteristics get affected when the medium of the environment changes, (2) how to reduce the pollution generated by a weapon, in an inexpensive way, (3) how we can change the quality of lands and grooves on the bullet just by altering the medium, and (4) what the pros and cons are of different environmental media.

**Impact Statement:** If the method described in the abstract becomes a proven exercise in firearms forensics, then the scientist will be eased in comparison, there will be reduction in the time for matching, quality of work will be improved, and the absence of a primer cup will not remain a big issue.

When gunfire takes place, the firearm leaves its marks on the bullet (i.e., on the projectile, the lands and grooves of the barrel-rifling of the firearm, the cartridge case, the breech-face marks, firing pin marks, and extractor-ejector marks). All these marks are of great importance. They are like the fingerprints of the firearm, which can be used to detect the firearm.

Breech-face marks are of great significance in firearms identification. Their significant nature escalates when the primer of the cartridge case is unavailable or pierced. The density of the breech-face marks changes with the change in medium in which the firearm is fired (i.e., water or air). Moreover, it also depends upon the metallurgical properties of the cartridge case. This presentation highlights the variation in density of breech-face marks when a firearm is fired in the air and the same firearm is fired under water. The technique used in this presentation may be helpful in those circumstances when breech-face marks are very light and are not enough to decide the identification and elimination. Furthermore, both projectiles of the bullet (in water-fired and in air-fired) are also compared. The behaviors of the firing pin and extractor-ejector are also observed regarding normal firing and submerged-firing. From submerged firing, we can have deeper, sharper, and cleaner breech-face marks and lands and grooves. Firing pin marks and extractor-ejector marks are relatively lighter and poor with respect to the normal test firing. Other benefits in submerged firing include bullet jackets are not burnt nor do they adhere to soot particle (in excess) because of the fluid film; this makes it convenient for the observer. The length of the water tank that was used in the experiment can be cut in half, so a smaller tank sufficed. Sound was drastically reduced, and smoke was absent because all the burnt gases were mixed in water.

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**Submerged Firearm Firing; Lands and Grooves; Identification Marks**

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**F114 A Quick Comparison of the Polygonal Rifling Using a 4K Resolution Microscope: A Field Examination Approach**

*John Wang, PhD\*, California State University-Long Beach, Artesia, CA*

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**Learning Objective:** After attending this presentation, attendees will learn of a novel method of comparing the polygonal rifling with the actual images. Using a quasi-experimental design, four pairs of fired bullets with the polygonal rifling (two pairs of .45 with an octagon profile and two pairs of 9mm with a hexagon shape) were compared by a 4 K resolution microscope in the field. The portable device was able to successfully measure the rifling widths, produce the rifling pitches, and display the rifling striations. These three indicators, which cannot be observed under a regular comparison microscope in the lab, now can be used to make a comparison decision on the difficult rifling for firearms examinations in the field.

**Impact Statement:** This presentation will impact the forensic community by providing a cutting-edge method with three new features: a 4 K resolution (4096 x 2160) or 8 million pixels, measurements of the rifling width and pitch by an auto-focus mode, and visible striation images all within five minutes.

One of the challenging issues in the field of firearms identification is to compare the polygonal rifling from certain brands of pistols because their lands and grooves have a rounded edge rather than the traditional rectilinear or angled segments (the land). Therefore, the polygonal rifling renders the observation and comparison extremely difficult by the traditional comparison microscope. Several other challenges are also encountered. First, some major firearm manufacturers have chosen the polygonal rifling as a new direction to better reduce both powder and copper fouling, increase velocity and accuracy, provide easier cleaning, and thus prolong the barrel life. To the firearm examiners, it implies that more and more fired bullets at scenes will have the polygonal rifling. Second, on the national GRC database, the lands and groove widths for this type of pistol is listed as 0.000, meaning “no reference available,” which can be translated that there is a lack of a national standard for comparison. Finally, due to the increase of shooting-related incidents in recent years, there is a strong need to provide a microscope that can be used for a field examination of fired casing and bullets, including those with the polygonal rifling.

This study employs a quasi-experimental design under a mock scene scenario where eight fired bullets in four pairs were placed on an indoor floor. The following results are reported: (1) the set-up and the adjustment time takes about five minutes; (2) the comparison of each pair requires about five minutes on average; (3) the microscope has an auto-focus mode to obtain a fast zooming with a one-button press; (4) the device provides a 4 K resolution that the image is four times clearer than that of a full high definition (FHD, 1080 x 1920) at 20X and 50X for better comparison and analysis; (5) the blurring rifling widths under the comparison microscope now become clearly visible and can be measured by an on-screen cursor in real time. The same method can be applied to measure the rifling pitch; (6) with a tilt lighting, the striations (the tiny hills) on the flat rifling allows an angled observation for a micro-tracing of unique markings on each pair of bullets. With the six new features, the author contends that this new 4 K resolution microscope should add a new promising approach for comparing the polygonal rifling from certain pistols in the field examination. The scientific functions provide for fast zooming, better imaging, and real-time measuring of the width and the pitch of the polygonal rifling.

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**Firearms Examination; Polygonal Rifling; 4 K Resolution Microscope**

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**F115 The 3D Correlations in Automated Ballistic Identification Systems Using the Evofinder® Ballistic Identification System**

*Logan Eickhoff, BS\*, Leeds Forensic Systems, Inc., Minneapolis, MN*

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**Learning Objective:** Attendees will learn how 3D images are generated using Automated Ballistics Identification Systems for sample visualization, virtual comparison microscopy, and automated identification for use in firearms and toolmarks examination.

**Impact Statement:** This presentation will help to provide insight into new technology in the field of firearms and toolmarks and show how it is currently being used for examination.

After attending this presentation, attendees will learn about new technology in the field of firearms and toolmarks and how labs across the country are currently using 3D technology. The 3D ballistic identification systems have a wide array of features, including the generation of 3D images that can be used for sample visualization, virtual comparison, and automatic identification within its database or those to which it has access. Visualization and comparison (also known as Virtual Comparison Microscopy) are integrated functions of the system and allow examiners to compare the digital sample representations, including sample and lighting position/orientation.

This presentation will address the standard features of all 3D ballistic identification systems and highlight some of the unique capabilities. These capabilities include the trace types used and setting search parameters in the software to determine the similarity between samples to help identify the firearm. These searches will display the results in tables to organize samples from most similar to least similar using the different labeled trace types and generate similarity coefficients in the list of correlations.

The 3D images can then be used for sample visualization and virtual comparison microscopy (VCM). VCM is a new technique that allows examiners to conduct examinations using software in a fashion similar to using a traditional comparison microscope. The automatic identification features allow the user to identify sample features for use by the system for comparison to other samples within the database, including the following: breech face impression, firing pin (striker) impression, ejector mark, land engraved area, groove impression, and slippage marks.

The 3D-generated images can be loaded into the VCM program and shared among examiners with the ability to change the lighting and orientation using different contrast methods and controls. The high-definition images can be stored digitally in a database and shared using the standard X3P file format.

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**Firearms; Ballistics; 3D Technology**

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**F116 The Attitudes of Infertile Italian Women Regarding Ethical and Regulatory Limits of Medically Assisted Procreation (MAP)**

*Andrea Cioffi, MD\*, Sapienza University of Rome, Rome, Lazio, ITALY; Camilla Cecannecchia, MD\*, Sapienza University of Rome, Rome, Lazio, ITALY*

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**Learning Objective:** We present data of considerable interest in the field of Reproductive medicine and its medicolegal and bioethical aspects. Our work frames a particularly delicate situation in Italy that may be interesting for attendees interested in bioethics and forensic gynecology

**Impact Statement:** In the field of forensic gynecology, our work is unique in its kind because, through questionnaires administered to infertile patients, it obtains the opinions of these subjects on the issues related to the law on the Medically Assisted Procreation in Italy. The article may favor an advance in the field of bioethical and forensic aspects of Reproductive medicine

In Italy, Medically Assisted Procreation (MAP) is regulated by Law 40 of 2004; this law is composed of 18 articles and has defined finalities, modes, and times of the regulated techniques.

Furthermore, in Italy, there is a national MAP Register, managed by the National Institute of Health. This is a database that collects the data of the structures authorized to carry out MAP techniques. It also records the number of embryos formed and children born with MAP techniques. These data are processed for the Minister's Annual Report to Parliament. This system allows constant monitoring of the progress of MAP techniques; in this way, it is also possible to consider the problems related to this field and the results achieved. Law 40 of 2004 for the type of issues dealt with, from the outset, has sparked a wide bioethical and legislative debate.

In fact, 15 years after the publication, this law has ended up under the judgment of a judge 38 times; of these, four times the Italian Constitutional Court has declared it unconstitutional.

Our objective is to outline the views of Italian women, with fertility problems, on Medically Assisted Procreation. We have collected the opinions of 448 infertile women. The items used in the questionnaire were created with a qualitative method based on the Medically Assisted Procreation's most important bioethics issues and on the basis of the limits imposed by law. The questions of the first part of the questionnaire were open; the second part was closed ("Yes/No"); for each method they were asked whether they would introduce a legal limit to prohibit it. The tests have been standardized with test-retest method. We found that much of the disputes that have, over time, been made to Law 40 of 2004 by Italian Courts, are shared by patients with infertility problems. Italian women do not share the legal limit for Medically Assisted Procreation in over 43 women, heterologous insemination with seed donation and egg donation. Moreover, our sample shows that Italian women do not share legal limit to pre-implantation genetic diagnosis and cryopreservation of embryos. Moreover, it emerges that many infertile Italian patients disagree with the Medically Assisted Procreation for homosexual couples.

It can be said that the current law governing MAP in Italy must be changed and it will be important—in the event of a legislative reform in Italy on the Medically Assisted Procreation—also consider the point of view of women with infertility problems.

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**Medically Assisted Procreation; Reproductive Medicine; Medical Ethics**

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**F117 The Role of Forensic Medicine in Addressing War Crimes: A Perspective From the Russia-Ukraine Conflict During the COVID-19 Pandemic**

*Andrea Cioffi, MD\*, Sapienza University of Rome, Rome, Lazio, ITALY; Camilla Cecannecchia, MD\*, Sapienza University of Rome, Rome, Lazio, ITALY*

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**Learning Objective:** We present an interesting work focused on the role of forensic medicine in war conflicts. This issue, particularly topical, is not sufficiently explored despite the problems related to an unclear definition of some war crimes at the international level and the insufficient definition of the role of the medical examiner in these circumstances.

**Impact Statement:** This presentation will impact the forensic science community by showing that it is necessary to fill some gaps in the forensic system applied to international law, war conflicts, and war crimes.

The International Criminal Court (ICC) has opened an investigation into the international crimes committed on Ukrainian territory. The ongoing Russian-Ukrainian war is a tragic opportunity for a necessary critical and scientific reading of the Rome Statute. The number and variability of crimes that can be considered “war crimes” requires the intervention of specialized professionals, including forensic doctors. In fact, in cases of suspected war crimes, medicolegal investigations are essential for clarifying the circumstances of death and/or violence in war contexts.

Sources of law and jurisprudence concerning international crimes, in particular war crimes, and the law of war have been consulted in order to better understand the role of forensic pathologists in such situations. Particularly, specific contents of the Rome Statute of the ICC, Geneva Conventions of 12 August 1949, and sentences of the International Criminal Court and the European Court of Human Rights concerning war crimes have been analyzed. In this work, we aim to critically analyze the contents of the ICC’s Rome Statute, with particular attention to the definition and listing of war crimes. Our objective is to assess whether the content of the Rome Statute and the Geneva Convention is useful to provide a correct and complete orientation of the medicolegal work in the context of war. We analyzed the various war crimes most frequently observed, with particular attention to “sexual violence” and its legal definitions (sexual assault, rape, and sexual abuse). We have identified all the problems inherent in the correct identification of the characteristics (lesions and circumstances) pathognomonic of certain war crimes. Our work highlights some gaps in the forensic system in the area of conflict management and war crimes. Filling these gaps is necessary for the proper application of international law.

We believe that is necessary to optimize and standardize the role of the forensic doctor in war scenarios, avoiding further underestimation of war crimes. Therefore, the forensic pathologists, and forensic experts in general, are the only professional figures specialized in providing scientific evidence of crimes compatible with war crimes. Their timely intervention and the standardization of their work—in association with a review of the deficient content found in the Rome Statute—is essential in order to allow the prosecution of international crimes.

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**War Crimes; Human Rights; International Law**

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**F118 A Wake-Up Call on the Deceptive Nature of Sodium Nitrite Suicides: A Case Series**

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**Learning Objective:** Attendees will learn the typical elements of suicide by sodium nitrite ingestion, which is a new and ever-increasing suicide mode. It is important to keep in mind the hypothesis of sodium nitrite intoxication for those deaths without any apparent cause and to assess the possible presence of suspicious powder at the crime scene to conduct specific toxicological analyses.

**Impact Statement:** This presentation will impact the forensic science community because the emerging phenomenon of sodium nitrite suicides represents a challenge for the forensic pathologist and the forensic toxicologist. Moreover, this suicidal trend highlights the need for stricter regulation of sodium nitrite sales as the use of sodium nitrite for suicidal purposes and sponsored on several websites is becoming one of the first-choice methods among the young.

Sodium nitrite is an odorless, white/yellow, crystalline inorganic compound, similar to salt at first sight. It is commonly used as an additive in various fields, from the food to the drug industry. Some cases of accidental intoxication have been described in the literature.<sup>1,2</sup> In past years, it has been used more and more for suicidal purposes, especially among the young, because it is sponsored as a “sweet death” on some websites and books. Sodium nitrite is widely used and cheap, so it is quite easy to be found, even on the internet.<sup>3</sup> Moreover, it is soluble in water and the lethal dose is low, as it has been estimated to be between 1 and 2.6g.<sup>4-6</sup> However, cases of sodium nitrite suicide are probably underrated because sodium nitrite is not part of routine toxicology screening.<sup>3</sup>

Ingestion of sodium nitrite leads to death by asphyxiation, because of the conversion of hemoglobin into methemoglobin. Methemoglobin does not have the ability to carry oxygen, thus leading to hypoxia and lactic acidosis.<sup>1,2</sup> Levels of methemoglobin in blood above 70% are usually fatal.<sup>1-3</sup> Moreover, sodium nitrite can also induce hemolysis and peripheral vasodilation.<sup>2-5</sup> Suspicion of sodium nitrite intake usually comes after evaluation of circumstantial data (e.g., the finding of a white powder near the body), which should induce pathologists to execute further analysis, in addition to the classic toxicological screening. The postmortem findings in such cases are unspecific, such as bluish hypostases and cyanosis of the lips and nail bed. Consequently, the diagnosis is based on the concentration of methemoglobin and nitrates, as nitrite has a half-life of about two minutes and is quickly converted into nitrate (NO<sub>3</sub>-).<sup>7</sup> In this presentation, the autopsic and toxicologic findings in four cases of suicide by sodium nitrite ingestion (three male, one female; mean aged 39.25 years, range 27–51 years old) are presented.

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**Suicide; Sodium Nitrite; Methemoglobin**

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**F119 Surveillance Fails: Suicides in Jails**

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**Learning Objective:** This study examines seven cases of suicide in jail. All seven people restricted had clinical risk factors for suicide and therefore were subjected to special control regimens. The aim of this study is to identify the circumstances of suicides highlighting those that can have adversely contributed to the failure of the prevention and the possible measures to improve the prevention of suicides in jail.

**Impact Statement:** This presentation will impact the forensic community by presenting a case series of suicide in prison regarding inmates subjected to various kinds of surveillance for clinical reasons. The high rate of suicides in inmates subjected to attention for clinical reasons highlights that the surveillance carried out by penitentiary police is not effective to prevent suicide. Our study points out the relevance of a multidisciplinary approach to these inmates and considers the methods most used to commit suicide, to prevent self-harming gestures during the sentence.

According to the Italian penitentiary legislation, inmates who have clinical risk factors for suicide are subjected to special control regimes. These are “surveillance by sight,” “great surveillance,” and “very great surveillance” and are carried out by penitentiary police. “Surveillance by sight” is a provision that is adopted for prisoners classified at high suicide risk. “Great surveillance” and “very great surveillance” are provisions adopted on medical advice in cases of inmates with fragile personalities and/or psychic pathologies according to their seriousness.

In our study, we examine seven cases of inmates who committed suicide despite the adoption of surveillance for clinical reasons. The first case concerns a 36-year-old male inmate from Romania. He had psychiatric disorders for which he was subjected to a regime of surveillance by sight, having already attempted suicide; he committed suicide using shoelaces to hang himself. The second case regards a 46-year-old Italian male with psychiatric disorders. He was under surveillance by sight for having already attempted self-harm; he used a sheet to hang himself. The third case regards a 20-year-old male inmate from Egypt. He had psychiatric disorders for which he was subjected to a regime of very great surveillance, not having already attempted suicide; he committed suicide using a rope to hang himself. The fourth case concerns a 35-year-old Italian inmate with psychiatric problems. He was subjected to great surveillance because of his past psychiatric illness, there were no documented suicide attempts; he used the drawstring of his trousers to hang himself. The fifth case regards a 24-year-old male inmate. He had psychiatric disorders and was subjected to very great surveillance for having already attempted suicide; he committed suicide using a piece of shirt to hang himself. The sixth case concerns a male from North Africa with psychiatric disorders; he was under great surveillance for having already attempted self-harm; he used a sheet to hang himself. The seventh case regards an Eritrean male with a substance use disorder. He was subjected to very great surveillance and there is no evidence of any other suicide attempts; he used a sheet to hang himself.

In all seven cases, on external examination, the only lesion is the sulcus on the skin, caused by the means used for hanging. Another sign present, typical of mechanical asphyxia, is the visualization of the subconjunctival petechiae. In all cases, the hypostasis is not typical of hanging because the corpses were discovered in a short while and placed supine on the floor to attempt resuscitation maneuvers. In correspondence of each sulcus, the autopsy showed the presence of hemorrhage in dermis, subcutaneous tissue, interstitial connective tissue, cervical muscles, and the laceration of the fibers of the neck muscles.

In all of these cases, the detainees were subjected to special surveillance regimes that had no effect on suicide prevention. It can be said that surveillance, understood as an active control by the penitentiary police only, fails to prevent suicides in prison. To prevent suicides, it would be advantageous to carry out a medical screening of all inmates with a multidisciplinary approach to frame the risk of self-harm even in the absence of known risk factors. It would also be advisable to pay particular attention to the forensic pathological point of view to study the methods of self-harm and the means used to make them unavailable to prisoners. This is in order to collect data on the most common types of suicide in prison and try to minimize the risk factors (including easily available objects) deriving from the prison environment.

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**Jail; Surveillance; Suicide**



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**F120 Exploring Potential Factors That Can Impact Students' Professional Identity in Forensic Science**

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**Learning Objective:** Attendees will learn about various factors affecting student retention in forensic science, such as the uncertainty of the future.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that increasing self-identification within forensic science could be paramount to encouraging more student engagement by searching for new opportunities and professional relationships, in turn encouraging retention.

There has been an increased interest in researching students' professional identity as a potential factor that can increase student retention. Professional identity is how an individual defines themselves within their profession. There is a gap in the literature investigating forensic students' professional identities. In 2015, Mancini et. al. validated and published the Professional Identity Status Questionnaire (PISQ-5D).<sup>1</sup> This survey measures five social constructs to discern how an individual might identify within their chosen field of profession. The original study primarily focused on undergraduate and master's program students within the field of psychology; however, in recent years, the survey has been modified and adapted for use in other professions such as nursing, social work, and STEM.

For this study, the survey language was modified to contain the phrase "professional in your field" as well. Open-ended questions were also added to the end of the survey to gather more in-depth data on how the students viewed themselves as professionals within the field of forensic science. Undergraduate forensic science majors in a senior-level course were given this modified version of the PISQ-5D to increase the understanding of how the students relate to their chosen profession of forensic science. Thematic qualitative analysis was used to analyze the open-ended responses.

This presentation will focus on the analysis of these open-ended questions to gain further insight into what may impact a student's professional identity in forensic science. The results from this study identified several themes among students. One such theme was that some students felt prepared and confident to call themselves a professional in forensics; however, there was a high degree of uncertainty within the students' confidence in identifying as a professional in the field of forensics due to multiple variables such as job insecurity and feelings of unpreparedness. Students mentioned finding themselves questioning a lifetime commitment to forensic science. The importance of this research will help identify key components that could help students connect within forensic science. As a result of increasing students' identity within their profession, this could help increase their self-connection to the forensic science field and encourage more student interaction through searching out new opportunities and relationships with others in forensic science, hopefully increasing retention.

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- <sup>1</sup>. Mancini, T., Caricati, L., Panari, C., & Tonarelli, A. (2015). *Professional Identity Status Questionnaire (PISQ-5d)* [Database record]. APA PsycTests. <https://doi.org/10.1037/t44250-000>.

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**Retention; Self-Identification; Connection**

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**F121 The Use of Barr Bodies to Determine Sex in Samples of Forensic Significance**

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**Learning Objective:** Attendees will learn how identifying the absence or presence of Barr bodies, X-linked condensed chromatin, in mammalian blood can be an effective screening method for determining sex in unknown samples. Attendees will learn what Barr bodies are, how Barr bodies can be used to differentiate between female and male mammals, and the method used to validate it as a reliable sex-determining screening method.

**Impact Statement:** This presentation discusses the use of Barr bodies as a potential screening method for determining sex in samples related to forensic science. In instances where formal testing is not readily available, criminal investigations are time-sensitive, criminal investigators want to narrow down suspects, or an evidence source needs to be confirmed by sex, this high-throughput and cost-effective method can be used as the first step in the investigative process. Additionally, extensive training is not required to successfully identify Barr bodies or prepare specimens, making it easily accessible to the experts and the novices in the field.

Barr bodies are condensed, inactivated X-chromosomes typically found in female mammals. Barr bodies can be used as substantial indicators of sex and can be found in all mammalian cells except sex cells. Previous research has shown that Barr bodies can be an aid in sex determination, particularly to the forensic science community, in buccal samples and hair shaft samples.<sup>1</sup>

For this study, the focus is on determining the reliability of detecting the absence or presence of Barr bodies in blood of unknown samples to provide an inexpensive and time-effective screening method to determine sex while determining how feasible this method is for a novice to use in this line of work. Collecting samples of blood from crime scenes and analyzing these samples under microscopes can be a non-invasive tool to determining or confirming the sex of a victim or suspect of a crime when each is of the opposite sex. To determine the efficacy of this method, male and female volunteers were recruited to provide blood droplet samples via a safety lancet prick. These samples were used to produce blood smears on glass microscope slides and have been viewed under a compound microscope at 100x magnification, after performing Giemsa staining, to detect the presence or absence of Barr bodies under blinded and unblinded conditions. The unblinded trials have shown Barr bodies present in all blood smears of female origin and < 3% in blood smears of male origin. In the unblinded trial, the sex of each sample was correctly determined 78% of the time. This non-invasive method of sex determination has the potential to simplify the standard protocol in criminal investigations where determining sex is essential to advancing in the investigation process in addition to alternative circumstances where the sex of an unknown sample is required to be identified.

**Reference:**

- <sup>1.</sup> Bhardwaj, Nandini & Bhat, Nitish & Gupta, Hitesh & Puri, Abhiney & Nangia, Rajat & Kaur, Rashmeet. (2020). Barr Bodies-A Spoonful Of Sex Chromatin. *International Journal Of Current Medical And Pharmaceutical Research*. 10.24327/23956429.ijcmpr202001838.

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**Barr Body Identification; Sex Determination; Screening Method**

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**F122 The Effects of Sleep Deprivation and Fatigue on Crime Scene Examination**

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**Learning Objective:** Attendees will learn about the effects of sleep deprivation and fatigue on human performance. A pilot study will be presented that explores how fatigue may negatively impact crime scene examination, documentation, and evidence collection.

**Impact Statement:** This presentation will impact the forensic science community by providing evidence that fatigued crime scene personnel perform worse than their rested counterparts. Agencies should take steps to minimize conditions that lead to sleep deprivation.

Inadequate sleep is known to negatively impact human cognition, performance, physical, and psychological health.<sup>1-4</sup> Studies of professionals in jobs requiring high-level decision-making, such as surgeons and pilots, have led to regulations in the number of hours worked since fatigue can lead to errors with life-or-death consequences.<sup>5-7</sup> Crime scene examiners are often subjected to sleep deprivation and fatigue. The purpose of this study was to examine the effects of sleep deprivation on performance during a mock crime scene investigation. Based on other sleep-deprivation studies, it was hypothesized that participants in the sleep-deprivation condition would exhibit a poorer performance in the mock scene investigation compared to participants in the control condition; specifically, they would collect fewer evidence items and make more errors when executing the protocol given to them.

The Institutional Review Board (IRB) -approved study was conducted using ten individuals ranging in age from 20 to 35 years old. Five participants were randomly assigned to the Rested Group and the other five were randomly assigned to the Sleep Deprived Group. The five participants in the Rested Group were required to have 7–9 hours of sleep the night before participating in the study. The five participants in the Sleep Deprived Group were to stay awake for 24 hours, with limited caffeine use, prior to participating in the study. The study was conducted by having all participants process a standardized mock scene of a burglary in their assigned state, either rested or sleep deprived. Participants were given one hour to process the scene. The scene contained ten pieces of evidence relevant to the crime and numerous items that were irrelevant to the crime. Participants were video taped during their examination and their performance was assessed.

The results indicate that the sleep-deprived group did worse in all aspects of the mock scene examination, including evidence collection and documentation. Rested participants collected more items of relevant evidence and fewer irrelevant items. Fatigued participants collected almost three times more irrelevant items and missed twice as many items of actual evidence as rested participants. Sleep-deprived participants did not execute their search methodically, often going over the same area multiple times, and failed to open drawers or look under furniture. Rested participants took the full hour to process the scene, while fatigued participants consistently finished before the hour was over. This study suggests that the quality and outcomes of scene investigation are negatively impacted when personnel are fatigued. A larger investigation is needed into the effects of sleep deprivation on the crime scene examiner community to ensure that such effects can be identified and mitigated.

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**Crime Scene Examination; Sleep Deprivation; Fatigue**

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**F123 Group Sexual Violence: The Experience of Careggi Hospital in Florence (2010–2021)**

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**Learning Objective:** After attending this presentation, attendees will have improved their knowledge from both a forensic and epidemiological point of view of the phenomenon of group sexual violence, which is still poorly investigated by the scientific community.

**Impact Statement:** This presentation will impact the scientific community by proposing analysis of the harmfulness due to group sexual assault. This can help forensic scientists in answering investigations' questions mainly related to the compatibility of lesions with sexual assault perpetrated by two or more offenders.

The phenomenon of sexual violence represents an issue of great social and legal importance. Tuscany has activated, since 2009, the so-called “Rose Code” in all hospital emergency rooms. This is a path dedicated to women and men who are victims of violence, abuse, stalking, and hate crimes, to create a time-dependent network of comprehensive protection and care for victims. The purpose of the “Rose Code” of Careggi Hospital in Florence is to intercept and refer victims of sexual violence to a proper pathway. Although sexual violence is highly scientifically investigated, the subcategory of group sexual violence is scantily reported and discussed, at least from a forensic perspective, as it is mainly studied in the psychopathological aspects of both aggressors and victims.

The purpose of the present study is to analyze, from an epidemiological and medicolegal point of view, the phenomenon of group sexual violence in order to provide a greater understanding of it, which would allow improvements of prevention of these crimes and the treatment of victims.

The Careggi Hospital case history from 2011 to 2021 was considered. Out of a total of 450 cases of sexual violence recorded by the Center, 45 cases of sexual assaults perpetrated by two or more offenders were included in the study.

To this purpose, a database was created containing: epidemiological data of each case, pertaining to both the victim (age, gender, ethnicity, etc.) and the abusers (number, age, ethnicity, gender, etc.), as well as the relationship, if any, between them and the victim. The location of the violence was also reported. Also described (in number and characteristics) were the injuries noted to the genitals and the rest of the victim's body, specifying (if possible) the manner of occurrence of the rape and the rest of the injuries. In addition, the number of gang rapes in the years studied (2011–2021) was measured, verifying the trend of the phenomenon during the SARS-CoV-2 pandemic.

The extent of genital and somatic injuries caused by two rapists was compared with those caused by a larger number of abusers. The overall lascivity and the compatibility with the alleged assault modality and number of offenders was discussed in light of previous scientific literature. The results of the study enable valuable conclusions about the trend of the phenomenon and improvements of medicolegal capability in evaluating the compatibility of the lesions with the assault manner and number of rapists.

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**Sexual Violence; Multiple Perpetrator Rape; Group Violence**

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**F124 Forensic Aspects in Death and Injuries Due to Elderly Neglect: Interpretative Criteria in the Acknowledgment of Elderly Maltreatment**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the impact of forensic science in elderly neglect cases.

**Impact Statement:** This presentation will have an impact on the forensic scientific community by demonstrating the social and health key role of the forensic pathologist not only in investigating the causes of death in cases of fatal neglect in the elderly, but also in preventing this form of violence.

In recent decades there has been a progressive increase in life expectancy, which has led to a progressive increase in the number of elderly people. A large number of these live alone at home in abandoned conditions. Elderly neglect occurs when someone fails to adequately care for an elderly person by failing to provide for his or her basic needs, both in terms of hygiene, nutrition, and therapy, and in psychological and social needs. We analyzed three cases, two of which were fatal, of elderly neglect. For the two fatal cases, a judicial inspection or health documentation analysis and autopsy were performed. For the third case, a forensic examination was carried out on the patient.

**Case 1:** A 79-year-old woman with multiple pathologies entered in the Emergency Department for accidental trauma. The patient presented with expired general conditions, cachectic and dehydrated. From the medical history, the doctors found that the accidental fall had occurred five days before the date of access to the hospital and after the necessary investigations, found a left femur multi-fragmentary pertrochanteric fracture and dorsal, sacral, and calcaneal pressure injuries. Despite the appropriate treatment, the patient died. Postmortem examination revealed massive pulmonary thromboembolism.

**Case 2:** A 98-year-old woman was found dead at her home. A judicial inspection was performed that revealed the completely burned corpse of the old woman following a fire that involved only one room of the apartment. The family members reported that the fire was accidentally caused by the proximity of the clothes to a stove near the victim. However, the analysis of the scene revealed the story incongruity and, moreover, the remains of woolen laces were found both on the chair armrests and in the garbage, bringing out the hypothesis that the old woman was tied to the chair. The autopsy revealed a decayed state, and toxicological tests confirmed death by carbonization.

**Case 3:** An 80-year-old woman accessed the Emergency Department for reported accidental trauma with no fractures. After providing the necessary treatment, the patient was discharged in stable clinical condition to a nursing home with a diagnosis of cognitive decay and gonarthrosis. During the two months of hospitalization, the family members checked for the onset of painful pressure injuries. A medical-legal examination was performed that revealed ulcerated and infected stage IV pressure sores in the sacrum, buttocks, and heels bilaterally with extensive soft tissue exposure. On her head was an alopecia area. The patient appeared depressed and anhedonic.

In the first two cases, the clinical history analysis revealed a family reality of abandonment in the elderly care. Postmortem examinations also revealed the patients' decay states. In the third case, the forensic investigation made it possible to detect the negligence in the health worker conduct who cared for the elderly.

These cases make it possible to detect that there is true death by negligence in the elderly. According to two recent systematic reviews, it is estimated that the elderly neglect phenomenon has a prevalence of 4.2% in community settings and 11.6% in institutional settings.<sup>1,2</sup> The need for a multidisciplinary approach in the care of the elderly to reduce the exponential increase in cases of elderly neglect should be emphasized. This could be achieved through the creation of an integrated operational protocol aimed at increasing control strategies in care areas by strengthening the medical, social, and family assistance services distributed throughout the territory in order to prevent fatal events.

**Reference:**

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**Forensic Sciences; Maltreatment; Elderly Neglect**

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**F125 The Chemical Analysis of Sexual Lubricant Residue: A Comparison of Swabs Used to Perform Medical Examinations Analyzed by Spectroscopic Techniques**

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**Learning Objective:** This presentation will help attendees understand the difference between types of medical swabs used for sexual assault examination, appropriate swabs for sexual lubricants collection, and the optimal solvent for extracting lubricant residues from swabs.

**Impact Statement:** This presentation will impact the forensic community by providing attendees with vital information about sexual lubricant collection and analysis as these are often encountered in sexual assault cases.

Although DNA analysis is the standard technique for identifying suspects in sexual assault cases, sexual lubricants have become an additional form of sexual assault evidence that should be considered. Sexual lubricant analysis can be used as an additional resource for linking a suspect to a victim through chemical profiling. The collection of sexual lubricants and DNA in sexual assault cases are typically done using swabs. Therefore, it is important to ensure that the swab-extracted lubricant sample can produce a similar chemical profile as the non-swab lubricant reference sample and to determine how significant the difference could be between the recovered residue and the reference.

This study evaluated different types of swab materials (i.e., cotton, polyester, rayon, and foam) purchased from three different manufacturers to determine which swab produced a similar chemical profile for the extracted recovered lubricant compared to the non-swab solubilized lubricant reference sample. A water-based and a silicone-based lubricant were used to evaluate the targeted swabs. These lubricated swabs were extracted in hexane, methanol (MeOH), and dichloromethane methanol (DCM-MeOH) to introduce a range in polarity for optimal extraction of compounds. All extracts were analyzed using Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy. Pearson correlation coefficients were used as a statistical technique to determine the similarity between the non-swab lubricant reference and the lubricated swab samples.

It was hypothesized that lubricant recovered from a cotton swab would produce a stronger correlation to the non-swab lubricant sample. However, in this study, it was determined that polyester and cotton fiber swabs produced the highest similarity scores when compared to the non-swab lubricant reference samples. It was also determined that DCM-MeOH-extracted samples produced a higher similarity score to the lubricant references and hexane had the lowest score. This study can be used in the future as a reference for sexual lubricant analysis.

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**Sexual Lubricants; Swab Analysis; Spectroscopy**

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**F126 The Ahoms—The Lost Dynasty of Northeast India: Reconstructing Ancestry Through Palaeogenetic Approaches**

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**WITHDRAWN**



## **F127 Proteomics Profiling From Skeletal Remains for Forensic Time-Since-Death Estimations: A Systematic Review and Future Probabilities**

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**Learning Objective:** After attending this presentation, attendees will be sensitized about the significance and current status of proteomic profiling in time-since-death estimations from skeletal remains retrieved in different forensic contexts.

**Impact Statement:** This presentation will impact the forensic community by informing attendees about an alternate method of postmortem interval estimation required in forensic death estimations. Proteomics as biomarkers of lifetime activities can also be scrutinized.

Time-since-death estimations of badly damaged and commingled human remains from forensic scenarios is the most challenging task faced by forensic pathologists in medicolegal death investigations. The recent advancements in instrumental and methodological armamentarium of forensic experts have revolutionized the field to make it more robust and efficient. Though the use of proteomic-based investigations in forensic anthropological context is in its infancy stage, the use of proteomic biomarkers in forensic casework has answered numerous difficult queries faced by the forensic community in courts of law around the globe, particularly in the past few decades.<sup>1</sup> The use of proteomics for postmortem interval (PMI) estimations is becoming a preferred method of investigating biological samples for forensic purposes. The study of the proteins and their rates of decay has proven to be one of the useful indicators to calculate the time-since-death intervals, most likely due to specificity, sensitivity, and accuracy of these biomarkers and owing to their requirement in comparatively lesser amounts for analysis. The postmortem degradation of the human body and thus estimation of PMI depends on various intrinsic and extrinsic factors. Proteome-based control studies have reported that the protein decay rate is faster during the first six months compared to the next six months, which further slows down with the passage of time. A potential protein biomarker, biglycan (a matrix protein), has been found directly associated with the PMI estimates.<sup>2</sup> Research on population-specific skeletal samples in controlled environmental conditions can provide substantial proteomic data, and new biomarkers are expected to change the investigation patterns for estimating PMI required in forensic death investigations.

In the present systematic review, the current status of the proteome-based research for time-since-death/PMI estimations from the human skeletal remains/bones and its future directions have been discussed. The scientific databases were searched using search engines like PubMed®, Clinical Key®, WoS®, and Google® Scholar using the terms forensic proteomics, protein decay, bones, skeletal remains, time since death, postmortem interval with the Boolean terms “and &” and “or.” The snowball technique of cross-referencing was used to identify more suitable articles. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed to include eligible research publications. Only nine eligible articles could be screened from a total of 234 searched articles using the above-mentioned search engines, though searching of eligible published articles will continue up to January 2023. All the full-text publications were scrutinized for the information relevant to the scope of this systematic review. The review will provide information about the pattern of protein degradations and protein biomarkers studied by different researchers and used to calculate the PMI estimates.

The human skeletonized remains kept in a non-controlled environment were found to have nearly 300 different proteins; 25% of them getting degraded within less than 20 years.<sup>3</sup> The time-dependent stability of the proteins was dependent upon their masses and their acidic nature. It has been reported that proteins having masses at least approximately higher than 30–40 and proteins that are acidic in nature were found more stable in bones, thus making these bone proteins suitable for estimating PMI.<sup>4</sup> Proteomic concentrations vary within the bones at the different sites, bone-specific proteins being found in abundance in the tibia compared to the femur bone.<sup>5</sup> Fresh iliac crest was found to have a higher number of proteins compared to the tibia bone of the same individual.<sup>6</sup> Estimating the PMI, degradation of the proteins and identifying bone-specific proteins have been widely evaluated in freshly skeletonized human remains wherein a functional protein (COBA2) has been tipped to be a potential biomarker for PMI estimation.<sup>4,6</sup>

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### **Forensic Proteomics; Forensic Anthropology; Time Since Death**

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**F128 Self-Amputation in Complex Suicides: A Comparison of Two Cases**

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**WITHDRAWN**

## F129 “Long COVID” Injury in Health Care Workers: The Biological Damage and Indemnity Compensation

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**Learning Objective:** After attending this presentation, attendees will have acquired a better insight on the importance of identifying a cause-and-effect relationship concerning the development of occupational Long COVID within a health care context, as a criterion to recognize the affected health care personnel an indemnity due to occupational accident.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of a proper surveillance of health care-related SARS-CoV-2 infection among the health care providers in order to guarantee adequate insurance coverage in case of permanent biological damage due to Long COVID syndrome.

Over the period of the COVID-19 spread, the development of sub-acute and chronic forms that prevent subjects from gaining back their original state of health in the absence of any other known disease has led to the emergence of a new pathological status defined as “Long COVID.” Since health care providers are more likely to be affected by COVID-19 compared to other occupational groups, the Italian insurance system against occupational accidents (INAIL) has implemented a medical-legal evaluation of the permanent and temporary biological damage related to SARS-CoV-2 infection developed within a health care system; the aim is to provide an indemnity benefit on behalf of health care providers who are exposed to a specific risk and thus presumably susceptible to a major incidence of Long COVID syndrome. It is a serious phenomenon, of which much is still unknown, including its causes. Given the rise in COVID-19 cases, there is a high probability that the prevalence of Long COVID is likely to be substantial.

In such a context, a retrospective investigation has been carried out within the University Hospital of Messina, aimed at evaluating the following data: percentage of health care providers (medical doctors, nurses, etc.) who contracted the COVID-19 infection and, among these, how many got infected within the occupational context; among this latter subgroup, the percentage of health care providers managed, from an insurance point of view, within a Long COVID indemnity context. Other characteristics considered included: age; sex; medical unit of affiliation; qualification; vaccine status (first, second, or third dose); first infection or re-infection; referred symptoms; and organ damage according to instrumental and laboratory data.

The ubiquitous spread of COVID-19, together with the annexed possibilities to get infected outside an occupational context, raised several problems concerning the ascertainment of the cause-and-effect relationship, especially from a medicolegal point of view. Consequently, the possibility of facing issues related to administrative and judicial appeals in the medium-long term cannot be excluded.

### References:

1. Zara G, Settanni M, Zuffranieri M, Veggi S, Castelli L. The long psychological shadow of COVID-19 upon healthcare workers: a global concern for action. *J Affect Disord* 2021; 294:220-226.
2. King CW et al. Persistent Symptoms among Frontline Health Workers Post-Acute COVID-19 Infection. *Int J Environ Res Public Health* 2022; 19(10): 5933.

### Long COVID; Occupational Accidents; Indemnity

### **F130 The Role of Fauna at the Crime Scene: A Risk of Contamination or a Possible Aid in Reconstruction?**

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**Learning Objective:** After attending this presentation, attendees will understand how the study of macro- and micro-fauna could help forensic pathologists in a comprehensive forensic investigation shed light on murders cases, accidents, or suicides, making the dynamic of the events and the nature of the injuries clearer.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility to determine with more accuracy the time and place of death with the help of forensic entomology and macro-fauna intervention's study of the crime scene, especially with the challenges of investigating doubtful deaths involving animals or larvae at the scene. For these reasons, we want to highlight how the intervention of micro- and macro-fauna at the scene could be a disturbing/confusing factor in the contamination of the crime scene or, if well understood, how it could become a key element in the reconstruction of the truth through the determination of the place, the elapsed time since death, and the modalities in which death occurred.

During the past few years, the study of micro- and macro-fauna in forensic pathology has had a fast development in the estimation of postmortem interval and species identification, especially about Diptera and family Calliphoridae.<sup>1</sup> Also, non-human models like pigs have helped us to understand how large vertebrate cadavers decompose in, for example, various environments, seasons, and after various ante- or postmortem cadaver modifications.<sup>2</sup> There are also cases in the literature where DNA fecal samples were collected at the crime scene and from the shoes of the suspect to see whether they could be linked, resulting in successful prosecution in some cases.<sup>3</sup>

We report five forensic cases with different death modalities: falling from great heights, death from firearms, natural cause of death, death from burns, and drowning at sea. In all the cases we examined, judicial inspections and autopsies were conducted. In cases where the entomological part was prevalent, we proceeded with laboratory analyses by entomology specialists, whereas in cases where the suspicion of macro-fauna contamination predominated, we proceeded with isolation of the animals and related analyses.

In the first case, we analyzed the scene of a young man found dead in an impervious cliff and in an advanced state of decay with the head separated from the body and postmortem injuries compatible with animal bites. In this case, it was important to clarify the nature of the head lesions and the possible compatibility with falling. In the second, we evaluated the death of a young man killed by multiple gunshot wounds in his home; the bloodstains analysis at the scene showed significant contamination due to blood traces attributable to the trampling of blood on the floor due to a dog present on the scene. In the third case, we evaluated a man found in a countryside covered with larvae of the same species and size. In this case, the forensic entomological analysis provided valuable clues on the evaluation of the postmortem interval, the place where the death occurred, and the nature of a dubious lesion on the head that, after microscopic analysis, was attributed to the activity of the micro-fauna. Also, in the fourth case, analyzing the death of a woman who died from burns inside her home, we found on the scene the presence of particular blood traces attributable to the presence of a dog, which also allowed the evaluation of the access and exit points of the house in forensic analysis. In these two cases, the possible source of contamination due to the presence of the dogs was immediately removed and examined. In the fifth case, we assessed the death of a young man by drowning at sea. In this case, it was important to clarify the role of micro-fauna growth in the submerged cadaver that colonizes the body and its clothing as an important source of information on postmortem submersion interval as well as the role of macro-fauna and the postmortem lesions it can create by producing much confusion for the forensic pathologist on the differentiation between the nature and type (vital or post-fatal injuries) of injuries for forensic purposes. Therefore, it is clear that the correct interpretation of the data can be helpful, with the intervention of forensic experts in this field (entomologists, veterinarians, and zoologists), in the resolution of cases in which the fauna plays a key role in the resolution of complex forensic cases.

#### **References:**

1. Gang Lei 1, Fei Liu 2, Peng Liu 1, Yuan Zhou 3, Tong Jiao 4, Yong-Hui Dang 5 A bibliometric analysis of forensic entomology trends and perspectives worldwide over the last two decades (1998-2017), 2019 Feb;295:72-82. doi: 10.1016/j.forsciint.2018.12.002. *Epub* 2018 Dec 11.
2. Szymon Matuszewski 1, Martin J R Hall 2, Gaétan Moreau 3, Kenneth G Schoenly 4, Aaron M Tarone 5, Martin H Villet 6 Pigs vs people: the use of pigs as analogues for humans in forensic entomology and taphonomy research, 2020 Mar;134(2):793-810. doi: 10.1007/s00414-019-02074-5. *Epub* 2019 Jun 17.
3. Vishal Somnay, Thomas Duong, Ray-Young Tsao, Joseph A Prahlow, Crime Scene Analysis Through DNA Testing of Canine Feces-A Case Report, 2020 Mar;10(1):56-61. doi: 10.1177/1925362120944743. *Epub* 2020 Sep 14.

#### **Forensic Sciences; Fauna; Postmortem interval**

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**F131 Current Status and Future Steps of Forensic Science in the Republic of The Gambia**

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**WITHDRAWN**

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**F132 The Contribution of Forensic Botany in Solving Cases of Traumatic Deaths With Dubious Dynamics**

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**Learning Objective:** After attending this presentation, attendees will know how forensic botany, particularly in cases of falls from heights or road investments, can be useful for correct reconstruction of the dynamics of the event.

**Impact Statement:** This presentation will impact the forensic scientific community by demonstrating the role of forensic botany for understanding complex dynamics and as judicial evidence.

Forensic botany has proven to be a constant support in crime scene investigations. In cases of traumatic deaths, such as falls from heights, the data collected at autopsy may not be sufficient to reconstruct the dynamics with certainty and thus the manner of the event. In this work, we describe the aid of forensic botany in dubious cases.<sup>1</sup>

Five cases were analyzed with crime scene analysis and autopsy. The botanical elements of the scene and the elements on the body were analyzed macroscopically and microscopically and subsequently compared.

In the first case of fall from height, the victim revealed the presence of leaves and thorns in the left hand and along the sleeve of the sweater. From the inspection, it was deduced that the point of loss of balance consisted of a very steep area, while about 1–3 meters further down, a second area was found characterized by numerous plant elements with dry shrubs and branches. The analysis of the plant elements present in this last area led back to the same botanical elements found on the sleeve and in the left hand of the victim. Furthermore, in that exact place, there were brambles that had a recent mechanical crushing. In the second case of fall from height, a girl was found dead in correspondence with a gazebo that had a damaged upper part, and not far from a separation hedge. Botanical elements compatible with the separation hedge were found on the victim's clothing and hair. The inspection of the home and the autopsy suggested how the victim, from the bathroom window of his home, fell to the ground in free fall, impacting against the hedge and the portion of the gazebo placed next to the hedge. In the third case, a woman was found lying on the banks of a dry stream. The body lay beneath a dock with rails. An evaluation of the access points of the area was carried out and two paths potentially reachable by the victim were identified. In this case, the plant elements collected along the two paths, called A and B, were compared with the plant elements found on the victim's clothes, so the exact path taken by the victim to reach the jetty was found. In the fourth case of falling, the victim was found on a floor below a retaining wall surmounted by a parapet. The body showed botanical elements scattered on the clothing with traces under the shoes. Between the parapet and the retaining wall there was a small natural ground, on which trampled gravel and vegetation of the same species as that found on the corpse could be observed. In the last case, a pedestrian accident involving a 6-year-old girl, an area of vegetation with shrubs near the point of impact was evaluated on the side of the road, which was altered as by probable mechanical crushing. Furthermore, multiple botanical elements identical to vegetated area were identified on the girl's body. The botanical evidence suggested, according to the analysis of the car, the likely point of impact and helped, together with the autopsy data, to define the dynamics of the event.

The cases described emphasize the careful evaluation of botanical elements on the victim and on the macroscopic and microscopic comparison, especially for the purpose of reconstructing the path taken or for defining the points of fall or impact on the ground in falling from height. These data can prove useful above all in dubious cases in which there is a discrepancy between the testimonies collected or when the circumstantial elements are scarce.

**Reference:**

- <sup>1.</sup> Aquila I, Gratter S, Sacco MA, Ricci P. The Role of Forensic Botany in Solving a Case: Scientific Evidence on the Falsification of a Crime Scene. *J Forensic Sci.* 2018 May;63(3):961-964.

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**Forensic Sciences; Forensic Botany; Crime Scene Investigation**

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**F133 Comparative Detection of Organic Peroxides Used in Improvised Explosive Devices Using Electrospray Ionization-Tandem Mass Spectrometry (ESI-MS/MS) and Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS)**

*Kayla Massari, BS, MA\*, Duquesne University, Pittsburgh, PA*

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**Learning Objective:** Attendees will learn how organic peroxide species commonly used in improvised explosive devices can form complexes with silver cations for more reliable detection using electrospray ionization-tandem mass spectrometry and liquid chromatography/triple quadrupole/mass spectrometry instrumentation.

**Impact Statement:** Organic peroxides have become a common agent in improvised explosive devices (IEDs) because they are accessible for purchase and are susceptible to extremely exothermic reactions due to the instability of the peroxide bond.<sup>1</sup> IEDs have been used in acts of terrorism such as the Oklahoma City bombing and the Olympic Park bombing, and the detection of the explosive component is necessary in terms of eventually identifying the perpetrator who had the means and materials to make the IED.<sup>2</sup> The use of electrospray ionization-tandem mass spectrometry (ESI-MS/MS) can be used to considerably decrease the amount of time and cost of materials required for organic peroxide detection in the context of IED investigations, while also maintaining detection reliability. Liquid chromatography/triple quadrupole/mass spectrometry methodology will also be employed in this study to directly compare and validate the findings of ESI-MS/MS experimentation.

The goal of this project was to develop electrospray ionization-tandem mass spectrometry (ESI-MS) coupled with collision-induced dissociation (CID) for the detection and identification of peroxide-based explosives. Benzoyl peroxide, dicumyl peroxide, and methyl ethyl ketone peroxide were used as model compounds in this study because they are prone to exothermic reactions. In previous studies within our laboratory, a variety of metal cations with a +1 charge, including lithium, sodium, potassium, rubidium, and silver, were complexed with the organic peroxide species to achieve reliable detection of these compounds. From these studies, it was concluded that the silver cation was the most effective at creating metal-peroxide complexes with characteristic CID fragmentation patterns for the organic peroxide species. In this study, benzoyl peroxide, dicumyl peroxide, and methyl ethyl ketone peroxide samples were collected using a swipe sampling method, which involves swiping the organic peroxide from a surface using filter paper. The rapidity and ease of the swipe sampling technique would make it favorable for use in the field for forensic technicians investigating a scene with suspected IED residue. Because silver cations readily complexed with the peroxide species in previous studies, silver-impregnated filter paper was used as the swiping substrate to create a positively charged silver-peroxide complex for detection. The swiped samples were then analyzed utilizing the ThermoFisher™ LTQ XL Linear Ion Trap Mass Spectrometer for ESI-MS experimentation. To complement these experimental studies, liquid chromatography/triple quadrupole/mass spectrometry (LC/QqQ/MS) was used to validate experimental findings with ESI-MS and to provide an alternative method of detection. The same swiped samples were analyzed using the Agilent® 1200 LC Stack and the Agilent® 6460 Triple Quad Mass Spectrometer, and these results were directly compared to results obtained using ESI-MS.

**References:**

- <sup>1</sup> Lu, K.-T.; Chen, T.-C.; Hu, K.-H., Investigation of the decomposition reaction and dust explosion characteristics of crystalline benzoyl peroxides. *Journal of Hazardous Materials* 2009, 161 (1), 246-256.
- <sup>2</sup> N/A IED Attack Fact Sheet: Improvised Explosive Devices. <https://www.dhs.gov/publication/ied-attack-fact-sheet> (accessed August 1).

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**Organic Peroxides; Improvised Explosive Devices (IEDs); Tandem Mass Spectrometry**



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**F134 Death From Heat: A Case Report and Literature Review of Fatal Heat Stroke**

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**Learning Objective:** This presentation will be interesting for the attendees as it analyzes the problem of heat stroke diagnostic difficulty. We present both our own case and a review of the specific literature, examining the theme in depth.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of immunohistochemistry for the diagnosis of heat-related deaths.

For forensic pathologists, it is very difficult to diagnose, with certainty, heat stroke deaths because the gross and histologic postmortem findings are not pathognomonic and biochemical investigations are not specific. Consequently, the diagnosis of heat-related death results from a combination of antemortem circumstantial data, investigative and forensic observations, and non-specific autopsy findings. We report a rare case of fatal heat stroke of an elderly woman diagnosed thanks to immunohistochemical reactions to HSP (Heat Shock Protein) in the absence of circumstantial evidence or other suggestive elements. A routine microscopic histopathological study was performed by using formalin-fixed paraffin-embedded tissue sectioned at 4µm and stained with hematoxylin-eosin and Weigert's elastic stain. In addition, immunohistochemical investigations of the scalp, brain, and skin of the left and right arm skin were performed utilizing monoclonal antibodies anti-heat shock protein (HSP 27, 70, 90).

Furthermore, we have summarized the results of a review of the literature on fatal heat stroke to compare the cases between them with that we reported. Our review shows that fatal heat stroke is also common in children who are forgotten in parked vehicles, especially on warm days and in the sun. Also, our review highlights that in adults, heat-related deaths are more frequently attributable to intensive physical exertion performed at high ambient temperature. The reviewed cases are related to young adults or adults and only rarely to elderly people. Our review demonstrates the infrequent use of biochemical investigations for the diagnosis of fatal heat stroke.

The aim of our work is, on the one hand, to report a rare case of heat stroke-related death and, on the other, to highlight the importance of immunohistochemistry for the detection of the cause of death in cases of suspected fatal hyperthermia and in the absence of alternative causes. We think that immunohistochemistry is the only specific examination available to the forensic pathologist to avoid the underestimation of heat-related deaths.

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**Heat Stroke; Hyperthermia; Immunohistochemistry**

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**F135 Humanitarian Forensics in the Era of Conflicts and Pandemic**

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**Learning Objective:** After attending this presentation, attendees will better understand the forensic humanitarian aspect in light of war-related conflicts and the devastation ravaged by the pandemic.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the current lacunae in the humanitarian forensic framework and the imminent need to rethink the ongoing practices to tackle the forthcoming problems with a more reliable and prepared infrastructure.

Forensic science as a discipline is seen to be occupied with the world of crime and legal disputes. As per the current scenario the role does not end here, and more importance is being given to the humanitarian aspect of the discipline as well. The major point of contemplation behind the humanitarian forensic action is to protect the dignity of the dead. Humanitarian forensics works with an agenda to ensure the proper identification and respectful handling of unknown human remains retrieved from the sites of mass disasters, natural calamities, war-related conflicts, terrorist massacres, and political genocide where many unidentified damaged or commingled human skeletal remains can be prevented from becoming missing persons.<sup>1</sup> All humanitarian emergencies either of the bygone era or impending necessitates the proper management and identification of casualties occurring worldwide. Humanitarian forensics deals with three major humanitarian functions arising out of circumstances involving mass disasters, armed conflicts, and migration.

This study set out to elaborate on the concept of humanitarian forensics with the role of prioritizing the humanitarian aspect that seeks accountability and restitution of the victims and the vulnerable individuals either dead or alive. Consequential instances of calamities and exigencies often result in identification of individuals who are missing or presumed dead from the recovered skeletal remains. Ethically, under such situations, dignified repatriation of the dead can help bereaved families and communities attain emotional closure and more. In addition, the psychosocial impact of the missing persons can be severe and, in contexts following armed conflicts and other situations of violence it is necessary to expedite the efforts for peace building.<sup>2</sup>

The need of the hour calls for sustainable and consistent humanitarian forensic action. This can be achieved through constant and meaningful commitment on the part of stakeholders involved and a continuous flow of resources to perform their duties. At a national level, more efforts are needed in terms of initiating capacity-building programs, encouraging technical training sessions, and casework management while at the same time keeping hold of the humanitarian aspect. Furthermore, collaborative opportunities need to be explored at the national and international level with organizations that are already actively working in the field of humanitarian forensics. Success in this noble field requires networking action taking place among different professional bodies for the sake of exchange of information, expertise, and the emergence of newer techniques to tackle the forthcoming challenges. At an academic level, experts and educational institutions need to carry out research and formulate outreach programs in tandem with the state authorities toward management of the dead in the aftermath of any disaster.

For instance, especially during COVID-19 and Russia-Ukraine war conflicts, there is a need for a more reliable central or international body with competence and expertise for dealing with possible biological attacks or bioterrorism and even for countries witnessing frequent occurrences of flash floods, cyclones, landslides, avalanches, etc., leaving dead bodies in its wake. Having easy access to central facilities in each state where natural disasters are a common occurrence can help reduce the burden of reported unidentified remains. Hence, a better understanding of the situations warranting an active role of humanitarian forensics keeping aware of factors like conflicts, pandemics, and religious and social constructs of a particular region will diminish the problems of the future.

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1. Cordner, S., & Tidball-Bin, M. (2017). Humanitarian forensic action—Its origins and future. *Forensic science international*, 279, 65-71.
2. The Palestine Yearbook of International Law, E. (Ed.). *Annual Report of the United Nations High Commissioner for Human Rights and Reports of the Office of the High Commissioner and the Secretary-General* (February 26, 2009). The Palestine Yearbook of International Law Online, 15(1), 265-268.

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**Humanitarian Forensics; War-Related Conflicts; Pandemic**

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**F136 The DNA Analysis of the Human Tissue Leaching From Different Soil Depths**

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**Learning Objective:** Attendees will learn how human DNA leaches into soil from decomposition. They will also learn if there is a difference in the rate of DNA leaching from different depths of loam soil. These rates will then be compared from five total extractions over the course of the year though the use of the DNeasy® PowerSoil® Pro Kit and quantified using qPCR.

**Impact Statement:** The analysis of DNA leaching into surrounding soil can have multiple impacts on the forensic science community. Since this type of analysis is relatively new, it can help specify the postmortem interval of buried human remains by determining the rate of leaching into the soil and depth. This research can also help determine the location of a body and if a body was moved after being buried. There have been many cases where victims were suspected of being buried and moved, but no way of proving it; this method could help determine that. Overall, analyzing the rate of DNA leaching at different depths can be beneficial in a multitude of ways.

Decomposition occurs in every living thing after death. It can be a great determiner in the forensic sciences as it helps in determining the time a person or living thing had died, based on factors such as soil composition changes and environmental factors.<sup>1</sup> Decomposition can become more complex when an individual is buried underground.<sup>2</sup> Environmental factors such as weather, temperature, and location may have different impacts depending on the depth of soil. When a body is buried, the DNA will leach into the surrounding soil at a rate determined by the depth and these outside factors.

This rate of DNA leaching at various depths is relatively unknown due to the lack of research. In the forensic science community, analyzing the DNA leaching from the soil at various depths can be beneficial in a multitude of ways. DNA leaching can help specify the postmortem interval (PMI), determine the location of a body, or identify if a body was buried in an area and moved to another location. Given that there are methods to find a body buried underground, such as ground-penetrating radars and cadaver dogs, there are not many ways to determine the length of time or confirm if a body was buried and moved. Determining the rate of DNA leaching from different depths in northwest Pennsylvania over a year can be beneficial in understanding how fast DNA can spread at different depths.

In this study, human tissue was obtained from a forearm belonging to a 55-year-old man that was freeze-thawed once. The amount of DNA leached into the surrounding soil will be analyzed by burying the tissue samples at 4 feet, 2 feet, and 1 foot, using loam soil and grow pots. The soil will be collected five times (1 month, 3 months, 6 months, 9 months, and 12 months) using a 4-foot coring device and placed in individually labeled manila envelopes. The DNA will be extracted using the DNeasy® Power Soil® Pro-Kit and quantitated using qPCR. The soil microbe will also be analyzed using the Model EM Soil Test Kit. Currently, the 1-month, 3-month, and 6-month soil samples have been collected. The analysis of the samples and future collections is still ongoing until February 2023.

**References:**

- <sup>1.</sup> Sangwan, P.; Nain, T.; Singal, K.; Hooda, N.; Sharma, N. Soil as a tool of revelation in forensic science: a review. *Analytical Methods* 2020, 12 (43), 5150-5159, DOI: 10.1039/D0AY01634A.
- <sup>2.</sup> Fiedler, S.; Graw, M. Decomposition of buried corpses, with special reference to the formation of adipocere. *Naturwissenschaften* 2003, 90 (7), 291-300, DOI: 10.1007/s00114-003-0437-0.

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**Decomposition; DNA and Soil Analysis; Postmortem Interval (PMI)**

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**G1 An Update on Standards Development Activities in Forensic Science: Legal Impacts Moving Forward**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the current state of discipline-specific standards within the forensic sciences and how these standards will impact the legal community going forward.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of the evolution and adoption of discipline-specific standards and their legal impact. This presentation will also increase awareness regarding training, tools, and resources that support standards implementation, compliance monitoring, and a broader understanding of standards by the legal community.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews standards and recognizes them on the OSAC Registry, which serves as a central repository of high-quality, consensus-based, technically sound standards. The legal system—criminal, civil, and administrative—must resolve controversies. Forensic science has long been at the forefront in answering complicated questions brought before the bar of justice. Forensic scientific standards, simply stated, enhance the value and usefulness of forensic science to the legal community. A good forensic science standard not only helps assure high-quality results in the laboratory, but also shows how work performed in accordance with the standard that is both well-grounded in theory and data and that it is presented within the boundaries of “the knowledge and experience of [the expert’s] discipline.”<sup>1,2</sup>

Standards address four principal features that are important to the legal system's utility of a given standard: (1) standards are written as clearly as possible, and without undefined technical terms and symbols, to enable lawyers and judges to grasp the main ideas and requirements; (2) standards describe in detail how the peer-reviewed and readily available scientific literature establishes the validity of the assumptions underlying the scientific tests and the interpretation of test results, (3) standards list the limitations of the tests and results and provide for expressions of the uncertainties in measurements and inferences drawn from them; and (4) standards include recommendations or requirements for the creation and retention of documentation of the test and the contents of reports, including the scientific limitations of the tests and related conclusions or inferences. Forensic scientific standards developed by practitioners working with other stakeholders address all the questions being asked by courts. Courts are concerned with both technical merit and legal importance. Courts are not able to assess the scientific merit of a standard on their own. Therefore, having a process that reviews whether a standard makes a case for the validity of the method and legal utility of the kinds of expert opinions that a standard contemplates is invaluable to the decision-making, truth-seeking function of the court.

During this presentation, updates related to standards development in forensic science will be presented and their legal impact will be discussed. Strategies for implementation and acceptance of standards within the legal community will be addressed. 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.

**References:**

- <sup>1.</sup> *Kumho Tire Co. v. Carmichael*, 526 U.S. 137, 148 [1999].
- <sup>2.</sup> *Daubert v. Merrell Dow Pharms, Inc.*, 509 U.S. 579, 592 [1993].

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**Forensic Science; Standards; Legal**

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**G2     How Relevant Are Forensic Science Standards to the Legal Process?**

*John Paul (JP) Jones, BS, MBA\*, National Institute of Standards and Technology, Gaithersburg, MD; Dana Delger, JD, Sundbyberg, Stockholm, SWEDEN*

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**Learning Objective:** Attendees will learn about the information gathered during an Organization of Scientific Area Committees (OSAC) data collection exercise on if and how forensic science standards are being used during the legal process.

**Impact Statement:** The federal government is investing resources to facilitate the development of forensic science standards for use in the criminal justice system. There are active efforts to encourage forensic science service providers to implement these standards in their operations. Discussion on if, and how, these standards are being used or referenced during the legal process can help prepare forensic scientists and attorneys for this engagement, thus impacting the forensic science community.

Since its official inception in 2014, the National Institute of Standards and Technology (NIST) -administered OSAC for Forensic Science has engaged in strengthening the practice of forensic science by facilitating the development of technically sound, science-based standards and promoting their implementation by Forensic Science Service Providers (FSSPs).

OSAC reviews and elevates specific standards to the OSAC Registry and promotes the forensic science community's use of these standards. OSAC interfaces directly with FSSPs and conducts annual surveys to determine the scale of implementation within the forensic science community. OSAC's first FSSP implementation survey was conducted in June 2021 when 46 standards were on the OSAC Registry. That survey indicated that 138 FSSPs had fully or partially implemented some of the standards listed on the OSAC Registry. OSAC's second implementation survey—conducted between June–August 2022—will assess FSSP implementation of 95 standards on the OSAC Registry. While much information is being gathered about how FSSPs are using standards, little information has been collected to date about how standards on the OSAC Registry, or other forensic science standards, are being used in legal proceedings. This presentation will describe the results of an information gathering effort with OSAC members and affiliates to better understand if and how standards are being used in legal proceedings.

This presentation will take a broad view of legal proceedings, to include plea discussions, admissibility hearings, trials, etc. As OSAC continues to support the implementation of standards by FSSPs, having a better understanding of how standards are used (or not) in legal proceedings can help identify where gaps exist between forensic scientists and legal practitioners. Also, this information can further inform stakeholders about the extent of forensic science standards implementation in legal proceedings, as well as how they may be better used.

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**Standards; Legal Proceedings; OSAC**

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**G3     The Organization of Scientific Area Committees (OSAC) Registry Standards in the Area of Reporting and Testimony**

*Lynn Garcia, JD\*, Texas Forensic Science Commission, Austin, TX*

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**Learning Objective:** This presentation will provide an overview of efforts within OSAC to focus on reporting and testimony documents. Users will understand how to obtain and track developments in the area of the OSAC Registry of great importance to the legal community, namely how forensic science information is presented in court. Attendees will also learn how to put these documents to use when litigating cases.

**Impact Statement:** This presentation will impact the forensic science community by helping to spread the message of OSAC Registry efforts to the legal community, where adherence to the standards can advance the presentation of forensic science information to end users (lawyers), judges and lay juries.

Since its official inception in 2014, the National Institute of Standards and Technology (NIST) Organization of Scientific Area Committees for Forensic Science (OSAC) has worked to advance the mission of strengthening forensic practice by facilitating the development of science-based standards through a formal Standard Development Organization (SDO) process, evaluating existing standards published by SDOs for placement onto the OSAC Registry, and promoting the use of the OSAC Registry-approved standards throughout the forensic science community.

While this effort is widely embraced by forensic science service providers and stakeholders of the criminal justice system in theory, the “how to” of practical implementation is far more challenging. This is true both at the laboratory gap analysis and training level as well as with respect to the education of lawyers and judges.

Further complicating the effort is the fact that the vast majority of criminal cases are prosecuted in state court. There is no national agency or regulatory body to act as the “owner” of the forensic science enterprise. As OSAC nears the end of its eighth year of operations, we are seeing the fruits of several years and thousands of hours of labor. By July 2022, there were 99 standards on the OSAC Registry of Approved Standards and, according to the most recent OSAC Standards Bulletin, there are several hundred standards at some point in the OSAC or related SDO process.

The purpose of this presentation is to help members of the legal community locate the OSAC Registry standards applicable to reporting and testimony. Attendees will also learn how to reference the standards during litigation to ensure appropriate communication of forensic science information to the end user. Attendees will gain an understanding of what standards related to reporting and testimony are in the pipeline. Attendees will also learn how to contribute to the process and how to submit comments or critiques for standards that may be lacking in sufficient specificity to be helpful to laboratories and the legal system alike. Finally, attendees will learn about efforts underway to develop resources at the state level for standards implementation as well as standard-specific training for lawyers and judges. Such efforts include the development of individual checklists and summary guides for laboratories and criminal justice partners.

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**OSAC Registry Implementation; Reporting Testimony; Vacuous Standards**

## **G4     The Organization of Scientific Area Committees (OSAC) Implementation: A Public and Private Perspective**

*Erika Ziemak, MSFS\*, Houston Forensic Science Center, Houston, TX; Hannah Gillis, MSFS\*, Bode Cellmark Forensics (Bode Technology), Lorton, VA*

**Learning Objective:** This presentation will provide insight into both the public and private laboratory perspectives on implementing standards published on the OSAC Registry. Presented by the Houston Forensic Science Center and Bode Technology, both labs will discuss the benefits of implementation, unique challenges faced, and paths forward.

**Impact Statement:** This presentation will impact the forensic science community by providing a realistic perspective from two forensic science service providers regarding implementation of standards published on the OSAC Registry. The perspectives will be provided by a private laboratory that performs forensic DNA testing and has multiple accreditations whereas the second will be provided from a public laboratory that has seven technical disciplines but only a single accreditation.

Tasked by Congress to develop standards for the forensic science disciplines within the United States, the National Institute of Standards and Technology created the OSAC.<sup>1</sup> OSAC has since worked with members of the forensic science community to publish over 90 standards across multiple forensic science disciplines that reside on the OSAC Registry with several more in development.<sup>2</sup> Standards development requires numerous manhours and can span the course of several years. The endless effort of some of the forensic science community's leading experts could be fruitless if laboratories all over the United States, across all disciplines of forensic science, do not embrace and implement these consensus-based efforts at standardization.

Forensic science service providers are tasked with monitoring the OSAC Registry to stay abreast of which standards have been published and are encouraged to participate in the public comment periods during standard development periods. They are then tasked with performing a gap assessment to gauge what tasks will need to be taken to comply with a published standard. Depending on the standard, these tasks can include minor revisions to Standard Operating Procedures (SOPs) to further define requirements that a lab may already be meeting, to major overhauls of SOPs and/or additional validation studies to meet requirements. Implementation of the standards is voluntary and only requires a self-declaration of implementation. Forensic science service providers have the choice to either fully implement a standard or partially implement a standard.

This presentation will provide insight from the perspectives of both a public and a private laboratory on implementing standards published on the OSAC registry. The Houston Forensic Science Center (HFSC) is a public laboratory that provides services spanning multiple forensic science disciplines such as, but not limited to, toxicology, biology, and friction ridge, while Bode Technology (Bode) is a private multi-accredited laboratory that focuses on the biology discipline providing forensic DNA services. Both HFSC and Bode are International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 accredited by the ANSI National Accreditation Board (ANAB) and the Texas Forensic Science Commission and faced similar but different challenges during implementation of standards. Implementing standards published on the OSAC Registry aligns with both HFSC's and Bode's quality missions to provide the highest-quality work to the communities each serves. As two of the first laboratories to commit to implementation of standards published on the OSAC Registry, in this presentation HFSC and Bode will discuss their implementation journeys as well as the benefits and challenges each laboratory has faced.

### **References:**

- <sup>1.</sup> [https://www.nist.gov/system/files/documents/2021/10/28/OSAC%20Charter%20and%20Bylaws\\_V1.9%20FINAL%2010-2721.pdf](https://www.nist.gov/system/files/documents/2021/10/28/OSAC%20Charter%20and%20Bylaws_V1.9%20FINAL%2010-2721.pdf).
- <sup>2.</sup> <https://www.nist.gov/organization-scientific-area-committees-forensic-science/osac-registry>.

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### **OSAC; Implementation; Standards**



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**G5 What's Happening (or Not?) With Forensic Science Training for Officers of the Court**

*Marna McLendon, JD, MFS\*, Arizona Office of the Attorney General, Scottsdale, AZ; John Paul (JP) Jones, BS, MBA\*, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Objective:** The goal of this presentation is to inform attendees about information regarding forensic science training specifically tailored to officers of the court. Attendees will also learn about new proposed programs and recommendations to enhance future trainings.

**Impact Statement:** The 2009 National Academy of Sciences (NAS) Report specifically recommended forensic science training for judges and lawyers.<sup>1</sup> This presentation will impact the forensic science community by providing an analysis of the relatively few programs that exist and will emphasize the need for additional programs. Several existing programs will be highlighted, along with recommendations from numerous judges and lawyers that have conducted such programs, enabling attendees to enhance their own training efforts.

The 2009 NAS Report highlighted the need for forensic science training for judges and lawyers noting, “Lawyers and judges often have insufficient training and background in scientific methods, and they often fail to fully comprehend the approaches employed by different forensic science disciplines and the strengths and vulnerabilities of forensics science evidence offered during trials”. While many prosecution, defense, and judicial associations (national and state) provide some education about forensic science at conferences and meetings, often the topics are identified by what is currently “of interest” to the legal community. There are very few educational programs with an established curriculum that includes the fundamentals of forensic science, as well as considerations for how it should be presented and challenged during pre-trial hearings and trial.

In 2021, the National Institute of Standards and Technology (NIST) was asked by Congress in a Joint Explanatory Statement accompanying the Consolidated Appropriation Act of 2021 (P.L. 116-260) to submit a plan for, “how to implement training to help officers of the court understand the science and concepts underlying the professional analyses of forensic experts.” To better understand the existing landscape, NIST began a project to identify forensic science training programs that serve judges and/or lawyers. Once these programs were identified, details of how they are administered, who provides the instruction, and what on-going resources they provide were captured. Additionally, lawyers, judges, law school professors, lab administrators, and scientists were interviewed to gain recommendations about what an ideal training program might look like in the future. What became apparent from this project is that lawyers want to know about a specific forensic science discipline when they need to—when they have a relevant case. Essentially, both lawyers and judges need the information when they need it. Even if lawyers and judges had received basic forensic science training at some point, a refresher on the current state of specific forensic science disciplines would be beneficial as they prepare a case for trial. Therefore, one recommendation is for training to be virtual and on-demand to maximize its accessibility.

A second recommendation is to provide law students with an opportunity to learn about science and the rules of admissibility during law school. Several programs have combined resources from law professors and science faculty to provide such course curriculum. This appears to be an exciting and productive collaboration that can provide solid foundations for future officers of the court.

In summary, having professionally produced virtual training that lawyers and judges can access—when they need it and on their own schedules—will be a benefit to these officers of the court. Furthermore, this on-demand training can be kept current and provide additional resources (e.g., journal articles, textbooks, list of experts) as well as a mechanism to contact the presenters. While virtual and on-demand training is recommended, it should be noted that local in-person training is also highly valued. This approach provides several advantages such as the opportunity to be physically present, experiencing the dynamics of the direct interaction with the presenter, and the ability to ask and build off others’ questions in the room. Regardless of the type of training, virtual or in-person, the content should be developed and delivered by those who know it best, which means pairing a scientist and a legal representative for the course. If you have the will to develop a virtual or in-person training program, follow some of these lessons learned to increase your impact.

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- <sup>1</sup>. National Research Council. 2009. *Strengthening Forensic Science in the United States: A Path Forward*. Washington, DC: The National Academies Press. <https://doi.org/10.17226/12589>.

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**Training in Forensic Science; Officers of the Court; Judges and Attorneys**

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**G6     The Scientific Literacy Project: A Collaborative Effort in Educating Legal Professionals on Forensic Evidence**

*Anthony Greiter, BS\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA; Stacy Renfro, MBS, Center for Statistics and Applications in Forensic Evidence (CSAFE), Ames, IA; Alicia Carriquiry, PhD, Center for Statistics and Applications in Forensic Evidence (CSAFE), Iowa State University, Ames, IA; Hal Stern PhD, University of California, Irvine, Irvine, CA; Chris Fabricant, JD, Innocence Project, New York, NY; Marika Meis, JD, Center for Appellate Litigation, New York, NY; Lynn Garcia, JD, Texas Forensic Science Commission, Austin, TX*

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**Learning Objective:** After attending this presentation, attendees will better understand the collaborative effort by the Center for Statistics and Applications in Forensic Evidence (CSAFE), the Innocence Project (IP), and a broad network of forensic experts contributing to the Scientific Literacy Project—a powerful agent of change working to promote forensic education for the legal community to help bridge the gap between law school curricula and traditional continuing legal education.

**Impact Statement:** This presentation will impact the forensic science community by educating attendees on the background and forward-looking plan for development of a valuable forensic education resource for the legal community, the Scientific Literacy Project. The Project plans to address some of the critical issues the legal community faces in understanding forensic evidence, including the lack of sufficient resources and tools available for lawyers.

In 2021, the IP and CSAFE founded the Scientific Literacy Project after identifying a critical need for forensic science education for legal professionals to help bridge significant gaps between law school curricula and traditional continuing legal education for lawyers and judges. The group has now developed an initial round of materials that is in the process of review and input from the forensic and legal community and has invited experts to provide feedback through a survey. The group collected input through its survey, revised materials, and plans to distribute the project materials to the broader legal community. The ultimate goal of the Scientific Literacy Project is to provide every lawyer and judge practicing criminal law in the United States access to sufficient tools required to understand and apply concepts underlying forensic evidence. The Scientific Literacy Project hopes to address systemic injustice and expand the already broad network of experts willing and able to contribute to making the Project a powerful agent of change for both the legal community and all who are impacted by the legal system.

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**Statistics; Lawyers; Forensic Education**

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**G7     Ten Things Every Court Should Know About Decomposing Remains**

*David Carter, PhD\*, Chaminade University of Honolulu, Honolulu, HI; Charlotte Carter, MS, Department of the Medical Examiner, Honolulu, HI; Sarah Sanger, JD, Sanger Swysen & Dunkle, Santa Barbara, CA*

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**Learning Objective:** After attending this presentation, attendees will have acquired a current understanding of, and approaches to, investigating decomposition cases and forensic taphonomy.

**Impact Statement:** This presentation will impact the forensic science community by providing a concise guide to decomposition and forensic taphonomy that can be used to aid decision-making by justice system personnel.

Medicolegal death investigation is an inevitable responsibility of forensic agencies around the world. The investigation of a death scene typically starts within hours of death when little to no decomposition has occurred. However, some death scenes are not investigated until remains have undergone marked decomposition; these are known as decomposition cases. Decomposition cases are not the norm, but they are an important subset of medicolegal cases. This presentation aims to serve as a concise summary of fundamental decomposition principles and a guide for more detailed learning.

1. The study of decomposition and forensic taphonomy is an accepted scientific discipline within the scientific community. It is documented in peer-reviewed journals and can be (and has been) subject to scientific testing and analysis. Scientists who practice in the area are well versed in the scientific literature, have evaluated human decomposition both in practical settings and in decomposition facilities, and have conducted research with animal remains.
2. Like most death scenes, decomposing remains are usually located indoors. However, most decomposition research is conducted outdoors and in laboratory settings. Twelve human decomposition facilities exist, with nine of them located in the United States. Several animal decomposition facilities are currently in use.
3. Decomposition cases should be investigated because decomposition can eliminate, mimic, and obscure evidence of criminal activity. For example, skin ruptures that result from the pressure of decomposition gases can resemble lacerations while postmortem color changes can obscure tattoos.
4. Taphonomy is a term that refers to the processes resulting in the decomposition and preservation of organic materials, including remains. Forensic taphonomy typically refers to an attempt to understand the decomposition and preservation of human or animal remains.
5. Decomposition is sometimes described as a series of stages. A universal series of stages does not exist, but stages are helpful to communicate information. One commonly used series includes five stages: Fresh, Bloat, Active Decay, Advanced Decay, and Skeleton.
6. Decomposition is regulated by three primary factors: environment, decomposer community, and resource quality. Environment includes the physical and chemical characteristics of the scene. Decomposer community refers to the biological characteristics of a scene and includes all of the organisms associated with the remains. Resource quality refers to the characteristics of the remains such as weight, trauma, and cause of death.
7. Temperature is often the most influential variable affecting decomposition. Temperature should be documented at every death scene because it can help explain many aspects of decomposition. Relative humidity and moisture can be as influential as temperature. Bodies can decompose quickly in humid climates while they tend to desiccate in dry climates.
8. Most decomposition is the result of decomposer organisms consuming the remains. The decomposer community can be organized into three general groups: arthropods and insects, microorganisms, and scavengers. Microorganisms are the only decomposers guaranteed to be present at a death scene because they originate from the remains and the environment.
9. Bodies buried in soil will often decompose slower than bodies placed on soil. However, buried remains can decompose faster than remains located indoors. The effects of other variables like trauma, clothing, wrapping, and placement in water require significantly more research to be understood properly.
10. The relationship between time and decomposition is poorly understood. It is possible to estimate the postmortem interval, but it is difficult and can require serendipity.

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**Decomposition; Taphonomy; Medicolegal**

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**G8 Forensic Genetic Genealogy: A Primer for Legal Professionals**

*Michael Coble, MFS, PhD\*, UNTHSC Center for Human Identification, Fort Worth, TX*

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**Learning Objective:** After attending this presentation, criminal justice stakeholders, especially lawyers and judges, will better understand the science behind the process to identify individuals using third-party genetic genealogy databases.

**Impact Statement:** This presentation impacts the jurisprudence community by providing a basic primer to non-scientists on the science behind the use of genetic genealogy for unsolved cases.

National DNA databases containing DNA profiles of convicted offenders and crime scene evidence have been a valuable tool for police investigations and public safety. In addition to the association of a person to a crime where there is no suspect, DNA databases can also provide case-to-case associations such as those committed by serial killers and or serial rapists. One major limitation of national DNA databases is that unless the offender is enrolled into the database, an association cannot occur. In an effort to increase the usefulness of DNA databases for investigative leads beyond the simple sample-to-person searches, forensic scientists have focused on the identification of close, first-order relatives (e.g., father to son or brother to brother) in database searches.<sup>1</sup> This application of “Familial Searching” has been effective at identifying individuals who have committed serious crimes such as the “Grim Sleeper” serial killer in California.<sup>2</sup> However, only a small percentage of states currently use familial searching.

Attempts to explore non-law enforcement databases for investigative leads initially focused on Y-chromosomal Short Tandem Repeat (Y-STR) associations to generate a paternal “last name.” Such was the case in the identification of the “Phoenix Canal Killer” in Arizona. The case, from the 1980s, involved the sexual assault, murder, and mutilation of two young girls and despite the crime scene profile of the unknown perpetrator in the Combined DNA Index System (CODIS) database, the investigation went cold. The accused killer, Bryan Patrick Miller, had his last name identified by searching a Y-STR haplotype developed from the crime scenes and searched in a publicly available genealogy database.<sup>3</sup> This allowed investigators to identify Miller, a suspect from the beginning of the investigation.

In 2018, investigators revealed the use of genome-wide autosomal Single Nucleotide Polymorphism (SNP) data, rather than the haploid Y-STR markers, to identify the “Golden State” killer, Joseph James DeAngelo.<sup>4</sup> In the Golden State killer example, a search of a third-party genetic genealogy database (GEDmatch; [www.gedmatch.com](http://www.gedmatch.com)) was used in that investigation. By examining blocks of DNA shared with distant relatives (third and fourth cousins), investigators were able to build an extensive pedigree that provided them with investigative leads that eventually led to the arrest of DeAngelo through an association of his STR profile to the evidence.

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**Forensic Genetic Genealogy; DNA Testing; DNA Database**

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**G9 Beyond the OMG Era: The Assessment of Forensic Genetic Genealogy as a Practical Investigative Tool**

*Colleen Fitzpatrick, MS, PhD\*, Identifinders International LLC, Fountain Valley, CA*

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**Learning Objective:** After attending this presentation, attendees will understand how modern Forensic Genetic Genealogy (FGG) has developed into a practical investigative tool.

**Impact Statement:** This presentation will impact the forensic science community by offering a practical characterization of the benefits and the limitations of FGG, to improve the assessment of which cases are the most likely candidates for resolution using the technique, tempering expectations and mitigating the risk of over-using a technique once regarded as a miracle cure for the common cold case.

FGG has been used over the past four years to solve hundreds of cold cases, many dating back decades. Each day the media reports still another violent crime being solved or a set of unidentified remains being identified through FGG. As more cases move forward to successful resolution, the capabilities of FGG are becoming more well characterized, tempering expectations and mitigating the risk of over-using a technique once regarded as a miracle cure for the common cold case.

The first agencies to follow up on the initial success of FGG were those who were willing to risk time and money on a brand-new investigative technique that was obviously a game-changer, but where the probability of success had not yet been established. However, as the catalog of FGG cases has expanded into the hundreds, a great deal more has been learned about why certain cases succeed, how long they take to solve, and the reasons why many cases are proving intractable. While the requirements placed by FGG processing techniques on the quantity and quality of a DNA sample are open to parametric study and are usually supported by a pre-processing quality check, much of the uncertainty associated with a case's solvability is dependent on the compatibility of the violent offender or unidentified remains with genetic genealogy database membership. This cannot be known *a priori*; however, it is possible to make general predictions about a case's solvability through statistical analyses of solve rates and solve times for cases that have been cleared, and by studying the characteristics of "FGG cold cases" that have yet to be resolved.

Such analysis is important to support both domestic as well as international casework. For example, developing more streamlined and therefore more efficient methods of addressing cases with high levels of endogamy here in the United States could lead to the development of best practices for using FGG on mass graves, where the surviving population and the victims exhibit high intermarriage. The same can be said for developing policies about target testing individuals who are not knowledgeable about DNA.

This presentation will present statistics on FGG solve rates, solve times, and other performance metrics as a function of database composition, that can lead to a more cost-effective and efficient use of FGG. It will provide insight into why and how cases have or have not been solved using FGG. The presentation will end with suggestions on how information revealed in even the most challenging FGG cases can provide investigative leads valuable to conventional investigations.

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**FGG; Genealogy; DNA**

## G10 Analyzing Homicide Cases Using Bayesian Networks

Luciano Garofano, PhD\*, Italian Academy of Forensic Science, Parma, Emilia-Romagna, ITALY; Laura Volpini, PhD\*, Sapienza University, Roma, Lazio, ITALY; Giovanni Acampora, PhD, University of Naples Federico II, Napoli, Campania, ITALY; Autilia Vitiello, PhD, University of Naples Federico II, Napoli, Campania, ITALY

**Learning Objective:** After attending this presentation, attendees will understand principles behind Bayesian Networks (BNs) and the necessary elements for their application in forensic investigations and in the criminal trial, where BNs are used to model relationships between hypotheses and evidence in legal cases.

**Impact Statement:** This presentation will have a strong impact on the forensic science community as it can augment traditional means of investigation and legal assessment by providing a systematic format and a mathematical reasoning to analyze hypotheses and evidence related to the criminal events.

A BN is a graph-based model in which the nodes represent variables, the arcs represent causal relationships between variables, and it is able to infer new knowledge by taking advantage of Bayesian reasoning and conditional probabilities. In the forensic context, variables could represent unknown hypotheses like “suspect murdered the victim” or observable evidence like “witness claims suspect was at scene of the crime.” Therefore, from this standpoint, a BN is a visual way to represent and communicate the relationships between different hypotheses and pieces of evidence related to a criminal event, but it has also an underlying calculus (based on Bayes’ theorem) that allows performing probabilistic reasoning about unknown hypotheses. To build a BN model acceptable in a legal case, an established idiom-based approach is used. This approach provides a generic method for translating legal cases into BNs by using so-called causal idioms. These idioms are the building blocks for legal reasoning. An example is the “opportunity and motive idiom” used to model the pre-conditions for guilt.

This report will present the Arce case as an example of the practical application of BNs in legal cases. Briefly, Arce is an Italian cold case reopened in 2016 thanks to new evidence from DNA analysis and 3D reconstruction of part of the supposed crime scene. This case concerns an 18-year-old girl, found dead on June 3, 2001, with her hands and feet bound with adhesive tape and wire and a plastic bag over her head, in a grove in the surroundings of Arce. The public prosecutor is convinced that the girl was killed in the Arce military barracks and then transported to the place where she was found. In this report, a BN has been designed to support or refute the hypothesis of the public prosecutor by relating all the evidence and witnesses acquired during the investigation, resulting in a more objective assessment of that hypothesis. The output of the model leads to say with high probability that the girl was in the Arce military barracks when she was killed.<sup>1-12</sup>

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### Investigations; Bayesian Network; Forensic Sciences

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**G11 Deciding Not to Decide: The Risks Associated With Inconclusives**

*Tiffany Roy, MSFS, JD\*, ForensicAid, West Palm Beach, FL; Michael Coble, MFS, PhD\*, University of North Texas Health Science Center Center for Human Identification, Fort Worth, TX*

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**Learning Objective:** After attending this presentation, criminal justice stakeholders, including lawyers, judges, and forensic/biology DNA analysts, will better understand the importance of assessing a DNA mixture for exclusionary purposes even where a DNA mixture does not lend itself to interpretation under the laboratory's standard operation procedures.

**Impact Statement:** This presentation impacts the scientific community by demonstrating the critical consequences in failing to accurately assess data for exclusionary purposes through investigative and criminal case examples and further demonstrates how probabilistic genotyping may mitigate some of these consequences.

The primary role of a DNA analyst in a forensic laboratory is to review and interpret data obtained from known and evidentiary samples generated by polymerase Chain Reaction (PCR) amplification of Short Tandem Repeat (STR) markers typed via Capillary Electrophoresis (CE). In order to do this in a scientifically reliable way, forensic DNA analysts must have extensive expertise in the principles of profile interpretation and an appreciation for the complexity of the samples and the possibility of missing data (allele dropout and other stochastic effects). As the number of contributors increases in a mixture and the quality of the profile decreases (due to low amounts of DNA, DNA degradation, PCR inhibition, etc.) it becomes increasingly more complex to make comparisons. This presentation seeks to reinforce what should be a commonly accepted principle among the forensic DNA community: that even when a DNA mixture does not lend itself to interpretation under the laboratory's standard operating procedure due to its complexity, the mixture should still be assessed for exclusionary purposes. Failure to assess mixture data for exclusionary purposes may result in a laboratory possessing but not communicating exculpatory information to the trier of fact, which risks depriving the parties of the opportunity to make important decisions about the criminal action with the best available information.

This presentation describes issues presented in the Texas Forensic Science Commission's Complaint NO. 21.54 filed by James Smiley. In April 2022, the Commission investigated Mr. Smiley's case and issued a final investigative report that included findings and recommendations related to DNA mixture interpretation and inconclusives. The presenters will highlight case examples such as Mr. Smiley's to demonstrate to attendees how to spot telltale signs of a laboratory failing to accurately assess data for exclusion and further demonstrate how this oversight can be properly mitigated with probabilistic genotyping software.

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**Forensic Biology/DNA; Complex Mixture Interpretation; Probabilistic Genotyping Software**

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**G12 Proving Distracted Driving Behavior Using Advanced iPhone® Forensics**

*John Carney, JD\*, Carney Forensics, Scandia, MN*

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**Learning Objective:** This presentation will briefly introduce the transformation in iPhone® forensics in the past two years and explain new, deeply probative extractions and the extraordinary recoveries of qualitatively new Apple® databases like KnowledgeC. It bears directly on the dynamics of the interface between the device and the user experience that go to distracted driving behaviors. The presentation will also show how iPhones® monitor which mobile app has control of the screen, microphone, and speaker and especially when. iPhone® forensics can recover mobile app metadata that serves as dispositive indicators of user inattention on the road.

**Impact Statement:** The presentation will impact attendees primarily tasked with trying criminal justice cases and trial lawyers who litigate personal injury cases involving wrongful death and catastrophic motor vehicle accident injuries. It will provide practical advice and effective forensic techniques for recovering compelling mobile evidence from late model iPhones® using advanced forensic techniques. And it will show credible demonstrative exhibits of material iPhone® evidence competently recovered from iPhone® databases and media to help attendees visualize what distracted driving looks like today.

Driver distraction is a specific type of driver inattention. Distraction occurs when drivers divert their attention from the driving task to focus on some other activity, like cell phone use and texting. According to the National Highway Traffic Safety Administration, in 2019 there were 387 fatal crashes reported as having cell phone use as a distraction, 13% of all distraction-affected fatal crashes. A total of 422 people died in fatal crashes involving cell phone-related activities as distractions.<sup>1</sup>

This presentation will focus on employing transformative, forensically sound inspections iPhone® forensics offers for jurisprudence purposes. It recovers heretofore undiscovered forms of digital evidence that either prove distracted driving behavior for plaintiffs and the government or the absence of which exonerates defendants or absolves them of liability.

The proposition of this presentation is to focus on a single iPhone® specimen device that becomes the cornerstone evidence upon which the case will turn to expose distracted driving behavior in motor vehicle accidents. An integrative approach involving diversified evidence from multiple sources is a best practice, but if the alternate evidence sources are unavailable, it is moot. Now it is possible to litigate successfully using a unitary device, if performed competently and guided by a smart evidence strategy with the latest iPhone® forensic tools and methods.

This presentation centers on the need for attorneys, paralegals, and investigators to become conversant in the data and artifacts inside the iPhone®. They need to understand how an iPhone® records events that shed light on what the user was doing at critical points immediately before and during a fatal crash.

The presentation will explore several forms of iPhone® evidence with attendees which go to the heart of distracted driving proof in both criminal and civil litigation:

- Pattern of life evidence showing insights into digital behaviors and frequency and location of routines
- Texting while driving
- Game playing
- Observation of social media and their feeds
- Capturing photographs and videos
- Activity and health data
- Internet browsing and Google® searches
- ScreenTime® app statistics, including iPhone® pickups

**Reference:**

- <sup>1</sup>. *Traffic Safety Facts Research Note, Summary of Statistical Findings*, National Highway Traffic Safety Administration, April 2021.

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**Distracted Driving; Texting While Driving; iPhone® Forensics**

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**G13 The Academy Standards Board (ASB) Standard Compliant Toxicology Report Content as a Tool to Support Informed Pre-Charging Communication and Testimony Planning**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the contributions to informed justice that could be made by expanding and strengthening discretionary informational content in toxicology laboratory reports to encourage communication between experts and litigators before charging decisions are made.

**Impact Statement:** This presentation will impact forensic sciences by encouraging early, informed communication between litigators and scientists to ensure that charging decisions are made based on an informed understanding of what the witnesses can testify to, consistent with emerging professional standards.

The United States continues to suffer from an opioid epidemic with over 105,000 deaths projected for 2022. Today's opioid market is dominated by illicit fentanyl and regularized evolution of a plethora of Novel Synthetic Opioids (NSOs). The opioids appear in combination with one another, with other NPS substances such as novel benzodiazepines, synthetic cannabinoids, with other stimulants, and with legacy drugs of abuse.

Criminal justice systems at state and federal levels have dealt with the rising opioid and polydrug death toll by applying the standard criminal justice response of identifying and prosecuting offenders who break laws. The sentencing enhancements of 21 U.S.C. Section 841 were increasingly used to charge and punish individual deemed responsible for opioid, fentanyl, and polydrug distribution resulting in death. The "death resulting" charging prototype has expanded to every state in the union, with the passage of new laws, and the extension of current law to allow enhanced charging and sentencing ranges for drug distribution that results in the death of the user. Drug delivery resulting in death is currently charged under theories ranging from First Degree Murder to Involuntary Manslaughter, with many states enacting particularized "drug delivery homicide" laws.

The theory of the charging decision in drug delivery resulting in death cases dictates the level and type of causation and intent that must be proven, but all cases are dependent to some degree on requisite proof that the drug or substance distributed caused or contributed to the user's death. Postmortem cases underlying those prosecutions now predominantly involve poly-substance intoxication, thereby creating a complex toxicological presentation that can only be properly understood with expert forensic toxicology and pathology interpretation. Prosecutors and attorneys, however, are regularly making charging and sentencing decisions based only on the numbers in a toxicology report and a Google® search on lethal ranges. Even experienced prosecutors with foundational understanding of toxicology are prone to believing that very high concentrations in blood or bodily fluid "must be" lethal or fatal. Once a person is charged, it is not typically until the case is coming on for trial, before the toxicologist is consulted for the first time; the unwinding of inaccurate assumptions and invalid conclusions is a very difficult and unpleasant exercise that puts the toxicologist under undue and unfair pressure. Moreover, once a plea agreement is tendered and accepted or a sentence is entered without toxicologist consultation, opportunity for clarification of toxicology reports is forfeited to the criminal justice system.

In 2019, the National Institute Standards and Technology (NIST) adopted American Standards Board (ASB) Best Practice Recommendation 037 that endorses scientifically acceptable practices in testimony relating to concentrations, pharmacokinetics, toxicodynamics, and toxicological calculations. In 2020, NIST adopted ANSI/ASB Standard 053 for Report Content in Forensic Toxicology, which provides for discretionary inclusion of "interpretative information to help the reader understand the meaning of detected analytes." Using toxicology reports taken from multiple sources, the presenter will discuss whether Standard 053 discretionary information is currently a customary inclusion in reports and will suggest informational inclusions that maintain the integrity of the scientist's opinion while also conveying "plain language" messaging to litigators regarding dangers in making assumptions and reaching uninformed conclusions, while also encouraging litigators to consult with the reporting toxicologist and expert toxicologists before charging decisions are made, plea bargains are accepted, and sentences are imposed.

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**Jurisprudence; Report Content; Expert Testimony**

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**G14 The Evidentiary Value of Human Remains Canine Behavior**

*Robert Sanger, JD\*, Sanger Swysen & Dunkle/Santa Barbara College of Law, Santa Barbara, CA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the scientific and legal significance of human remains canine behavior.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of the scientific and legal aspects of human remains canine (cadaver dog) behavior; specifically with regard to what behavior is of legal significance before a court or jury.

Human remains canines or cadaver dogs are trained in tandem with their handlers. In theory, the dogs are trained to help locate areas in which human remains have been deposited. The training involves a human handler and dog doing field exercises in which human remains are concealed. The dogs are trained to exhibit certain behavior when they sense the presence of human remains. The behavior is, in theory, unique to sensing human remains, as opposed to other stimuli, and is identifiable by the handler. This is generally called an alert.

There is a significant dispute in the scientific literature as to how and why dogs alert in response to training and, specifically, in response to suspected human remains. Dogs, with superior olfactory capabilities, are able to discern volatile organic compounds that are emitted by decomposing animal remains. For this to be valid evidence of the detection of human remains, the dogs would have to make a distinction between other volatile organic compounds, including those emitted by remains of decomposing non-humans. It is not known to science what particular volatile organic compounds, of the roughly 400 involved in human decomposition, are specifically responsible for the canine reactions, if any.

In controlled scientific testing, even the best-trained dog and handler combinations have false positive and false negative results. Dogs alert on non-human tissues, other stimuli or nothing at all. Similarly, dogs sometimes fail to alert when human tissue is concealed. While handlers claim that a body that was present but then removed could still cause a valid alert, the scientific studies indicate that any lingering volatile organic compounds would dissipate after a period of time, perhaps 48 hours under ideal conditions. There are also studies that have identified subtle, even unintentional, behavior on the part of handlers that can cause a dog to display alert behavior.

Dog alerts or other dog behavior is, therefore, not actual evidence of the presence of human remains. The evidence is in the results. That is, if a dog alerts, it may be cause to investigate further. If evidence is found, that would be the evidence admitted in court. If no evidence is found, then alert or not, there is no evidence. This is not intended to be disrespectful of the volunteers who train their dogs and often provide a public service by going to disaster scenes and sometimes finding a human victim. The question here is what is of evidentiary value before a court or jury.

The case law on the subject is sparse. Analogies are drawn to dog tracking cases, which are different. In tracking cases, the proffered evidence is that a dog followed a recently deposited scent which eventually led to a suspect. That is different than a cadaver dog alert that did not locate any physical evidence. In the tracking cases, admissibility is dependent on making a foundational showing of the proficiency of the dog and handler but also on a showing that there is reliable corroborating evidence that the actual person found was related to the crime. There is a dearth of case law on human remains dog alerts where no human remains were found, but the weight of the science supports exclusion of the alerts themselves. More study is needed in this area.

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**Human Remains; Cadaver Dogs; Canine Evidence**

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**G15 “Uninvested” Human Capital: How Much Is an Abortion?**

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**Learning Objective:** This presentation will allow reflection and propose a standardization criterion on the issue of quantifying compensation in the event of abortion due to the fault of third parties in order to propose homogeneous criteria for quantifying the damage caused by abortion.

**Impact Statement:** This presentation will impact the forensic science community by serving as a key aspect of quantifying compensation in the event of abortion.

Human capital is defined as a mix of innate skills and knowledge acquired by investing in one’s training; a real “capital” that pays off in the long term.

In the Italian legal system, the human being is recognized as a “person” from the moment of birth, that is, from natural or induced detachment from the mother’s body. This determines the acquisition of personal rights of the person. Necessarily, therefore, a fetus would not have such rights; yet it has its own innate “potential.”

Can the death of a fetus be considered differently from the death of a “person?”

This report will present a retrospective study in which the attached motivations of 31 sentences of the Italian Courts were analyzed. These sentences were found on the Portal of Telematic Services (PST) of the Ministry of Justice, using the search terms “abortion” and “harm.” The determinants of compensation profiles for non-pecuniary damage linked to abortion were investigated. It emerged that in terms of compensation, the death of the fetus represents a circumstance that is judged differently from the loss of a born child and in a non-homogeneous way.

The damage from loss of the parental relationship is the damage deriving from the loss of a neighbor and from the condition of existential emptiness that derives from it in the family. Compensation for this damage in the event of abortion due to third-party responsibility presents a non-uniform recognition in the sentences of the Italian jurisprudence, but in any case, it is almost always recognized in a partial way, since the emotional relationship with the lost person is defined only as potential.

Is the economic estimate of abortion affected exclusively by objective and standard criteria or is it influenced by a subjective evaluation? Is it possible to find standard criteria that act as guidelines to quantify the loss of that human capital in progress?

The authors try to answer these questions by analyzing what already exists in the Italian and international context (France, Germany, England, Spain, United States), to propose homogeneous criteria to quantify the damage caused by abortion (gestational period, any gynecological pathologies, parental relationship, age of the parents, existence of any other children of the couple, any previous abortions).

It is recommended that medicolegal death investigators become familiar with the criteria for quantifying abortion damage.

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**Abortion; Compensation; Human Capital**

## G16 An Analysis of the Utilitarian Ethical Foundation of Genetic Profile Databases in the European Court of Human Rights

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**Learning Objective:** After attending this presentation, attendees will have an overview of the jurisprudence of the European Court of Human Rights (ECHR) in relation to DNA databases.

**Impact Statement:** The consolidation of the arguments contained in ECHR jurisprudence can be of great importance for the debate about DNA Databases (DNAD) around the world, mainly due to the theoretical-legal consistency of the analysis of the theme that is consolidated in this Court. The discussion on controversial points such as types of passive offenses of collecting biological samples to feed DNAD and the length of time genetic profiles remain in these databases can be substantially increased by considering the ECHR jurisprudence.

There are countless examples of the contributions of DNAD to criminal prosecution in many countries. However, several bioethical and legal aspects of these repositories remain subject of discussion in various legal systems, such as the possible utilitarian ethical foundation. Thus, based on the analysis of ECHR jurisprudence, this research aims to examine whether there is an interface between utilitarianism and DNAD. Furthermore, it sought to define whether the eventual utilitarian arguments adopted by the ECHR would survive the theory of Human Rights and the principle of human dignity. A survey was carried out on ECHR decisions concerning the use of DNA testing and DNAD, considering its Case-Law Guides. Mentions to the term “DNA” were identified in the Guides related to articles 6, 7, and 8. Then, cases that had a criminal aspect were searched, arriving at the following list: *Saunders v. United Kingdom*, 12/17/1996; *Van der Velden v. Netherlands*, 12/07/2006; *S. and Marper v. United Kingdom*, 12/4/2008; *W.v. Netherlands*, 01/20/2009; *Peruzzo and Martens v. Germany*, 06/04/2013; *Aycaguer v. France*, 06/22/2017. Based on the analysis of these judgments, it was found that the ECHR developed a coherent jurisprudence regarding DNAD.

The understanding developed by the ECHR is that such repositories fulfill several relevant functions, in the interests of public security, such as protection of rights and freedoms; crime prevention; identification of their authors; assistance in excluding wrongly identified individuals from the list of suspects as possible perpetrators of criminal offences; and encouraging the reduction of recidivism, as they can deter those who know they are in the genetic profile database.

States have “margin of appreciation” to establish the contours of their DNAD. As long as they are provided for and “in accordance with the law,” insofar as they pursue one or more of the legitimate objectives legally established and take into account the seriousness of the crimes, they are proportionate and “necessary in a democratic society.” The decisions studied sought to reconcile the effectiveness of genetic profile databases, recognized as of high importance by the ECHR for the protection of society, with an approach based on respect for individual rights, which can, ultimately, be characterized as a “sophisticated” form of utilitarianism. In decisions related to Genetic Profile Banks, the utilitarian arguments adopted by the ECHR not only subsist but are reinforced when confronted with the theory of Human Rights and the principle of human dignity. In this way, the recognition by the ECHR of the ambivalent character of human dignity stands out, as its judgments seek to balance the two sides of this principle with the aim of reconciling freedoms and responsibilities; rights and duties; in short, the individual perspective with the community one; in order to promote the increase of general welfare in harmony with human rights.

It is concluded that the arguments built in the decisions of the ECHR are of great importance for the debate on DNAD, mainly due to the theoretical-legal consistency of the analysis of this theme that is consolidated in its jurisprudence. The discussion on controversial points such as types of offenses passive of collecting biological samples to feed DNAD and the length of time genetic profiles remain in these databases can be substantially increased by taking into account the ECHR jurisprudence.<sup>1-26</sup>

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## ECHR; DNA; Human Rights

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### **G17    Discussing the Establishment and Use of DNA Databases in Light of the European Convention on Human Rights and the European Court of Human Rights Decisions**

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**WITHDRAWN**



## G18 Discussing the Use of Artificial Intelligence in the Interpretation of DNA Mixture Profiles in Terms of Constitutional and Human Rights Laws

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**Learning Objective:** In routine forensic DNA analysis, complex mixtures are difficult to interpret and there is yet no consensus in the forensic biology community on handling such profiles. In 2010, the United States Scientific Working Group on DNA Analysis Methods (SWGDM) published guidelines to help solve mixed DNA interpretation. The SWGDM guidelines (paragraph 3.2.2) mention that validated computer interpretation methods can be used for “probabilistic genotyping.” There are currently more than a dozen softwares available, grouped under one roof and referred to as “probabilistic genotyping” (PG) systems: EuroForMix, DNASTatistX, STRmix™, MixSep, and TrueAllele Casework™, and such. The interpretation of mixed profiles with artificial intelligence tools is controversial in several aspects, both in American and European law. In this study, the use of artificial intelligence in analyzing mixed DNA samples is discussed in the context of the rights mentioned above.

**Impact Statement:** The use of artificial intelligence in probabilistic genotyping is controversial in European and American legal systems. In United States law, it is stated that probabilistic genotyping software may fall within the scope of “due process” in the 5<sup>th</sup> Amendment of the United States Constitution and the “right to confrontation” in the 6<sup>th</sup> Amendment. In addition, the importance of auditability, transparency, and consistency principles is emphasized in the presentation of evidence within the scope of the due process clause. In Europe, there is an opinion that this software violates Article 6 of the European Convention on Human Rights, which regulates the principle of a fair trial. Accordingly, if the software in question is used, the “presumption of innocence,” “right to confrontation,” “right to defense,” and the “principle of equality of arms,” which is revealed by the interpretation of this article, are violated. In this study, the use of artificial intelligence in analyzing mixed DNA samples is discussed in the context of the rights mentioned above.

Routine forensic DNA analysis is based on a comparison of suspects’ DNA and the DNA obtained from the crime scene. As a result, suspects are excluded or included in the case. Probabilistic calculations are made in cases if the suspect is included. The interpretation of single-source DNA profiles is easier than mixed or low-copy-number DNA profiles. These complex mixtures are difficult to interpret, and there is yet no consensus in the forensic biology community on handling such profiles.

In the evaluation made in the context of United States law, it is stated that it is not known exactly how the software works, since trade secrets cover the source codes of the software in question. For this reason, it is stated that probabilistic genotyping software may fall within the scope of “due process” in the 5<sup>th</sup> Amendment of the United States Constitution and the “right to confrontation” in the 6<sup>th</sup> Amendment. In addition, the importance of auditability, transparency, and consistency principles is emphasized in the presentation of evidence within the scope of the due process clause. According to the same clause, it is stated that the defendants should be allowed to refute the negative information and evaluations about themselves. Although American courts accept the necessity of allowing refuting negative information against them, it isn’t very optimistic about requests for the disclosure of source codes.<sup>1-9</sup>

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### Interpretation of DNA Mixture; Use of Artificial Intelligence in DNA Mixture Profiles; Constitution Law

**G19 Dismissals of Driving While Intoxicated (DWI) Offenses in New Jersey**

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**Learning Objective:** Attendees will learn how DWI offenses are handled in New Jersey and what causes exist for their dismissal.

**Impact Statement:** This presentation will impact the forensic science community by introducing a discussion of DWI cases from a procedural standpoint.

According to the National Highway Traffic Safety Administration (NHTSA), every year more than 10,000 lives are lost due to impaired driving. Thanks to various campaigns put in place to make citizens more aware of the importance of DWI, the rate of fatalities has decreased by more than 30% in the past 30 years; however, the numbers continue to remain high.

In the State of New Jersey, in 2018, there were 564 deaths involving impaired driving, and more than a quarter of them involved alcohol. Even though there are significant penalties associated with them, DWIs are not considered a criminal offense in New Jersey; they are handled as traffic offenses and, therefore, the court procedures associated with them reflect this status. In past years, it has been noticed that a significant number of arrests for DWI in New Jersey end up in dismissals; this happens in all counties and the reasons for that are not evident. This is something that has been observed in other states as well, and several Departments of Transportation and Traffic Safety have deemed it important to investigate and have created task forces dedicated to the monitoring of these offenses. With the aim of gaining a better understanding of the court handling process of DWI cases, the New Jersey Statewide Traffic Safety Project, in collaboration with Kean University, has started collecting data from all counties to investigate the causes for DWI dismissals.

The goal of this project is to produce a report illustrating the dispositions of DWI cases in New Jersey as well as the reasons on which those dispositions are based; the collection of the data will continue for three years. The report will not have any identifying information and is not intended to be a statistical analysis. Here we will present the preliminary data of this report.

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**DWI; DUI; Traffic Investigations**

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**G20 Femicide: Scientific and Juridical Elements to Protect Vulnerable Victims**

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**Learning Objective:** After attending this presentation, attendees will have a clearer understanding of the criminological and legal implications of femicide and the possible juridical means to fight violence against women.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview of the scientific and juridical components of femicide and the possible strategies for a more effective approach to defending vulnerable individuals.

Femicide is a category of murder introduced by several authors and the media, but not recognized by the Jurisprudence, to indicate the intentional murder of a woman or girl by a male individual, usually their partner, motivated by the gender of the victim. The laudable intent to identify a social and criminological problem must, however, be examined from a legal point of view to identify whether the possibilities exist for its definition or whether there may be other instruments suitable for the defense of vulnerable victims.

From a juridical point of view, it is necessary to ensure that every person is equal before the law and is entitled to the equal protection of the law without discrimination (e.g., art. 7 Universal Declaration of Human Rights: art. 3 and 37 Italian Constitution). Creating a specific victim's category based on gender and/or biological sex would undermine this principle of equity, which has been enshrined on several levels. However, suppose it is impossible to protect women with *sic et simpliciter* institution of a gender-specific homicide, the law highlights the possibility of recognizing aggravating circumstances for those who commit a crime against a vulnerable individual. An example is offered by the aggravating circumstances of "impaired defense" (art. 61 Italian penal code), which increase the severity of the criminal act.

Furthermore, one of the purposes for the femicide proposal is the stigmatization of the reasons that lead some men to murder their female partners. An analysis of the phenomenon demonstrates how it is historically rooted in a patriarchal concept of a society where women are often seen as the property of their partners or families. The criminological classification of femicide as a multifactorial criminal phenomenology should not be underestimated. In case a new regulation to protect women would be developed, the reasons underlying the perpetrator's motive must also be considered, as well as the understanding of the nature of violence used by the male aggressor against the female victim. Moreover, it should be analyzed whether, in femicide, the criminal attitude is statistically connected to a partial or total reduction of the mental capacity. On the contrary, it could result from a conscious intention unrelated to any mental disorder and driven by a particular evil nature of the culprit (Italian p.c., art. 85, 88, 89, 108).

Discrimination and hate crimes have to be stigmatized and flagged in modern society. At the same time, the conception of femicide as a juridical category should be rejected according to the legal equity mentioned above. Nevertheless, legislation can still offer the possibility to protect women and legally mark out any violent action against them perpetrated for the simple reason of their gender and biological sex. The critical analysis of the drives that lead to the murder of the woman can ascribe the motive to the category of trivial reasons (art. 61 Italian p.c.), thus constituting a further aggravating circumstance for the offender. The latter aspect is particularly significant for its implications in protecting women and partners of any gender within a relationship characterized by inherent violence. Identifying the desire for possession and control of the partner, regardless of their gender, as a trivial reason allows more inclusive protection of any vulnerable individual victim of crimes within relationships. The authors present a synthetic examination of the legal means that could increase the culpability of a criminal act against female and vulnerable subjects. These means (e.g., aggravating circumstances) would allow victims protection without the need to establish a new category of murder (femicide), potentially in contradiction with the principles of equity established by international laws and the Universal Declaration of Human Rights.

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**Homicide; Hate Crimes; Gender-Specific Crime**

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**G21 Improving Forensic Science Education Within a Law School Curriculum**

*Shelby Kmidowski, BS\*, Duquesne University, Hamburg, NY*

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**Learning Objective:** The goal of this presentation is to educate attendees on the gap of forensic science teachings to the legal community. Then, they will be shown an example of how forensic science courses can be incorporated into a criminal law curriculum and the effectiveness based on the completed trials.

**Impact Statement:** This presentation will impact the forensic community by bridging the gap between forensic science and the legal communities, which can improve the criminal justice system as a whole.

Many, if not most, criminal lawyers and judges do not have a background in the forensic sciences.<sup>1,2</sup> Because of this, judges may struggle to determine what type of forensic evidence can be admissible in court and base their admissions on past cases. Lawyers struggle to clearly communicate forensic evidence to juries during the course of a trial. Many studies have already assessed where lawyers then gain their forensic science knowledge, most commonly through Continuing Legal Education courses or through trial experience.<sup>3</sup> Very rarely does their knowledge come from the implementation of forensic science courses within a law school curriculum.

To address this issue, an online module program on forensic science, which contained several different topics such as DNA and toxicology was developed via Canvas to determine if the modules increased criminal law students' knowledge on different forensic sciences. To gauge the students' knowledge before and after the educational material, a pre- and post-test was utilized to determine if any knowledge was gained throughout the course of the module. Both the tests were the same set of questions to better statistically analyze the scores. To prevent participants from using their pre-test to complete the post-test, they were unable to see their responses as well as the correct answers to the questions. The participants were also required to complete knowledge checks about halfway through the module to ensure they were grasping the forensic concepts confidently. The pre- and post-test scores should show the importance of teaching basic forensic science techniques to criminal law students.

If forensic science is incorporated into law school curriculums, law students will be better equipped to handle forensic evidence in court quickly upon becoming a licensed and practicing attorney rather than gaining this knowledge throughout their career.

**References:**

- <sup>1.</sup> Forensic Science & Law Program, Duquesne University, Pittsburgh, PA 15282, USA.
- <sup>2.</sup> Wecht Institute for Forensic Science, Duquesne University, Pittsburgh, PA 15282, USA.
- <sup>3.</sup> Administrative Offices of the Pennsylvania Courts, Western District, Pittsburgh, PA 15282 USA.

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**Criminal Law; Forensic Evidence; Curriculum**

**G22 Criminal Courts and Experts: The Admissibility of the Scientific Evidence in Criminal Procedures and the Transposition of the *Daubert* Standard in French Law**

*Alice Despierres, MS\*, NIMES, Languedoc-Roussillon, FRANCE*

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**WITHDRAWN**

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**G23 The National Institute of Standards and Technology (NIST) Scientific Foundations of Forensic Firearm Examination: A Discussion of Validation and Proficiency Tests and the Theory of Identification**

*Robert Thompson, MSFS\*, National Institute of Standards and Technology, Gaithersburg, MD; Theodore Vorburger, PhD, National Institute of Standards and Technology, Highlands Ranch, CO; Wayne Arendse, MSc, Omega Laboratories, Inc., Mississauga, ON, CANADA; John Butler, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Steven Lund, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Heather Waltke, MFS, National Institute of Standards and Technology/Stratavia, Crompond, NY; Gregory Klees, BA, Bureau of Alcohol, Tobacco, and Firearms, Beltsville, MD*

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**Learning Objective:** The goal of this presentation is to introduce the attendees to the Association of Firearm and Toolmark Examiners (AFTE) Theory of Identification, its principles, and its practice among expert examiners. The theory is compared to performance studies (proficiency and validation tests) of examiner accuracy and confidence and how those results compare to the statements in the theory. Attendees will be introduced to our proposed additional content to include its practice by the specialists.

**Impact Statement:** This presentation will impact the forensic science community by offering an updated and expanded theory of identification to include the specialty as practiced. The approach may also be a guide for research in other impression comparison evidence theories and tenants, including “white” box studies.

In 1992, the AFTE proposed the “Theory for Identification.” Briefly, the theory describes the basic principles of the uniqueness of the tool surfaces of the firearm that produce toolmarks onto fired cartridge components (bullets and cases). It states that a proficient examiner comparing toolmarks will be able to determine the source of the toolmarks if sufficient agreement is observed. Subsequently, if there exists sufficient microscopic agreement, the examiner may report a conclusion that the chance that a different firearm source producing toolmarks with such observed agreement is a “practical impossibility.”<sup>1</sup>

Our review of the results of firearm examiners’ performance in validation and proficiency tests demonstrates a measurable and low false positive error rate. A discussion regarding how these results may affect the profession’s theory of identification will include proposed additional content for the current theory. The proposal will have a following “in practice” component to align the Theory of Identification’s theoretical basis more fully to the results of professional practice measured in studies. Research investigating the subjective confidence of the firearm examiners’ opinion of firearm source show a tendency toward overconfidence compared to the ground truth of the examined test samples.<sup>2</sup> There are limited studies that pertain to forensic firearm examination examiner opinion confidence metrics; however, other diagnostic sciences in medicine have investigated expert opinion confidence and the basis for such. A discussion of these studies may form the basis for research into what cognitive processes affect the examiner confidence in examination result opinions. Such future research may be best designed as “white box” studies. This presentation will describe the results of validation studies and proficiency tests that were reviewed by the authors; how the practitioner authors in the team developed a “challenge method” to estimate the difficulty of tests in this study; and how this method could be used for research in the future studies of examiner performance.

**References:**

- <sup>1.</sup> *AFTE Theory of Identification* | AFTE.
- <sup>2.</sup> Mattijssen, E., Witteman, C. L. M., Berger, C. E. H., Brand, N. W. & Stoel, R. D. 2020. Validity and reliability of forensic firearm examiners. *Forensic Sci Int*, 307.

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**Theory of Identification; AFTE; Scientific Foundation**

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**G24 The Trouble With Inconclusives in Firearms**

*Lynn Garcia, JD\*, Texas Forensic Science Commission, Austin, TX*

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**Learning Objective:** Attendees will learn about the results of a Texas Forensic Science Commission review into reporting and testimony in the discipline of firearm and toolmark examination. This presentation will discuss strategies for making reporting and testimony in firearms more transparent and clear to end users, including lawyers and judges.

**Impact Statement:** The presentation will impact the forensic science community by discussing problems with presentation of firearm and toolmark evidence that risk contributing to miscarriages of justice and offering practical ways to improve the communication of this information based on extensive research and collaboration.

On October 6, 2021, the Innocence Project (IP) filed a complaint with the Texas Forensic Science Commission requesting that the Commission “investigate and report ‘the integrity and reliability’ of toolmark and firearms analysis . . . as used in criminal proceedings.” The IP asked the Commission to set “appropriate limits” on the conclusions of firearm and toolmark examiners in traditional bullet-to-firearm matching testimony and determine what conclusions—if any—can be proffered in other toolmark assays. The Commission reviewed the IP’s request and complaint and sought input from many external resources within the legal, statistics, and practitioner communities. The result was a report that provides new perspectives on how the community should communicate comparison results in the firearms discipline. The report pays special attention to the difficulties practitioners face in reaching an exclusionary result due to the limitations of the test method itself. Often, examiners reach an inconclusive finding because there are insufficient markings left to make a determination. The problem is that with a category as broad as “inconclusive,” it is difficult to know how juries and judges hear that information. Various possible approaches to reporting are discussed, including the use of likelihood ratios. The transition to likelihood ratios is not without precedent in the forensic community, with DNA analysts having made that move with the introduction of probabilistic genotyping software. However, significant challenges are present in communicating the significance of likelihood ratios appropriately to a lay jury. A not insignificant challenge is that while DNA analysts historically have had some background and exposure to concepts in statistics, firearm and toolmark examiners have not. Indeed, a common perspective among practitioners is that firearms examination has nothing to do with statistics, as results have historically been reported using qualitative language.

This presentation will summarize the various considerations and report on recommendations made by the Commission to advance the conversation on reporting and testimony in the discipline, both within Texas and nationally.

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**Firearms Toolmarks Limitations; Reporting Testimony; Forensic Science Commission**

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**G25 Results From a Review of Scientific Foundations of Firearm Examination**

*Theodore Vorburger, PhD\*, National Institute of Standards and Technology, Highlands Ranch, CO; Steven Lund, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Heather Waltke, MFS, National Institute of Standards and Technology/Stratavia, Crompond, NY; Gregory Klees, BA, Bureau of Alcohol, Tobacco, and Firearms, Beltsville, MD; Wayne Arendse, MSc, Omega Laboratories, Inc., Mississauga, ON, CANADA; James Yen, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Shannan Williams-Mitchem, MA, National Telecommunications and Information Administration, Washington, DC; John Butler, PhD, National Institute of Standards and Technology, Gaithersburg, MD; Robert Thompson, MSFS, National Institute of Standards and Technology, Gaithersburg, MD*

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**Learning Objective:** After attending this presentation, attendees will have seen a comprehensive summary of the existing data that provides a scientific foundation for the practice of firearm examination in forensic science.

**Impact Statement:** The forensic science community will better understand the strengths and limitations of firearm examination as a pattern discipline using scientific methods.

The National Institute of Standards and Technology (NIST) is conducting reviews of the scientific foundations of several forensic science disciplines. These reviews seek to answer the question: “What empirical data exist to support the methods that forensic science practitioners use to analyze evidence?” They attempt to consolidate information supporting the methods used in forensic analysis and identify knowledge gaps where they exist. The current review of firearm examination follows reviews of DNA mixtures, digital evidence, and bite mark analysis, which have been completed or drafted. The project team has evaluated a wide body of published literature and other materials devoted to firearm examination. A bibliography database has been developed containing 925 references (as of July 2022). The draft report includes historical perspectives of the field, a review of scientific critiques, studies of advanced methods of 3D acquisition and objective algorithmic analyses, and a preliminary statistical model for design of error rate tests, but the primary emphasis is placed on the widely used method of comparison microscopy.

Toward this end, all known literature and materials that contain data for error rates for identification and exclusion of matching bullets and cartridge cases based on microscopy comparisons have been included. The focus will be on 20 reports of interlab studies, method validations, and proficiency tests. We classify these works into four categories depending on the experimental designs (for example, on whether each question posed in a study is independent and uncorrelated from the others or whether each identification or other decision changes the a priori probability of other decisions). The false positive error rates for these vary from 0% to 8.2%, but for a majority of the studies, the false positive error rate lies between 0.08% and 1.5%, for studies reported so far. Others are known to be in process in this active field of investigation.

In addition, six other factors are considered as possible influence factors on the values of measured error rates. These include:

- The region of interest—bullet land engraved area, breech face impression, etc.
- Factors affecting the size of the study—number of questions, number of firearm types, and number of participants
- Whether or not known pairs are included as examples and standards
- Whether or not the study includes consecutively manufactured firearms
- Whether questions are declared to participants or covertly included in case work
- Test difficulty

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**Firearm; Examination; Scientific Foundation**

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**G26 Junk Science and the American Criminal Justice System**

*Chris Fabricant, JD\*, Innocence Project, New York, NY*

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**Learning Objective:** After attending this presentation, attendees will gain insight from an insider's perspective into the heart of a broken American criminal justice system where junk science is just one piece in maintaining the status quo, including insight from author M. Chris Fabricant's recently released, award-winning book, *Junk Science and the American Criminal Justice System*.

**Impact Statement:** This presentation impacts the forensic science community by demonstrating the perspective of the premier junk science wrongful conviction investigative and litigation organization—the Innocence Project—that ushered forensics through a time that saw statements such as “one-to-a-million chance” as acceptable science into in a new era of reliability, validation, and, most of all, the accountability we expect to see today.

In 2012, the Innocence Project began searching for prisoners convicted by junk science, and three men, each convicted of capital murder, became M. Chris Fabricant's clients. In his book *Junk Science and the American Criminal Justice System*, Fabricant gives an insider's perspective into the heart of a broken American criminal justice system where junk science is just one piece in maintaining the status quo. Fabricant chronicles his fight to overturn the wrongful convictions and to end the use of the bad science that destroyed these men's lives and many others' lives. The quest took many turns and the story weaves together courtroom battles over faulty forensics from Mississippi to Texas to New York City and beyond. The book describes a journey into the heart of a broken, racist system of justice and the role forensic science plays in maintaining that broken system. Fabricant describes the evolution of junk science from the perspective of some of the Innocence Project's most notorious cases.

In addition to some of the interesting case studies demonstrated in his book, Fabricant's career documents the slow but steady evolution of the field of forensics as a whole and the difficult challenges and barriers the Innocence Project, lawyers, judges, other stakeholders, and forensic scientists themselves have faced in evolving and moving forward from duplicitous times. While no one will ever know the true number of wrongful convictions in America brought on by junk science, together we can assist the criminal justice community in understanding how to prevent such injustices as we move forward, away from faulty ideas and junk science and toward sound, supportive data, even where that information may unravel centuries of unreliable forensic science.

**Reference:**

- <sup>1.</sup> Fabricant, M. Chris., *Junk Science and the American Criminal Justice System* (2022).

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**Innocence; Courts; Wrongful Convictions**

**G27 Lindbergh Case: Hauptmann's Wrongful Murder Conviction**

*Lise Pearlman, BA, JD\*, Oakland, CA; Pete Speth, MD, Forensic Consultations, Wenonah, NJ; Jamie Benvenutti, BA, St. Louis, MS*

**Learning Objective:** This presentation provides attendees with freshly reevaluated 1930s evidence that Bruno Richard Hauptmann was wrongly convicted of murdering Charles Lindbergh, Jr., the son of aviator Charles Lindbergh. The evidence supports the conclusion that the toddler didn't die when, how, and where Hauptmann was charged with the homicide.

**Impact Statement:** This presentation will impact the forensic community by providing proof of police, prosecutorial, and medical professionals' misconduct resulting in Hauptmann's wrongful conviction for "the crime of the century." It reinforces the importance of ethics in scientific and medical evidence to minimize miscarriages of justice.

On March 1, 1932, police announced Lindbergh's son was kidnapped from the aviator's New Jersey farmhouse. On May 12, 1932, the toddler's largely bloodless remains were found in nearby woods. After the coroner claimed the body, the New Jersey State Police removed abundant surrounding materials for analysis by Squibb Biological Laboratories.<sup>1</sup> The detailed Squibb Report arrived with a cover letter from the lab's Director: "the results were practically barren of any significant clues ... of value in the [homicide] investigation ...."<sup>2</sup>

Hauptmann was arrested in New York in September 1934 for extortion after police found him with nearly \$14,000 in ransom money. Thereafter, Hauptmann was extradited to New Jersey to face murder charges. Prosecutor David Wilentz opened by arguing Hauptmann invaded Lindbergh's farmhouse by climbing a ladder to the second-floor nursery. Hauptmann allegedly placed the sleeping toddler in a burlap bag he accidentally dropped while descending the ladder, killing the toddler instantly.<sup>3</sup>

The murder charge contradicted Dr. Charles Mitchell's May 14, 1932, medical report opining "the child could have been killed and left at the point where it was found for a period of from seven to ten weeks," (i.e., March 3 to 24, 1932.) Mitchell testified at a different trial in 1933 that the skull fractures could not have resulted from a two-story fall into mud.<sup>4,5</sup> Nonetheless, at Hauptmann's trial, the State did not produce Mitchell's May 14, 1932, report. Instead, assistant defense attorney Fred Pope overheard Mitchell being coached to testify the child's death was instantaneous on March 1 at the Lindbergh farmhouse.<sup>6</sup> Mitchell testified accordingly. During closing arguments, Wilentz switched theories and violated due process. Wilentz argued Hauptmann attacked the toddler in his crib with a chisel, crushing his head, before bagging and dumping the body in the woods. However, no blood was found on the chisel, the crib, or the ground outside the nursery.<sup>7</sup> In 1977, the New Jersey State Police reexamined the Squibb Report and proclaimed it reaffirmed that the body decomposed where discovered—as Wilentz alleged in prosecuting Hauptmann.<sup>8</sup> Recent careful review of the Squibb Report proves otherwise: Squibb technicians reported finding no blood on the child's clothing, the dirt and leaves they examined, nor the burlap bag.<sup>9</sup> Forensic expert reevaluation of written and photographic evidence (including the Squibb Report, Mitchell's medical reports, contemporaneous police reports, corpse photographs, and witness statements, trial testimony, and weather reports) negates both theories on which Hauptmann was convicted of murder.<sup>10</sup>

Today, unlike in 1935, defendants can discover material exculpatory evidence possessed by prosecutors or their agents.<sup>11</sup> Yet then as now, it was illegal for the prosecutor to knowingly present false testimony.<sup>12</sup> Wilentz pursued two different false murder theories and suborned perjury from Mitchell to convict Hauptmann. Squibb Laboratory Director Anderson had to know the Squibb Report disproved both prosecutorial murder theories.

The New Jersey Attorney General and State Police committed serious misconduct to convict Hauptmann of murder; Mitchell testified falsely at Hauptmann's trial; Director Anderson misrepresented that the Squibb Report provided no valuable insight into the homicide and the State Police perpetuated the cover-up. Ergo, Hauptmann's conviction was a travesty of justice.

**References:**

- <sup>1</sup> The Squibb Report, reprinted in *The Lindbergh Kidnapping: Suspect No. 1, The Man Who Got Away*, [Regent Press 2020] Appendix B. In 1989, the Squibb Laboratories merged into Bristol, Myers Squibb.
- <sup>2</sup> May 27, 1932, letter of Director John F. Anderson, M.D. to N.J. State Police Supt. Col. H. Norman Schwarzkopf, p. 3; The Squibb Report, supra, n.1 at p.442.
- <sup>3</sup> Wilentz opening statement, untitled list of proposed witnesses' testimony and exhibits, New Jersey State Police Museum (NJSPM).
- <sup>4</sup> Dr. Mitchell's May 14, 1932, death report for Charles Lindbergh, Jr. has disappeared but the text was retained by the FBI. *FBI Summary Lindbergh Report* (NY-62-3057) 110 (Autopsy) [www.archive.org](http://www.archive.org).
- <sup>5</sup> Charles Mitchell Testimony, *U.S. v. Gaston Bullock Means and Norman Whitaker*, Supreme Ct. of D.C., 265, May 9, 1933, NJSPM.
- <sup>6</sup> Michael Melsky, Vol. II, 22, n.77 quoting George G. Hawkes (1951) *Trial by Fury: The Hauptmann Trial*, Princeton Univ. Sr. Thesis, 112. n. 74 (interview of Fred Pope March 3, 1951), NJSPM.
- <sup>7</sup> Suspect No. 1, supra, n.1, "The Squibb Lab Report" pp.96, 383-84.
- <sup>8</sup> L77311932 Lindbergh Evid. Review Phase 1(April-June 1977) Report, p.9, NJSPM.
- <sup>9</sup> Suspect No. 1, supra, n.1, pp.383-84 and Appendix B.
- <sup>10</sup> Ibid., Declaration of Peter Speth, MD., Appendix C, pp. 460, 470.
- <sup>11</sup> *Brady v. Maryland*, 373 U.S. 83 (1963); *Kyles v. Whitley*, 515 U.S. 419 (1995).
- <sup>12</sup> *New Jersey Revised Statutes RS 2:157-1* [Perjury and the Subornation of Perjury].

**Medical Evidence; Prosecutorial Misconduct; Professional Responsibility/Ethics**

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**G28 Amendments to FRE 702: Some Teeth for *Daubert*?**

*Donald Shelton, PhD, JD\*, University of Michigan-Dearborn, Saline, MI*

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**Learning Objective:** After attending this session, attendees will be familiar with the proposed amendments to the Federal Rule of Evidence governing the admissibility of forensic science evidence and how they will change *Daubert* hearings before judges.<sup>1</sup>

**Impact Statement:** The proposed amendments to FRE 702 will affect how forensic science experts testify at *Daubert* hearings and how attorneys and judges can fulfill their duties under the new rule. Many states may revise their rules of evidence accordingly.

It has been 30 years since the *Daubert* decision ostensibly changed the rules for the admission of forensic science evidence and made judges the gatekeepers for such evidence. *Daubert* and the subsequent *Kumho* case led to a new Federal Rule of Evidence 702 adopted in 2000: Rule 702. Testimony by Expert Witnesses 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: (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 the principles and methods to the facts of the case.<sup>2</sup>

Many states that adopted *Daubert* changed their rules of evidence to correspond. But many judges have not performed the gatekeeping role demanded by *Daubert*. In many cases, judges, relying on *stare decisis*, simply held that a *Daubert* hearing on admissibility was not required because many forms of forensic science evidence have been routinely admitted in the past. Some even used the concept of judicial notice to keep admitting such evidence without any regard for *Daubert*. In cases where judges even considered a *Daubert* challenge, judges reversed the burden of proof and required the opponent to prove that the evidence was not reliable enough to be admitted. Others simply refused to perform their gatekeeping duty by finding that any concerns about the reliability of forensic science evidence would be left to the jury. Because of those abuses, amendments to FRE 702 have been proposed by The Judicial Conference of the United States’ Committee on Rules of Practice and Procedure. The proposal has gone to the entire Judicial Conference, and if approved in the September 2022 meeting, will be sent to the Supreme Court. The current proposed amended rule provides: FRE 702 current revision

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: (1) 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; (2) the testimony is based on sufficient facts or data; (3) the testimony is the product of reliable principles and methods; and (4) the expert has reliably applied expert’s opinion reflects a reliable application of the principles and methods to the facts of the case.

The amendment addresses many of the prior abuses. First, it makes clear that the burden to prove admissibility is on the proponent of the evidence, just as it is for all other evidence admissibility issues. Second, it clarifies that it is the court, and not the jury, that must decide whether the evidence is based on reliable science and a reliable application of that science. Finally, it requires the proponent to demonstrate admissibility using the preponderance of evidence standard of “more likely than not.” These amendments to FRE 702 could finally put some “teeth” into *Daubert* and convince reluctant judges to perform their gatekeeping responsibilities for forensic science evidence. The many states that have adopted *Daubert* may well amend their rules of evidence accordingly.

**References:**

- <sup>1.</sup> *Daubert v. Merrell Dow Pharmaceuticals*. US Supreme Court 509.U.S.579,113S.Ct.2786, 125L. Ed.2d 469. 1993.
- <sup>2.</sup> *Kumho Tire Co. v. Carmichael*, 526 U.S. 137.

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**Admissibility; *Daubert*; Evidence**

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**G29 You See Plastic Wrap, I See Aluminum Foil: Improving Transparency by Bridging the Communication Gap Between Scientists and Attorneys**

*Darina Griffin, JD\*, Massachusetts State Police Crime Laboratory, Maynard, MA; Lynn Schneeweis, MSFS\*, Massachusetts State Police Crime Laboratory, Maynard, MA; Kristen Sullivan, MSFS, Massachusetts State Police Crime Laboratory, Maynard, MA*

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**Learning Objective:** After attending this presentation, individuals will better understand the challenges associated with developing a culture of transparency and effective communication in forensic science that meets the unique needs of the various parties in the judicial system.

**Impact Statement:** This presentation will provide insight to both the forensic and legal communities that may assist jurisdictions to understand the information and documentation available in, and therefore more clearly articulate what is needed from forensic laboratories to improve communication and transparency within the legal system.

The past decade has brought about many changes to the forensic science community, including technological advancements, more scientifically robust validation and methods, and more stringent and expansive accreditation and certification requirements for laboratories and analysts, respectively. The impetus for such change has arisen from a variety of sources: legislative mandates, case law, and demands to “fix” forensic science when errors or so-called scandals are identified, to name a few. The call to “strengthen forensic science” has been a persistent one and, in many ways, the field of forensic science has responded favorably by striving to understand and subsequently address the constructive criticism and develop a culture of continuous improvement. While strengthening the “science” in forensic science may seem like a tall order, from the scientist’s perspective, it is arguably the easier criticism to address. Instead, one of the most challenging areas that has emerged in the quest to improve forensic science is increasing the transparency and improving communication between forensic laboratories and the legal system. As forensic scientists, there is an inherent responsibility to provide information and documentation of the work that is done in the laboratory and to effectively communicate the meaning and/or significance of the information being provided. However, the challenge lies with identifying what information is actually being sought when the request to provide “anything exculpatory” or “any and all associated information and documentation” is made of the laboratory. The solution that seems immediately obvious is to provide anything and everything in the laboratory’s custody, thereby eliminating the chance of not providing information that ultimately is deemed to be important to either party. However, anyone familiar with the volume of documentation maintained by a laboratory understands that would simply result in a “data dump” to the attorneys, much of it potentially unnecessary, and increase the frustration for those on the receiving end.

The questions, therefore, remain: what does it mean to be transparent and where is the balance between providing everything that is important to a case but not every piece of information or documentation in the laboratory’s custody? To answer those questions, it is imperative that scientists and attorneys attempt to bridge the natural communication gap that exists between them. This presentation will examine these questions from the scientist and attorney perspectives and provide examples of where this communication and knowledge gap can be improved, with the goal of promoting a culture of transparency and effective communication between all parties in a criminal case.

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**Transparency; Communication; Relevancy**

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**G30 Building the Bridge: Increasing Legal Awareness of the Importance of Forensics**

*Barbara Hervey, JD\*, Texas Court of Criminal Appeals, Austin, TX; Carson Guy, JD, Texas Court of Criminal Appeals, Lampasas, TX*

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**Learning Objective:** After attending the presentation, attendees will appreciate the multi-targeted approach the Texas Court of Criminal Appeals takes, through its grant programs, to address the need for a thorough understanding of forensic science as it impacts the legal system. Attendees will also understand the need to begin teaching a forensic science core curriculum in law schools so future attorneys are better prepared to handle cases involving forensic science upon obtaining their licenses.

**Impact Statement:** With but a few examples of success from the intersection of science and law, the scientific community can understand why a hardcore focus on training, translating good science into life-changing legal events, must continue.

The Texas Court of Criminal Appeals administers multiple grants for the purpose of providing Continuing Legal Education (CLE) courses, programs, and technical assistance. Constituents consist of judges, court personnel, prosecuting attorneys, prosecutorial staff, and criminal defense attorneys who regularly represent indigent defendants in criminal matters. The Court awards money to grantees, conditioned on their justifications for receiving funds, to be applied to important principles of law and science. Although the grantees serve particular participants in the criminal justice system, the Court believes in across-the-board training to ensure that participants are “on the same page” when it comes to science. Occasionally, the Texas Court of Criminal Appeals also hosts across-the-board, tuition-free training opportunities for constituents and others in the legal community, such as law enforcement and scientists. Speakers have presented in these forums on topics such as DNA, arson, false confessions, and toxicology. Many of these seminars were successful joint collaborations. For example, the Court works extensively with the Texas Forensic Science Commission (TFSC) and other collaborative agencies in the development of learning opportunities.

In 2008, the Court created the Texas Criminal Justice Integrity Unit (Unit) to review the strengths and weaknesses in the Texas criminal justice system. As part of its work, the Unit hosted training events and assisted in the creation of numerous pieces of legislation. For example, legislation requiring the adoption of a model eyewitness identification policy and allowing for small counties to store biological material at Texas Department of Public Safety facilities were passed into law after being examined by the Unit.

**Forensic Science Core Curriculum:** While educating participants in the criminal justice system is critical, it is insufficient by itself. Law schools need to offer students a core forensic science course, teaching legal principles governing forensic science with an overview of different fields of forensics and how to apply it in the courtroom. The Court, working with the TFSC and others, emphatically supports such an effort and is optimistic such a project will be accomplished in all Texas law schools. Legal rules and principles include relevant rules of evidence (103, 702, 703, 705, etc.), Article 11.073 (Texas junk-science writ), and legal precedent such as *Daubert v. Merrell Down Pharmaceuticals, Inc.*, 509 U.S. 579 (1993), *Kelly v. State*, 824 S.W.3d 568 (Tex. Crim. App. 1992), and *Nenno v. State*, 970 S.W.2d 549 (Tex. Crim. App. 1998).

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**Training: Forensics; Core Curriculum**

## **G31 A Forensic Science Informational Video Can Help Mock Jurors**

*Devon LaBat, MSc\*, Florida International University, Miami, FL; Deborah Goldfarb, JD, PhD, Florida International University, Weston, FL; Jacqueline Evans, PhD, Florida International University, Miami, FL; Nadja Schreiber Compo, PhD, Florida International University, Miami Shores, FL; Cassidy Koolmees, MSc, Florida International University, Aurora, CO; Gerald LaPorte, MSFS, Florida International University - Global Forensic and Justice Center, Williamsburg, VA; Kevin Lothridge, MS, Florida International University, Largo, FL; Laura Shambaugh, PhD, Florida International University, Doral, FL*

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**WITHDRAWN**



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**G32    *Brady* and Forensics: Disclosing Favorable Scientific Evidence**

*Robert Smith, JD, Texas Forensic Science Commission, Austin, TX*

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**Learning Objective:** Attendees will learn the core legal concepts surrounding the disclosure of information favorable to an accused and how those principles apply in the context of a forensic analysis.

**Impact Statement:** The presentation will impact the forensic science community by highlighting areas where disclosure methods have failed in the forensic science field and discuss methods to increase transparency.

Sixty years ago, in the landmark case of *Brady v. Maryland*, the Supreme Court held the state violates due process when it fails to disclose information in its possession that may be favorable to a defendant.<sup>1</sup> Courts have expanded and interpreted this core concept in a vast array of contexts, including its application in the field of forensic science.

Crime laboratories and forensic analysts create and store reams of scientific information related to a forensic analysis and often produce a summary report for use in the criminal justice system. This presentation will discuss various types of favorable information that may not be disclosed in these summary laboratory reports, and the high risks involved to the forensic science community and criminal justice stakeholders when favorable evidence contained in the laboratory casefile is not disclosed.

The information included in this presentation was derived from legal opinions applying the principles of *Brady* and its progeny to the field of forensic science and from case examples encountered by the Texas Forensic Science Commission. The research indicates information favorable to an accused fails to be adequately disclosed in many instances, for various reasons, and additional strategies should be employed by crime laboratories and analysts to facilitate the proactive dissemination of exculpatory, mitigating, and impeaching evidence or information. Robert Smith is a former career prosecutor and current staff attorney at the Texas Forensic Science Commission.

**Reference:**

<sup>1</sup>. *Brady v. Maryland*, 373 U.S. 83 (1963).

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**Favorable Information; Disclosure; Transparency**

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**G33 Challenging Retinal Hemorrhage Testimony in Court**

*Kathleen Pakes, JD\*, Wisconsin State Public Defender, Madison, WI; Patrick Lantz, MD, Department of Pathology/Wake Forest School of Medicine, Winston Salem, NC*

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**Learning Objective:** This session is geared toward lawyers who are challenging expert opinion related to the significance of retinal hemorrhages in Abusive Head Trauma/Shaken Baby Syndrome (AHT/SBS) cases. The session will discuss pretrial (*Daubert* and *Frye*) challenges as well as cross examination strategy related to the significance of retinal hemorrhages in child abuse evaluations and testimony.

**Impact Statement:** This presentation will provide lawyers (primarily family defense and criminal defense lawyers) with background information helpful for challenging the forensic reliability of retinal hemorrhage opinion testimony.

Lawyers are often tasked with challenging the admissibility of expert testimony or cross-examining expert witnesses. In AHT/SBS cases, this may include challenges to, and cross examination of, the Vitreoretinal Traction (VT) theory of Retinal Hemorrhage (RH) development during alleged acceleration-deceleration episodes (shaking). This session will discuss RH development, child protection theory related to the significance of RHs in a child abuse diagnosis, and illustrate that RHs are a non-specific marker of intracranial pathology.

The medical literature has documented that RHs, including severe retinal hemorrhages, develop in response to various conditions and contexts such as high-altitude exposure, carbon monoxide poisoning, extra-axial bleeding/raised intracranial pressure (Terson syndrome), natural disease processes, and birth. The medical literature further recognizes that with infants, severe RHs develop in the context of complicated medical conditions and confounding factors, including hypoxia, cardiopulmonary resuscitation, anemia, reperfusion injury, raised intracranial pressure, and congenital and acquired coagulopathies. The child protection literature recognizes that the development of severe RHs correspond with the seriousness of intracranial pathology; that the pathophysiology of RHs is obscure; and that the effect of confounding factors on the pathophysiology and appearance of RHs has not been well studied. Critics of the child abuse literature note that many studies are limited by circular reasoning and/or selection bias (i.e., clinicians have been trained to associate RHs with abuse [VT theory/shaking], therefore, clinical data reflects a preferred association, not actual causation). For these reasons, many authors (legal and medical) have argued that RHs are not a reliable marker of trauma, RHs are not a reliable marker of a specific mechanism of trauma (shaking), and RHs are not a reliable marker of the amount of force (violent shaking).

This session will discuss the Swedish Agency for Health Technology Assessment and Assessment of Social Services (SBU) systematic review of the medical literature, which concluded low-quality evidence supports the triad theory, and the responding Consensus statement. In addition to discussion of the legal and medical criticisms of the use of RHs as a marker of abuse, this session will discuss *Daubert* and *Frye/Kelly* factors such as general acceptance and the relevant scientific community (biomechanical engineers, forensic pathologists, child protection clinicians) and whether the RHs-are-a-marker-of-abuse theory should survive a *Daubert* or other reliability challenge. Attendees will gain a basic understanding of anatomy and pathophysiology related to RHs. Attendees should also leave with an understanding of the limitations of the child abuse clinical diagnostic process, including the subjective nature of that process, the application of invalidated general causation beliefs to specific causation diagnosis, the unreliability of clinical child abuse data, and how bias affects that process.

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**Retinal Hemorrhages; *Daubert/Frye*; Admissibility Challenges**

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**G34 Drug Recognition Experts: Unreliable Expertise**

*Steven Hernandez, BA, JD\*, Hernandex Law Firm, Toms River, NJ; Gil Sapir, JD, Chicago, IL; Jay Zager, Parkland, FL*

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**Learning Objective:** After attending this presentation, attendees will understand the questionable scientific issues of Drug Recognition Experts (DRE).

**Impact Statement:** The presentation will impact the forensic community by illustrating the evidential unreliability of quasi-medical expert police officers as DREs.

A DRE is a police officer preliminarily trained to recognize drug-induced impairment of drivers, including alcohol intoxication. The results are used to prosecute Driving Under the Influence/ Driving Under the Influence of Drugs (DUI/DUID) cases.

Police officers without formal scientific training enroll in a seven-day course designed to teach them about the characteristics and effects of seven different drug categories on all major systems in the human body.<sup>1</sup> They are not trained in blood testing for drug intoxication, nor do they receive training in pharmacology. Training entails an assessment protocol, Standard Field Sobriety Tests (SFSTs), observational “clinical signs,” physiological measures (gaze nystagmus, blood pressure, heart rate), a medical condition/treatment interview, medication history, and a subject’s physical appearance to formulate the Drug Influence Evaluation (DIE).<sup>2</sup> Ergo, “Doctor Cop.”<sup>3</sup>

DREs receive brief training in the DIE and “field experience.” To date, the DIE has not been subjected to sufficient scientific study to permit its use as a reliable and valid protocol to determine if an individual is impaired as a result of ingestion of a controlled substance. The DIE is derived from SFSTs employing psychophysical tests to determine probable cause during pre-arrest assessment of individuals suspected of driving under the influence of alcohol.

The title’s inclusion of “expert” was created by the International Association of Chiefs of Police for the imprimatur of credibility.<sup>4</sup>

Current scrutiny and litigation concerns:

- Is DRE testimony admissible as expert testimony;
- Does DRE/DIE evidence qualify as scientific evidence subject to judicial gate-keeping;
- Is the DRE/DIE a valid scientific protocol;
- Are DREs performing medical diagnostic tests and interpreting clinical signs and symptoms without sufficient training or experience—not as technicians or observers;
- Should DRE/DIE protocols and reports include confirmatory diagnostic testing; and
- Can DREs impartially, credibly, and reliably determine specific drug-induced impairment?<sup>5</sup>

The evidential admissibility standards of *Frye* or *Daubert* apply to DRE testimony.<sup>6</sup> Occupational training, experience, or previous testimony are not equated with expert witness status.<sup>7</sup> The courts are not bound by *stare decisis*, especially concerning valid science.<sup>8</sup>

The DRE conducts a medical observation and judgmental evaluation using science, without a required reported corroborating confirmatory blood or urine analysis, to conclude a person is under the influence of one or more of seven specific drug categories.<sup>9</sup> The DRE does not possess sufficient expertise to resolve a person was under the influence of drugs or ascertain the specific category of drugs causing the impairment.<sup>10</sup> The unreliability of DRE protocols are not obvious, understandable, or known to the average lay juror.<sup>11</sup>

The National Highway Traffic Safety Administration (NHTSA) issues requests for scientific studies to test the validity and reliability of the DIE and DRE training. The process and procedures are scientifically unreliable. Furthermore, DRE opinions cannot be supported by the disfavored “to a reasonable degree of scientific certainty” standard.<sup>12</sup>

The DRE’s expertise is tantamount to junk science. “‘Junk science’ is the mirror image of real science, with much of the same form but none of the same substance.”<sup>13</sup> Junk science or pseudo-science routinely consists of flawed, unreliable, exaggerated, and sometimes fabricated testimony, usually founded on questionable methodology.

A high degree of reliability is necessary where a person’s freedom is concerned. Courts should not accept DRE/DIE evidence until the process and procedures are truly scientifically reliable. DRE drug intoxication claims must be validated and reported with standard confirmatory blood or urine analysis (GC/MS). The DRE’s opinion is dangerous “junk science” and its moniker of “expert” is fictitious.

## References:

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4. Cliff J. Vanell, "Is 'DRE' Extinct? Or What's in a Name?," *The DRE (Newsletter)* (September-October 1990),2; The International Association of Chiefs of Police (IACP); On March 25, 1992, the Technical Advisory panel (IACP Highway Safety Advisory Committee) approved the self-proclaimed term "Drug Recognition Expert." Cliff J. Vanell, "What's in a Name?," *The DRE (Newsletter)* (March-April 1992),10.
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6. *Frye v. United States*, 293 F. 1013 (D.C. Cir. 1923); *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 509 U.S. 579, 595 (1993).
7. *Bogosian v. Mercedes-Benz of North America, Inc.*, 104 F.3d 472, 477 (1st Cir. 1977); *Thomas J. Kliner, Inc. V. Lorillard, Inc.*, 878 F.2d 791, 800 (4th Cir. 1989) cert. denied, 493 U.S. 1073 (1990).
8. *Fox v. Snow*, 6 N.J. 12, 23 (1950).
9. Central Nervous System (CNS) Depressant, CNS Stimulant, Hallucinogens, Dissociative Anesthetics, Narcotic Analgesics, Inhalants, and Cannabis.
10. "These procedures do not, generally speaking, disclose what specific drug or drugs the subject has used ... What the procedures will do, however, is to disclose (with reasonable accuracy) the category or combination of categories that produce distinguishable indicators visible to a qualified DRE." *Instructor Guide: Drug Recognition Expert Course*, supra, n.1, at p.2.
11. *State of New Jersey v. Odom*, 116 N.J. 65, 71 (1989).
12. DRE Instructor's Manuel uses "reasonable accuracy," supra, n.10, p.2; Science Bench Book for Judges, 2nd Ed. pp. 32,33, 263, National Judicial College, Reno, Nev., 2020; Gil Sapir, Legal Aspects of Forensic Science, chp. 1, in "Forensic Science Handbook," vol.I, 3rd ed, p.12, n.109, Richard Saferstein and Adam Hall, ed., CRC Press, 2020.
13. Peter W. Huber, *Galileo's Revenge: Junk Science in the Courtroom*, Basic Books/Harper Collins Publishers: New York, 1991;2.

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## Drug Recognition Expert; DUI/DUID; Dr. Cop

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**G35 Experts? We Don't Need No Stinking Experts!**

*Raymond Valerio, JD\*, Queens District Attorney, Kew Gardens, NY; Melissa Mourges, JD, New York County District Attorney's Office, New York, NY*

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**Learning Objective:** Forensic science is under attack as never before. This presentation will explore these attacks on forensic expertise and the perils to criminal justice if these critiques are taken to their logical conclusion.

**Impact Statement:** This presentation will impact attendees by confirming the value of forensic science, defining the relevant scientific community, and explaining how certain tactics have diluted the value of forensic science in the criminal justice system.

Forensic science is under attack as never before. Academics and other commentators who proudly call themselves “anti-expert experts” make the incoherent claim that their complete lack of practical forensic training and experience in any discipline renders them uniquely qualified to critique every forensic science discipline. Bolstered by the demonstrably false President’s Council of Advisors on Science and Technology (PCAST) claim that all pattern-matching forensic disciplines involve measurement science, or metrology, these anti-expert experts denounce the so-called “foundational validity” of every forensic conclusion. They claim there are not enough forensics studies and those that do exist are poorly designed. Anti-expert experts consider the low error rates proving practitioners are accurate when they describe a match to be “suspicious” and urge an outlier statistical approach to sharply increase the appearance of error.

They decry with equal fervor forensic software that allows computers to make lightening quick calculations and sort through millions of candidates, as well as human analysts who make “subjective” judgments.

They claim without evidence that casework forensic practitioners are motivated solely by money while the anti-expert experts are beyond those grubby considerations. A forensic discipline is considered a gold standard when it excludes someone but suspect when it inculcates. They urge match conclusions be limited to “cannot be excluded.” Those statements are of zero practical use to a juror, as the defense will always point out the thousands or millions or tens of millions of other guns or fingers or shoes or tires with similar class characteristics that also cannot be excluded.

The only possible goal is to make forensic evidence so innocuous as to be useless to the finders of fact or to keep it out of the courtroom altogether. Courts have said—and the law is clear—that only relevant scientists have the right to opine on the acceptance of a forensic methodology. Yet, it seems like anti-expert experts are gaining ground. This presentation will explore these attacks on forensic expertise and the perils to criminal justice if these critiques are taken to their logical conclusion.

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**Anti-Expert; Relevant Scientific Community; Admissibility**

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**G36 Hidden Wounds: A Trauma-Informed Approach to Sentence Reduction**

*Anna Sofine, MD\*, USC Institute of Psychiatry and Law, Los Angeles, CA*

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**Learning Objective:** After attending this presentation, attendees will understand: (1) why sentence enhancements are a target of reform; (2) the growing demand for forensic mental health specialists to evaluate and help determine whether childhood trauma, victimization and/or mental illness were contributing factors in a crime; and (3) challenges faced by the forensic psychiatry community in conducting these types of evaluations.

**Impact Statement:** This presentation will impact the forensic science community by assisting the expert witness in navigating the challenges faced when determining if prior victimization, mental illness, and/or childhood trauma are contributing factors in a criminal act.

In a 2014 national survey, it was found that “prisons and jails have become America’s ‘new asylums.’ The number of individuals with serious mental illness in prisons and jails now exceeds the number in state psychiatric hospitals tenfold. ... Such individuals are vulnerable and often abused while incarcerated.”<sup>1</sup> In response to this crisis, many states around the country are implementing criminal justice reform to incorporate consideration of mental illness, trauma, and its various facets when sentencing. This presentation will discuss the expanding role of the forensic mental health expert in providing evaluations for the Court by looking at California’s Penal Code (PC) §1385, amended in January of 2022, as a model of trauma informed sentencing.

Sentence enhancements are used to extend a defendant’s total time served. Such enhancements can be related to a defendant’s prior criminal history or to aggravating factors related to the criminal act, such as being armed with a weapon in the commission of a crime. One of the most well-known enhancements in California is the Three Strikes Law, in which a judge can sentence a defendant to between 25 years-to-life for a third conviction. As such, a defendant may have decades added to their sentence, in comparison to another defendant without enhancements. As of September 2016, 79.9% of California state prisoners had at least one sentence enhancement, and 25.5% of prisoners had three or more enhancements.<sup>2</sup> Additionally, in California it was found that “common enhancements are applied disproportionately against people of color and people suffering from mental illness.”<sup>3</sup> Moreover, “people suffering from mental illness are also overrepresented among people currently serving life sentences under the Three Strikes law for nonviolent crimes.”<sup>3</sup>

Prior to the amendment of PC §1385, it was at the discretion of judges to dismiss proposed sentence enhancements. However, as can be seen in the statistics above, this was rarely done, in part due to vague wording of the penal code. The now-amended Penal Code §1385 clarifies when judges should dismiss enhancements and provides several direct mitigating factors for dismissal, such as when mental illness, prior victimization, and/or childhood trauma were contributing factors to the current offense.

The amended bill calls upon “a qualified medical expert” to assist the judge in determining whether these factors are relevant to the case. Specifically, questions that may be posed to the forensic mental health expert include: (1) did the “defendant display symptoms consistent with the relevant mental disorder at or near the time of the offense,” (2) did the “defendant’s childhood trauma substantially contribute to the defendant’s involvement in the commission of the offense,” and (3) did “prior victimization substantially contribute to the defendant’s involvement in the commission of the offense.”<sup>4</sup>

The mental health expert may face challenges when answering the above questions due to the novelty of the amendment. This presentation will provide practice guidelines on how to best approach this type of evaluation and discuss approaches to potential challenges that may arise.

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**Trauma; Victimization; Sentencing**

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**G37 A Crash Course in Forensic Psychiatry**

*Vivian Shnaidman, MD\*, Jersey Forensic Consulting LLC, Princeton, NJ*

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**Learning Objective:** In this presentation, attendees will learn how to use and understand a forensic psychiatric evaluation. They will learn the jargon of psychiatry, understand how to best format a question for their psychiatric expert, and how to understand and interpret reports and testimony that they receive and hear.

**Impact Statement:** The forensic science community will greatly benefit from this specialized training in understanding how to use this very common type of forensic expertise. Many judges and lawyers instinctively know that there is some sort of psychiatric or mental health issue in a case, but often they are stymied when it comes to formulating the appropriate question and understanding the expert's response. The ability to understand our forensic experts is critical. Translating psychiatric expertise into easy-to-understand-and-follow plain English will help anyone who has ever encountered a psychiatric defense or issue of any kind.

A Crash Course in Forensic Psychiatry is exactly what it sounds like. Participants with no previous knowledge or experience of psychiatry will learn how to work with a psychiatric expert, how to formulate a psychiatric-legal question, how to understand the reports and testimony, and even how to properly pronounce some of the words they use without having ever heard them before. We will go over the presentation of common psychiatric disorders, the concept of insight, and the mental status examination. The idea of landmark cases will be covered, although we will not be exploring these cases in detail. Rather, the attendees will learn how to apply this knowledge to any case that includes psychiatric, psychological, or any other mental health testimony or issues.

The Mental Status Examination is equivalent to the physical examination in regular medicine. Individuals in the legal arena can usually recognize when someone is mentally ill, but have never been taught exactly how to identify what the specific signs and symptoms of mental illness are and how to identify them. We will learn how to assess someone who shows up for a legal matter and how to decide if a legal defense or some other legal issue must be explored.

Forensic Psychiatry is very different from general psychiatry. We will learn what goes into a forensic psychiatric report and what to do when an expert presents a net opinion report. We will briefly cover the case law concerning net opinions.

Different jurisdictions have different laws concerning expert testimony and different statutes that apply to various legal matters. Although we clearly cannot cover every statute and case law in the whole world, we will learn how to identify the relevant statute/case law and how to interact with our experts so they can answer a relevant question and be helpful to the case.

Experts vary. We will go over what makes a good expert, how to avoid hired guns, and how to best leverage our experts toward an outcome that reflects truth and justice.

Sample cases will be shared and discussed with the group, and we will use video presentations to help identify which experts are good and less good.

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**Forensic Psychiatry; Expert Witness; Hired Gun**



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**G38 The Admissibility of “Child Sexual Abuse Accommodation Syndrome” in Criminal Cases**

*Donald Shelton, PhD, JD\*, University of Michigan-Dearborn, Saline, MI*

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**Learning Objective:** After attending this presentation, attendees will understand the legal and scientific issues affecting the admissibility of “Child Sexual Abuse Accommodation Syndrome” (CSAAS) in criminal cases, particularly under the standards established in the *Daubert* and *Kuhmo* cases.

**Impact Statement:** Attorneys handling cases of alleged child sexual abuse will be familiar with the issues surrounding the admissibility of CSAAS testimony. Behavioral science experts will understand the limitations of their testimony about CSAAS in alleged child sexual abuse cases.

Behavioral science issues are a large part of the national wrongful conviction data. In wrongful convictions for child sex abuse, “perjury or false accusation” is the leading cause of wrongful convictions at 85% (258/302) while “mistaken witness” accounted for another 12% (37/302).

Initially, there was a question whether *Daubert*, or even *Frye*, applied to the behavioral sciences. It was resolved in federal courts and *Daubert* States in *Kumho Tire Co. v. Carmichael* applying *Daubert* to all experts. Prosecutors have sought to present expert testimony concerning a syndrome they claim is consistent with the behavior of alleged child victims.

The American Psychological Association (APA) *Diagnostic and Statistical Manual of Mental Disorders*, (DSM) previously defined “syndrome” but that definition has been dropped from the current *Manual*. A “syndrome” is not a diagnosis but “a recognizable complex of symptoms and physical findings which indicate a specific condition for which a direct cause is not necessarily understood.”

In 1983, Roland Summitt proposed a theory to describe children’s reactions to sexual abuse and dubbed “Child Sexual Abuse Accommodation Syndrome” (CSAAS). Prosecutors use CSAAS to bolster child witnesses who failed to report or even denied or recanted abuse statements. Prior to *Daubert*, some courts admitted CSAAS and others excluded it. The important question is whether CSAAS survives *Daubert*.

Most agree that it is not ethically or legally permissible to testify that CSAAS symptoms indicate that the abuse occurred. Summitt later clarified that CSAAS is not a diagnostic tool. Courts agree. Some nevertheless allowed CSAAS to “explain” or “rehabilitate” child testimony.

A major problem is that the concept is clinical rather than scientific. Therapists are trained to make subjective assessments of subject responses and base conclusions on anecdotes. Anecdotal conclusions can be useful for therapists but are contrary to a scientific method. Allowing testimony based on anecdotal “experience” is not *Daubert* forensic science. But using *stare decisis*, many judges still admit CSAAS, citing general acceptance and witness qualifications.

CSAAS does not meet *Daubert* standards. There is no established scientific basis. It is not testable. There is no ability to replicate or control a test. There can be no error rate. The observations are of children that the observer regards as victims. In 2005, the Second Circuit addressed a claim of ineffective defense counsel for not contesting CSAAS testimony. In *Gersten v. Senkowski*: “[E]ven a minimal amount of investigation into the purported ‘Child Sexual Abuse Accommodation Syndrome’ would have revealed that it lacked any scientific validity for the purpose for which the prosecution utilized it: as a generalized explanation of children’s reactions to sexual abuse, including delayed disclosure and blurred memory.”

Ten years later, the Kentucky Supreme Court held in *King v. Commonwealth*: “The validity of the [CSAAS] theory was not self-evident in 1985 and it is not self-evident today. . . . To be clear: we have never ruled the theory to be inadmissible because it is demonstrably wrong; rather, we have ruled it inadmissible because no one has offered proof of its validity. . . .”

In *State v. J.L.G.*, the New Jersey court held that “expert testimony about CSAAS in general, and its component behaviors other than delayed disclosure, may no longer be admitted at criminal trials”, stating “ . . . it is important to note that CSAAS stems from observations made in clinical practice—not systematic scientific study.”

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**Behavioral Science; CSAAS Admissibility; Child Sexual Abuse**

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**G39 Qualified Immunity for Forensic Experts: Should Qualified Immunity be Granted? An Interactive Session**

*Stephanie Domitrovich, PhD, JD\*, Senior Judge of Pennsylvania, Erie, PA; William Nuzum, JD, BS\*, Supreme Court of Ohio, Vincent, OH*

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**Learning Objective:** The objectives of this session are to inform forensic expert witnesses as to instances where qualified immunity may or may not be granted. Attendees will be challenged to analyze a series of hypothetical case scenarios that are then revealed as being actual court cases. The attendees will be asked to interact with the faculty, by assuming the role of a judge, to analyze the hypotheticals and explain their rationale for denying or granting qualified immunity. After discussion, faculty will reveal the actual court decision upon which each hypothetical is based. Attendees will be able to ascertain when qualified immunity is granted or not granted by various courts.

**Impact Statement:** This presentation will provide attendees in the forensic science community with caselaw that will demonstrate when forensic experts can be provided with qualified immunity and when the courts have denied forensic experts qualified immunity. The attendees will learn about the important policy reasons justifying qualified immunity for forensic science experts who make critical and yet split-second life changing decisions.

This session will give attendees an opportunity to interact with case examples about qualified immunity. Sweeping in scope, qualified immunity is designed to immunize all but the plainly incompetent expert or those who knowingly violate the law. The attendees will learn the important policy reasons behind qualified immunity for forensic science experts. Qualified immunity applies equally to all governmental employees and not just law enforcement officers. As result of recent high-profile incidents involving uses of force, there is a heightened call to reexamine best practices for permitting qualified immunity for others. Bills have been introduced as a call for greater oversight and review, re-allocation of policies and priorities, and systemic reforms to law enforcement. Qualified immunity is one of the most hotly debated topics. However, said calls for changes to qualified immunity can involve short-sightedness, especially in view of forensic science experts. Calls to eliminate qualified immunity may result in excess judgments which in turn result in higher taxes, decreased services, a profound impact on secondary insurance market, and may adversely impact recruitment and retention of forensic science experts.

Expert testimony from forensic science experts is essential in civil and criminal court proceedings. Despite forensic science experts providing their opinions on reliable methodology; however, said experts can still be sued and face potential liability. Jurisdictions have granted expert witnesses qualified immunity from civil claims. Qualified immunity protects government employees performing discretionary functions from civil liability if their conduct does not violate plaintiffs' clearly established constitutional rights. Although qualified immunity is an affirmative defense, plaintiffs have the burden to demonstrate by closely analogous case law, the defendants have violated plaintiffs' clearly established constitutional rights. A review of the forensic science expert's developed and established policies and procedures manuals, for instance, used by personnel in said offices are subject to review in said cases. The faculty for this session will review with attendees the latest cases in this area of qualified immunity with detailed information derived from the underlying cases. The faculty will use actual reported cases to challenge attendees to interact with the faculty and other attendees, by assuming they are the judge whose responsibility is to admit testimony of the expert witness. At the conclusion of each scenario, the faculty will disclose the actual decision of the trial court judge as to each issue on qualified immunity.

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**Qualified Immunity; Shields Experts; Immunity**

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**G40 Challenging the Forensic Scientist's Credibility in the Courtroom: Issues of Ethics and Implicit Bias—You Are the Judges! An Interactive Session in Forensic Science and the Law**

*Stephanie Domitrovich, JD, PhD\*, Senior Judge of Pennsylvania, Erie, PA; William Nuzum, JD, BS\*, Supreme Court of Ohio\*, Vincent, OH*

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**Learning Objective:** The objective of this session is to identify issues of ethics and bias that can be used by trial counsel to challenge the credibility and admissibility of expert testimony of a forensic scientist. Expert witnesses in the courtroom must abide by their own ethical code and not be influenced in their interpretations of scientific and forensic evidence by bias, whether explicit or implicit. The presenters will use actual reported legal cases as the basis for a series of hypothetical situations in which issues of ethics and bias are used by trial counsel to challenge the credibility and admissibility of expert testimony of a forensic scientist. The attendees will be asked to interact with the faculty, by assuming the role of a judge, to analyze the hypotheticals and explain their rationale for admissibility of the testimony. After discussion, faculty will reveal the actual court decision upon which each hypothetical is based. Attendees will be able to apply this knowledge in their legal practice when called upon to examine the credibility of forensic scientists in cases in which the admissibility of the expert's testimony should be examined for ethical lapses or bias.

**Impact Statement:** This presentation will reinforce the importance for attendees to have a thorough knowledge of the Code of Ethics of their expert's field of knowledge. It will demonstrate how violating elements of the ethical expectations can affect an expert's credibility as a witness and affect the admissibility of their testimony in specific cases. Moreover, this session will highlight and reinforce that biases, whether explicit or implicit, can affect the credibility and admissibility of an expert's testimony at trial. This session will increase the confidence that forensic scientists perform their duties in an unbiased and ethical manner.

This session will give attendees information and tools to encounter lapses in ethical conduct and undue influence of bias faced by forensic scientists in the performance of their professional duties. The faculty for this session will inform or remind attendees of the different ethical obligations codified in the code of ethics for their discipline of forensic expertise. The faculty will also examine how biases, whether explicit or implicit, can affect admissibility of expert testimony in court. The faculty will utilize hypothetical scenarios based on actual reported cases to challenge attendees to interact with the faculty and other attendees, by assuming they are the judge whose responsibility is to admit testimony of the expert witness. The hypothetical scenarios will challenge attendees to find ethical lapses or witness bias in the fact statements of each hypothetical that could affect admissibility of testimony of the expert witness. If such lapses are identified, attendees will be challenged to determine if it would impact the credibility of the witness to an extent that the testimony would not be permitted by the judge. At the conclusion of each scenario, faculty will disclose that actual decision of the trial court judge on admissibility of the testimony. This highly interactive session will enable attendees to apply their knowledge of ethics and bias to learn how the conduct of forensic scientists affects their credibility as a professional.

As professionals, forensic scientists must conduct themselves by codes of professional conduct in forensic science, their professional disciplines, and their employers, whether in the public or private sector. To be a competent professional, every forensic scientist's conduct must be performed in a manner beyond reproach. Ethical conduct becomes acutely important when the scientist's conduct is scrutinized in minute detail by counsel in the courtroom when that scientist is called upon to testify. It can affect admissibility of their testimony in a particular case and could affect their credibility in future cases. The scrutiny of counsel can extend beyond the codes of ethical conduct to factors that can demonstrate the professional opinion of the forensic scientist is compromised by bias, whether explicit or implicit. Even implicit or unconscious bias, if it exists, can affect the credibility of a witness and admissibility of that witness's testimony. These are factors of which forensic scientists and counsel must be aware. They must always examine their professional conduct and motivations. This session will prepare attendees to be well informed of these obligations and counsel to be better able to cross-examine an expert witness in a particular forensic discipline.

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**Ethics; Implicit Bias; Expert Testimony**

## H1 “There’s a First Time for Everything”: Using Digital Dental Equipment in a Mortuary

*Camilla George, MSc, LLM\*, DentArtWork Dental Care, Lechlade, England, UNITED KINGDOM*

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**Learning Objective:** After attending this presentation, attendees will understand some of the main principles of DVI and how this was instrumental in the seamless teamwork that enabled a successful identification.

**Impact Statement:** This presentation will impact the forensic science community by showcasing the importance of planning and more planning prior to execution of what was a “mini DVI.”

Two people were reported missing following a major fire in the United Kingdom that was declared a Disaster Victim Identification (DVI). A team was formed with a common goal to safely recover and identify both individuals. The coroner headed meetings online to keep all disciplines involved up to date as the recovery was not a straightforward process. The scene of the incident will be shown along with the layout of the building and the issues that occurred during the recovery. The recovery took over a month and the identification took place during a wave of Covid-19. Acquiring the Antemortem (AM) records was done well in advance of the Postmortem (PM) identification. This allowed me to check all information and make sure all radiographs taken were sent to me before the PM.

This presentation is from the point of view of a “newbie” to forensics and the DVI process. I qualified with a Masters (Distinction) in Forensic Odontology in 2013. Depending on where you live and work in the United Kingdom, odontology cases can be plentiful or rare. After attending my first AAFS and ASFO, forensic odontologists welcomed and encouraged me to keep up my skill set and attend courses. On meeting and listening to the experienced odontologists, I knew exactly who to contact when I was contacted for this case of burnt remains. The international community of forensic odontologists I met and kept in touch with were so helpful. I felt supported and ready to do my first DVI.

As a young odontologist, this case demonstrates the importance of knowing there are many experienced “odonts” at home and away who are happy to advise, support, and encourage you to go forward and find your path in forensic odontology.

The key to this case was planning, planning, and more planning!

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**Forensic Odontology; DVI; New to Forensics**

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**H2 A New Algorithmic-Based Model for Sex Estimation in Forensic Identification**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of dental dimorphism in forensic sex estimation, of the applications of Artificial Intelligence (AI) in identification processes, and of the new perspectives of 3D Geometric Morphometric Analysis (GMA).

**Impact Statement:** This presentation will impact the forensic science community by proposing a new sex estimation method that combines an Artificial Neural Network (ANN) with GMA on occlusal dental crown surfaces. The proposed approach may be a new and conservative method for forensic analysis in pre-adolescent skeletons when genetic or morphologic data do not suffice for sex diagnosis.

Morphological examination of dental crowns is an efficient tool for sex determination as teeth are highly resistant to unfavorable conditions and dental sexual dimorphism influence both teeth size and shape. Previous studies demonstrated that GMA can be useful to evaluate the 3D occlusal surfaces of molars and premolars for sexual determination, but it requires the processing and combination of a very significant number of variables and values, from which the need for a validated algorithmic approach.<sup>1,2</sup>

Upper right premolars and first molar of 230 Caucasian Italian subjects (115 males and 115 females) aged from 12 to 63 years were included into the study. The teeth were intact and untreated. An operator used the Viewbox 4.0 software to place and digitize the reference points (landmarks and semi-landmarks) on the occlusal surface of the teeth. Within the GMA, the shape and size variations between males and females were studied by General Procrustes Superposition (GPS) and the Principal Component Analysis (PCA). The data obtained was used to create an ANN trained on 75% of the sample, while the remaining 25% was used to test the discriminatory and predictive potential of the ANN.

Accuracy rates yielded by the method in classifying sexes resulted in 94% for the training sample and 82% for the test sample. On the test sample, the sensitivity value was 93% for females and the specificity 93% for males, while positive and negative predictive values were 78% and 89% for females and 89% and 78% for males, respectively. The combined use of GMA with ANN has shown an excellent ability to classify by sex compared to odontometric and morphometric traditional methods, highlighting that females are more likely to be correctly classified, while males were more likely to be not misclassified.<sup>3</sup>

The proposed method can represent a reliable and affordable tool for estimating sex based on dental features. Future research is ongoing toward a complete automatic landmark placement that will allow for a consistent reduction of processing time compared to both previous traditional and the here-proposed approach.

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**Geometric Morphometric Analysis; Artificial Neural Networks; Odontological Sex Determination**

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**H3 A Case Report of an Unusual Use of Dental Identification in a Homicide Case**

*John McDowell, DDS, MS\*, University of Colorado, Dental Medicine, Castle Rock, CO*

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**Learning Objective:** After attending this presentation, attendees will understand how important dental evidence can be in the identification of a homicide victim and how that can relate to collection and analysis of dental evidence recovered at or near the purported crime scene.

**Impact Statement:** Dental identifications have always been important to all the various branches of the judicial system. The instant case is unusual as it demonstrates how a single tooth was important in establishing that dental evidence collected near a purported crime scene was valuable to establishing that a single tooth could have associated with only one individual. That single tooth was established to be a tooth that was missing from the recovered remains of a homicide victim found several miles from the possible site of the homicide. Specific dental identifiers and DNA were used by law enforcement and the coroner's office to firmly establish the victim's identity.

In 2017, a nude body of an adult female was discovered by the side of an interstate highway in Douglas County, CO. No identifying documents were recovered with the remains, making identification initially impossible. Upon autopsy, the remains were shown to demonstrate significant evidence of inflicted trauma, including facial injuries that included multiple facial and lip lacerations, dental subluxations, and one missing maxillary lateral incisor. Full-body radiographic images showed that there were no maxillary lateral incisor tooth or tooth fragments located in the mouth, oropharynx, upper airway, lungs, esophagus, stomach, or in the skin or hair of the upper body. Despite an extensive search of the recovery site, the missing lateral incisor was not found. Law enforcement searched an area approximately 25 miles away from the recovery site where it was believed the homicide may have occurred. Law enforcement personnel were able to recover a single tooth in the trash located near the site where the homicide possibly occurred. That maxillary lateral incisor tooth was shown through dental anatomic comparison to the empty socket, similar staining on the adjacent teeth, similar dental anatomy of the contralateral maxillary incisor retained in the maxilla of the victim, and DNA analysis to have been the tooth of the homicide victim.

Extensive pre-trial exhibits to include radiographic images (prepared with the assistance of the division of Oral and Maxillofacial Radiology at the University of Colorado School of Dental Medicine) of the jaws were prepared to demonstrate to and instruct the jury that this maxillary lateral incisor could only have come from the remains recovered by the highway. Some of the exhibits used at trial will be shown to those individuals attending this oral presentation. Through oral testimony and anatomic demonstrations to the jury, it was made clear that this maxillary lateral incisor was avulsed at or around the time of the assault, resulting in death of the recovered remains.

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**Dental Anatomy; Oral and Maxillofacial Radiology; Dental Identification**

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## **H4 Looking Forward: From Periapical Film Identifications to Cone Beam Computed Tomography (CBCT) 3D Volume Identifications**

*Robert Wood, PhD\*, Ontario Forensic Pathology Service/OCCO, Brighton, ON, CANADA; Taylor Gardner, BSc\*, Toronto, ON, CANADA*

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**Learning Objective:** After attending this presentation, attendees will be cognizant of the changes of postmortem and antemortem dental imaging. Additionally, attendees will be aware of the step-by-step process to manipulate medical CT DICOM image sets into usable postmortem images for the purpose of identification.

**Impact Statement:** The presentation will impact the forensic science community by providing a simple overview on how utilizing multiple modalities of available digital radiographic data can expedite scientific identifications that allow deceased individuals to be returned to their loved ones in a timely manner.

Human identification by dental comparison of antemortem to postmortem radiographs has been long established to be one of the most useful scientific methods to determine the identity of a deceased individual. Since Dr. Walkhoff captured his first dental radiograph in 1896, dentists have been exposing, storing, and maintaining dental films of their patients.<sup>1</sup> In 1987, the introduction of the first digital radiograph machine brought the gradual transition of dental radiographs collections by dental practitioners from film to digital sensor, and hardcopy patient file to electronic folders.<sup>2</sup> Dentistry has adapted a form of CT scanning that uses a cone-shaped beam and is thus termed Cone Beam Computed Tomography (CBCT). CBCT is presently being used in many aspects of dentistry, including osseointegrated implant planning, orthodontics, endodontics, investigation of pathology, and assessment prior to complex dental extractions.<sup>3</sup>

As technology evolves, so too does our digital communication and data retention. The ease of access of this information at our fingertips is critical to help expedite the identifications and releases of deceased persons.

Warranted antemortem images can be both requested and received electronically, eliminating the need for couriers and often the on-site attendance of a forensic odontologist, except in complex cases or multiple fatality incidents. Radiographic images can be shared and received via password-protected, high security, cloud-based portals, allowing us to receive these images at an earlier interval. With the primary goal being the most efficient method of scientific identification, using new and pre-existing technology comparisons can be performed very quickly and can allow for positive identification prior to postmortem examination in some cases.

Postmortem CT data sets are obtained of each case at the author's institution. A dental comparison can be undertaken by harvesting of anatomically important data from medical CTs or intra-oral full mouth series of radiographs. The CT data does require manipulation with commercially available CBCT software used in dental applications to provide postmortem pantomographic-type images, sectional views, three-dimensional rendered images to compare to available antemortem records in real time. Following the autopsy, intra-oral, digital full mouth series can also be exposed and can be directly compared to antemortem radiographs. The benefit of utilizing CT data is it avoids any biological/body substance or radiation exposure to the operator. Currently medical CT imaging for postmortem dental identification comprises between 30–40% of case loads in the authors' catchment area.

The authors will present several cases that highlight how various modalities are used to transition the science of dental identifications and expedite turnaround time for identifications using the traditional method of periapical film identification to a CBCT 3D Volume identification using commercially available software while still maintaining a high level of quality and confidence in the system.

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### **Forensic Odontology; Cone Beam Computed Tomography (CBCT); Body Identification**



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**H5 The Effect of Burning on Dental Tissue: A Macroscopic and Microscopic Investigation**

*Michal Peer, MSc\*, Dental Anthropology Lab, Tel Aviv University, Aurora, CO; Rachel Sarig, DMD, PhD, Dental Anthropology Lab, Tel Aviv University, Tel Aviv, HaMerkaz, ISRAEL*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how exposure to heat impacts enamel and dentin tissues.

**Impact Statement:** This presentation will help lay the groundwork for the potential development of forensic techniques that can be used to assess burnt human remains more accurately.

Due to the prevalence of burnt human remains in various cases of forensic investigation such as vehicle fires, homicide, suicide, and the use of fire to obstruct forensic evidence, it is imperative to analyze and understand the changes that occur to human remains due to exposure to heat. In addition, teeth are one of the more common skeletal elements for forensic identification and are resistant to high temperatures due to their structural strength and protected location. Therefore, the intersection of these two factors should be more highly examined than it has been in the past. A better understanding of the processes that occur during burning will allow for the development of more accurate methods for the purposes of forensic identification of burnt remains. This study examined 58 maxillary molars that were burned in a Cole-Parmer StableTemp Box Furnace at the temperatures corresponding to carbonization (400°C) and calcination (700°C).

Teeth were scanned in the Shmunis Family Anthropology Institute using micro focus X-ray computed tomography system (225kV 225W reflection target, 0.1mm aluminum filter, 17.2µm, 220kV, 77µA). The teeth were also analyzed by means of an XRD Bruker® D8-Discover diffractometer equipped with a Linxeye-XE linear detector, using reflection geometry with a 0.5mm collimated parallel beam. All teeth underwent these examinations both prior to and post-burning. All statistical analysis was performed using IBM® SPSS Statistics 22.

Teeth burned at 400°C displayed both an increase (27.5%) and decrease (72.5%) in dentin volume, each of which was found to be statistically significant ( $p < 0.001$  and  $p < 0.05$ , respectively). Teeth burned at 700°C consistently displayed a decrease in root volume, which was statistically significant ( $p < 0.001$ ). Teeth burned at 700°C showed a statistically significant increase in dentin shrinkage in comparison to teeth burned at 400°C ( $p < 0.001$ ).

Teeth burned at 400°C displayed both an increase (37.9%) and decrease (62.1%) of hydroxyapatite crystal size, each of which was found to be statistically significant ( $p < 0.05$  and  $p < 0.001$ , respectively). Teeth burned at 700°C displayed a majority decrease (78.5%) in hydroxyapatite crystal size, which was found to be statistically significant ( $p < 0.001$ ). Teeth burned at 400°C showed a significantly higher rate of crystal shrinkage than teeth burned at 700°C ( $p < 0.05$ ).

Based on the above results, it can be seen that both of the main types of dental tissues underwent similar changes in size due to burning. At the carbonization stage, a combination of increased and decreased size can be found with there being a complete or majority decrease in size during the calcination stage. While this supports previous work regarding dentin shrinkage due to burning, it contradicts the research on hydroxyapatite crystals, which determined that crystals shrink after reaching calcination.

This research allows a greater understanding of how teeth behave when they are burned, which can assist in determining the series of peri- and postmortem events. In addition, dentition is considered to be as unique as fingerprints, and it can be used to identify the individual. When the changes in teeth due to burning are better understood, it will allow for the development of more accurate methods of forensic assessment.

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**Burnt Teeth; Micro-CT; X-Ray Diffraction (XRD)**

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## **H6 A Forensic Provenance of Ajnala Skeletal Remains From Strontium Isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) Analysis of Their Dental Enamel Samples**

*Jagmahender Sehrawat, PhD\*, Panjab University, Department of Anthropology, Chandigarh, Chandigarh, INDIA; Vaughan Grimes, PhD, Memorial University, St. John's, NL, CANADA*

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**Learning Objective:** After attending this presentation, attendees will be informed about the significance of stable isotope analysis as fingerprints of geographic affinity of an individual.

**Impact Statement:** This presentation will impact the forensic community by informing attendees that strontium stable isotope analysis can help in provenancing (particularly geographic origin and migration patterns) of unknown human remains retrieved from forensic or bioarchaeological context.

**Introduction:** The utilization of stable isotope concentrations for provenance of unidentified human remains is a relatively new field of inquiry in forensic anthropological investigations. Strontium isotope concentrations ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) have been widely used for provenance purposes in forensic science, bioarcheology, and paleo-environmental sciences.<sup>1</sup> The radiogenic strontium isotope ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) composition of human dental tissues (enamel and dentine) can be used to estimate dietary patterns, childhood locale, residential mobility histories, and, hence, geographic provenance of an individual.<sup>2-7</sup> Thousands of badly damaged skeletal remains (along with more than 9,000 human teeth) reportedly belonging to 282 Indian soldiers of a specific regiment were unearthed from an abandoned well at Ajnala (Amritsar, India).<sup>7</sup>

**Methods:** Due to logistic and funding constraints, 47 mandibular molars randomly selected from Ajnala skeletal assemblage were processed for strontium isotope analysis in the present study and the calculated concentration values were compared with published isotope data to assess local or non-local status and mobility patterns of Ajnala skeletal remains. Standard protocols were followed for sample preparation, analysis, and quantification of Sr isotopic values in the studied dental samples.

**Results:** The average  $^{87}\text{Sr}/^{86}\text{Sr}$  value estimated from enamel was found 0.722392, with the majority of Sr isotopic values concentrating in the range of 0.72100 to 0.727000; though a few samples were also characterized by relatively low (0.715938) as well as high  $^{87}\text{Sr}/^{86}\text{Sr}$  ratio (0.727949); similarly, the mean Sr isotopic concentration from dentin was 0.722776 (minimum 0.719707 and maximum 0.724072). Comparative analysis of isotopic values revealed that the most individuals buried in Ajnala have  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures close to ground water of the Gangatic plain (less radiogenic  $^{87}\text{Sr}/^{86}\text{Sr}$  ~0.716); most likely originating between the Allahabad and Varanasi (Uttar Pradesh) region, whereas individuals with higher values ( $^{87}\text{Sr}/^{86}\text{Sr}$  ~0.7200) probably resided in the Bihar, coastal Orissa and West Bengal, and some northeastern Indian states where the ground water is characterized by relatively more radiogenic water.

**Discussion and Conclusions:** Though the Sr isotopic values cannot be used for establishing positive identity of Ajnala victims, the results definitely corroborate other forensic odontological observations that Ajnala victims belonged to Eastern Uttar Pradesh (Awadh), Bengal, Bihar, coastal Orissa, and some northeast states of India. The accurate interpretation of  $^{87}\text{Sr}/^{86}\text{Sr}$  for forensic purposes will require efficient bioavailable baseline data for human remains from different Indian regions to estimate spatial variations as current Sr isoscapes are based on bedrock or vegetation samples. The detailed results will be presented in the textual, graphical, tabular, and diagrammatic forms in this presentation.

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### **Forensic Provenance; Ajnala Skeletal Remains; Stable Isotope Analysis of Strontium**

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**H7 Murder! Who Does the Grillz Fit?**

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**Learning Objective:** A unique and challenging case will be presented where forensic odontology was utilized to determine if a custom dental grillz found at a crime scene belonged to the victim or could be an important piece of evidence in finding the murder suspect.

**Impact Statement:** The presentation will impact the forensic science community by presenting a different dental comparison using digital dentistry where a piece of dental jewelry discovered at a crime scene became central evidence in a murder investigation.

Dental identification cases are the most common type of case a typical forensic dentist is involved with. Most of them involve taking postmortem radiographs of the remains of a deceased individual and comparing the latter to the antemortem radiographs of the suspected victim. However, not all comparisons always follow that pattern.

The advent of digital dentistry has benefited all aspects of dentistry, from taking digital impressions to computer-aided designing and milling of dental prosthetics. In forensics, this has created a digital database of sorts, where dentists and labs who utilize the digital technology often have the cases backed up on hard drives. Access to these archived cases can often be a key piece of information for forensic dentists. The same digital technology can also be utilized even if the archived original information is not known or found.

After a dental grillz, a removable dental jewelry worn over the teeth, was located at a crime scene, the investigators had questions: Was it the victim's? If it did not belong to the victim, could it help identify a suspect in a murder case? A simple question it seems. However, we may recall the trial of O.J. Simpson and the same type of question surrounding the infamous glove.

The different steps leading to the arrest and trial of a suspect will be presented and the various reasons why one could not simply try the dental jewelry on the suspect's dentition will be discussed. The use of digital superimposition to determine for which dentition the grillz was fabricated and the comparison process will be demonstrated.

Discussing the interdisciplinary collaboration and how, by thinking outside the box and using digital dentistry, it was possible to build a solid and unbiased case. You will also discover how the evidence was used in court during the preliminary hearing and the final outcome of the case.

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**Dental Comparison; Grillz; Murder**

## H8 The Applicability of Cameriere's Third Molar Maturity Index on a French Orthopantomogram and Computed Tomography Scan Sample

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**Learning Objective:** The goals of this presentation are to inform attendees of what consists of Cameriere's dental radiologic age estimation method on the living, its interest on the determination of the crucial age of 18, how the third molar maturity index of this method works and how to assess it, this method's sensibility and specificity on orthopantomograms of our sample and moreover on computed-tomography scans which is an unpublished applicability, and the clinical applicability of the method for assessing the age of 18 years.

**Impact Statement:** The presentation will impact the forensic science community by underlining the importance of dental age estimation methods, with a highlight on the tool that 3D examinations represent in age estimation methods. Moreover, by analyzing Cameriere's method, the forensic science community will be able to shade this method, as every method of age estimation should be used with caution.

**Introduction:** Due to the increase of migration in recent years, age estimation of unaccompanied minors became a forefront challenge in forensic science. Dental methods are particularly interesting for age estimation as the third molar is still in development at the crucial age of 18. In 2006, Cameriere improve a new method for assessing adult age based on the relationship between age and the third molar maturity index I3M, which is related to the measurement of the open apices of the third molar on orthopantomograms. The index is then compared to a cut-off value of 0,08 to discriminate if an individual is adult ( $I3M < 0,08$ ) or minor ( $I3M \geq 0,08$ ).

**Goal:** The goal of our study was to evaluate the applicability of the Cameriere's method on Computed Tomography (CT) scans on a French juvenile and young adult population and compare the results on orthopantomograms of the same individuals.

**Materials and methods:** Our sample consists of 200 previous examinations realized in the radiological department of a French University hospital between 2007 and 2020. Each patient had an Orthopantomograms (OPG) and a cranial CT scan for medical purposes: The left (38 - FDI Word Dental Federation numeration) and the right (48) third molar maturity index I3M was assessed on orthopantomograms as the sum of the width of the inner sides of the two open apices, divided by the tooth length. The measurement of the third molar maturity index calculated by Cameriere on OPG was adapted in a similar way on scanners. Exclusion criteria were decayed teeth, broken teeth, fracture of the mandible angle concerning 38 or 48, presence of osteosynthesis material, absence of radicular edification, illegible radiographs, roots duplication that led to a final sample of 74 orthopantomograms and 63 scans. The sensibility, the specificity, and a misclassified rate were evaluated for testing the concordance between chronological age and estimated age in both OPGs and CT scans. Intra-observer reproducibility and inter-observer reproducibility measurements were studied using the concordance correlation coefficient.

**Results and discussion:** The test on our 74 OPGs present a concordance between chronological age and estimated age described by sensibility of 78,57%, specificity of 89,74% and misclassified rate of 18,03% on 38 and sensibility of 78,79%, specificity of 91,67% and misclassified rate of 17,78% on 48. Results on CT scans present a concordance between chronological age and estimated age on 38 described by sensibility of 77,78%, specificity of 94,12%, misclassified rate of 16,98%. Concordance between chronological age and estimated age on 48 is defined by sensibility of 75%, specificity of 93,75% and misclassified rate of 19,23%. The intra-observer and the inter-observer agreements higher than 90% on 38 and 48 and on both orthopantomograms and CT-scans emphasize an excellent similarity between the two measures of the same observer and an excellent similarity between the measures of the two observers. Moreover, the concordance between orthopantomograms and scans on 38 and 48, higher than 90%, points to an excellent similarity between our results on orthopantomograms and scans on 38 and 48. It indicates that there is no precision gain or loss of information by assessing the I3M on CT scans instead of orthopantomograms.

**Conclusions:** This study reveals the applicability of the Cameriere's method on CT scans on a French sample, which was not tested yet in the literature. Moreover, the results are similar to results on OPGs of the same individuals. Due to several limits as individuals' misclassification and anatomical variability of the third molar, we issue a few reservations about the use of the method in clinical practice.<sup>1-21</sup>

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## Forensic Odontology; Age Estimation; Radiology

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**H9 Dental Age Assessment of United States Black and White Children: Performance Reliability of Harris and McKee**

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**Learning Objective:** Attendees will learn how reliable the data reference set previously published by Harris and McKee performs in dental age assessments of United States Black and White children.<sup>1</sup> They will also learn how often the chronologic age is predicted within one standard deviation, within two standard deviations, and within three standard deviations of the estimated age. Last, they will learn what effects the exclusion of certain teeth has on the accuracy of the calculated estimated age.

**Impact Statement:** Harris and McKee is currently the only published reference data set for United States White and Black children for dental age assessment.<sup>1</sup> No previously published reliability studies have been found, therefore this research will help demonstrate the reliability of Harris and McKee's reference data set. Using reliable reference data sets is important for forensic dental age assessments to ensure the estimated age is accurate. This presentation also evaluated how often the chronologic age is captured by the age interval at specific standard deviation intervals, and it shows that the chronologic age is falling within certain intervals more often than statistically expected. This result shows the forensic science community that dental age assessment with this methodology and reference data set is reliable for assessing chronologic age in Black and White children in the United States.

Medicolegal authorities utilize forensic dental age assessment of children for the purposes of establishing the biologic profile to assist in human identification, answering questions related to immigration, and answering questions used to substantiate eligibility for social benefits. The goal of the study was to assess the performance reliability of the child dental age assessment data previously published by Harris and McKee in a geographic population congruent with the Harris and McKee research population as well as in two additional geographic populations. A total of 432 dental panoramic radiographs were obtained and utilized from three geographic locations in the United States: Memphis, TN; Knoxville, TN; and Saint Louis, MO. All radiographs were staged by two separate individuals using the modified Moorrees, Fanning, Hunt staging system as outlined by Harris and McKee. Age assessments were then performed for all radiographs. Five age assessment types were performed: with all teeth staged, without canines, without third molars, without maxillary second molars, and without all maxillary teeth. Results were reported by various categorical groupings. The results indicated Harris and McKee accurately estimates known chronologic age across the ancestral, sex, and geographic categories and is effectively concordant with chronologic age. The results also indicated the known chronologic age fell within one standard deviation and two standard deviations of the estimated age more than the statistical expectation for most categories. Excluding canines from the age assessment showed a higher accuracy in estimating chronologic age, therefore exclusion of these teeth should be considered when performing age assessments with this methodology. Excluding third molars shows the lowest accuracy in most of the categories and, therefore, should be included in age assessments for children nearing adolescence. Exclusion of all maxillary teeth captured the known age within one standard deviation at the highest rate. Standard deviation was calculated using simple averaging and would be more statistically appropriate when calculated using sum of squares.

**Reference:**

<sup>1</sup>. Harris, E.F. and McKee, J.H., Standards for blacks and whites from the middle southern United States, *J. Forensic Sci.*, 1990 Jul;35(4):859-72.

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**Dental Age Assessment; Age Estimation Accuracy; African American Children**



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## **H10 The Dental Age Estimation of Skeletal Remains: Interfacing the Shareware Program Gimp With Kvaal Dental Age Estimation (DAE) Quicksheets**

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**Learning Objective:** The goals of this presentation are to demonstrate the Kvaal age assessment technique blended with DAE Quicksheets; within this protocol, to think “outside the box” in applying computer software integration; and finally, to realize this technique’s successful implementation via fingerprint analysis/verification.

**Impact Statement:** Dental age assessment of forensic remains is definitely not an exact science. However, it can be a very helpful tool to the forensic team in the information gathering process. The ultimate goal, as with any forensic identification query, is to obtain an exact identification.

The science of age estimation through analysis of deceased dentition and its degradation has expanded within the past few decades. A variety of testing procedures have come to the forefront. They are varied and diversified in protocol. Certainly, no individual technique has been hailed as the best. They are, however, very useful in the forensic identification “toolbox.” One such platform is the Kvaal tooth age-estimating technique.<sup>1</sup>

Developed by Sigrid I. Kvaal et al., it focuses on the very heart of the tooth, the dental pulp, and how it changes over time.<sup>1</sup> The change zeroed in upon is secondary dentin deposition, thus effecting a volumetric reduction in the pulp chamber size—a change that is measured and compared with statistical documentation for the dentition in question. This technique has been accepted to be accurate within accepted standard deviations and forensic protocols. However, the Kvaal analysis requires many ratio measurements of the tooth via the imported digital radiograph. Thus, it is very time consuming, depending upon the software utilized to achieve this task.

Once these ratio measurements are obtained, Dr. Derek Draft has assisted greatly to achieve rapid final Kvaal results. The Kvaal ratios are entered directly into his Microsoft® Excel®-based Draft Age Estimation “DAE” Quicksheets. Age estimates accompanied with standard deviations are automatically generated.

Within modern computer protocols, Adobe® Photoshop® appears to be the research “standard” to obtain the many necessary Kvaal measurements from digital radiographs. This is what Dr. Draft has recommended within his Quicksheets release. Understanding that Kvaal depends solely upon ratios to accomplish its goal, another software option was investigated to interface with this technique. Gimp is a shareware program that provides most, if not all, of the options of Adobe® Photoshop®.

Adobe® Photoshop® relies upon a “millimeter accurate” dental digital radiograph to be imported into the program. The millimeter standardization must remain stable throughout the process. Gimp allows for simple image import. The size of the image and millimeter importation standardization is not significant with Gimp. The measurement reference within Gimp is the pixel, the basic unit of a digital image. This is ideal with the Kvaal ratio-driven technique. Gimp interfacing with Kvaal provides the same successful outcome as Adobe® Photoshop® with less effort.

**Results:** Gimp is demonstrated to be a viable alternative to Adobe® Photoshop® within the Kvaal tooth age-estimation platform. The findings can then be placed within the Draft DAE Quicksheets, achieving the same outcome. In this case, an outcome that was verified by the medical examiner viz. fingerprint analysis.

**Conclusion:** The Kvaal tooth age estimation technique has been blended with the Adobe® Photoshop® software integration standard. An alternative shareware program, Gimp, may be substituted successfully, and perhaps proving to be easier to manipulate. The demonstrated case results are shown to be accurate via definitive fingerprint identification of the victim.

### **Reference:**

- <sup>1.</sup> Sigrid I. Kvall, Kristin M. Kolltveit, O. Thomsen, Tore Solheim, Age estimation of adults from dental radiographs, *Forensic Science International*, July 1995.

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### **Dental Age Estimation; Computer Shareware Programs; DAE Quicksheets**



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## **H11 The Development of Third Molars and Its Implications on Age Estimation in the Hispanic Population**

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**Learning Objective:** Following this presentation, attendees will understand the influence of sex variations in dental development of third molars. This will be presented in a form of research study that compares the average age at assessment of crown and root formation stages in the maxillary and mandibular third molars between females and males belonging to Hispanic population.

**Impact Statement:** This presentation will impact the forensic community by demonstrating variations observed in development of third molars between females and males in the Hispanic population. It also emphasizes the importance of using population and sex-specific dental reference standards for reliable age estimation.

**Background:** Age estimation is an integral part of forensic practice. It is mostly employed to estimate the age of children who do not possess authentic birth documents, those who falsify the age when seeking asylum, or those who are involved in criminal offenses. Considering the influence of ethnicity on dental development, it has been emphasized to use population specific standards while conducting age estimation. Several studies have demonstrated the importance of ethnicity and third molar development and, therefore, developed reference standards specific to a population.<sup>1-3</sup> Most recently, population-specific dental reference standards have been reported for Hispanic children in Texas.<sup>4</sup> Although the relationship between ethnicity and third molar has been well established, such data is not available for Hispanic population, particularly on younger children.

**Aim:** To evaluate the development of third molars in Hispanic children and analyze sex variations in dental development.

**Methods:** In total, 600 dental panoramic radiographs belonging to healthy children of Hispanic ethnicity were obtained from a teaching hospital in San Antonio, TX. The sample comprised an equal number of males and females in each age group ranging from 6 to 17 years. A single trained and calibrated examiner (JJ) scored all the third molars in the maxillary and mandibular arches based on Demirjian's classification system (A to G). The mean Age at Assessment (AaA) for each stage of development along with the number, and standard deviation were calculated. Statistical significance was set at  $p < 0.05$  and independent sample *t*-test was used to compare the AaA for individual stages of development for each third molar, separately for females and males.

**Results:** Females demonstrated advanced development in all third molars by an average of 0.15 years, except for mandibular right third molar where no difference was observed (0.01 years). The difference in AaA was not statistically significant for most of the tooth developmental stages in both maxillary and mandibular third molars ( $p < 0.05$ ).

**Conclusion:** Similarity in third molar development was observed between females and males belonging to Hispanic population and this finding should be taken into consideration during age estimation proceedings.

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**Age Estimation; Third Molar; Hispanic**

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**H12 The Impact of an International Reference Data Set and Ancestral Data Sets on Dental Age Estimation**

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**Learning Objective:** This presentation will demonstrate the differences in Dental Age Estimation (DAE) of a single United Kingdom White subject when using ten ancestral-specific Reference Data Sets and comparing these estimates with an International Reference Data Set (RDS) of 16,330 subjects constructed from the combination of the ten ancestral RDS. A single subject of known age is used for all the estimates.

**Impact Statement:** The differences in Dental Age Estimation (DAE) using different Ancestral or Ethnic RDS will draw attention to the need for Specific RDS when conducting Dental Age Assessments. The combination of all 10 RDS to create an International RDS offers a suitable alternative when there are no specific ancestral data sets. These limitations and difficulties of interpretation associated with this international RDS outweigh the interpretative difficulties of applying an alternative RDS for age estimation.

**Introduction:** Dental Age Estimation uses the Age at Assessment (AaA) of pre-defined stages of developing teeth. The system with the highest level of Inter-Rate Agreement (IRA) is that described in 1973.<sup>1</sup> This comprises 8 stages of development, the identity of which are assessed using precise anatomical descriptions.

**Materials and methods:** A total of 10 international studies in which the Demirjian Tooth Development Stages (TDS) provided summary statistics for the Age at Assessment (AaA) of children and adolescents aged from 4 years to 26 years. These summary data comprised n-tds, x-tds, and sd-tds. All the data from research projects using identical TDS and identical statistical management of the raw data. Thus, 10 worksheets each comprising up to 256 sets of summary statistics were entered into an Excel® Workbook. These data sets were then combined to create an International Data10 worksheet by using the expression =(‘Afro-Trin’!B60+Kuwait!B60+Malta!B60 ... ..)/COUNTA(‘Afro-Trin’!B60,Kuwait!B60,Malta!B60 ... ..) [... .. is for hidden information] The Excel® Expression has been truncated—the important feature is the COUNTA function that cumulatively totals a cell if it is NOT empty.

The DAE of a single subject was calculated using each of the separate Ancestral data sets and the Dental Age from the International RDS comprising over 16,000 subjects

**Results:** The International worksheet comprises data from 10 separate RDSs and combines the data from the 10 separate Ancestral data sources. For clarity of presentation only the mean value for the DAE from each RDS data is reproduced in this abstract. The subject used for DAE is the example from the paper on Expressing Uncertainty.<sup>2</sup> In addition, only the mean value in years is presented here. The data to be presented will include n-tds, x-tds, and sd-tds.

Afro-Trin 9.00, International 9.13, Sudan 9.50, Qatar 9.58, Hispanic (Texas) 9.68, South Korea 9.74, White UK 9.78, Israel 9.81, Kuwait 9.86, South China 10.13, Malta 10.3

For all the above values the DA was calculated using the example for the RDS of each ancestral study and the International RDS. The youngest DA is for The Afro-Trinidadian RDS and the oldest is for the Malta RDS.<sup>2</sup> The estimates cover a range of 1.31 years. The differences were tested for statistical significance using the “immediate statistics” procedure in STATA.<sup>3</sup> Most of the ancestral-derived DAs were statistically significantly different from the International data set estimates.

**Conclusion:** It is important to select a suitable statistically and ancestrally concordant RDS when performing Forensic Dental Age Estimates. In the absence of a specific RDS the use of an International RDS is recommended but care must be taken with the interpretation of the outcome value for DAE.

**References:**

1. Demirjian A, Goldstein H, Tanner JM. A New System of Dental Age Assessment. *Human Biology*. 1973; 45(2): 211-227.
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3. StataCorp Stata Statistical Software. Release 15. College Station TX. USA

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**International Reference Data Set; Ethnic DAE; Ancestral Age Estimation**

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**H13 The Impact of Ancestral Reference Data Sets of Third Molars on Dental Age Estimation**

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**Learning Objective:** This presentation will demonstrate the differences in Age at Assessment (AaA) of third molar Ancestral Reference Data Sets (RDS).

**Impact Statement:** This paper will confirm the importance of specific third molar ancestral data sets for Dental Age (DA) at the 18 year threshold and show the range of values that need to be considered for a forensic Dental Age Assessment

**Introduction:** The mandibular third molar has developed a pivotal role in the estimation of age at the threshold between childhood (minor status) and adulthood (majority status). It is more accurately considered as a threshold assignment; that is, the individual is either below or above the age of 18 years. One of the difficulties with comparing data from different ethnic groups is that Reference Data Sets (RDS) use different staging systems, different methods of expressing the variation, and different statistical methods. The most important development stage is H, which is the final stage. A further difficulty is that few studies have censored the Demirjian Stage H data. This leads to a maximum value for Stage H determined by the upper age limit of the sample rather than an upper age estimate based on appropriate censoring of the Stage H data. Thus, there are widely varying values for the mean AaA. The consequence of this is when Probability Estimates (PE) are based on the summary statistics of Stage H, the probability value is usually too high in disfavor to the subject of interest.

To enable realistic age comparisons of the influence of ancestry, it is appropriate to compare ethnic groups using the penultimate stage of development, Stage G.<sup>1</sup> This is because there is a biological natural entry age and exit age for the stage. The variation associated with this conforms to a normal distribution curve.<sup>2</sup>

**Materials and methods:** Only DAE papers using the Demirjian TDSs were used for assessment. Within these papers, the summary statistics for The Lower Left Third Molar and the AaA data for Demirjian Stage G were extracted.

**Results:** The mean value only for the AaA of Stage G is presented. This is for clarity of presentation. The values are in decimal years—n-tds and sd-tds are omitted for clarity of presentation.

German 21.20, Southern China 20.10, Australia 18.65, Turkey 18.30, Scandinavia 17.60, East China 17.39, Brazil 16.30, Afro Trinidad 16.10.

The eight examples of the AaA for specific ancestral groups have been chosen to represent the range of values reported in the literature.

**Discussion:** The different studies show the extent to which ancestral groups differ in dental development. An important underlying issue is that all these studies, plus others not reported in this abstract, exhibit markedly different values for the average AaA for specific ancestral or ethnic groups. There is the possibility that these variations are partly influenced by sampling error. Further work will be needed to explore this potential source of error.

**Conclusion:** It is important to select a suitable statistically and ancestrally concordant RDS when performing Forensic Dental Age Estimates. In the absence of a specific RDS, the use of an International RDS is recommended but care must be taken with the interpretation of the outcome value for Dental Age Assessment.

**References:**

- <sup>1.</sup> Demirjian A, Goldstein H, Tanner JM. A New System of Dental Age Assessment. *Human Biology*. 1973; 45(2): 211-227.
- <sup>2.</sup> Roberts GJ, McDonald F, Andiappan M, Lucas VS. Dental age estimation (DAE): data management for Tooth Development Stages including the third molar. Appropriate censoring of Stage H, the final stage of tooth development. *Journal of Forensic and Legal Medicine*. 2015; 36: 177-184.

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**Ancestral; Third Molar; Threshold Assignment**

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## **H14 The Applications of X-Ray Microtomography (Micro-CT) in Studying Age-Related Tooth Morphological Changes: A Scoping Review**

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**Learning Objective:** After attending this presentation, attendees will have gained an understanding of the current application of micro-CT in studying age related tooth morphological changes.

**Impact Statement:** This presentation will impact the forensic science community by providing an overview of how far micro-CT has been applied in studying age-related changes in dental hard tissue, which is crucial in the fields of forensic odontology as well as biological anthropology. This presentation will also outline the research gaps and research opportunities that can be taken into consideration in future forensic and anthropological research.

Age estimation is an important niche in the forensic sciences in instances of identification for legal purposes as well as disaster victim identification. As teeth are made up of the most durable tissues in the human body, they act as excellent age indicators as they are resistant to decay, environmental factors, and external influences. Conventional tooth-based age estimation relies on invasive factors such as sectioning and staining, which are unpreferable when the tooth is required for evidence in case of legal procedures or archaeological preservation. Micro-CT, first introduced in the 1980s, is a non-intrusive, non-invasive, high-resolution imaging technique that allows for the development of accurate three-dimensional models as well as the acquisition of quantitative data. Although there is ample literature of micro-CT technology in studying tooth morphology in the areas of dentistry and the biological sciences, studies focusing on age-related changes of tooth morphology and age estimation based on micro-CT imaging remains lacking.

Based on the questions: “How has micro-CT technology been applied in studying age related tooth morphological changes?” and “How has micro-CT technology been applied in age estimation studies?”, the authors conducted a scoping review, based on studies that has applied micro-CT technology in studying tooth-based age-related changes and/or age estimation, both in human and animal dentition.

The authors used the framework proposed by Arksey and O'Malley and the PRISMA-ScR checklist to guide the scoping review.<sup>1,2</sup> The five steps followed were: (1) proposing the research question, (2) identifying relevant studies, (3) selecting eligible studies, (4) documenting the evidence selected, and (5) summarizing the results.

A pilot search of four databases (PubMed®, Medline® Complete via Ebsco, Scopus®, ScienceDirect®) identified 247 articles that matched the keywords used. Authors further identified relevant studies by screening the title and abstract, excluding duplicates, studies that did not use micro-CT technology, did not use dentition as primary sample, and studies that did not focus on age-related changes and/or age estimation; 18 full-text articles were identified as eligible and were included in the scoping review.

Out of the 18 research articles, 14 studies (77.7%) looked at age-related changes, while only 4 studies (22.3%) performed age estimation analysis. Furthermore, 7 studies (38.8%) utilized animal dentition, while 11 studies (61.2%) used human teeth. An overview of the parameters used to study age-related changes and to estimate age revealed a variable of tooth characteristics; such as tooth shape, tooth length, cusp height, crown height, root canal length, root canal width, ratios between different areas of the teeth, dental pulp cavity, distance between pulp cavity and tooth surface, formation of secondary dentin, apical constriction, and root dentin calcification.

The findings of this scoping review highlight the extent micro-CT is used in studying tooth-based age-related changes, as well as the effectiveness of micro-CT in age estimation studies. Based on the findings, this review serves as a guide for future forensic age estimation studies.

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- <sup>1.</sup> Arksey, H. and L. O'Malley, Scoping studies: towards a methodological framework. *International Journal of Social Research Methodology*, 2005. 8(1): p. 19-32.
- <sup>2.</sup> Tricco, A.C., et al., PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann Intern Med*, 2018. 169(7): p. 467-473.

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### **X-Ray Microtomography; Forensic Odontology; Age Estimation**

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**H15 Validation of Automated Dental Age Assessment**

*Salem Altalie, DDS, MSc\*, Abu Dhabi Police GHQ, Deba, Sharjah, United Arab Emirates*

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**Learning Objective:** After attending this presentation, attendees will be informed about the validation of using automated dental age assessments by different levels of dentists' expertise.

**Impact Statement:** This presentation will impact the health care community by acknowledging the use of technology in age assessment.

Inter-examiner reliability is low when examining cases, especially those above 22 years old and reaches inequivalent conclusions. Therefore, innovated digital model of age assessment applied by Abu Dhabi Health Services (SEHA) and Emirates Health Services (EHS) in the United Arab Emirates (UAE) has shown a better way of age verification and avoiding confirmation bias.

Identifying the biological age is needed to assess the chronological age calendar of living persons. Some reasons are evaluating asylum seekers, starting an orthodontics treatment plan, preventing child recruitment, and identifying athletes in competition. However, undefined workflow, outdated data, and limited training and certification reduce inter-examiner reliability. After a disagreement in age estimation reports, which were communicated to the legal authority, a decision was formed to restructure age assessment using technology. A solution is a pathway process of steps and procedures for age assessment that improves the practice based on international standards. Both evaluations and collaborations are established based on consistent criteria that will enhance quality control to reduce human errors.

Thirty experts from 20 countries, including SEHA and EHS health care facilities from the United Arab Emirates, have been trained on the digitized assessment. Each dentist tested five cases that consist of matching a pattern of teeth cluster—*atlas technique*—represents the deciduous dentition age groups from birth to 5 years old. Secondly, rating a stage of specific value—*scoring technique*—represents mixed dentition from 6 to 14 years old and wisdom dentition from 15 to 21 years old. Finally, evaluating the level of degenerative changes using radiographic and macroscopic analysis—*measuring techniques*—which represents the permanent dentition of 22 years old and above.

The validation method targets the consistent application of age assessment methods (standard procedure) by different dentists. The authorities seek to assess the age to reach the level of fitness for the purpose. After dentists tested all cases in routine and digital examination, the results of digital prediction are higher in precision and faster in the process. Software with the learning algorithm helps find patterns in the data such that the input parameters correspond to the target. Parameters of this model are a combination of data and information that has been previously verified for age prediction. So, experts in machine learning constantly have optimized these parameters (algorithms) for better predictions.

The future of computer vision in dentistry is promising, with the capability to integrate other functions and make it easier to perform even crucial tasks. However, the intention here is to detect the alignment of experts using digitized age assessment. Examiners can do multiple tests or re-do a single test that qualified for a good result. Validation shows consistent age estimation reports because the digitized checklist, alert, and constraints helped the examiner to reduce reliance on memory and minimize errors. Machine learning (ML) will provide a range for more thoughtful planning, managing, and evaluating a case. ML will give the gift of time to focus on the case and work faster with the aid of reliable software.

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**Age Assessment; Quality Control; Computer Vision**

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**H16 The Accuracy of Age Estimation and Assessment of the 18-Year Threshold Based on Second and Third Molar Maturity in Korean and Japanese Populations**

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**Learning Objective:** Through this presentation, attendees will learn about Lee's method, which is an age estimation method using the development of the second and third molars and will be able to recognize the possibility of population difference in age estimation.

**Impact Statement:** The results of this study will be used as a scientific basis for the need to apply the method derived from their own population data to be assessed for more accurate estimation.

This study aimed to validate Lee's age estimation method and assess the 18-year threshold in Korean and Japanese populations. We evaluated the maxillary and mandibular second (M2) and third molars (M3) in 2,657 orthopantomograms of the Korean and Japanese populations aged 15–23 years ( $19.47 \pm 2.62$  years for Koreans,  $19.31 \pm 2.60$  years for Japanese), using Demirjian's criteria. Dental ages of both Korean and Japanese panoramic radiographs were estimated according to Lee's method, and correlations between chronological and dental ages were analyzed. Classification performance was calculated based on the 18-year threshold. The relationship between developmental stage and chronologic age was analyzed using multiple linear regression. Our results revealed that Lee's method was appropriate for estimation in the Korean population, and the calculated sensitivity, specificity, and accuracy for Korean population data were also enough accurate in applying for assessing legal threshold in Korean population. However, when the Lee's method was applied to the Japanese population, a lower value of correlation coefficients between estimated and chronological age and lower specificity were observed. Population differences were observed predominantly in the stages of root development (stages F and G) of M2s and M3s in both jaws and more frequently in females than in males. In the multiple linear regression between developmental stage and chronological age, lower values of the coefficients of determination were observed in the Japanese population than in the Korean population.

In conclusion, the Lee's method derived from the Korean population data is suitable for Koreans; however, it may be unsuitable for Japanese juveniles and adolescents. This study was conducted with the samples from single institutes of both Korea and Japan, generalization to population tendency should be cautious, and future studies with samples from multiple institutions should be conducted to support the results of this study. Future Japanese population-based studies with larger sample sizes are also warranted to improve the accuracy of dental age estimation and confirm the developmental pattern of teeth in the Japanese population.

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**Dental Age Estimation; Population Study; Accuracy**



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**H17 Intraoral Digital Record Assessment as Forensic and Legal Evidence: From a Paradigm to Reality**

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**Learning Objective:** Comprehensive and accurate methods for intraoral record data should be the alternative to traditional methods of intraoral data, such as stone models. Dental identification is the first choice for human identification, and the methodology applied should be friendly for frequent use. It will allow immediate results and analysis following the quality of the report.

**Impact Statement:** In extreme forensic scenarios, such as arm conflicts or legal cases, needed long-term storage and real-time sharing of digital record methods are the election.

In extreme forensic scenarios, such as arm conflicts or mass disasters catastrophes, the oral cavity is a database for forensic identification that is associated with legal issues. The registration of dental records is mandatory in several European countries. In modern dental clinical practice, digital scanners have become common health devices to record the individual's dental status.

The study intends to present a digital methodology for intraoral digital records in forensic pathology procedures. A quasi-experimental study was performed in the Central Branch of the National Institute of Legal Medicine and Forensic Science, Portugal. The intraoral scanner i7000 (Medit, Lusobionic, Portugal) was selected as a wireless device, and the Canon 5D-Full Frame equipment was used to record intraoral postmortem status. Data were compared according to team expertise (number, time required, and training) and intraoral information (teeth number, morphologic, dimensions, color, and metrics). The two methodologies were compared to establish the one that is more suitable to optimize human identification in corpses.

The scanner allowed two-dimension and three-dimensional reconstruction findings while the photography can only concede two-dimension reconstructions. The intraoral scanner allowed full arcade and inter-arcade registrations when confronted against a limited mouth opening, characteristic of cadaveric rigidity. On the contrary, intraoral photography did not permit the registration of all oral structures. The superimposition of intraoral data for the scanner and the photography were not equal. The scanner displayed high accuracy in position matches between postmortem and antemortem intraoral records of the anatomical structures (up to 90%,  $p < 0.01$ ), whereas the photographic method contained minor information, thus lower efficient matches were obtained. Furthermore, photography requires color and dimension calibration through the mandatory use of scales and color checkers, which the scanner did not require due to hitsis primer calibration prior to its use. In addition, both methods in a digital medium can permanently store data and share it instantly.

The scope of the intraoral scanner expands clinical purposes, allowing the forensic record of oral cavity with morphological details. It can be performed by one expert, recording general and specific anatomical features of the oral cavity. Teeth and soft tissues, namely palatine rugae, were recorded to perform identification, as an adequate and an accurate alternative to traditional records.

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**Human Identification; Forensic Dentistry; Forensic Pathology**



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## **H18 Postmortem Interval (PMI) Estimation on Dental DNA Studied by Next Generation Sequencing (NGS): A Pilot Study**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the impact of forensic science in elderly neglect cases.

**Impact Statement:** This presentation will have an impact on the forensic scientific community by demonstrating the social and health key roles of the forensic pathologist not only in investigating the causes of death in cases of fatal neglect in the elderly, but also in preventing this form of violence.

**Introduction:** In recent decades, there has been a progressive increase in life expectancy, which has led to a progressive increase in the number of elderly people. Many of these live alone at home in abandoned conditions. Elderly neglect occurs when someone fails to adequately care for an elderly person by failing to provide for his or her basic needs, both in terms of hygiene, nutrition, and therapy and in psychological and social needs.

**Materials and methods:** We analyzed three cases, two of which were fatal, of elderly neglect. For the two fatal cases, a judicial inspection or health documentation analysis and autopsy were performed. For the third case, a forensic examination was carried out on the patient.

**Case 1:** A 79-year-old woman with multiple pathologies entered the Emergency Department for accidental trauma. The patient presented with expired general conditions, cachectic and dehydrated. From the medical history, the doctors found that the accidental fall had occurred five days before the date of access to the hospital and after the necessary investigations, found a left femur multi-fragmentary pertrochanteric fracture and dorsal, sacral, and calcaneal pressure injuries. Despite the appropriate treatment, the patient died. Postmortem examination revealed massive pulmonary thrombo-embolism.

**Case 2:** A 98-year-old woman was found dead at her home. A judicial inspection was performed that revealed the completely burned corpse of the old woman following a fire that involved only one room of the apartment. The family members reported that the fire was accidentally caused by the proximity of the clothes to a stove near the victim. However, the analysis of the scene revealed the story's incongruity and, moreover, the remains of woolen laces were found both on the chair armrests and in the garbage, bringing out the hypothesis that the old woman was tied to the chair. The autopsy revealed a decayed state, and toxicological tests confirmed death by carbonization.

**Case 3:** An 80-year-old woman accessed the Emergency Department for reported accidental trauma with no fractures. After providing the necessary treatment, the patient was discharged, in stable clinical conditions, to a nursing home with a diagnosis of cognitive decay and gonarthrosis. During the two months of hospitalization, the family members checked for the onset of painful pressure injuries. A medical-legal examination was performed that revealed ulcerated and infected stage IV pressure sores in the sacrum, buttocks, and heels bilaterally with extensive soft tissues exposure. On her head was an alopecia area. The patient appeared depressed and anhedonic.

**Results:** In the first two cases, the clinical history analysis revealed a family reality of abandonment in the elderly care. Post-mortal examinations also revealed the patient's decayed state. In the third case, the forensic investigation made it possible to detect the negligence in the health worker conduct who cared for the elderly.

**Discussion and conclusions:** These cases make it possible to detect that there is a true death by negligence in the elderly. According to two recent systematic reviews, it is estimated that the elderly neglect phenomenon has a prevalence of 4.2% in community settings and 11.6% in institutional settings.<sup>1,2</sup> The need for a multidisciplinary approach in the care of the elderly to reduce the exponential increase in cases of elderly neglect should be emphasized. This could be achieved through the creation of an integrated operational protocol aimed at increasing control strategies in care areas by strengthening the medical, social, and family assistance services distributed throughout the territory in order to prevent fatal events.

### **References:**

1. World Health Organization (2020) *Elder abuse*. <https://www.who.int/news-room/fact-sheets/detail/elder-abuse>. Accessed 15 July 2022.
2. Yon Y, Mikton CR, Gassoumis ZD et al (2017) Elder abuse prevalence in community settings: a systematic review and meta-analysis. *Lancet Glob Health* 5:147–156.

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### **Forensic Sciences; Maltreatment; Elderly Neglect**

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## H19 Teeth Hold Clues: A Unique Positive ID Case of Burned and Fragmented Remains

*Megha Shah, DDS\*, Smile 360 Family Dentistry, Alta Loma, CA; Janice Klim Lemann DDS, Odontologist Riverside County Sheriff and Odontologist SBD County Sheriff, Redlands, CA*

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**Learning Objective:** Attendees will see and learn from an actual case without DNA and with minimal remaining tooth structure that a positive ID can be achieved.

**Impact Statement:** This presentation will impact the forensic community by giving them more information from an actual case. Evidence is foundation in forensic dentistry. The case also highlights the importance of how each and every tooth and tissue details help to achieve answers in ID cases.

A common method used to perform Dental Identifications (IDs) is the of comparison of known Antemortem (AM) radiographs to unknown Postmortem (PM) radiographs. This presentation is a dental ID case of a homicide victim from Riverside County in Southern California. It shows how using Photoshop®, a positive dental ID was able to be achieved after days of burning at three different locations and scattering of the fragmented remains. In such cases, it becomes extremely difficult and challenging to identify all the evidence and not miss any vital information to find the answers.

The AM radiographs used for this dental ID comparison had been taken before restorative fillings were placed and did not show any wisdom teeth. All the anterior teeth in both arches had been lost and/or burned in the fires PM, rendering the AM images of them useless. The remaining teeth and/or roots recovered were all posterior bicuspid or molars. Of these, only tooth #2 and a root-fragment of tooth #3 had enough information and were able to be compared. Every tooth has a unique and distinct anatomy, both internally with the pulp chamber and canals, and externally with a specific outline and shape of the crown and roots. All aspects of this individualized anatomy were considered in the analysis of tooth #2 and the root-fragment of #3, when the AM radiographs were compared side-by-side with the PM radiographs on PhotoShop® software. Additionally, using PhotoShop®, tooth #2 and the root-fragment of #3 were isolated from the PM radiographs. The overlay formed from them was superimposed over the AM radiographs, and a positive ID was confirmed. Later the homicide case was presented in court and an expert witness explained the dental ID report and process used to the jurors.

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### Burned Fragmented Remains; Tooth Roots and Overlays; Positive Dental ID

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## **H20 Report Writing Using Word® Developer: A Streamlined and Systematic Approach to Comprehensive Documentation**

*Peter Marsh, DDS\*, HCIFS, Manvel, TX; J. Carol Geer, DDS, HCIFS, Richmond, TX*

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**Learning Objective:** After attending this presentation, attendees will be aware as to how to activate and use Word® Developer to generate comprehensive identification reports in minimal time while at the morgue.

**Impact Statement:** This presentation will impact the forensic science community by showing attendees how to generate time savings and make resulting reports easier to understand while prompting completeness.

A novel method for writing dental identification reports by using the Microsoft® Word Developer tab to create templates using Cunningham's Tooth (Universal System 1) and a modified WinID nomenclature will be shown. We produced this template to decrease the amount of time spent generating a report while in the morgue and have reduced the time by over 50%. The resulting reports are more comprehensive, detailed, and include all pertinent information. While using this template, you are provided with prompts so no distinctive features will be omitted. In the development of this template, every effort was made to incorporate all the data included in the published ABFO guidelines.

Most odontologists use Microsoft® Word as a routine word processor but are unaware of the optional Developer tab included in most versions after 2007. This optional tab provides access to form controls and other programming-type controls. By activating the tab, both reports and forms can be created in order to take advantage of the repetitive aspects that appear in each report. These include the originating agency, the authors, the type of identification, the introduction, the antemortem and postmortem exhibits, the discussion, the conclusion, and the opinion and disclaimer.

The Developer tab in Word® provides the user the ability to insert the date, use spaces for text, insert pictures, place drop-down boxes for various data, create check boxes, and includes a design mode customizing text. Data is available that corresponds to every case. The extraneous data that is unnecessary is easily removed. Information that is contained in the form prompts the user to discuss distinctive and remarkable aspects and features found in each case.

By using this template, your identification reports will demonstrate professionalism and thoroughness with a more efficient use of time. The end result will be a comprehensive, high-quality report that is concise and easily readable and interpreted.

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### **Identification; Template; Report**

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## H21 Standards Development Activities in Forensic Odontology

*Kenneth Aschheim, DDS\*, New York, NY*

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**Learning Objective:** After attending this presentation, attendees will better understand the status of discipline-specific standards related to the field of forensic odontology.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities within the field of forensic odontology. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards 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.

This presentation will discuss standards development updates related to Forensic Odontology. These include the recent documents added to the OSAC Registry to be sent to an SDO and include OSAC 2021-N-0030, Terminology for a Suspected Pattern of Dental Origin, which provides terms and definitions for describing a suspected pattern of dental origin. In addition, another document, ISO CD 20888 Dentistry—Terminology for forensic orodental data, has been forwarded to the American Dental Association SCDI Subcommittee 14 on Forensic Odontology Informatics for incorporation into ANSI/ADA 1058-2010D Forensic Dental Data Set an OSAC-approved Registry standard.

The OSAC Forensic Odontology Subcommittee is currently working on two new documents. The first is a Proposed Standard for the Photographic Documentation of Suspected Pattern of Dental Origin. This document will provide the best practice recommendations for the photographic documentation of these patterns by a trained forensic specialist for future characterization and analysis. The second document is a standard for the Education and Training in Forensic Odontology, which provides an overview of the minimum educational, training, and competency requirements to practice Forensic Odontology. The presentation will continue with a discussion of those standards being developed internationally by ISO/TC 106 Dentistry on Dentistry—Designation system for tooth developmental stages as well as three ISO/TC 272 Forensic Sciences documentation on the Analysis, Interpretation, and Reporting of Forensics data and its relevance to Forensic Odontology. Finally, 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.

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**Standards; OSAC; ADA**

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**H22   Bitemark Photographic Evidence: Recognizing the Limits**

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**Learning Objective:** The authors will present a bitemark case they were involved in and educate the attendees on the issues that were present with the photographic evidence, how to evaluate the photographic evidence, and how to know the evidentiary limits of photographic evidence.

**Impact Statement:** This presentation will impact the forensic science community by educating the attendees about the limits of photographic evidence and how to evaluate photographic evidence they may receive from individuals involved with the case.

As forensic dentists, one area of our expertise we are often involved with is the evaluation of patterned injury evidence and providing an opinion if the injury was potentially caused by either human or animal teeth. Our opinion and evaluation of the injury can have a significant impact on the case for both the victim and the suspect. Therefore, it is prudent that we understand the limits of the photographic evidence to help prevent us from making erroneous assessments.

With pattern injuries, proper early photographic documentation of the injury is ideal as healing can occur on living individuals and postmortem changes can occur on deceased individuals. Taking evidence photographs with quality cameras can be done by many individuals who have received proper training; however, we often see photographs taken by other individuals such as emergency room personnel, case workers, or family members of the victim who have not had the training or have access to the proper equipment. While they do the best they can with what they have, the resulting photographic evidence can greatly limit our ability to analyze the injury.

There are many factors to evaluate when we have been provided photographic evidence taken by other individuals: type and quality of the camera and lens, the resolution of the image, any angular or other distortion, quality of lighting, sharpness of the image, and proper use of a reference scale.

If a pattern injury is determined to be a bitemark by the forensic dentist and he/she would like to do a comparison to suspect(s) dentitions, does the quality of the photographic evidence allow for a proper 1:1 comparison?

The authors will present a case they were involved in where the early photographic evidence taken was less than ideal; however, it was the best documentation of the injuries to the victim. They will review the photographic evidence received and show the attendees the issues with the images and how to properly analyze the data file.

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**Bitemark; Evidence; Photography**

## II Internal Graze Gunshot Wounds

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**Learning Objective:** After engaging with this presentation, attendees will have been introduced to a proposed new term to describe a unique type of skin gunshot defect referred to as an “internal graze wound.” The term is used to describe a skin injury resulting from an internal projectile wound that travels beneath the skin in a relatively tangential trajectory, without exiting.

**Impact Statement:** This presentation will impact the forensic science community by introducing a new term to describe a specific type of gunshot injury of the skin, wherein a non-exiting skin injury is produced by a projectile traveling beneath the skin in a tangential fashion. We present a series of example cases of what we describe as “internal graze wounds.”

In medicolegal autopsies involving gunshot wounds, forensic pathologists must account for all firearm injuries. This includes describing projectile entrance, exit, and graze wounds, as well as other aspects of the wounds, such as evidence of gunshot residue, projectile pathways, and whether or not projectiles are recovered. Especially when multiple gunshot wounds are present, initial confusion is not uncommon when, in the context of radiologic examination and external body examination, one attempts to correlate all wounds and account for all projectiles.

In clinical medicine, the “bullet rule” is a mathematical formula used by some trauma surgeons and emergency department physicians to account for bullets that have entered the body and either exited or remained within the body. This relatively simplistic rule states that the sum of the number of bullet wounds on the skin surface (entrances and exits) and the number of projectiles detected on imaging studies should always be an even number. There are a variety of situations that may explain why the bullet rule is “broken,” including the presence of incomplete or partial exit wounds. These and other phenomena are well-known by forensic pathologists in explaining the initial confusion that sometimes exists with gunshot wound cases at autopsy.

Another phenomenon that can lead to confusion or discrepancy when attempting to account for all projectiles, entrance wounds, and exit wounds is the presence of what we refer to as “internal graze wounds.” We define an “internal graze wound” as a partial- or full-thickness skin defect produced by a projectile traveling beneath the skin in a relatively tangential fashion as part of a wound pathway within the body. Although the skin is injured, the projectile does not exit at the site of injury. Instead, it remains within the body, continuing to travel to a location away from the internal graze wound, where it either exits the body or remains within the body. The “internal graze wound” is somewhat analogous to a traditional graze wound, where a projectile traveling outside of the body strikes the skin from above (or outside) in a tangential fashion, without entering the body. With the internal graze wound, the bullet is already traveling inside of the body, and it disrupts the skin in a tangential fashion from below (or inside), rather than from above, without exiting the body. Internal graze wounds can also involve exposed mucous membranes, including the eyes. We present this series of cases not because they are necessarily unique or unusual, but as a means by which we can introduce proposed new descriptive nomenclature within the forensic pathology and firearms injury lexicon.

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### Forensic Pathology; Firearms; Internal Graze Wound

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**12 One Nail Drives Out Another: An Atypical Suicide**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the importance of distinguishing self-inflicted injuries (suicide) from accidents and homicides in head trauma caused by nail gun injuries.

**Impact Statement:** This presentation will impact the forensic science community by presenting the first case in Italy of an autopsy performed in a case of head trauma caused by self-inflicted nail gun injuries.

A nail gun, or nailer, is a form of hammer used to drive nails into wood or other materials. It is usually driven by compressed air (pneumatic). Penetrating brain injury resulting from nail gun use is an unusual event and represents a medicolegal problem: it may be difficult to distinguish accidental deaths from suicide attempts or even homicide.

In July 2021, a 61-year-old man was admitted to the emergency room of our hospital with a diagnosis of head trauma. Neurological examination showed a state of coma (CGS 5), absence of normal pupillary light response and blood vomiting. Relatives reported that the patient had no psychiatric history but in the past few months he had experienced deflection of mood tone due to COVID isolation and loss of his job. It was also referred that the man was found lying on his garage floor, but nobody knew what had happened. Physical examination revealed wide bruising areas and swelling of the soft tissues on both temporal regions of the head. The wounds were highly hemorrhagic, oval, 0.5cm long, and surrounded by a rectangular imprint abrasion. Eleven wounds were found on the right temporal region and six on the left.

CT scan showed massive subarachnoid hemorrhage both on cerebral hemispheres and the skull base, extensive bilateral temporal hemorrhage, and endoventricular blood rate in the fourth ventricle. At least ten foreign bodies with metallic density were placed through the skull reaching the temporal lobes of the brain. Three days later, the patient died. A radiological examination and a head-only section of the cadaver were performed. X-ray scans showed 12 foreign bodies with metallic density placed through the right temporal region of the skull and 9 through the left temporal region. At the opening of the skull, hemorrhagic infarction of both temporal muscles was found. Twelve nail heads completely crossed the right temporal bone reaching the intracranial cavity. Four other nail heads were stuck in the left temporal bone, through the sphenoid, reaching the brain parenchyma. The brain was diffusely hemorrhagic, especially the temporal lobes. An inspection of the garage was carried out, during which a bloodstained nail gun was found. DNA investigations were performed to examine fingerprints and make blood DNA comparisons.

Given the compatibility of the DNA results and the absence of defense injuries on the cadaver, death was attributed to head trauma caused by penetrating nails. Police investigations allowed the authorities to declare that all the elements were consistent with suicide. To our knowledge, this is the first case reporting the macroscopic data of head and brain nail gun injuries. Radiological examination can be helpful in showing the exact location of the nail, but the authors firmly believe that the cadaver dissection, especially the head section, is crucial to identify the nature and the extension of such unusual lesion, thus giving much more information about the mechanism of death and the circumstances in which it occurred.

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**Nail Gun; Suicide; Brain/Head Injuries**



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**I3 A Case of Autoerotic Death by Electrocution**

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**Learning Objective:** This presentation will inform attendees of electrocution as a rare cause of autoerotic death and of the associated investigative and autopsy findings of electrocution.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of electrocution as a rare cause of autoerotic death and the associated investigative and autopsy findings.

Autoerotic death is defined as an accidental death that occurs when a device used to enhance sexual stimulation causes unintended death. Autoerotic deaths by mechanisms other than asphyxia are rare. One study reviewing 50 years of published autoerotic deaths between 1954 and 2004 reports that only 15 of 408 cases (3.7%) were caused by electrocution.<sup>1</sup> Electrocution injury occurs when a passage of a substantial electrical current through tissues causes skin lesions, organ damage, and/or death.<sup>2</sup> Electrical current enters the body at one point and leaves at an exit point, both of which can leave thermal burns due to the high resistance of skin. The resistance greatly decreases when skin is wet, and the current subsequently increases per Ohm's law ( $V=IR$ ). Although injuries associated with low voltage ( $< 1,000$  V) current may be subtle or even absent, electrical marks or Joule burns characterized by charred, blistered, and hyperemic skin may be evident at autopsy. The mechanism of death in low voltage injuries is most commonly cardiac dysrhythmia, followed by respiratory paralysis from spasm of the diaphragm, and rarely by paralysis of cardiac and/or respiratory centers in the brainstem.

Described here is a sudden death of a 66-year-old man with a history of chronic alcoholism, illicit drug abuse, and chronic obstructive pulmonary disease who was found unresponsive in his mobile camper. His girlfriend discovered him lying prone and nude on his bed with a light bulb and attached lamp inserted into the anus. The lamp was plugged in, and the electricity was on. She reported feeling an electrical shock when she touched the decedent in an attempt to begin cardiopulmonary resuscitation. In addition, the decedent was covered in an oily substance, and an open bottle of vegetable oil was found near the body. Occupants of a neighboring camper reported they heard "screaming and hollering" coming from the decedent's camper on the day of the incident. Although the decedent was not known to engage in autoerotic behavior, the scene and investigative findings were consistent with an accidental autoerotic electrocution.

Autopsy examination demonstrated evidence of electrocution. The right aspect of the forehead had a  $2\frac{1}{2} \times 2\frac{1}{2}$ -inch area of gray-brown parchment-like skin with foci of singed epidermis and singed hair. The anorectal margin had a thin, dry, blackened strip with foci of disrupted mucosa, the perianal skin and anal mucosa were gray-brown, dry, and leathery extending up to 1 inch distal to the pectinate line, and histologic sections of the anus showed changes of the mucosa and submucosa consistent with electrocution, including streaming elongated epithelial nuclei and dermoepidermal separation. Other autopsy findings of note included hyperemic rectal mucosa with scattered contusions, hepatic cirrhosis, focal moderate atherosclerosis of the left anterior descending coronary artery, and an abdominal tattoo of lightning bolts. Postmortem toxicology testing of femoral blood detected methamphetamine (459.0ng/mL). Death was attributed to autoerotic electrocution. Manner of death was an accident. This case is an unusual autoerotic death caused by electrocution and highlights associated investigative and autopsy findings.

**References:**

- <sup>1.</sup> Sauvageau, A.; Racette, S.; Autoerotic deaths in the literature from 1954 to 2004: a review. *J Forensic Sci.* 2006 Jan;51(1):140-6.
- <sup>2.</sup> Saukko, P.J.; Knight, B. *Knight's Forensic Pathology*. CRC Press; 2016.

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**Autoerotic Death; Electrocution; Electrical Marks**

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**I4      A Case of Mechanical Asphyxia Due to a Bowling Pinsetter Machine and a Brief Review of the Literature**

*Adam Gonzalez, MD\*, Travis County Medical Examiner, Manor, TX; Lucas Wieck, MD, Travis County Medical Examiner, Austin, TX; J. Keith Pinckard, MD, PhD, Travis County Medical Examiner, Austin, TX*

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**Learning Objective:** After attending this presentation, attendees will understand some principles of mechanical asphyxia resulting from work-related deaths involving bowling alley pinsetter machines, identify characteristic injury patterns from such injuries, and realize the importance of scene investigation in understanding asphyxial deaths.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of a rare workplace injury and reinforce the importance of aggregating information from all aspects of the forensic investigation to accurately understand asphyxial deaths.

In 2020, there were approximately 4,000 deaths due to work-related accidents. Aside from transportation injuries, work environment hazards make up a significant portion of worker deaths, with machinery-related deaths being well-represented in these statistics.

The decedent was a 37-year-old man who was found unresponsive in a standing position with his neck caught in a pinsetter machine at the bowling alley at which he was employed. Emergency medical services responded and encountered great difficulty with freeing him from the machine. He was then transported to the emergency department but was unable to be resuscitated. At autopsy, a patterned abrasion on the skin of the decedent's neck corresponded to the piece of machinery. This finding was associated with contusions of the underlying soft tissues, including the strap muscles of the neck, as well as a fracture of the right greater horn of the hyoid bone. There was hemorrhage within the right cricothyroid muscle and adjacent soft tissue of the anterior larynx and trachea. The location of these injuries correlated with a photograph documenting the decedent's position as he was lodged in the pinsetter machine prior to his extraction. Minute petechial hemorrhages were also noted on the decedent's bulbar and palpebral conjunctiva. There were no injuries of the cervical spine or head; thus, the cause of death was certified as mechanical asphyxia.

A preliminary search of the literature and the Occupational Safety and Health Administration (OSHA) Fatality Inspections Database revealed several injuries associated with these machines; specifically, there were 12 reported deaths due to becoming caught or crushed and there were 2 fatal electrocutions reported. In addition, there were numerous instances of non-lethal traumatic injury attributed to these machines. Fatalities by pinsetter machine, however unique and rare, continue to be a workplace hazard. The necessity of improved workplace safety measures will be briefly discussed.

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**Mechanical Asphyxia; Pinsetter; Occupational Safety**

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**I5      An Unusual Suicide With Multiple Gunshot Injuries to the Head From Two Different Weapons**

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**Learning Objective:** After attending this presentation, attendees will have a better knowledge of the autopsy findings of suicide from multiple gunshot injuries to the head.

**Impact Statement:** This presentation will impact the forensic science community by discussing the differential diagnosis between homicidal and suicidal multiple gunshot injuries to the head. The presented case will show the specific characteristics of the wounds, their interpretation, and how a bullet, usually considered non-fatal, can result in the death of a person.

The presence of multiple gunshot wounds, especially head wounds, is always a solid reason to suspect homicide. Multiple self-inflicted gunshot wounds to the head are rare and usually challenge the forensic pathologist in determining the manner of death. Such cases should be considered homicides until proven otherwise.

A 35-year-old man was found dead on the floor in the bathroom of his apartment in a sitting position. A pool of blood surrounded the body. There were numerous bloodstains, and two different weapons were found next to the body. One was loaded with rubber bullets. The police suspected a possible homicide due to the ambiguous crime scene, even though the apartment was secured. The neighbors reported having heard two different shots from an interval of approximately one minute. The clothes were soaked with blood, mostly on their frontal aspects. The external examination showed one entrance wound at the base of the chin with a muzzle imprint. At autopsy, the wound track traveled upward, involving the oral cavity, the facial sinuses, and the forehead on the left next to the midline of the face, where an exit wound was found on. No vital structures were injured. The second entrance wound was localized in the right temporal region and showed a muzzle imprint, consistent with a hard-contact gunshot wound. No exit wound was found. The wound track traveled to the left involving the brain, where smashed fragments from the rubber bullet were found. Based on the available evidence, the manner of death was determined to be suicide, despite the two headshot injuries. Supportive for this conclusion was the result from the autopsy, which showed that the first shot—the one on the base of the chin—did not immediately incapacitate the victim, so he could act and shoot himself a second time.

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**Multiple Gunshot Injuries to the Head; Suicide; Rubber Bullet**

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**I6 An Assault on Coagulation: A Complex Suicide by Ingestion of Warfarin and Stab Wounds**

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**Learning Objective:** After attending this presentation, the attendees will be able to better appreciate the implications of a careful examination of the scene and corpse in cases of death resulting from self-harming acts.

**Impact Statement:** The presentation will impact the forensic community by sharing the experience gained during the postmortem investigation of the death of a 65-year-old man, which occurred because of repeated warfarin ingestion and cut injuries.

Although suicide represents a current social problem, complex suicides constitute a rare occurrence, accounting for only 1.5–5% of the total. They are called complex as they occur because of more than one suicide method. Based on the timing, it can be divided into primary (characterized by simultaneous mechanisms) and secondary (characterized by consecutive mechanisms); similarly, according to the premeditation of the executions of the multiple modalities, it can be divided into planned and unplanned. The reported case concerns the death of a 65-year-old man following anticonservative acts that took place through multiple and polymorphic methods in his apartment. The subject was lying on his back on the bedroom floor, heavily smeared with blood and with multiple cuts, none apparently injuring arteries or veins; also, the bed was largely bloody as well as the adjacent floor. On the dresser, many packs of medicines were stacked, including some of which were warfarin. The house was generally untidy, characterized by shattered glass tables and various objects lying on the ground. The floors were heavily smeared with blood, sometimes in drops and sometimes representing footprints. A kitchen knife with a serrated blade stained with blood was on the counter.

The autopsy was requested by the prosecutor to establish the cause of death and to trace the demise to a suicidal or homicidal dynamic. The external examination of the cadaver revealed the presence of widespread cuts to the upper limbs and right knee associated with the presence of ecchymotic areas. The evaluation of the distribution of the cut injuries highlighted the involvement of self-attacking areas and, therefore, to trace the dynamics to suicidal acts. At the autopsy, subdural and subarachnoid intracranial hemorrhage, abdominal soft tissue hemorrhage, and renal medullary hemorrhage were detected. Dissection of the upper limbs identified signs of vitality and confirmed the integrity of arterial and venous vessels. In consideration of the diagnostic suspicion and the findings highlighted, samples of biological fluids were performed to carry out toxicological investigations; organ and tissue samples were also taken for subsequent histological investigations. Toxicological investigations revealed the presence of warfarin in a concentration equal to 17.5mcg/ml, higher than therapeutic values. Histopathological examinations confirmed the vitality of the skin lesions and the presence of blood infiltration in the brain and kidneys.

At the end of the investigation, the cause of death was identified as a cardiocirculatory insufficiency due to intracranial, abdominal, and cutaneous hemorrhages in the context of warfarin acute intoxication and superficial stab wounds at the level of the upper limbs. In conclusion, the experience gained demonstrates the usefulness of an accurate crime scene investigation to identify all the elements useful for achieving a correct interpretation of death. In fact, the search for further elements in addition to the stab wounds to justify apparently unjustifiable bleeding made it possible to highlight this dual suicidal method.

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**Complex Suicide; Scene Investigation; Forensic Pathology**

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**I7 Complex Deaths: Murder or Suicide? A Report of Three Cases**

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**Learning Objective:** This presentation aims to establish how to reach a differential diagnosis between suicide and homicide, especially in cases assessed as complex suicides, in which the use of multiple methods of self-injury could make the manner of death in doubt.

**Impact Statement:** This presentation will affect forensic pathologists by bringing attention to complex suicides that are likely to be potentially mistaken for homicide. The cause of death in these cases should be investigated with a comprehensive autopsy, a detailed examination of the scene, consideration of any history of prior suicide attempts, leaving a suicide note behind, and any diagnosis of psychiatric illnesses and chronic illnesses.

Complex suicides represent 1.5–5% of all suicides reported in the literature.<sup>1</sup> The use of many potentially lethal methods to complete suicide is called a “complex suicide.”<sup>2</sup> These cases can be classified as planned and unplanned complex suicides. In planned complex suicides, two or more methods cooperate simultaneously to ensure that death will occur even if one method fails. In unplanned complex suicides, the method of suicide changes as the previous one failed.

Complex suicides can often mislead researchers due to their combined use of multiple methods; in these cases, the distinction between a suicide and a homicide constitutes a crucial problem for forensic pathologists.<sup>3</sup> Careful examination of the crime scene, evaluation of the victim’s medical history, and a complete autopsy are required to differentiate between complex suicide and homicide.

This study aimed to discuss the characteristics of three complex suicide cases observed in the period between 2016–2021 at the Department of Forensic Sciences of the University of Foggia.

The first case is a 33-year-old female discovered lying in the woods. Near the body, there was a bottle of muriatic acid and a plastic bag in shreds. External examination showed slight burns around the mouth and strangulation marks around the neck. The autopsy revealed hemorrhagic infiltration of the neck muscles and signs of erosion of the tongue and esophageal mucosa with gastric perforation. Death was attributed to a combined mechanism of caustic ingestion and asphyxiation by strangulation.

The second scenario concerns a 55-year-old Romanian man discovered in his incompletely burned apartment. Near the body, there was a kitchen knife covered with blood and the corpse had a large cut wound with intestinal loops protruding from the abdomen. The autopsy revealed soot on the mucous membranes of the upper airways and no other lesions. His death resulted in hemorrhagic shock from exsanguination due to the cut wound on the abdomen, in association with carbon monoxide intoxication.

The third case concerns a 48-year-old man who suffered from psychiatric disorders and whose body lay on the ground beneath his bedroom window. External examination showed mutilation of the right testicle and several injuries on the penis and the scrotum. The autopsy also revealed multiple cranial and thoracic traumatic lesions. Death resulted from a combined mechanism of exsanguination due to the cut wound on the testicle and polytrauma by precipitation.

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**Complex Suicide; Multidisciplinary Approach; Murder**

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**I8      Dating Traumatic Brain Injury With a Dura Mater Immunohistochemical Assay: An Ongoing Study**

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**WITHDRAWN**

## **19 The Decapitation of a Motorcyclist Wearing a Full-Face Helmet in a Crash Against the Guardrail: A Case Report**

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**Learning Objective:** A case of complete decapitation of a motorcyclist, wearing a full-face helmet, following a high-energy impact against a guardrail is presented. After attending this presentation, attendees will be exposed to an uncommon lesion after a crash. In this regard, decapitation is an unusual event in forensic medicine. Complete decapitation in the context of traffic accidents has been occasionally mentioned in high-speed crashes.

**Impact Statement:** This presentation will impact the forensic science community by serving as a rare event of fatal crash investigation. This unusual case is compared with other cases reported in the literature to analyze how decapitation occurs in cases of an impact between the human body and a guardrail.

Decapitation consists of the complete separation of the head from the body.<sup>1</sup> It is an uncommon event in forensic practice and can be intravital or postmortem.<sup>2,3</sup> Accidental decapitation can be the result of an explosion, industrial, or road traffic accidents, including those of motorcyclists.<sup>1</sup> Head and neck injuries are the principal cause of fatalities in motorcycle accidents.<sup>4</sup> To prevent these kinds of traumatic injuries and to reduce their seriousness, the use of a protective helmet is already widespread and mandatory throughout the Western world.<sup>3,5</sup> However, they cannot guarantee complete safety. In certain situations, in fact, the mass of helmet can increase the risk of neck injuries, resulting in some cases of decapitation.<sup>6</sup> Furthermore, in cases of impact with the road surface and roadside safety barriers, helmets do not protect the midsection (C3–C5) of the cervical spine, which is the most vulnerable area to traumatic fracture.<sup>3</sup>

A case of complete decapitation of a motorcyclist who was riding his motorcycle wearing a full-face helmet is described. A 55-year-old man lost control of his motorcycle and bumped his neck into an iron double-blade guardrail, producing a complete separation of the head from the body that continued to slide on the road. When the police arrived, they found the body of the driver on the road near the curb, while his head, completely separated from the body, lay several meters away in the center of the road between the road lanes.

The Judicial Authority required an autopsy. The head, which included the facial anatomy and the skull, was separated from the rest of the body at the level of the neck at the C2–C3 cervical tract. Examination of the skin around the decapitation points of both the head and trunk revealed intense infiltration of blood in the subcutaneous soft tissues around the injury, mostly on the left side. In particular, the tissues of the neck showed lacerations and the lower portion of the head appeared separated at the level of the hard palate structures that remained adherent to the body. The skullcap and facial skeleton presented multiple fractures. There was subarachnoid bleeding in the right side of brain while the basilar skull, left side of brain, cerebellum, and brainstem were absent. The tongue, esophagus, and trachea were undamaged. The spinal cord was transected at the T1 level, the T7 spine was fractured, and there were compound fractures of almost all ribs, especially in the left side.

In forensic literature, few cases of decapitated motorcyclists are reported. Nine cases of decapitation of motorcyclists are cited of which three were complete and in three cases there was use of full face helmet.<sup>3,5,7-10</sup> In six cases, a direct impact was considered the cause of decapitation but in only one case was the impact on a roadside barrier.<sup>1,3,5,8-10</sup> The analysis of presented case suggests a strong direct impact of the neck of the victim with part of the metallic structure that caused the complete amputation of the cervical tract through a side-to-side directional force, causing tissue avulsion in the front part of the neck corresponding with the edge of the helmet and the victim's complete decapitation. This lacerating trauma that affected the neck was probably favored by the presence of the helmet, which remained attached to the guardrail on the left side while the body was pushed in the opposite direction, probably by the motorcycle where it remained stuck.

Forensic disciplines have a role in identifying high-risk locations and dangerous behavior and could help to promote the adoption of measures to improve rider safety.

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### **Decapitation; Motorcycle; Full-Face Helmet**

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**I10 Forensic Aspects of Overkilling in Femicides: A Comparison of Forensic Case Series**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the dynamics in femicides with overkilling.

**Impact Statement:** This presentation will impact the forensic science community by providing a more detailed profile of the psychology of the killer in cases of overkilling.

Femicide refers to the extreme form of violence against someone belonging to the female gender. A recent review of available literature on the topic shows that, based on geographical region, the definition of this phenomenon may vary.<sup>1</sup> The United Nations estimates a total of 87 000 women were intentionally killed in 2017, of whom 58 % were killed by intimate partners or family members. In these instances, the murder often happens with an evident disproportion between the excessive amount of violence used by the killer and what's necessary to perpetrate the crime, configuring a phenomenon known as overkilling. This work compares six cases of femicide with overkilling. In all the cases, the scene was analyzed, and an autopsy was completed.

The first case reports the death of a woman who passed because of cerebral hemorrhage, multiple skull base fractures, and fractures of the left arm due to multiple shots of both single-load and multiple-load firearms. The second case, linked to the first, concerns the death of a man who died from perforation of heart, bowel, and left kidney due to four shots from a single-load firearm. The third case is the death of a woman by traumatic shock due to encephalic trauma in a victim beaten with multiple blows, both blunt force and from an unusual-edged weapon. The woman was partially dismembered by the killer. The fourth case is a femicide in which the victim died from multiple-edged weapon blows aimed mainly at the thorax and the head. The wounds caused a fatal traumatic shock. The victim was subsequently thrown off a cliff to hide the body. The fifth case regards a woman killed by her former partner; the victim showed multiple cut injuries to the thorax and multiple blunt force lesions to the head, which caused skull rupture, with multiple fractures and cerebral hemorrhage. The crime's peculiarity was the adoption by the killer of two different weapons used simultaneously at different parts of the victim's body. The sixth case is a matricide. The body showed multiple-edged weapon blows to the head and thorax, followed by incisions of the blood vessels at the wrist carried out with the use of a cutter.

The analysis of the cases mentioned above shows the presence in all the crimes of at least one of the following elements: multiple types of weapons used and/or multiple body parts where the injuries had been inflicted. Whenever identifiable, the killer was found to be male. The cases show a great level of ferocity. In the cases reported, the number and extent of the injuries shows the intent not only to kill the victim but to destroy it until it is annihilated with uncontrolled violence. Most of the time, the victim had a relationship to the killer. This finding is supported by most of the literature as a predictor for overkilling. The review of the literature and the cases presented show how the discussed topic still need more research; this would be of great help to depict a more detailed connection between the victim and the aggressor and to identify possible predictors of femicide, which may provide the possibility of preventing such crimes.

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**Forensic Sciences; Overkilling; Femicide**

## **111 Intracranial Hemorrhage/Thrombosis in Infancy: Trauma or Not?**

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**Learning Objective:** After attending this presentation, attendees will better understand two rare cerebrovascular abnormalities and their associated neuropathological findings and forensic implications.

**Impact Statement:** This presentation will impact the forensic science community by raising awareness of two cerebrovascular abnormalities to aid in their differential diagnosis with traumatic head injury in the setting of intracranial hemorrhage in an infant.

We present two cases of infants with intracranial hemorrhage/thrombosis due to cerebrovascular abnormalities that should not be mistaken for traumatic head injury.

**Case 1:** A two-month-old girl presented to hospital with projectile vomiting, fever, decreased responsiveness, and tremors. Neuroimaging showed extensive intraventricular hemorrhage, hydrocephalus, midline shift, inferior herniation, and no osseous abnormalities of the calvarium. A skeletal survey was negative for healing or acute fractures. Hospital ophthalmology exam revealed multiple bilateral retinal hemorrhages extending to the ora serrata, and clinicians became concerned about inflicted head trauma. Death was pronounced on hospital day five. Autopsy revealed no significant injuries, and postmortem respiratory and spinal fluid cultures were negative.

Neuropathologic examination of the brain, dura mater, spinal cord with cervical spine en bloc, and right and left eyes was conducted. Abundant intraventricular hemorrhage was observed within the lateral ventricles, and the choroid plexus of the right lateral ventricle harbored an irregular proliferation of thin blood vessels of variable caliber with minimal intervening stroma, consistent with a choroid plexus angioma. Observed mass effect sequelae included displaced intracranial contents in the spinal cord subdural space, spinal cord infarcts, dorsal root ganglia/nerve root hemorrhages, pituitary infarct, and retinal hemorrhages. Global hypoxic-ischemic changes with widespread acute neuronal necrosis were observed throughout the cerebral cortex, brainstem, and cerebellum, consistent with decreased cerebral perfusion. Venous sinus thromboses were noted, consistent with brain edema and mechanical compression of dural sinuses resulting in venous stasis. There was no evidence of infection, trauma, or neoplasm.

Choroid plexus angiomas have the potential to rupture and cause spontaneous primary intraventricular hemorrhage. These lesions are most often located in the lateral ventricles, and up to 40% may be bilateral.<sup>1</sup> Choroid plexus angiomas are typically sporadic, commonly associated with cardiovascular anomalies, and may be associated with Sturge-Weber Syndrome and leptomeningeal angiomatosis.<sup>1</sup>

**Case 2:** A three-month-old boy was found unresponsive, swaddled in an infant swing, approximately two hours after he was last known alive. Death was pronounced at the scene after unsuccessful resuscitative efforts. Autopsy revealed an atraumatic scalp and skull with prominent leptomeningeal vasculature over the right temporal lobe and left parietal-occipital regions. Postmortem CSF cultures were negative, while lung and blood cultures grew mixed flora consistent with postmortem transmigration/agonal spread of skin and gastrointestinal tract organisms. Medical history included hospitalization for two weeks at birth for respiratory distress, but pediatrician visits documented a well-appearing, vigorous infant since discharge.

Neuropathologic examination of the brain and dura mater was conducted. The investigation revealed diffuse involvement of the dural, leptomeningeal, intraparenchymal, and intraventricular vasculature by antemortem thrombus with associated scattered acute infarcts and subarachnoid hemorrhage. An irregular cluster of thrombosed vessels in the right lateral ventricle was suggestive of a vascular malformation. Other findings included bilateral chronic subdural membranes. There was no evidence of acute trauma, significant inflammation, infection, vasculitis, or neoplasm.

The neuropathologic findings are consistent with cerebral sinovenous thrombosis (CVST), an exceedingly rare cerebrovascular disorder affecting children that involves thrombosis of cerebral veins that can result in increased intracranial pressure, cerebral ischemia, and infarcts. CVST has a variety of etiologies, including acute systemic illness, meningitis, coagulopathy, hemoglobinopathy, or congenital disease, among others.<sup>2</sup> Disorders of coagulation occur in approximately one-third of children with stroke, and inherited coagulopathies are associated with increased risk.<sup>3,4</sup> Of note, the infant's mother was known to have heterozygous alpha thalassemia and sickle cell trait; however, the infant's status was unknown.

These two cases highlight the importance of a thorough gross and histopathologic exam to elucidate the etiology of intracranial hemorrhage and thrombosis in infants, as what may appear at first glance to be trauma-related can have a much less sinister explanation.

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### **Cerebral Sinovenous Thrombosis; Choroid Plexus Angioma; Pediatric Neuropathology**

## I12 Live(r) and Let Die: A Murder Case of a Woman Suffering From Alcoholic Hepatopathy

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**Learning Objective:** After attending this presentation, attendees will be aware of a peculiar type of traumatic death, producing small vascular lesions, in which the patient's medical history was fundamental to investigate and to define its causes.

**Impact Statement:** This presentation will impact the forensic science community by underlining the importance of a person's medical history to determine their cause of death when the autopsy alone is inconclusive.

Cirrhosis is a world-wide major cause of death and disability. It may produce severe systemic abnormalities, especially in the coagulation cascade.<sup>1</sup> For this reason, after a traumatic injury, liver cirrhosis can impact the outcomes of the lesions increasing the risk of mortality and complications.<sup>2-4</sup> In these cases, even minor traumatic insults can be lethal, as happened in the following case report.

In an abandoned construction site, a corpse was found by the police following an anonymous tip. In a metal site hut, used by some homeless for accommodation, a female body was discovered, laying on a mattress and tucked into some blankets. At the scene inspection, ordered by the prosecutor, the corpse revealed multiple and widespread ecchymosis, especially in the dorsal region. A forensic autopsy was requested and performed, including histological and toxicological investigation. An important hematic effusion was found in both pleural cavities (about 1.2 liters), together with dorsal costal fractures. Furthermore, a massive hemoperitoneum was discovered (about 3 liters) and majors blood infiltrates were detected on the right hypochondrial and lumbar regions, as well as on the ipsilateral renal capsule. Hepatic parenchyma showed nodularity and increased consistency; its histological examination revealed severe subversion of the organ structure with numerous and widespread parenchymal nodules, major fibrosis, and steatosis. A blood alcohol concentration of 3.23g/L was measured, thus supporting a severe alteration of the woman's mental state at the time of death. On this basis, it was possible to ascertain the cause of death as a severe hemorrhagic shock. From the police investigation, it was revealed that the corpse belonged to a homeless woman, living irregularly on the Italian territory with her partner. The man used to beat the woman up. He confessed to a friend that, a few days before, he hit her hard with several punches on her back; the following morning he could not wake her up, so he quickly ran away from the country. The investigations proved that the woman was a known alcoholic and was at least twice hospitalized for severe hematomas due to small trauma: a blood test revealed important clotting disorders due to her alcoholic liver disease. Given the absence of macroscopic and microscopic internal lesions (besides the costal fractures) and her medical history, the source of the bleeding was attributed to small vessels ruptures, from both the intercostal and renal vessels, caused by the woman's partner's multiple hits in the setting of coagulation disorders due to the alcoholic cirrhosis.

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### Homicide; Cirrhosis; Coagulation Disorders

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**I13 Men as Victims of Intimate Partner Violence: A Ten-Year Retrospective Study**

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**Learning Objective:** After attending this presentation, the attendees will have a better knowledge of the most common patterns of injuries and characteristics associated with intimate partner violence against men.

**Impact Statement:** This presentation will impact the forensic science community by examining in detail a phenomenon that is underreported and rarely discussed.

Intimate partner violence is a particular type of interpersonal violence in which violence is performed between individuals involved in an intimate relationship. According to the World Health Organization, this includes “any behavior within an intimate relationship that causes physical, psychological or sexual harm to those in the relationship.” Conventionally, intimate partner violence is referred to as violence against females. However, this type of violence can also be directed toward men, although this phenomenon is highly underestimated. A current or former partner commonly performs intimate partner violence, but other subjects can be involved.

The study aims to describe the characteristics of physical intimate partner violence against men reported over ten years in the Department of Forensic Medicine and Deontology in the city of Sofia, Bulgaria. The medicolegal reports of male victims between 2012–2021 were analyzed. No limits for age, race, or cause of death were imposed. The following information was obtained: the age of the victim; marital status (ex or current spouse or cohabitant); alleged manner of attack and the weapons used to kill/injure the victim; type of injuries and their localization over the victim's body.

Our study aims to raise awareness of the problem of men being victims of domestic violence since this type of violence is usually associated with women. The preponderance of female victims of intimate partner violence visiting medical examination centers is attributed not only to a higher number but also to a higher likelihood among women to report incidents of violence to formal institutions, resulting in more support services targeting women. Conversely, male victims tend to underreport violent offenses due to a sense of shame and fear, injuries considered to be “minor,” and a lack of information and appropriate support. A thorough discussion of the results will be presented to attendees.

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**Intimate Partner Violence; Domestic Violence; Male Victimization**

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**I14    Necks, Saws, and Video Tape: Using Surveillance Footage to Determine Manner of Death**

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**Learning Objective:** After attending this presentation, attendees will recognize key features of a saw-related suicide and identify how video footage can be used effectively to correlate the manner of death with the observed patterns of injury.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how a rare type of suicide can be appropriately classified by conducting a thorough investigation.

One of the medical examiner's central functions is to assign manner of death. The large majority of deaths can be categorized as natural, accident, suicide, or homicide, with a relatively small subset being undetermined. This latter category is typically reserved for deaths where no reasonable cause of death can be identified, or where multiple manners are possible, and the medical examiner lacks sufficient information to assign only one.

Deaths from saws are unusual, and suicides by band saw are rare, with only a few reported instances in the literature. Given the ever-present possibilities of accident or foul play, ruling a band saw death as a suicide is potentially challenging.

**Case report:** A 23-year-old man was discovered in the back of a butcher shop, where he was employed. His mouth was sealed with duct tape. He was nearly decapitated by a clean axial incision through the neck. An apparently contiguous coronal incision was also present, separating the anterior and posterior head into roughly equal portions. His body lay next to a specialized commercial band saw used to cut meat. In the absence of any additional history or findings it would be difficult to classify the manner of this death, particularly because of the presence of duct tape and the extent of the injuries. Homicide, suicide, and possibly even accident would be viable considerations.

The decedent's past medical history was notable for depression with two known previous suicide attempts. Video surveillance footage from the butcher shop was also obtained, which clearly showed the decedent running his own neck through the meat saw in an axial plane. He fell backward when his body went limp, creating the coronal hemisection of the head. The footage allowed the medical examiner to correlate the mechanics of the suicide with the injuries seen at autopsy and confidently assign the correct manner.

For deaths where the manner may at first appear undetermined, it is critical to obtain additional information related to the decedent, as well as circumstantial and/or scientific evidence related to the death. Camera surveillance systems have been in use since the 1940s, and in recent years their use has proliferated due to technological advancements and effective marketing. Add to this the ubiquity of smart phones with video recording and it becomes inevitable that more deaths will be recorded. This may obviously be of benefit to medical examiners and death investigators but obtaining and interpreting such footage poses its own difficulties.

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**Band Saw; Case Study; Manner of Death**



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**I15 Sharp Force Fatalities: The Application of Bayes' Theorem to Evaluate the Manner of Death**

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**Learning Objective:** After attending this presentation, attendees will learn our approach to reporting relevant opinions in the field of forensic pathology in a logically correct way to investigators and to courts of law.

**Impact Statement:** This presentation will impact the forensic science community by serving as a theoretical and practical example of the implementation of the Bayes' theorem into daily casework in a series of sharp force fatalities.

The role of forensic science may be defined as the provision of relevant opinion to help answer questions to investigators and to courts of law. The Bayes' theorem provides an approach to quantitate and logically convey the strength of the expert's evidence, but forensic pathology seems slow to adopt these principles that are widely known and embodied in forensic genetics.<sup>1,2</sup> In this presentation, using data from a systematic review to obtain large data sets, we show how Bayesian analysis can be applied in a series of sharp force fatalities to assess the manner of death.

A review of the literature was performed for cross-sectional studies of sharp force fatalities. Four studies were included, for a total of 879 sharp force fatalities: 207 suicides and 672 homicides.<sup>3-6</sup> Data extracted from individual studies were pooled to provide larger data sets. Likelihood ratios (LR) were calculated for individual characteristics whose frequencies significantly differ between homicides and suicides, namely place of death, place where the weapon was found, type of sharp object used, number and localization of wounds, toxicological findings, and psychiatric morbidities of the victim. The same approach was applied in six sharp force fatalities cases, three homicides and three suicides, showing that the use of pooled data is the premise for having a statistical significance in the analysis of the LR. Moreover, certain individual characteristics, such as psychiatric comorbidity, weigh more in favoring a hypothesis. Finally, the combination of individual characteristics, considering the independence and dependence of the variables, increases the value of the LR, even if it can be lower than in forensic genetics. In conclusion, in order to be able to extensively apply the Bayesian theorem in the field of sharp force fatalities, as in other violent fatalities, in light of the research findings, it will be necessary to expand the case series and targeted cross-sectional studies, standardizing data collection.

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**Forensic Pathology; Sharp Force Fatalities; Bayes' Theorem**



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**I16 Shotgun Deaths in Maryland: A Ten-Year Review**

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**Learning Objective:** This presentation will provide attendees with information regarding the trends in shotgun-related deaths in the state of Maryland over the past ten years. The data will focus on manner of death, demographics of the decedents, and location of bodily injury.

**Impact Statement:** The presentation will bring awareness to the use of shotguns in all manners of death and what demographic groups are most affected.

According to the CDC, 2020 was a record-setting year, with 45,222 gun-related deaths nationwide. While handguns predominate as the weapons most frequently used in firearm fatalities, shotgun deaths are not uncommon and have been implicated as a weapon of choice in more multiple fatality incidents. In early July 2022, two homicides by shotgun occurred in Baltimore, MD, less than one week apart.

Data was compiled from the Office of the Chief Medical Examiner, located in Baltimore, the state's lone medical examiner facility.<sup>1</sup> From January 2012 to July 2022, there were a total of 492 shotgun-related deaths (data for the remaining part of 2022 will be included in the final presentation). In 83% of the cases, the manner of death was suicide; the next most common manner of death was homicide, occurring in 16% of cases. Assessment of demographics of the decedents revealed most suicide deaths were White males (81% of total cases) and most homicide victims were African American males (51% of total cases). Females represented a small fraction of shotgun-related deaths (49 deaths in total). White females comprised 15% of homicides and 5% of suicides; African American females were homicide victims in 9% of cases. Incidents among females of other racial groups were exceedingly rare. The geographic distribution of deaths varied, but suicides were more concentrated in the rural areas, while homicides had higher frequencies in the urban and suburban sections of the state. The age of the decedents ranged from 14 years to 95 years old. For the homicide fatalities, injury to the torso was the cause of death in 72% of cases, while injury to the head was the cause of death in 80% of suicide cases. A spike in shotgun wound homicides in 2018 included a mass fatality resulting in five deaths.<sup>2</sup> Our results complement the few previous studies examining shotgun fatalities.

To conclude, shotgun-related deaths in Maryland largely involved White and African American males, with varied geographic and age distributions. While unable to be concealed, individuals may prefer shotguns due to ease of availability, relatively cheap cost of ammunition, and, in most states, lack of a permit requirement to own. Due to the variability of shotgun-compatible ammunition and wound patterns observed with shotgun fatalities, it is important for forensic pathologists and medicolegal investigators to remain familiar with these deaths and their distribution, particularly considering the current gun epidemic and dynamic gun restriction legislation.

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**Shotguns; Homicide; Suicide**

## I17 Astroglial and Microglial Reactions in Fatal Head Injury Decedents: A Survival-Time Correlation Study

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**Learning Objective:** After attending this presentation, attendees will have better understanding of the survival-time dependent alterations in astroglial GFAP and microglial CD-68 in fatally injured human brain tissue.

**Impact Statement:** This presentation will impact the forensic science community by updating the existing knowledge regarding survival-time dependent alterations in astroglial GFAP and microglial CD-68 in fatally injured human brain tissue.

Traumatic brain injury (TBI) is a major public health problem that is the leading cause of loss of mankind potential across all regions of the globe. Determining TBI as a cause of death during autopsy (aided with computed tomography) is a relatively easy task in routine forensic practice. Although it is challenging to determine the time span between TBI and time of death (survival time) of the deceased, it is significant in medic-legal investigations.<sup>1,2</sup>

The present study included 100 decedents, out of which 64 died due to closed TBI, 24 due to non-TBI (blunt trauma abdomen, etc.), and 12 control cases (without any injury). Corpus callosum and gray-white matter interface were collected and examined for histopathological grading and of expression of glial fibrillary acidic protein (GFAP) and CD-68 using immunohistochemical analysis. A younger population (20–29 years) was more vulnerable for traumatic events in both sTBI and non-TBI group. A higher number of males are more likely to be subjected to traumatic insult compared to females. The majority of patients were in deep coma (56.3%) with verbal response being the most hampered parameter.

Maximum changes were revealed in corpus callosum with greater histopathological grade compared to gray-white matter junction. Correlation of final histopathological score with patient survival time among sTBI patients revealed maximum changes in the chronic phase (>10 days) (mean score: 11.1) compared to the patients who died in the sub-acute (3–10 days) and acute phases (0–3 days) (mean score: 9.9 vs. 7.5). Minimal changes were observed in T1a (0–6hrs) (mean score: 6). The difference between various time cohorts was statistically significant.

Extensive gliosis was noted in cases with sTBI, which is more pronounced (Median: 139.5) as compared to non-TBI patients (Median: 44.5). None of the patients in the control group showed reactive gliosis. The difference in extent of gliosis was statistically significant among sTBI vs. non-TBI and control groups. The extent of gliosis was higher in the sub-acute phase of patient survival (Median: 183). However, non-significant difference for astrogliosis was observed among acute vs. sub-acute vs. chronic phase.

Compared to corpus callosum, astrogliosis was observed to be increasing with survival time from acute (T1) to chronic phase (T3) in gray-white matter interface (median 179.5 to 325.5). The majority of cases in the sTBI group (67.2%) showed macrophage proliferation, which was more prominent in the gray-white junction. Only 1 (4.2%) case in the non-TBI group exhibited this feature, while none of the cases in the control group showed activation of microglial cells. The difference in microglial reaction among sTBI vs. non-TBI vs. control group was statistically significant.

There was a significant increase in CD-68 positivity with survival time. The majority of the decedents who had survived up to the chronic phase 'T3' (>10 days) showed microglial/macrophage reaction with CD-68 positivity (83.3%), while only 56.7% in acute phase 'T1' (0-3 days) showed microglial proliferation.

Reactive astrocytic and microglial proliferation can be appreciated in decedents who died in the first three days. In the present study, significant inflammatory response was noted even in patients who died within six hours of injury. Thus, GFAP and CD-68 immunostaining may be utilized for the assessment of head injury patients in association with survival time. Forensic pathologists may utilize CNS tissue-specific biomarkers, which helps to illustrate the time-dependent changes in cellular mechanisms after severe brain insult in humans.<sup>3,4</sup> This further establishes the utility of pathological examinations in forensic practice, especially for unknown bodies with dubious histories.

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### Traumatic Brain Injury; Astroglial; Microglial

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**I18 Bigger Weapons Do Not Kill You More: A Dart Through the Head Is Sometimes Not Enough!**

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**Learning Objective:** After attending this presentation, attendees will be aware of the characteristics of the injuries produced by a particular weapon: the crossbow. They will also be informed about the effects of dart lesions and the possibility of non-lethal injuries, even on vital organs such as the brain.

**Impact Statement:** This presentation will impact the forensic science community by detailing the peculiarity of dart lesions and by clarifying how to distinguish them from firearms injuries, using not only the forensic findings but also radiological images and circumstantial evidence.

The crossbow is an ancient weapon, initially used in war, then replaced by firearms. It is now used for hunting and sports.<sup>1</sup> In Italy, it is not mandatory to have specific authorizations to buy it and use it. This instrument could be used as a weapon in homicides and suicides; it may happen that the injury produced by the dart is not lethal, even if inflicted on a vital organ such as the brain.<sup>2-4</sup>

On a late March afternoon, a man was found walking down his home stairs by his relatives, in a confused state and wearing only a pair of trousers improperly. Noticing some blood stains in the bathroom, they asked the man if he fell, and he confirmed their suspicion with a nod. An emergency team was called; the doctor discovered two small lesions on the left and right parietal regions of the head and ascertained a GCS of 12 and the presence of continuous purposeless movements. Therefore, the man was taken to the nearest hospital and was subjected to a cerebral CT scan, which revealed signs of bilateral frontal bleeding and swelling between the two cutaneous lesions, associated with pneumocephalus in the subarachnoid space. The radiological picture was interpreted as a possible lesion inflicted by a projectile. The day after, the neurological examination showed global aphasia and obtundation, hence another cerebral CT scan was taken, which detailed a decrease of the pneumocephalus and of the bleeding, because of an initial physiological recovery. The following week, the third CT scan showed a complete resorption of the internal lesions, thus, given the unaltered clinical picture, the patient began neurorehabilitation. At this stage, finally, the prosecutor requested a forensic investigation to clarify the nature of the injuries and the dynamic of the event. In the man's house, the police found several firearms and a crossbow, as well as a single damaged dart in the trash. Furthermore, it was discovered that, in the past few months, the man was diagnosed with a severe form of depression and had to be subjected to a psychiatric visit the same day of the accident. At the medicolegal visit, performed a week after the trauma, two small head scars were described: on the right temporo-parietal region, a wedge-shaped lesion of 0.4cm length on both sides; on the left fronto-temporal region, a triangular lesion of about 0.4 x 0.4 x 0.2cm. Given the patient's medical history and the characteristics of the injury, an attempted suicide was suspected; it was necessary to discern if the lesion was caused by a firearm or a crossbow. Even if a firearm injury characteristic varies depending on the distance of the shot, it is well known that the effect of the passage of a bullet in an organ produces not only local lesions, but also long-range damage.<sup>5</sup> Dart injuries are caused by a combination of cutting and stabbing of the organ, penetrating as deep as a bullet but without its kinetic energy and, consequently, without the long-range damage previously explained. Therefore, according to circumstantial evidence, the patient's clinical condition, and the radiological findings, it was possible to conclude that the man was not lethally injured by a dart, shot at close distance with a crossbow.

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**Crossbows; Attempted Suicide; Head Injury**

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**I19 Burial at Sea**

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**Learning Objective:** After attending this presentation, attendees will better understand the characteristic injury patterns seen in postmortem shark predation.

**Impact Statement:** This presentation is beneficial to the scientific community as it is a unique presentation that has not been previously commented on in the scientific literature to the best of the authors' knowledge. Determining an accurate cause and manner of death is often predicated on making sense of incomplete information. This requires prudent consideration of the facts gathered through the collaboration of individuals and agencies. In forensic cases involving trauma, establishing whether the injury occurred antemortem or postmortem can dramatically influence the cause and manner of death.

A fisherman approximately 4.5 miles off the east coast of Florida found a body floating face down. The Coast Guard recovered the remains of a 48-year-old White male. Autopsy revealed 50–60% soft tissue loss, lacerations, abrasions, contusions, incised bloodless wounds, amputation of the upper extremities, and partial truncal evisceration. The head was intact with no significant external trauma; interestingly, an acute liquid right subdural hemorrhage was noted. Was this a homicide tossed into the sea, an accidental boat injury, perhaps a suicide? Many questions surrounding this case needed to be resolved.

Findings at autopsy are characteristic of postmortem shark predation. Shark bites share many distinctive features such as punctures from teeth, extensive tissue loss, and even fractures of large bones.<sup>2</sup> Additionally, a series of ragged-edged, roughly parallel lacerations that may overlap to form a crescent or arc shaped perimeter are frequently appreciated. Often two distinct series of arcs caused by the parallel rows of teeth around a mass of tissue that has been completely excised are found.<sup>3</sup> Bite marks on the bone are typified by roughly parallel linear grooves.

Shark predation of postmortem tissue lacks vitality as is observed by the complete absence of hemorrhage or erythema of the wound edges.<sup>2</sup> Shark's triangular teeth come to a point at the apical aspect and have fine serrations along the edges. In addition to punctures caused by the apical end, the serrations can leave multiple, more delicate linear striations on bone.<sup>1</sup> Sharks often violently shake their heads from side to side after biting tissue, whereby the serrated teeth first puncture and then act as a saw to cut through tissue. This thrashing back and forth motion was the likely explanation for the subdural hematoma identified at autopsy.

Detectives later discovered that the decedent had suffered from amyotrophic lateral sclerosis and had died three days prior. It had been his wish to be buried at sea. His spouse fashioned a body bag from heavy canvas material, added weights totaling 150 pounds and lashed everything together with rope. She placed the unembalmed decedent on ice and made the two-day drive to Fort Lauderdale to meet with the boat captain who drove them roughly three miles offshore and he was buried at sea.

This case is a reminder that decedents have much to teach us if we choose to be receptive.<sup>1</sup>

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**Shark; Postmortem; Burial**

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**I20     Decapitation and Crushing by Combine Harvester: An Infernal Machine**

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**Learning Objective:** Following the advent of agricultural industrialization, more and more accidents occur in the workplace every year. The injuries produced by agricultural machines can be widely destructive. In investigating such cases, it is fundamental to know how the machines work to interpret the lesion features correctly. This work proposes an unusual case of a fatal decapitation caused by a combine harvester, a sophisticated, self-propelled farm machine with a header unit for crop cutting and a threshing unit to convey cut materials. After attending this presentation, the attendees will understand that accidents with agricultural vehicles can produce highly devastating and specific injuries that depend on the type of machine.

**Impact Statement:** This work highlights the importance of forensic inspection at the crime scene to understand the compatibility between injuries and machinery action. Moreover, in this case, the collaboration between pathologists and engineers was essential to recreate the dynamics of this rare decapitation accident by a combine harvester.

A 54-year-old man was found decapitated in a wheat field atop the header of a combine harvester, trapped between the cochlea and the cutter bars of the machine's head. His right elbow was flexed ~90 degrees, the forearm resting beneath his torso. The left upper limb was situated entirely under the trunk. Both legs were mixed with fragmented vegetation, wedged between the rollers, and the header was extensively covered in blood. According to his son's deposition, the worker had intended to harvest the wheat. Upon external examination, the head was severed at the level of the first cervical vertebra. The cut was clean with infiltrated margins, likely inflicted by a sharp and highly kinetic blade. Soft tissues of the trachea and esophagus appeared fully transected. There were numerous displaced and open fractures of the left arm, while the right arm showed diffuse bruising but was intact. Both lower limbs were severely mutilated, reflecting the rotational force applied to the longitudinal body axis, and the left leg was amputated. A bleeding penetrating wound to the back was additionally noted. A second forensic inspection found the completely mangled head and left leg remnants in the grain tank and straw walker, respectively.

A combine harvester is a sophisticated, self-propelled farm machine with a header unit for crop cutting and a threshing unit to convey cut materials.<sup>1,2</sup> The cochlea is a rotating element (152 revolutions per minute) along the longitudinal axis located in the header.<sup>3</sup> A straw walker ultimately separates the chaff from clean grains, transporting the latter to a storage tank.<sup>4</sup> In this case, all observed injuries were compatible with the mechanics of the cochlea, which severed the neck in an oblique plane, decapitating the man through one fast rotation. His legs were also consumed, and the violent rotary action caused severe longitudinal axis mutilation.

Our reconstruction of the event indicates that first the man hit his back in a pointed element of the header, producing the penetrating wound; then he rolled off, falling into the cochlea that decapitated him and destroyed the extremities by its rotating movement.

Complex and grossly contaminated lacerations, severe friction burns, crush injuries with the fracturing of digits, significant amputations, skin avulsions, and soft-tissue tears are characteristic in this setting. Similar accidents have been reported to date, but decapitation by combine harvester has yet to be described.<sup>5-6</sup>

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**Decapitation; Combine Harvester; Work Accident**

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**I21 A Review of Violent and Unnatural Deaths in Which the Manner of Death Is Undetermined**

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**Learning Objective:** After attending this presentation, attendees will understand that violent and unnatural deaths with undetermined manner of death are largely due to the increasing complexity of violent and unnatural deaths and are rarely re-categorized despite review.

**Impact Statement:** There is currently very limited literature available characterizing violent and unnatural deaths with undetermined manner of death. This presentation will impact the forensic science community by characterizing a rare subset of undetermined deaths.

According to the United States Bureau of Justice Statistics, in 2018, there were more than 1.3 million deaths referred to medical examiner offices, with 605,000 accepted for further investigation; a small portion of those deaths were classified as undetermined.

The standard death certificate reports both a cause and manner of death. In most states, the acceptable options for manner of death classification are natural, accident, suicide, homicide, and undetermined. A manner or cause of death is reported as undetermined or could not be determined in the forensic setting when evidence equally supports multiple conclusions despite a thorough autopsy and investigation.

Between January 1, 2021, and December 31, 2021, at the West Tennessee Regional Forensic Center, there were a total of 1,731 autopsies and 575 external exams performed. Of these, there were 138 cases with either manner of death (74 cases) or both cause and manner of death (64 cases) marked undetermined. Strikingly, 21 of the 74 cases with only the manner of death undetermined (36% of undetermined manner cases) had a violent or unnatural cause of death, with 12 gunshot wound cases, 8 blunt force trauma cases, and 1 stab wound case.

Of the 64 deaths with undetermined cause and undetermined manner of death, there were no cases involving violent or unnatural causes. The aim of this study was to review the violent or unnatural cases with undetermined manner of death to determine if any of the cases could be reclassified.

The cases were reviewed by at least two board-certified forensic pathologists and one forensic pathology fellow. Components of the case that were reviewed include histology, toxicology, scene and autopsy photos, police investigative information, and medical records (if relevant). Any additional investigative information that had become available in the interim was also reviewed. The reviewing pathologists were then given the option to agree, disagree, or state additional information is required. After review, there was minimal variation from the original case classification.

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**Undetermined Manner of Death; Violent Deaths; Unnatural Deaths**



### **I22     A New Approach on the Timing of a Dynamic Process: The Pulmonary Thromboembolism**

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**WITHDRAWN**



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**123 Certification of Deaths of Persons With Reported Histories of Seizures or Epilepsy**

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**Learning Objective:** After attending this presentation, attendees will be aware of how often seizures, epilepsy, and seizure-like activity are reported by Medical Examiner Investigators (MEIs) in death investigation narratives and the overall frequency of these factors as a cause of or contributing factor to death.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the importance of obtaining an accurate medical history. It will reveal how often a reported history of seizures is medically pertinent through comparison of stated seizure history to certified cause of death/contributory factors.

Many individuals report a history of seizures, though often the etiology and even the true diagnosis is unclear to their families. The National Association of Medical Examiners (NAME) has published a position paper recommending guidelines for the investigation and certification of deaths in persons with epilepsy, most of which fall under the jurisdiction of the medical examiner/coroner.<sup>1</sup> This study investigated the frequency at which decedents with a reported history of seizures/epilepsy have this condition listed as a cause of death or contributing factor in their death.

The electronic death investigation database was queried for all cases in which the MEI narrative contained “seizure” and/or “epilepsy” that were investigated and certified by the Medical Examiner’s Office between January 2018 and December 2021. These cases were manually reviewed to confirm that the decedent had a reported history of either of those conditions. The cause of death fields (Parts I a-d and Part II) of the death certificate for the same time period were then searched for the same terms. The results of the first and second queries were compared to investigate the frequency at which these conditions were contributory to death. Furthermore, decedent demographics, extent of postmortem examination, manner of death, and injury circumstances (if applicable) were documented.

A review of MEI death investigation narratives revealed 487 decedent cases. Eighty cases were excluded, as seizures or epilepsy were either listed as a pertinent negative or an anti-epileptic medication was mentioned without a documented history of epilepsy or a seizure disorder. Additionally, 76 decedents had a history of terminal seizure (e.g., cardiac arrest with seizure-like activity noted at or around the time of the event). The majority of cases (331 decedents) had a documented reported history of seizure/epilepsy that was either mentioned by witnesses or family or reported in medical history that was available to the MEI.

A review of death certificates certified by the Medical Examiner’s Office revealed 78 individuals with seizures and/or epilepsy listed as contributory to death. These included 2 of the 76 of those classified as having a terminal seizure (2.6%) and 70 of 331 of those with documented reported history of seizure/epilepsy (21.1%). An additional 6 decedents not captured on the initial query of the MEI death investigation narrative were discovered via death certificate review.

Deaths of individuals with a history of seizures or epilepsy often fall under the jurisdiction of the medical examiner/coroner. In this study, roughly one-fifth of those decedents with that reported history had it either cause or contribute to their death. Acquisition of records regarding the seizure timeline, etiology, characteristics, and other pertinent information helps corroborate witness/family statements and aids in the accurate certification of cause and manner of death in persons with epilepsy.

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**Seizure; Epilepsy; Cause of Death**

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**I24 Neuropathology of Middle Meningeal Artery Embolization for Subdural Hematoma**

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**Learning Objective:** The goal of this presentation is to increase the understanding of the potential progression of subdural bleeding post-middle meningeal artery embolization.

**Impact Statement:** This presentation will impact the forensic science community by leading to a better understanding of the re-bleed of subdural hemorrhage in the cases of blunt impact of the head observed during the forensic autopsy.

**Background:** Embolization of the middle meningeal artery (MMA) has been employed over the past decade as a minimally invasive procedure to reduce the risk of re-bleeding of traumatic subdural hematoma (SDH). Published reports of the direct neuropathological tissue examination following this procedure are few.

**Objective:** Our goal was to outline the neuropathologic findings following MMA embolization among decedents with SDH who were autopsied at the New York City Office of Chief Medical Examiner to ascertain: (1) the presence and distribution of embolic material in the MMA branches; and (2) whether there was any acute re-bleeding of the SDH.

**Materials and methods:** Over a 13-month period, the Neuropathology Service at our agency examined two cases of MMA embolization macroscopically and microscopically with consideration of available medical records and other autopsy findings.

**Results:** Case 1 is a 71-year-old man with a history of metastatic small cell carcinoma of the lung, treated with irradiation, including to the brain, with resultant parkinsonian symptoms. Thirty-seven days prior to death, he fell and had SDH, treated 4 days later with bilateral MMA embolization. He had another fall 2 weeks after the embolization procedure, and a CT scan at that time showed persistent bilateral frontal convexity subdural hematomas. He died 33 days post-procedure from choking on a bolus of food. Autopsy revealed radiation-related gliosis and neuronal loss in the substantia nigra, and cerebrovascular disease. There were bilateral convexity organizing SDH, consisting of diffuse, dark red-brown neomembranes measuring less than 3mm in thickness, with slightly granular adherent red blood. Microscopy confirmed intact and degenerating red blood cells, and established an early neomembrane formation including microvessel formation, fibrin deposition, macrophage infiltration, and iron deposition. The embolization material was seen within a subset of branches of the right and left MMA and was focally associated with giant cell reaction. Case 2 is a 62-year-old man with a history of alcohol use disorder, esophageal cancer, and chronic kidney disease who was admitted to the hospital with blunt impact injury of the head following an assault. A CT scan showed a 9mm acute SDH along the left inferior frontotemporal convexity. On hospital day 16, he experienced a decline in mental status, and repeat CT showed interval increase in the SDH to 18mm. He underwent left burr hole creation and insertion of a subdural evacuation port system, followed by embolization of the ipsilateral MMA 3 days later. His subdural hemorrhage stabilized clinically, but he continued to decline due to his other medical comorbidities, including urinary tract infection and aspiration pneumonia, and died 3 months after post-embolization. Autopsy confirmed chronic SDH with neomembrane formation over the bilateral convexities, most pronounced on the left inferior frontotemporal surface. The dura and neomembrane measured up to 5mm in thickness, with focal recent hemorrhage. Microscopically, embolization material was present within the MMA branches, and in neovessels of the neomembrane, as well as free in the subdural space, with associated foreign body giant cell reaction.

**Discussion:** In two decedents autopsied 1 to 3 months following MMA embolization for traumatic SDH, we found neuropathologic evidence of intra-arterial foreign (embolization) material in a subset of dural artery branches. Moreover, focal recent hemorrhage was superimposed upon old and organizing neomembranes, indicating a component of re-bleeding, at least in part from neovessels already having arisen from the middle meningeal artery before embolization (although additional bleeding from bridging veins [i.e., the cause of the initial SDH, cannot be excluded]). Our findings contribute to the understanding of the potential progression of subdural bleeding post-MMA embolization. Additional neuropathologic analyses of such cases are required.

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**Middle Meningeal Artery; Trauma; Subdural Hemorrhage**

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**I25 COVID-19-Related Myocarditis: Are We There Yet?**

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**Learning Objective:** Attendees will learn the effects on myocardium of COVID-19 infection in a rare case of fulminant myocarditis after a complete histopathological study.

**Impact Statement:** This presentation will impact the forensic science community by presenting a rare case of a histologically confirmed myocarditis with evidence of SARS-CoV-2 myocardial infection through quantitative RT-PCR in a patient who likely died from COVID-related fulminant myocarditis

Since the pandemic outbreak, both a higher mortality among patients with previous cardiovascular disease and the potential cardiac involvement related to COVID-19 have been reported. Although severe COVID-19 primarily occurs with fever and very serious respiratory symptoms, an elevated incidence of myocardial injury, as defined by blood levels of cardiac biomarkers above the 99<sup>th</sup> percentile upper reference limit, has been observed. The rise of cardiac biomarkers is associated with cardiac dysfunction and malignant arrhythmias, representing an independent predictor of worse prognosis and fatal outcome. The exact mechanisms beyond the biomarker's elevation have been long debated.

To date, it is still unclear whether SARS-CoV-2 may cause a myocarditis-like disease through cardiac infection and direct cytotoxicity or if myocardial damage is attributable only to an excessive proinflammatory response as well as to a mismatch between oxygen demand and supply as previously observed in other critically ill patients. Apart from the lack of robust pathological evidence of myocarditis due to SARS-CoV-2 direct myocardial damage, clinical diagnosis of myocarditis in COVID-19 patients is challenging due to overlapping symptoms and signs between myocarditis and other forms of myocardial injury, the lack of electrocardiographic/echocardiographic changes, and the difficult access to CMR for many patients positive for SARS-CoV-2.

The death of a COVID-19 unvaccinated 30-year-old woman without significant medical history with symptoms of fever, chills, persistent cough, headache, and sore throat is presented. An empirical treatment with oral amoxicillin-clavulanate and paracetamol was started and a nasopharyngeal swab sample for SARS-CoV-2 testing was positive. The physical examination performed by the physician was unremarkable; the peripheral oxygen saturation at rest and during the six-minute walk were 98% and 100%, respectively. Two days later, the patient experienced a sudden onset of profuse sweating and cold extremities and alerted the emergency medical service. The patient was found to be afebrile, with normal vital signs. The patient refused to be transferred to the Emergency Department (ED) for further care and monitoring. The following day, the patient and her husband presented to a local hospital because of clinical deterioration, consisting of progressive dyspnea and consciousness impairment. On arrival at the ED, the patient's condition suddenly precipitated, and she developed pulseless cardiorespiratory arrest. After 20 minutes of unsuccessful attempts at cardiopulmonary resuscitation (CPR), reanimation maneuvers were stopped.

To establish the precise cause of death, a complete postmortem examination was performed. Gross examination revealed the presence of heavy, congested, and edematous lungs (right 630gr, left 500gr). Pulmonary embolism was excluded. The heart weighed 300gr, with subepicardial petechiae in the anterior and posterior wall of the left ventricle. The coronary examination was unremarkable as well as the valve apparatus. Gross examination of other organs was normal. Samples of organs were fixed in formalin, then embedded in paraffin. Massive pulmonary edema and mild interstitial lymphocytic infiltration were observed bilaterally. Features of diffuse alveolar damage were excluded as well as pneumocytes hyperplasia and fibrin thrombi. The heart presented hypercontraction of the myocytes. Mild infiltration of macrophages and lymphocytes as well as erythrocyte extravasation were observed in the myocardial interstitium. One-micron sections were processed for immunohistochemistry staining: antibodies against SARS-CoV-2 spike protein were tested in heart specimens in which focal lymphocyte infiltration was identified at histological analysis. Infiltrates were further characterized by immunohistochemical staining for CD68, CD45, CD3, CD8, and CD4 performed on an automated staining device. Lymphocytic infiltration was characterized from a predominance of CD4+ T lymphocytes. *In situ* hybridization (ISH) was performed. Finally, real time-polymerase chain reaction (RT-PCR) was extracted from 8µm sections, which were deparaffinized and digested.

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**COVID-19; Myocarditis; Autopsy**

## I26 Is It Really a Channelopathy? A Systematic Review of the Alterations Found at Autopsy in Brugada Syndrome

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**Learning Objective:** This presentation will show and discuss the results of a systematic review on the histopathological features recurring in Brugada syndrome (BrS) cases. The core message of the presentation will be that, albeit BrS is not a classical channelopathy from a forensic point of view (having recurring microscopic alterations), current evidence advises against its reclassification (for instance, as a cardiomyopathy) and stresses the importance of a multidisciplinary forensic approach to avoid missed diagnosis and misdiagnosis.<sup>1</sup>

**Impact Statement:** This presentation will have an impact on the forensic science community as the observation of structural heart anomalies at autopsy does not justify the exclusion of BrS as a possible diagnosis, and, on the other hand, the identification of these findings in some cases highlights the potential complex interplay between these structural alterations and ion channel dysfunction.

Brugada Syndrome (BrS) is traditionally classified as an inherited cardiac channelopathy and thus as a congenital anomaly that should not show microscopic or macroscopic alterations at forensic autopsy. Fatal BrS cases are of great medicolegal interest, because this syndrome is a leading cause of sudden cardiac death in young healthy adults and its diagnosis may be easily missed. Indeed, its first clinical manifestation is represented by fatal arrhythmias, that can be triggered by licit and illicit drugs and can be prevented through specific interventions. Albeit it is classified as a channelopathy, BrS may be associated with ill-defined/slight histologic abnormalities at the autopsy (in particular, myocardial inflammatory infiltrates and fibrosis). These findings are not specific and often difficult to interpret, and, without a molecular autopsy (i.e., postmortem genetic testing), the correct diagnosis is not possible. Since some microscopic features can mimic arrhythmogenic cardiomyopathy (ACM), some authors suggested that BrS could be a phenotypic variant of ACM.

We performed a comprehensive review of the literature following PRISMA criteria focusing on the reported macroscopic and microscopic structural alterations in BrS, observed in explanted hearts, autopsy samples and endomyocardial biopsies. The aim was to evaluate if the current classification of BrS as a channelopathy is appropriate from a pathological point of view. Our systematic review found that there currently is not enough evidence to reclassify BrS as a cardiomyopathy. At the same time, because of the risk of missed diagnosis or misdiagnosis, our evidence strongly stresses the importance of a multidisciplinary approach to sudden deaths. In particular, when suggestive microscopic alterations (like inflammation and/or fibrosis in the myocardium of the right ventricle outflow tract) occur, the pathologist should carefully analyze them and indicate second-line testing, like molecular autopsy (performed through next-generation sequencing). This recommendation is important also for research purposes, because research on BrS has traditionally focused on its clinical aspects but a better understanding of its possible microscopic features (like chronic inflammation) may have a relevant translational value, making it possible to develop new primary prevention interventions and new therapies.

### Reference:

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### Brugada Syndrome; Sudden Cardiac Death; Molecular Autopsy

## 127 Subnuclear Renal Tubular Vacuoles in Alcoholics

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**Learning Objective:** Attendees will learn that subnuclear renal vacuoles can be seen on renal histology and how they are related to ketoacidosis. They will learn what types of cases exhibit these changes and how specific they are for ketoacidosis in deaths in alcoholics.

**Impact Statement:** This presentation will impact the forensic science community by showing the characteristics of alcoholic deaths at autopsy associated with subnuclear vacuoles, how these histologic changes are related to deaths in alcoholics and their specificity for deaths from alcoholic ketoacidosis.

Subnuclear vacuoles in the proximal renal tubules have been reported as a histologic sign of ketoacidosis.<sup>1-4</sup> First reported in diabetic ketoacidosis, they have also been reported in other ketoacidotic conditions, including alcoholic ketoacidosis, starvation, and hypothermia.<sup>2,5,6</sup> Ketoacidosis at autopsy can be diagnosed by examination of blood and vitreous ketone bodies, particularly betahydroxybutyrate (BHB).<sup>7</sup>

**Aims:** To examine the incidence of subnuclear vacuoles in deaths of alcoholics and their specificity for deaths from ketoacidosis. To also determine what demographic, biochemical, and pathologic findings are associated with subnuclear vacuoles.

**Methods:** A retrospective analysis of alcoholic deaths examined at autopsy at the Eastern Ontario Forensic Pathology Unit between 2017 and 2020 was undertaken. Deaths in patients with poorly controlled diabetes mellitus, diabetic ketoacidosis, and hypothermia were excluded, along with decomposed bodies, deaths under 18 years, and those with prolonged hospitalization. Each case underwent vitreous humor biochemical analysis, including electrolytes, glucose and BHB, postmortem blood HbA1c, as well as liver and renal histology. Where analytic data were absent, these cases were also excluded. Histology of the kidney was graded for renal tubular vacuolation on a score of absent (0), scanty (1), and easy to identify (2). Liver histology with additional Masson trichrome stains was graded for steatosis on a scale from absent to severe, and for fibrosis from absent to cirrhosis. The presence or absence of glycogenated hepatocyte nuclei was also scored. Demographic characteristics and blood ethanol concentrations were recorded, along with BMI, heart weight, liver weight, and combined kidney weights. Cases with and without vacuoles were compared with respect to these demographic, biochemical, and pathologic findings.

**Results:** One hundred thirty-three cases were analyzed: 63 cases had no renal vacuoles, 45 had scanty vacuoles, and 25 had easily identified renal vacuoles. Grade 0 median BHB was 1.39mmol/L (range 0.14-5.4), grade 1 was 1.31mmol/L (range 0.22-3.57), grade 2 was 2.06mmol/L (range 0.67-18.02). Of deaths certified as alcoholic ketoacidosis, 1 case had no renal vacuoles (1.6% of grade 0), 4 cases had scanty vacuoles (8.9% of grade 1 cases), and 8 cases (32%) had grade 2 vacuoles. Grade 2 vacuoles were seen in non-ketoacidosis deaths but were most commonly seen in deaths classified as complications of alcoholism (including ketoacidosis) compared with other types of death.

Lower vitreous sodium (139 vs. 142mmol/L;  $p=0.005$ ), higher vitreous BHB (1.50 vs. 1.39mmol/L;  $p=0.04$ ), more severe hepatic steatosis, and more severe hepatic fibrosis were seen in cases with vacuoles (grades 1 and 2) compared to cases without vacuoles (grade 0). No statistically significant differences were found between the two groups when the other recorded variables were compared. Following logistic regression, vitreous sodium (OR 0.95), vitreous BHB (OR 1.44), and severe hepatic steatosis (OR 4.04) were found to be independent predictors for subnuclear vacuoles.

**Conclusions:** Vacuoles were commonly seen in the deaths of alcoholics. While typically reported in deaths from diabetic and alcoholic ketoacidosis, vacuoles may be seen in non-ketoacidotic deaths, including those scored as easily identifiable. When vacuoles were present, the liver typically had severe fatty change on histology; vitreous sodium was lower, and vitreous BHB was higher. While previous episodes of non-fatal ketoacidosis cannot be excluded, the presence of renal vacuoles alone cannot be used to diagnose fatal ketoacidosis at autopsy. As grade 2 vacuoles are more commonly seen in alcoholic ketoacidosis, when seen on histology, measurement of BHB levels in blood or vitreous should be considered to determine if fatal ketoacidosis was present.

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### Alcoholic; Kidney; Histology



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**I28     Updates in Normal Organ Weights: A Review of 4,000+ Cases From 2009 to 2014**

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**Learning Objective:** Attendees will be aware of updated reference material for autopsy organ weights. They will understand the limitations of prior reference tables, the methods used to create the updated tables, and advantages/limitations to the updated tables. Additional, previously unpublished data will be included in the presentation to reinforce these learning objectives.

**Impact Statement:** Accurate reference tables are important for reliable reporting of routine autopsy information. Dissemination of this robust reference material is important for the production of high-quality autopsy reports that draw accurate comparative conclusions about such data as organ weights.

Organ weights are an essential part of autopsy analysis. Deviations from normal organ weights provide important clues to disease processes. The quality of such inferences, however, depends in large part on the reliability and accuracy of the reference tables. There are at least 14 commonly used organ reference tables, and the most frequently used tables typically date to the mid-century. It is well established that, for many organs, normal organ weights vary directly with body weight and yet there have been significant changes in height and weight distributions in the past 50 years. Additionally, historic standard organ weight tables relied heavily on hospital-based populations or on tissue bank material that presented a significant selection bias. While some groups have tried to update the tables from forensic files, these have suffered from small sample sizes.

Herein, we provide an updated reference table of organ weights based on contemporary sources and a large sample size. Organ weights, body weight, and body length were collected from 4,197 carefully screened autopsies performed on adults in a combined forensic and hospital autopsy setting for decedents aged 18 years or older from 2009-2014. Grossly abnormal organs (visibly edematous, putrefied, deformed, injured, etc.), and patients hospitalized for more than two days were excluded. There were no BMI criteria, in order to reflect data which is representative of the population rather than the desirable target values of the population. All cases were performed with 24 hours of death.

Of the 4,197 decedents in the study, 2,999 (71.5%) were male and 1,198 (28.5%) were female. There was a higher percentage of women in the lower weight categories. Two reference tables were generated, first by sex and body weight ranges and second by sex and age range categories. Height and body weight data in this study reflect the well-recognized increases in both variables, but most particularly in body weight, seen during the past decades. The study data show a strong positive association between organ weight and body weight for the heart, liver, and spleen. There is a similar but weaker association between body weight and the weight of the lungs and kidneys. Brain weight is independent of body weight but shows a strong negative association with age. Even when controlling for body weight, men's organs are heavier, except for the weight of the liver, which is comparable in men and women. The current study suggests that, for some of the commonly weighed organs, there has been an increase in median organ weight when compared with existing references.

The tables presented provide an updated reference tool that is useful to autopsy pathologists in forensic and hospital settings.

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**Autopsy; Reference Material; Organ Weights**

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**I29     Discordance of Reported History and Diagnosis of Mental Illness in Suicides**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the impact mental health diagnoses can have on vital health care statistics concerning suicide.

**Impact Statement:** This presentation will impact the forensic science community by analyzing the importance of coroner/medical examiner access to medical records when completing a death certificate for a suicide.

Suicide is a manner of death that the medical community often attributes to mental illness. Coroners/medical examiners investigate these deaths by documenting the evidence found in an examination and in reports from the loved ones of the deceased. After obtaining this information, the death is categorized into one of five manners of death. In cases of suicide, friends and family often report that their loved one experienced some form of mental illness prior to their death. However, coroners/medical examiners seldom have access to medical records to confirm these reports, and therefore are unable to collect accurate data on the correlations between mental health diagnoses and the completion of suicide. The portion of the death certificate detailing contributing factors to a death is rarely utilized, in most cases being left blank.<sup>1</sup> Especially in cases of suicide, coroners and medical examiners do not have the necessary resources to complete this portion of the certificate with accuracy and confidence. A standardized system of access to medical records would benefit the medicolegal professionals who are responsible for completing the death certificate and furthermore benefit vital health statistics on the rates of suicide in those with diagnosed mental illness. For this study, data was collected on the decedents with a manner of death of suicide investigated by the Jefferson County Coroner/Medical Examiner's Office (JCCMEO) from January 2013 to May 2021. The JCCMEO database contains "narrative reports" of circumstances surrounding deaths as well as pertinent medical history (including reports of mental health, if applicable). This is often obtained from family members and friends. The University of Alabama at Birmingham (UAB) Hospital electronic health record was then queried for diagnoses of mental illness. This study is limited in that it includes only the decedents who had records of a previous visit to UAB Hospital and therefore had the opportunity to be diagnosed.

A total of 758 patients with a manner of death of suicide were identified in the JCCMEO system from January 2013 to May 2021. Thirty-seven percent (278) of these have a medical record at UAB. Of those 278, 2% were reported to have PTSD while 6% had a diagnosis of PTSD documented at UAB. Forty-one percent were reported to have depression, while only 38% had an official diagnosis documented. Concerning bipolar disorder, 7% were reported to JCCMEO and 9% were diagnosed. When comparing the individuals who had a reported history of mental illness and a documented diagnosis, very little concordance was seen (1% for PTSD, 21% for depression, and 4% for bipolar disorder).

The JCCMEO, and many medical examiner offices across the country, only have access to the reported history of decedents when completing the death certificate. Our findings show that these reports often do not align with the diagnoses of medical professionals at our institution, which is a major tertiary care facility for the county but not the only source of health care. Access to electronic medical records would allow for greater confidence when tasked with assessing the contribution of a mental health illness on suicide completion. Including mental health diagnoses as a contributing factor to suicide on a death certificate would benefit health statistics and offer a more accurate representation of the effects of mental illness in our society. Additional studies concerning the confidentiality of this portion of the death certificate would ensure privacy for a decedent's family.

**Reference:**

- <sup>1</sup>. Hoff, C. J. B., & Ratard, R. (2010). Louisiana death certificate accuracy: a concern for the public's health. *The Journal of the Louisiana State Medical Society*, 162(6), 350+.

**Suicide; Mental Illness; Death Certificate**



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**I30 Fatal Injuries in Traffic Accidents: A Retrospective Analysis of 294 Medicolegal Autopsies at the University Hospital of Montpellier**

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**Learning Objective:** The goal of this presentation is to identify the main causes of death in fatal motor vehicle accidents, detect possible differences in injury pattern between victims, and show the utility of the AIS as a valuable tool for classification of lethal injuries.

**Impact Statement:** This presentation will impact the forensic science community by showing the utility of systematic autopsies after fatal motor vehicle accidents (MVA) and establishing an international protocol for using the Abbreviated Injury Scale (AIS) score as a valuable tool of forensic comparison (in traumatic deaths).

**Introduction:** A motor vehicle accident (MVA) victim is someone who gets injured in a traffic crash. A motor vehicle accident involves at least one vehicle on a lane open to public traffic, regardless of the causal event, except in the cases of intentional acts of suicide or homicide. All deaths occurring immediately or within 30 days of the accident will be considered an MVA death. Road safety is an important public health issue since the overall cost of road safety is in the range of billions of euros.

**Aim:** The aim of the study was to identify fatal injuries involving victims of motor vehicle accidents.

**Material and methods:** Two hundred ninety-four autopsy cases were analyzed and for each of the lesions described the AIS score (Abbreviated Injury Scale, score used to assess the severity of the lesions) was calculated. They are considered fatal from stage 4 to 6 (life-threatening lesions).

**Results:** Thoracic (90.1%) and cranio-cerebral (84%) traumas were the most encountered. All victims respected this distribution except the motor bikers in whom the abdomino-pelvic region was the second-most affected part of the body. All the cyclists had cranio-cerebral injuries, but they were mostly found in pedestrians if we compare all the victims. The most serious injuries were most commonly found in car drivers and then pedestrians.

**Conclusion:** Our autopsy data is consistent with those of the literature concerning the predominance of fatal thoracic and cranio-encephalic lesions during motor vehicle accidents. A complete injury assessment during these deaths is now essential to confirm data of judicial investigation, to deepen the understanding of the accidents, and to study preventable deaths. We will discuss the importance of using the AIS score. It could be a valuable tool for documentation and comparison of fatal injuries. Documented causes of death should systematically be included in the accident report.

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**Forensic Autopsies; Motor Vehicle Accidents; Fatal Injuries**

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**I31 Fatalities at the Astroworld® Music Festival in Houston, Texas**

*Hannah Jarvis, MRCS\*, Harris County Institute of Forensic Sciences, Rosharon, TX; Merrill Hines, MD, Harris County Institute of Forensic Sciences, Houston, TX*

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**Learning Objective:** After attending this presentation, attendees will have learned about the mass fatality event that occurred at the Astroworld® Music Festival In Houston, TX, in 2021 to include factors that contributed to the disaster, autopsy findings, and a review of the cause and manner of death.

**Impact Statement:** This presentation will impact the forensic science community by reviewing a mass fatality event, including a timeline, and discussing the role of the medical examiner, challenges encountered, autopsy findings, and mechanism of death associated with compression asphyxia.

The Astroworld® Music Festival is an annual outdoor music festival held in Houston, TX, since 2018. In 2021, the two-day music festival opened on November 5, with 50,000 tickets sold. Fans began lining up at 5:00 a.m. and by 8:15 a.m. police in riot gear were requested due to unruly crowd behavior. At 9:15 a.m., the main entrance gate was breached by a crowd surge, and over the course of the morning the atmosphere became increasingly volatile, resulting in multiple injuries requiring medical attention. By mid-afternoon, multiple other checkpoints had been breached. Concertgoers positioned themselves within four viewing areas around the main stage. Each area had one way in, one way out, and was contained by metal barriers on three sides. Shortly before Travis Scott took to the stage at 9:00 p.m., an uncontrolled surge of people entered these contained viewing areas, resulting in a tightly packed crowd, which worsened as the music began. This push combined with the rotations of concertgoers created a swirl of motion that moved throughout the crowd, resulting in eight fatalities and hundreds of injuries. Two concertgoers who were resuscitated later died in the intensive care unit. Seven of the fatalities occurred in the south quadrant viewing area, thought to be one of the most tightly packed.

Of the ten fatalities, seven were male and three were female; and the average age was 19.6 years, ranging from 9 to 27 years. Four were White, three were Hispanic, two were Asian, and one was Black. The average height was 67.95 inches (range: 60 to 73 inches); and the average weight was 225.1 pounds (range: 83 to 498 pounds). Eight cases were pronounced dead at the hospital following resuscitative efforts. Two cases had a survival interval of 6 and 9 days, respectively. Toxicological analysis revealed illicit substances (cocaine and methamphetamine) with ethanol in one case; one case had sertraline; and the two cases that were hospitalized showed therapeutic drugs. All cases underwent autopsy examination, and all demonstrated findings such as facial plethora, petechial hemorrhages, and cutaneous blunt trauma (abrasions, contusions, lacerations). The cause of death was compression asphyxia in all ten cases, with combined drug toxicity of cocaine, methamphetamine, and ethanol as a contributory cause of death in one case. The manner of death in all cases was Accident.

The management of a mass fatality event in a medical examiner's office requires a prepared plan of action and coordinated implementation. Initial circumstantial information may be sparse and evolving; accurate identification of decedents and notification of families is crucial. Mass fatalities related to crushing events in a crowd have previously occurred in sporting and religious festivals, notably at the Hillsborough Football Stadium disaster in 1989 (96 fatalities) and in 2013 during a Hindu festival in Navratri, India (115 fatalities).<sup>1</sup> Compression asphyxia occurs when a force is applied in the anteroposterior plane of the body, resulting in an inability of the lungs to expand, progressively causing hypoxemia. This increase in intrathoracic pressure also results in reduced venous return to the heart, causing reduced cardiac output, and hypotension. This combination can result in loss of consciousness—which may be as rapid as one or two minutes—cardiac arrest, and subsequent death. Sustained compression while standing, due to pushing and people leaning on each other, and in the majority all of the fatalities, was compounded by collapse and subsequent compression while on the ground. Many lessons have been learned from Astroworld® and other mass fatality crowd events, such as the need for a plan to manage crowds and the risks individuals pose to one another.

**Reference:**

- <sup>1</sup>. Nolan JP, Soar J, Cary N, et al. Compression asphyxia and other clinicopathological findings from the Hillsborough Stadium disaster. *Emergency Medicine Journal* 2021;38:798-802.

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**Astroworld®; Mass Fatality Event; Compression Asphyxia**

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**I32 Female Homicide Victims in Cook County, Illinois: A Retrospective Review**

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**Learning Objective:** After attending this presentation, attendees will have better knowledge of the characteristics of female homicides in a large metropolitan area to emphasize the relevance of this phenomenon and its social implications.

**Impact Statement:** This presentation will impact the forensic science community by providing information including the incidence, demographic data, causes of death, patterns of injury, associations between victim and perpetrators, and risk factors associated with female homicides.

Female homicide is a broad term that includes any homicide in which the victim is a female. This category also includes “femicide,” which conventionally describes hate crimes against women simply because of the gender roles assigned to women. In broader terms, such killings can be divided into those perpetrated within the family and those committed outside the family sphere. While men are usually considered the perpetrators of gender-related killings of women and girls, this is not the case in all situations. Female homicide, especially gender-based violence, is a significant public health concern and a violation of women’s human rights that affect communities worldwide. The features of this phenomenon are incredibly heterogeneous among countries.

The Cook County Medical Examiner’s Office files were searched for homicides in female subjects between January 2017 and January 2022. No limits for age, race, or cause of death were imposed. Only homicides were included in the search criteria. Information regarding the cases, including characteristics of victims and perpetrators, circumstantial and postmortem data, was obtained from the investigative reports, autopsy reports, and toxicological studies. A total of 527 cases were found in the Cook County Medical Examiner’s Office database. The study population included female subjects with a mean age of 33 years (age range: 0–96). Three hundred eighty-three victims were Black, 138 were White, 1 was Asian, 1 was Native American, and 4 were “others.” The causes of death included gunshot wounds (352 cases), sharp force (48 cases), asphyxia (29 cases), blunt force (22 cases), combined (49 cases), and others (27 cases). A full postmortem examination was performed in each case.

Homicides involving females have distinct features compared to homicides in other subjects. This phenomenon is likely to be underestimated due to social and cultural reasons. Moreover, collecting correct data about femicide is challenging since, in many cases of homicide, the necessary information is missing, there is no reported victim-perpetrator relationship, or motives for the homicide are unknown. Thus, some deaths are considered female homicides instead of “femicides.” Knowing the features of such events may help in preventing certain risk factors associated with violence against women. This study will illustrate distinct features of homicidal deaths in female subjects in a large metropolitan area. Information regarding different groups’ characteristics and potential perpetrator-victim relationships will be investigated to determine the patterns of injury, the circumstances of the event, and other aspects in this specific population. A thorough discussion of the results will be presented to attendees.

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**Female Homicide; Femicide; Gender-Based Violence**

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**I33     A Five-Year Retrospective Analysis of On-The-Job Deaths in Shelby County, Tennessee**

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**Learning Objective:** This presentation will give attendees an overview of the cause and manner of death for on-the-job deaths in Shelby County, TN, which includes the city of Memphis.

**Impact Statement:** The intent of this study is to impact the forensic science community by providing greater insight into the challenges of accurate reporting of job-related deaths and illustrate specific criteria to better determine job-related deaths versus deaths that simply occur while at work.

In the United States, thousands of people die at work each year. This study defines on-the-job deaths as fatalities caused by the job function and/or work environment. Despite the national decline in fatal work-related injuries, the United States Bureau of Labor Statistics reports an increase in work-related fatalities over the past three years in Tennessee.<sup>1</sup> Tennessee remains above the national average for such deaths, with Shelby County having the highest reported incidence.<sup>2</sup> This study analyzes specific data for reported work-related fatalities in Shelby County over a five-year period in which an autopsy was performed at the West Tennessee Regional Forensic Center. Analysis was performed to characterize each death by cause and manner, along with review of law enforcement/investigative agency and death scene investigation to determine if the death was due to job function and/or workplace environment.

Data was collected from the Death Investigation and Decedent Information (DIDI) database for Shelby County from 2017–2021 in which the death was labeled as on-the-job, and an autopsy was performed. A total of 88 cases were identified and sorted according to manner of death, cause of death, and how the injury occurred. The distribution of the manner of death was as follows: 51 accidents, 23 homicides, 12 natural, and 2 suicides. Deaths that fit the study criteria were as follows: 41 accidental deaths, 23 homicidal deaths, and 1 suicidal death. Blunt force injury-related deaths were the most common (49%), followed by gunshot-related deaths (36%). The majority of blunt force injuries occurred due to falls (14) or motor vehicle/machinery-related incidents (12). Multiple falls were associated with improper safety gear, and in half of the cases involving motor vehicle collisions, the decedents were unrestrained. Additional accidental deaths had varying causes including drowning, electrocution, and thermal injury. All but one of the homicidal deaths were due to gunshot wound(s), with one death due to sharp force injury. This study revealed the many complexities of determining job-related deaths and the need to formulate stringent criteria in classifying cases as being on-the-job deaths. Furthermore, added research with similar study criteria over a longer timeframe would be beneficial.

**Reference:**

- <sup>1.</sup> U.S. Bureau of Labor Statistics. (2022, March 15). *Fatal occupational injuries in Tennessee - 2020: Southeast Information Office*. U.S. Bureau of Labor Statistics. [https://www.bls.gov/regions/southeast/news-release/fatalworkinjuries\\_tennessee.htm](https://www.bls.gov/regions/southeast/news-release/fatalworkinjuries_tennessee.htm).
- <sup>2.</sup> National Council for Occupational Safety and Health. (2016, April). *Tennessee Workers: Dying for a Job*. <https://nationalcosh.org/>.

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**On-the-Job; Workplace; Accidental**

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**I34 A Fatal Abdominal Trauma Following a Fall From Height: Trauma Score, Mortality Meeting, and the Forensic Pathologist**

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**Learning Objective:** After attending this presentation, attendees will know about the trauma score and further understand the role and importance of forensic pathologist in mortality meeting on fatal abdominal trauma cases involving the liver.

**Impact Statement:** This presentation will impact the forensic science community by discussing trauma scoring in fatal abdominal trauma cases and the role of a forensic pathologist in mortality meetings involving abdominal trauma-related deaths.

The first trauma scale that was used to quantify the severity of trauma was the Abbreviated Injury Scale (AIS). In patients with multiple injuries, the Injury Severity Score (ISS) is used. To calculate the ISS, the highest AIS from the three most severely injured areas is taken and their squares are added up. The Revised Trauma Score (RTS) uses physiological parameters like respiratory rate, systolic blood pressure, and Glasgow coma scale score. The TRISS method combines anatomical injuries (ISS), physiological derangement (RTS), and the patient's age.<sup>1</sup>

Liver trauma cases are classified as per the American Association for the Surgery of Trauma (AAST) scales. This score includes measurement of the lacerated wounds and hematomas and the degree of involvement of hepatic lobes and Couinaud segments. There is also a World Society of Emergency Surgery (WSES) classification that divides the liver injuries as minor, moderate and severe.<sup>2</sup>

Trauma cases coming to emergency or surgery department are given a trauma score called Trauma Injury Severity Score (TRISS) to calculate the expected probability of survival. The TRISS method is calculated to find out any disparity between expected survival and actual survival. These trauma cases are later reviewed in morbidity and mortality meetings. The forensic pathologist is an integral part of the trauma surgery team. Trauma surgeons can translate an autopsy description of injuries into a quantitative injury score. This in turn helps them to analyze retrospectively how severely the decedent was injured.

A case of fall from height is described wherein the decedent sustained head injury, chest trauma, and penetrating injury to the abdomen. GCS score was 15/15. The injuries to the liver and its vessels were surgically treated accordingly. There was a gradual rise in serum urea level from 4th day onward until his death on the 11th day. A liver function test showed raised values for the different parameters like bilirubin, AST, ALT, etc. Despite following the proper management protocol, the patient succumbed to the injuries on the 11th day. A mortality meeting was convened, and this case was discussed to analyze the treatment pitfalls and rule out medical negligence if any. The autopsy findings were corroborated with the surgical team's findings.

**References:**

- <sup>1</sup> Rogers CB, Devera R. The Forensic Pathology of Liver Trauma. *Acad Forensic Pathol.* 2018;8(2):184-191.
- <sup>2</sup> Coccolini F, Coimbra R, Ordonez C. Liver trauma: WSES 2020 guidelines. *World J Emerg Surg.* 2020;15:24.

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**Trauma Score; Mortality Meeting; Forensic Pathologist**

**I35 Injury Pattern in Electric Scooter Accidents: A Case Report**

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**Learning Objective:** After attending this presentation, attendees will understand the injuries characteristic in accidents involving electric scooter use and the role of helmet use in reducing the fatality rate.

**Impact Statement:** This presentation will impact the forensic science community by helping to assess new risks for the community; in particular, it will emphasize the need to extend the helmet obligation to all ages, to reduce the number of e-scooter-related deaths and injuries.

**Background:** Currently, one of the most important health and social policy issues concerning all countries is the problem of road accident rates. Traffic is one of the most important risk factors. For this reason, ridesharing companies have been launching electric scooters in Rome since June 2019 with the aim of reducing car traffic.<sup>1</sup> In the absence of relevant legislation, the risk is an increase in deaths due to electric scooter crashes.<sup>2-4</sup>

**Case report:** The report presents a case study of a man who was driving a shared electric scooter when it collided with another vehicle.

The event was reconstructed, and a frontal collision between the car and the e-scooter was determined to be the most likely scenario. The patient's right leg was the first to be struck in the accident with the automobile. The man then hit his head against the windshield before falling to the ground, according to the dynamics. Autopsy and toxicological analyses were conducted. External examination revealed small, lacerated injuries to the head and chest, and a fractured femur. Autopsy demonstrated severe head-brain trauma characterized by fractures of the vault and skull base associated with subdural, subarachnoid, and intraparenchymal hemorrhage. Toxicological analyses were negative for both substances and alcohol.

**Discussion:** E-scooters are one of the best choices for reducing pollution in major metropolitan areas like Rome; as a result, the circulation of these vehicles should be supported on the one hand, while prevention campaigns should be implemented on the other.

Maximum speed reduction is not enough: in this case study, the speed was not high, even though the impact resulted in severe craniofacial injuries. Compulsory helmet use and access to well-regulated bicycle lanes can reduce fatalities in all cases where high-speed crashes are not involved.

The scooter sharing companies, for example, should provide a box in which to keep the helmet to encourage use. In Italy, although some limitations have been introduced by legislation, helmets are only compulsory for people under 18 years old.<sup>5</sup>

Finally, this case report emphasizes how the obligation of using helmets must be extended to all ages to reduce the risk of an increasing the number of deaths. Compulsory helmet use can reduce fatalities in all cases where high-speed crashes are not involved.

**References:**

1. M. inf. Gabinetto reg. Decreti. R. 0000229 (2019) [https://www.interno.gov.it/sites/default/files/allegati/allegato\\_alla\\_circolare\\_n.\\_300-a-1974-20-104-5.pdf](https://www.interno.gov.it/sites/default/files/allegati/allegato_alla_circolare_n._300-a-1974-20-104-5.pdf) Accessed 04 Jun 2019.
2. Faraji F, Lee JH, Faraji F, MacDonald B, Oviedo P, Stuart E, Baxter M, Vuong CL, Lance SH, Gosman AA, Castillo EM, Hom DB. Electric scooter craniofacial trauma. *Laryngoscope Investig Otolaryngol.* 2020;3:390-395. <https://doi.org/10.1002/lio2.380>.
3. Störmann P, Klug A, Nau C, Verboket RD, Leiblein M, Müller D, Schweigkofler U, Hoffmann R, Marzi I, Lustenberger T. Characteristics and Injury Patterns in Electric-Scooter Related Accidents-A Prospective Two-Center Report from Germany. *J Clin Med.* 2020;5:1569. <https://doi.org/10.3390/jcm9051569>.
4. Toofany M, Mohsenian S, Shum LK, Chan H, Brubacher JR. Injury patterns and circumstances associated with electric scooter collisions: a scoping review. *Inj Prev.* 2021;5:490-499. <https://doi.org/10.1136/injuryprev-2020-044085>.
5. Decreto legge 10 settembre 2021, n. 121. Disposizioni urgenti in materia di investimenti e sicurezza delle infrastrutture, dei trasporti e della circolazione stradale, per la funzionalità del Ministero delle infrastrutture e della mobilità sostenibili, del Consiglio superiore dei lavori pubblici e dell'Agenzia nazionale per la sicurezza delle ferrovie e delle infrastrutture stradali e autostradali. (21A06656) <https://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:decreto.legge:2021-09-10;121> Accessed 10 september 2021.

**E-Scooter Accidents; Helmet; Forensic Pathology**

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**I36 The Categorization of Autopsies as a Mechanism to Identify Possible Human Rights Violations**

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**Learning Objective:** This is a new methodology to interpret autopsies from victims who alleged extrajudicial executions in order to give the criminal justice another type of analysis regarding the causes and the context of those deaths. Especially, in those cases which have doubts if it happened in duty and combat or not.

**Impact Statement:** This could be a proposal to connect other disciplines, expertise, and findings for the criminal investigation of human rights violations. The conclusions of an analysis from 496 autopsies showed some particularities in those bodies and the occurrence of deaths, and these conclusions could be used on other scenarios of violence and war.

In Colombia, there is a transitional justice with an open file of unlawful deaths alleged to the Colombian Armed Forces. EQUITAS decided to develop research to contribute in the judicial investigation collecting 496 victims and 297 files of extrajudicial execution cases, using information from the autopsies.

In this research, the probability of survival is understood as a possibility to deduce if one person who was reported as killed in action could be attended by health personnel despite the injuries reported in the autopsy and the conclusion of death.

The development of this methodology is based on the scale from the International Committee of Red Cross (ICRC) to determine the triage of each injured civilian or soldier in war. It was necessary to construct a methodology because the autopsy does not register detailly the circumstances of injuries as a medical report at a hospital. EQUITAS' team took some principles of the wound score as the entrance and exit of the wound, if there is fracture or penetration of the dura, pleura, peritoneum or peripheral vessel, and the existence of bullets or fragments of them and their methodology of classification and defined the following basic scale:

- Very low
- Low
- Medium
- High
- Very high

The range of that scale used the number of injuries, their location, and if there were osteological damage and/or vital tissue damage, based on the ICRC referring to the body regions (head and neck, chest, extremities, and pelvis) and the damage of osteological tissue or vital organs and vessels. In consequence, the categories of analysis permit us to obtain the possible damage or not of those injuries in order to group each person in that scale to identify the serious injuries.

The analysis of this information permits us determine that 65% of the data had very low and low probabilities of survival, meaning the inflicted injuries could accelerate death. The results showed that each case has at least one severe injury in any of the regions of the body, including fracture and affection of vital organs.

On the other hand, 29% of the sample showed high and very high probabilities of survival. The characteristics of those deaths reported injuries in head and neck or abdomen with or without damage in vital organs, without fractures.

However, it is important to mention that there are some limitations of the data that came from the differences in findings reported in the autopsies, the details, and description of the evidence. There is a lack of pathologists who carry out the autopsies; in some places, hospital doctors must autopsy some of those bodies. It implies that some of the documents do not have all the information they must report. For this reason, it is possible to say that there are cases with high probabilities of survival because there is a lack of information in the location of the injuries or in the characteristics of the affected tissues or vital organs.

In any case of those limitations, this scale is relevant in the judicial investigation because, through this methodology, it is possible to find elements for considering if these persons could survive. The jury and the investigators can consider if those deaths were produced out of duty or if those people could receive medical attention because they were injured in action.

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**Extrajudicial Execution Cases; Probability of Survival; Autopsy**



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**I37 Mafia Organizations in Italy: Useful Markers in Attributing Murders to Specific Groups**

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**Learning Objective:** This presentation aims to depict the feature of mafia organizations in the north of Apulia, a region in southern Italy. The criminal organizations of this region have been differentiating over the past years, acquiring their distinct features compared with classical mafia organizations that make them immediately recognizable.

**Impact Statement:** This presentation shows that, alongside features shared by contiguous crime organizations, typical markers help distinguish the groups. By a punctual study of these features, searching for these specific signs, it is possible to discern with high probability to which criminal group a mafia murder is attributable. This is important to guide the prosecutor in collecting evidence during the inquiry to resolve the case.

Despite a global decline in homicides in Italy, including those caused by the mafia, the rate of mafia murders in the north of Apulia (i.e., the province of Foggia) is still very high. Since the so-called Bacardi massacre in 1986, the Apulian mafia organizations in the province of Foggia split into new criminal groups characterized by a strong sense of belonging to their clan. This led to the generation of different criminal organizations (the Foggian society, the Cerignolan mafia, the Garganic mafia) with different criminal modalities from other Italian mafia organizations (i.e., camorra, 'ndrangheta, or Sicilian mafia).

Some essential features characterize the homicides committed by these criminal groups. First, all homicides are committed exclusively by firearms. Most of the murders are carried out in places with potential witnesses (such as city centers), while the remaining are in isolated places (such as the countryside or state roads). The anatomical areas most involved in the shots are the skull and the back. This distribution implies an evident willingness to kill the victims. Often the victims have a blow to the back of the head: it coincides with the so-called coup de grace, especially for murders perpetrated with handguns. Another peculiarity of homicides committed by the Apulian mafia is to shoot the victims in the face to disfigure them (as an utmost insult).

Moreover, the short time available in crowded places to carry out the murders and the resulting high mortality rate suggests that the killers are experienced people familiar with firearms. Victim sex is another constant in mafia killings: 96% of victims are male. Moreover, in the Apulian mafia, no known women lead the clan.

However, despite some common characteristics, nodal differences exist between the various criminal groups' actions. First, the members of Foggian society did not use shotguns, commonly used in Gargano mafia killings. Another essential difference is in the fate of corpses. Often in the Gargano mafia, the bodies are destroyed (e.g., by burning) or disappear, often thrown into karstic caverns widespread all over the territory. The coup de grace marker has been found in many murders of the Gargano mafia, mainly directed at the skull to blow it up. An ambush preceding the killing is also frequent in Gargano mafia.

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**Mafia Murders; Gargano Mafia; Shotguns**

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## I38 Identifying Stress Factors and Related Coping Mechanisms Among Forensic Pathologists

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**Learning Overview:** The goal of this study is to identify the five most commonly reported work-related stress factors and the five most common coping mechanisms among retired and working forensic pathologists across the United States.

**Impact to the Scientific Community:** This research study will impact the forensic science community by observing national stress factors and coping strategies among forensic pathologists in order to provide medical examiner offices suggestions to improve employee wellness and potentially adopt stress-reduction programs in the workplace.

Forensic pathology is a critical science that bridges medicine and law. A forensic pathologist conducts autopsies to determine the cause and manner of an individual's death, which is helpful in solving forensic cases and criminal cases and discovering hereditary diseases.<sup>8</sup> They also may be responsible for contacting family members or next of kin of the deceased and for presenting their findings or conclusions in court. Although forensic pathology is a vital field in science and law, there is a national deficiency of active forensic pathologists. This may be due to the lack of awareness given to the health and wellness of the workforce, which is at risk in a high stress-inducing work environment.<sup>7</sup> Forensic pathologists are consistently subjected to stress due to overwhelming caseloads and a lack of adequate staffing.<sup>3,5,6</sup> While there have been studies conducted in the United States to examine occupational stress and potential coping mechanisms,<sup>4</sup> these reports focus on forensic scientists and not forensic pathologists specifically who may be more likely to develop stress due to their direct work with death.

In order to identify what work-related factors cause forensic pathologists the most stress and how have they chosen to cope with their stress, this study conducted a survey collecting both qualitative and quantitative data from members of the American Academy of Forensic Sciences (AAFS).<sup>1</sup> After completion, this study identified workload, inadequate staff, dissatisfaction with administration, court processes, and lack of time to complete duties as the most recurring themes of stress, which are supported by the quantitative analysis as well. The coping mechanisms most often mentioned by the participants include exercise, time with family and friends, communicating their stress, complete separation from work, and media use.

After identifying these stressors and coping strategies, the participants of the study listed potential practices or programs that could be instated in order to mitigate workplace stress. From this study, suggestions can be made to medical examiner offices. This includes incorporating programs involved in loan forgiveness to reduce medical student loan debt or enhancing communication among staff and leaders.<sup>2</sup> Dedicating rooms in medical examiner offices for stress management techniques and implementing an annual training course for leaders to better manage and support their employees is suggested. These stress-reduction practices and techniques can potentially lead to greater employment and retention rate of forensic pathologists and improve quality of work.

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### Forensic Pathology; Medical Examiner; Stress and Coping Mechanisms

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**I39 Strengthening Forensic Pathology Opinions With the Bayesian Approach for Evidence Interpretation: From Theory to Practice**

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**Learning Objective:** The general aim of this presentation is to show how forensic pathologists can benefit from the Bayesian approach for evidence interpretation. To this end, we present real-life casework that illustrates how the theoretical framework of the Bayesian approach can be applied practically and which challenges forensic pathologists may face while doing so. We specifically aim to provide practical advice and discuss (among others) how the forensic pathologist's role is dependent on the phase of the investigation; how to formulate hypotheses; and how to assign and report the likelihood ratio.

**Impact Statement:** This presentation provides practical examples of how the Bayesian approach for evidence interpretation can be applied in forensic pathology casework. This will help forensic pathologists adopt this method in their daily work, helping them to adhere to the current standards of interpreting and reporting forensic evidence, and thereby aiding them in increasing the usefulness of their opinions.

In many fields of forensic science, the Bayesian method of evidence interpretation is now the standard for interpreting and reporting evidence. Its use has also been discussed in forensic pathology literature, showing that a Bayesian approach can have important benefits for forensic pathologists.<sup>1,2</sup> However, the principles associated with the Bayesian approach (e.g., the difference between “investigative” and “evaluative” opinions and the use of the likelihood ratio) are much less applied in forensic pathology than in other fields of forensic science. This could be explained by a lack of understanding of how the theory of the Bayesian approach is best applied practically. To address this issue, we present various real-life scenarios to illustrate how a forensic pathologist can benefit from the Bayesian framework.

First, we show how the role of the forensic pathologist changes over the course of an investigation, and how this requires a shift from an investigative mode to an evaluative mode of evidence interpretation. This shift influences the type of opinions a forensic pathologist can provide, but also has an impact on the risk of bias and context-information management.

Second, we demonstrate how the Bayesian approach helps to identify the key issues in a case, and how this leads to formulating the two hypotheses that are required to provide a likelihood ratio. The discussion specifically focuses on why this may be challenging for forensic pathologists, and how to deal with such challenges.

Third, practical examples are used to illustrate how the forensic pathologist can provide an opinion using the likelihood ratio, and why this opinion does not necessarily require large amounts of numerical reference data. At the same time, we demonstrate how the use of a likelihood ratio aids the forensic pathologist to remain in their role as expert witness and within their area of expertise, and how it helps to ensure that the opinion is well-understood.

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**Forensic Pathology; Bayesian Evidence Interpretation; Reporting**

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**I40 Providing Opinions on the “Degree of Force”: Problems and a Solution**

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**Learning Objective:** When providing evidence, forensic practitioners are often asked to comment on the amount, or “degree” of force that is associated with an injury. Such opinions are, however, considered difficult, if not impossible, due to a variety of theoretical and practical issues. Attendees of this presentation will develop a better understanding of these issues and will be provided with an alternative approach, based on the Bayesian method of evidence interpretation.

**Impact Statement:** This presentation presents an overview of the several problems associated with questions relating to the “degree of force.” It also provides an alternative way to answer such questions. This will help forensic science provide better, more meaningful evidence.

When providing evidence, forensic pathologists and anthropologists are often asked to provide an opinion on the amount, or ‘degree’ of force required to cause a specific injury. Such questions on the ‘degree of force’ are considered difficult, if not impossible to answer due to a variety of theoretical and practical challenges, and a commonly accepted approach to provide the best answer is currently not available. This presentation provides an up-to-date discussion of the various challenges and limitations pertaining to opinions on the degree of force. Also, a potential solution is provided. This will aid forensic practitioners in translating their findings in the most informative, meaningful way.

First, the relevance of the question within the framework of criminal justice will be shortly discussed. This enables an understanding of the two assumptions that the question implies. One, that forensic pathologists or anthropologists can reliably infer the amount of applied force from their observations. Two, that the amount of applied force informs a choice between various scenarios of intent. The second part of the presentation focuses on these premises in more detail, by describing the currently available empirical evidence. More specifically, it discusses the outcomes of a literature review of the various types of studies that investigated the relation between applied force and injury morphology. It is concluded that most studies may be relevant as a means of understanding the biomechanical underpinnings of injuries, but that important shortcomings hamper their application in forensic casework. It is therefore unlikely that a forensic practitioner can provide a reliable opinion on the amount of applied force, based on injury morphology alone.

In the last part, it is argued that these limitations do not, however, mean that a forensic pathologist or anthropologist cannot add anything of value to the discussion. The application of a Bayesian approach to evidence interpretation helps to circumvent many of the problems. With examples, it is shown that this approach helps the practitioner to provide more relevant and specific evidence to the members of the court.

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**Degree of Force; Forensic Pathology; Bayesian Evidence Interpretation**

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**I41 The Impacts of Governing Agencies: A Comparison of Differential Resources in the Medicolegal Death Investigation System Patchwork**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how data from the 2018 Census of Medical Examiner and Coroners (CMEC) show a difference in resources available to a Medical Examiner or Coroner (MEC) office based upon its governing agency, and how key MEC community resources are critical to mission and operation.

**Impact Statement:** The presentation will impact the forensic science community by creating greater awareness of a governing agency's effect on MEC office resources and how this information can inform budgets and staffing decision, and organizational structuring.

In the United States, medical examiners and coroners (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 role, they are organized in disparate systems with varying makeups of governing functions, staffing, caseload, budget, and access to resources.

The 2018 CMEC was conducted by RTI on behalf of BJS (2017-MU-CX-K052) from June 2019 through March 2020.<sup>1</sup> The 2018 CMEC questionnaire was developed based on review and update of the 2004 CMEC, expert panel review and pilot testing. Mixed mode collection of data was conducted by mail, online, and email. A total of 1,648 MECs submitted 2018 CMEC surveys, for an overall response rate of 80.9%. RTI conducted an analysis of the 2018 CMEC data using the publicly available file. The 2018 CMEC data provided by the responding offices was examined based upon governing agency in terms of budgets, staffing levels (including any full-time employees, autopsy pathologists, and death investigators), caseloads, and percentage of reported cases accepted and autopsied. This presentation will highlight how key metrics of agency type, population, budget, staffing, and caseload are interrelated. Participation in national data collections and databases, like Combined Offender DNA Index System (CODIS) and National Missing and Unidentified Persons System (NamUS) will also be discussed.

The findings show that over half of MECs were self-governing (51%), and the remainder were governed by public health agencies (15%), law enforcement agencies (13%), district attorney offices (6%), forensic science agencies (4%), and other agencies (11%). Notably, the results show that independently operated MEC offices often have access to better resources when compared to offices governed by other agencies. Specifically, independently operated MECs had the highest median budgets and the highest levels of access across all 11 forensic functions or services. Although they have the highest proportion of offices operating without any full-time staff and full-time investigators, independent MECs also appeared to have more capacity overall. Differences across the other types of governing agencies will be highlighted.

Overall, MECs—regardless of governing agency—continue to lag in access to crucial resources needed to adequately perform death investigations and properly serve their jurisdictions. The differences found herein may reflect, at least in part, that where an MEC office resides has them beholden to the priorities of their governing agency. To that end, given this national patchwork, this presentation is intended to spur further discussion about how to move toward a more equitable, stronger medicolegal death investigation community that can better support public health and public safety.

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**Medical Examiner and Coroner Offices; Resources; Governing Agency**

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**I42 Standards Development Activities in Medicolegal Death Investigation and Forensic Pathology**

*J. Keith Pinckard, MD, PhD\*, Travis County Medical Examiner, Austin, TX*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the documentary standards development process and the current state of affairs of documentary standards within the fields of forensic pathology and death investigation.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of how standards are developed, and the players involved in standards development in the medicolegal death investigation realm, as well as their implementation.

The Organization of Scientific Area Committees (OSAC) for Forensic Science 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."<sup>1</sup> OSAC has many discipline-specific subcommittees that conceive of and draft standards to be submitted to Standards Developing Organizations (SDOs) that put them through defined, consensus-based processes to vet and publish them. OSAC then reviews those finalized standards for placement 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 consensus-based, documentary standards are not appropriate for medical practice. 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 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 OSAC Subcommittee, draft standards that have been sent to an SDO for further development, and standards to be considered for placement on the OSAC Registry. In addition, priorities for future standards development will be discussed.

**Reference:**

- <sup>1</sup>. <https://www.nist.gov/organization-scientific-area-committees-forensic-science>.

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**Forensic Pathology; Medicolegal Death Investigation; Standards**

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**I43 Postmortem Findings in Three Cases of Vaccine-Induced Death: The Autopsy Role in Vaccine-Induced Thrombotic Thrombocytopenia (VITT) Diagnosis and Comprehension of the Pathophysiology**

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**Learning Objective:** After attending this presentation, attendees will have acquired a better insight on the importance of forensic investigations as essential complementary approaches in the achievement of a definite diagnosis of Vaccine-Induced Immune Thrombocytopenia and Thrombosis (VITT) as cause of death

**Impact Statement:** This presentation will impact the forensic science community by highlighting the contribution of postmortem immunohistochemistry in the elucidation of the pathophysiological mechanisms underlying VITT onset.

Since the onset of the COVID-19 pandemic, the need to contain SARS-CoV-2 spread has led to the implementation of primary prevention campaigns through the production of a few vaccines, such as ChAdOx1 nCoV-19 (AstraZeneca®)—a chimp recombinant vector coding for viral Spike glycoprotein—and Ad26.COV2.S (Janssen®/Johnson & Johnson®)—a human recombinant vector coding for viral Spike glycoprotein as well. Soon after its release, the AstraZeneca® vaccine in particular raised controversies concerning the ratio between risks and benefits due to several reports of Vaccine-Induced Immune Thrombocytopenia and Thrombosis (VITT), a thrombotic disease occurring within 5 to 20 days from vaccine administration, which involves not only cerebral venous sinuses but also visceral vessels.

On a pathophysiological basis, the clinical evidence of anti-Platelet Factor 4 (PF4) antibodies in subjects who hadn't been previously administered heparin, has led to postulate that, similar to heparin-induced thrombosis, negatively charged components of the vaccine could bind the PF4 expressed on the platelet's surface, thus leading to the production of immunological complexes responsible for both a massive B-lymphocyte response and platelet activation with subsequent thrombosis. In such a context, forensic investigations have proven useful not just in corroborating the immuno-mediated nature of VITT onset, but also in the comprehension of its pathogenesis.

On such basis, we here present the postmortem findings from three subjects (two males and a female) who died 16, 24, and 19 days respectively following vaccine administration. In line with the antemortem results from radiological investigations, autopsy and histology showed evidence of multiple thrombosis involving both cerebral venous sinuses and several visceral vessels. The VITT diagnosis, already made antemortem on the basis of platelet count reduction, and D-dimer and anti-PF4 antibodies increase, has been further confirmed by histologic and immunohistochemical investigations, the latter also providing a better definition of the immune infiltrates within the thrombosed sites, and thus contributing to shedding light on the pathophysiological mechanisms underlying this pathology.

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**COVID-19 Vaccine; VITT; Forensic Investigation**



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**I44 Thrombosis Patterns of COVID-19 Vaccine-Induced Thrombotic Thrombocytopenia (VITT): “Order and Method” in Forensic Pathology**

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**Learning Objective:** Attendees will learn the importance of operating, in cases of suspected VITT and post-vaccine deaths, an algorithm for probabilistic stratification of causal link between the disease and the vaccination.

**Impact Statement:** This presentation will analyze case histories of rare events of VITT post-vaccine anti-COVID-19 and the importance of implementing a rigorous methodology that provides laboratory tests, research of antibodies anti-PF4, postmortem investigations completed by immunohistochemical analyses, and a WHO algorithm to classifying AEFI and detect a causal link with the COVID19-vaccine.

Vaccine-induced immune thrombotic thrombocytopenia (VITT) is a rare and severe group of adverse effects that may appear after the administration of Covid-19 vaccination. This syndrome is associated with unusual thrombosis in cerebral, portal, and mesenteric veins, and is characterized by severe thrombocytopenia and positive antibodies against platelet factor 4 (PF4), activating platelets through their FcγRIIa receptors. Prognosis is not yet well defined. The estimated mortality rate has been calculated to be less than one death in a million. However, considering that thrombosis most commonly involves atypical sites such as cerebral veins, a rapid identification of this syndrome is crucial to prevent morbidity and long-term mortality.

We analyzed five cases of death, which were clinically and temporally suspected of being linked to the development of VITT and therefore to the administration of the COVID-9 vaccine. Specifically, symptoms developed between 6 and 27 days after vaccine. Previous diseases (such as thrombocytopenia, kidney failure, and hypertension) affected two of them. Presentation symptoms were similar (headache, fever, neurological signs) and death occurred between 3 and 16 days later. In three cases, antibodies to PF4 were dosed in the blood. In four cases, an autopsy was performed, while in one case, it was not performed because the organs were harvested for graft. Postmortem investigation has detected in all cases cerebral hemorrhage and signs of thrombosis of the encephalic veins. Immunohistochemical investigations were also performed with the aim of better clarifying the tricky pathophysiological mechanism of the disease. Finally, in all cases, the algorithm proposed by WHO was adopted in order to analyze the causal link between AEFI and the COVID-9 vaccine.

In conclusion, we recommend following this practice based on both clinical diagnosis of VITT, through the study of laboratory parameters and research of antibodies anti-PF4, postmortem investigation, completed by immunohistochemical analyses. This allows us to deeply understand the possible pathophysiological mechanisms of fatal effects. Lastly, this presentation underlines the importance of using the algorithm that WHO proposed to classify and establish a clear cause link between vaccination and the adverse event.<sup>1-7</sup>

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**Thrombosis; Vaccine; VITT**

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**I45     A Multidisciplinary Analysis of Partial Human Remains Found Under the Sea in Venezuela, Investigated With an International Collaboration During the COVID-19 Pandemic**

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**Learning Objective:** After this presentation, attendees will have a better insight into the complexity of forensic analyses required when partial and highly decomposed human remains are found underwater and how a multidisciplinary international collaboration can enable more information to be obtained for further investigation

**Impact Statement:** This presentation will impact the forensic community by showing how forensic services of different countries have overcome the difficulties and limitation of the COVID-19 pandemic by collaborating remotely, making communication, dedication, and resilience key to solve crimes.

Venezuela is a South American country with a high rate of violent crime, including homicide and kidnapping, as well as property crimes such as robbery, often accompanied by extreme levels of violence. Venezuela is bounded to the north by the Caribbean Sea and the Atlantic Ocean, with a coastline of 2,800km, favoring different types of transnational crimes, such as piracy and drug and human trafficking. In Venezuela, forensic science services are provided by the National Service of Medicine and Forensic Sciences (Servicio Nacional de Medicina y Ciencias Forenses [SENAMECF]); however, they currently do not specialize in water and underwater investigation.

At the beginning of March 2022, fishermen discovered human remains approximately 15 meters below the surface, near Cubagua Island (Nueva Esparta state, northeast Venezuela). The remains were found wrapped in robes and a fishing net, fixed to the bottom of the sea with two anchors; the remains were incomplete, partially skeletonized, with the soft tissues in adipocere. A male undergarment was found in association with the remains. The robes, the fishing net, the anchors, and the majority of the bones were colonized by acorn barnacles.

The investigation of this case required the collaboration between SENAMECF in Venezuela and underwater forensic experts based in Western Australia. A multidisciplinary analysis comprehensive of complete medicolegal investigation, anthropological assessment (biological profile, pathology and traumatology), bone collagen analyses, adipocere analyses, diatom test, barnacle analyses, hyperspectral analyses of the garments, and a DNA profile was performed to identify the remains, the cause of death, the postmortem interval and the postmortem submersion time. Adding to the complexity of the case, it was necessary for some of these analyses to be performed remotely, as some of the evidence could not leave the country and the restriction of COVID-19 prevented the experts from travelling overseas.

Although the identity of the subject is currently still unknown and the case is still open, the present case provides an example of professional collaboration that overcomes the restrictions due to the global pandemic and showcases the importance of sharing knowledge in a developing forensic discipline. Furthermore, the multidisciplinary approach toward this case, which saw the application of the most up-to-date techniques in underwater forensics, provides guidelines for investigating similar cases in the future.

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**Postmortem Investigation; Underwater Forensics; Remote Analyses**

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**I46 The Final Frontier: Human Decomposition in Low Earth Orbit**

*Sherri Damlo, MS\*, University of Florida, Seattle, WA; Lerah Sutton, PhD\*, OPEN Health, Seattle, WA; Craig Damlo, MSc, Blue Origin, Seattle, WA*

**Learning Objective:** After attending this presentation, attendees will better understand the concerns that exist surrounding alterations of decomposition artifacts in the unique ecology of the space environment.

**Impact Statement:** This presentation will impact the forensic medicine community by introducing attendees to the concept of space taphonomy, assessing timelines of body retrieval, and examining how forensic medicine practitioners can influence the future of human space exploration and space permanence. It will stress the importance of the evolution of space medicine to include the field of forensic pathology for the development of proactive, not reactive, protocols for death investigation.

Spaceflight is not without risk, and less-rigorous medical screening and access to space for all weaken existing risk-mitigation strategies. Such access also represents progress in the exploration and democratization of space. Governments and commercial enterprises plan for health and safety issues, but no such mandatory requirements exist for the procedures of managing natural, sudden, homicidal, or accidental deaths aboard closed space systems or following exposure to space. The first death in low Earth orbit is likely to result in public scrutiny and international attention, so these entities must be prepared to properly manage such an occurrence.

In April 2022, the National Aeronautics and Space Administration updated its rationale for human space flight technical standards to include the capability of space systems to collect forensic evidence, contain biohazards, and consider jurisdictional matters within the space environment as they relate to the handling of deceased crew. Despite such a request, a huge gap exists in our understanding of the process of human decomposition in microgravity, anaerobic, and closed environments.

This presentation will explore our current understanding of the physiologic issues known to occur in astronauts aboard space stations that could affect the rate and process of decomposition. A literature review will also be presented of terrestrial analogs similar to the environment of low Earth orbit by comparing and contrasting the aspects of each. These analogs, such as various anaerobic, arid, and thermal cycling environments, will be examined to inform on how one might expect human decomposition to progress in a closed space habitation and in the remains of those exposed to the space environment. Important endogenous variables that could affect the rate of decomposition, such as preexisting disease, use of certain medications, and core body temperature, are also reviewed.

Discussion is provided on how one might conduct an experiment, with particular attention given to challenges completely unique to a space environment that cannot be reliably replicated on Earth. Final thoughts are given with regard to future considerations necessary to prepare for a death event in the environments of low Earth orbit unique to those from the terrestrial ecosystem, including assessment of timelines for potential body retrieval.

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**Decomposition; Jurisdictional Authority; Microgravity**

### I47     **Unique Medical Identity Card (UMID): The First DNA Database and Identification Portal in India for the Identification of Unidentified Bodies and Missing Persons**

*Chittaranjan Behera, MD\*, Forensic Medicine, AIIMS, New Delhi, Delhi, INDIA*

**Learning Objective:** After attending this presentation, attendees will understand the development and functioning of the first DNA database and identification portal in India for identification of unidentified bodies and missing persons.

**Impact Statement:** This presentation will impact the forensic science community by providing attendees with the traditional forensic approaches for the identification of unidentified dead bodies and missing persons in India, as well as the current usage of DNA databases and identification portals for the same purpose.

Identification of missing persons and unidentified dead bodies has been a well-documented global problem in recent years. Many nations, like the United States, United Kingdom, and Australia, currently have DNA databases in place to address this issue. In India, approximately 50,000 unidentified dead bodies are recovered every year; 80–90% of them are left unidentified afterward because the current procedures and protocols being followed are insufficient to determine their identities. Apart from these, more than 300,000 people are reported missing annually. There is a need for a DNA database and an integrated system of identification processes for the identification of these unidentified bodies and to solve the issue of missing persons.

We created an identification portal and DNA database for unidentified deceased corpses that were autopsied at the Department of Forensic Medicine and Toxicology, AIIMS, Delhi, India. Biological samples from unidentified dead corpses and a thorough phenotypic description of the deceased are gathered with the investigating officer's informed consent. While the genotypic data produced by STR analysis is only accessible internally, the phenotypic data kept in the database is accessible to the general public. Claimants may browse through the URL (<http://umid-aiims.icmr.org.in/>), and if they wish to claim an unidentified dead body, they may approach as per the given guidelines. The current DNA database includes only autosomal STRs profiles for each cadaver, and a pattern-searching-based program has been developed for the database search. The claimant's STR profile was run through the questioned DNA database to look for a potential match. If positive, the investigating officer in that case is informed for necessary action. However, it also supports Y-STR- and mtDNA-led database searches. Y-STRs and mtDNA information will be updated regularly in the next phase of the project, which is being implemented in an additional six centers across India.

Our identification portal is India's first-ever identification portal for both phenotypic and genotypic data of unidentified deceased. Until July 2022, it consisted of the information of 510 individuals. In a pilot study, two cadavers were successfully identified using STR profiles. This project's success can also lead to a pioneering national DNA database of unidentified and missing persons in India.

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#### **DNA Database; Unidentified Dead Bodies; Short Tandem Repeats (STRs) Profiles**

**I48 A Rare Case of Cardiac Tamponade by the Surgical Correction of a Hiatal Hernia**

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**Learning Objective:** This work presents a rare case of cardiac tamponade following anti-reflux surgery. This complication, although rare and many times unexpected, can often result in the death of the patient. The attendees will understand the possible complications of surgical correction of hiatal hernia, which, although rare, have a not-negligible mortality rate.

**Impact Statement:** Considering a potential cardiac tamponade after surgery involving the esophageal hiatus is essential, especially if sutures or staples are placed in this region. Only in this way can signs of this life-threatening complication be recognized in time and their fatal consequences averted using adequate management. Raising awareness of this complication is essential because it rapidly leads to circulatory failure and, with inadequate management, to death. This case and the literature findings suggest that avoiding graft fixation in the treatment of hiatal hernia, especially using metal helicoidal tacks, will undoubtedly decrease the risk of highly mortal cardiac tamponade.

Cardiac tamponade is characterized by blood accumulation in the pericardium, resulting in compression of all cardiac chambers and compromising the entire circulatory blood flow. In anti-reflux surgery, cardiac lesions are caused mainly by stitches, while the instruments used to place the stitches and the abdominal access type do not seem relevant. The risk related to mesh fixation to the diaphragm from the abdominal side is known, as the thickness of the diaphragm ranges between 1.5–5.4mm.<sup>1,2</sup> During surgical procedures, the patient positioning associated with the induced pneumoperitoneum causes the diaphragm to be tighter and, therefore, closer to the pericardium, especially at the central tendon, where the thickness is between 2.9 and 3.0mm.<sup>3</sup> The depth of penetration of the various tacks available on the market ranges between 3.7 and 7.0mm. Consequently, the diaphragm thickness will be the primary determinant for the outcome of surgical treatment of hiatal hernia. In the tack manufacturers' instructions for use, the deployment of tacks for mesh fixation in the vicinity of the diaphragm is contraindicated.<sup>1,2</sup> Despite manufacturers' recommendations, it can be very tempting to use tacks in complex hernia repairs to prevent a recurrence.<sup>4</sup> Mesh augmentation was shown to decrease the recurrence rate of complicated/large hiatal hernias, and this is because surgeons prefer to use mesh, overlooking the possible risks involved when fixing a graft or putting stitches IN the diaphragm.<sup>5</sup>

This work presents the case of a 70-year-old woman admitted to the surgical department to undergo anti-reflux surgery after being diagnosed with a hiatal hernia. The procedure was carried out by repositioning the herniated structures, followed by mesh and graft-reinforced hiatoplasty. The day after the surgery, the patient started to show hypotension and hemodynamic changes. Ecographic investigation showed a small laceration of the pericardium and the heart's right ventricle with consequent cardiac tamponade by a helical metal-tipped clip inserted on the diaphragm. The woman underwent surgical removal of the clip and suture of the myocardium, but despite efforts by medical staff, the patient died shortly after.

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**Cardiac Tamponade; Hiatal Hernia; Anti-Reflux Surgery**

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**I49 An Uncommon Cause of Gastrointestinal Bleeding in the Forensic Setting**

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**Learning Objective:** After attending this presentation, attendees will be aware of the uncommon cause of gastrointestinal bleeding and the importance of morphology and immunohistochemistry in diagnosis of follicular lymphoma.

**Impact Statement:** This presentation will impact the forensic science community by revealing underlying cause of death by autopsy by being aware of morphologic features of hematologic malignancies.

Sudden death associated with gastrointestinal hemorrhage account for a small but significant percentage of autopsies in the forensic setting. Well-recognized causes of gastrointestinal bleeding include esophageal varices, black esophagus, gastritis and peptic ulcer, intestinal perforations, and intestinal obstructions. We describe a forensic case of a 55-year-old man who presented with abdominal pain and rectal bleeding. Abdominal CT showed hepatic steatosis and central mesenteric adenopathy. Endoscopy demonstrated a friable, hemorrhagic proximal jejunal mucosa, which was biopsied. Despite medical treatment, including blood products, his hemoglobin declined to 4g/dl and he died three days after presentation without a diagnosis. The coroner authorized postmortem examination and the decedent was autopsied two days after death at the Medical University of South Carolina. The autopsy was able to identify mesenteric lymphadenopathy and an area of mucosal nodularity with focal erosion in the proximal third of the jejunum. The ileal and rectosigmoid mucosae were hemorrhagic. No lesions were identified in the stomach or duodenum. Histology confirmed lymphocytic infiltrates in follicular/nodular aggregates distorting the jejunal mucosa, infiltrating the mesentery and effacing mesenteric nodal tissue. Limited mucosal acute inflammation was discernible at the site of jejunal erosion. Immunohistochemistry of the lymphoid infiltrates in the jejunum was positive for CD20, CD10, and BCL2 and was negative for CD5, consistent with follicular lymphoma. Additional findings at autopsy included cardiomegaly (460 grams), acute bronchopneumonia, hepatic steatosis, nephrolithiasis, and a wrinkled splenic capsule. Toxicology of hospital blood obtained near the time of original admission was presumptively positive for caffeine.

Although the gastrointestinal tract is the most common extranodal site of involvement in lymphoma, presentation as gastrointestinal hemorrhage is uncommon. Additionally, despite postmortem artifacts inherent to autopsy specimens, the authors were able to classify the lymphoma type via morphologic appearance and immunohistochemical stains. The authors hope this case reinforces the utility of the autopsy as a gold standard in diagnostic medicine.

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**Gastrointestinal Bleeding; Follicular Lymphoma; Autopsy**

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**I50     Cassette Agitation as a Time-Saving Method to Improve Lung Insufflation and Histology**

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**Learning Objective:** Attendees will learn a possible time-saving method to improve the quality of lung insufflation and histology by cassette agitation in formalin. This can be applicable to those in surgical pathology as well as forensic pathology.

**Impact Statement:** The forensic science community can benefit from time-saving methods to improve the quality of lung insufflation and histology in autopsy cases or even surgical pathology cases.

**Background:** Assessment of postmortem lung histology is often obscured by artifactual atelectasis. Traditionally, this is remedied by whole lung insufflation. While effective, it is a time-consuming procedure that uses large amounts of formalin. Thus, it is poorly suited for the high pace environment within a medical examiner's office. An alternative technique has been colloquially described among pulmonary pathologists whereby a piece of tissue can be quickly insufflated using agitation in formalin. To this end, we aim to examine and test this technique and evaluate its utility in medicolegal autopsies. To our best knowledge, this has not been previously examined in medical literature.

**Method:** Three autopsies were randomly selected from the docket at an academic hospital. Seven pulmonary sections were taken from two of the cases. Six underwent an intervention, and one was left unaltered to act as a negative control. In the intervention arm, one was insufflated, one was crushed, one was crushed then agitated, one was agitated three times, one was agitated for three seconds, and one was agitated for ten seconds. In the third autopsy, six sections were collected: three peripheral and three central. One section from each region was submitted as is, one was agitated, and one was insufflated. Specimens were agitated one by one in Tissue-Tek Paraform Cassettes. All the sections were then assessed for the percentage of alveolar expansion and given a grade using the following: grade 1—0 to 25%, grade 2—26 to 50%, grade 3—51 to 75% and grade 4—76 to 100%. The sections were also assessed on the presence or absence of edema and any other intralveolar material.

**Results:** In our initial cohort, the crushed sections had the lowest percentage of alveolar expansion with an average grade of 1. Sections taken without intervention had an average grade of 3. Crushed and agitated sections averaged a grade of 3. Insufflated and agitated specimens averaged a grade of 4. Pulmonary edema and intra-lveolar hemorrhage were observed in the sections with no difference between agitated and unaltered sections. No recognizable artifact was observed in agitated sections, and there was no difference between peripheral versus central sections.

**Discussion:** Our initial data demonstrates that agitation can greatly improve alveolar expansion, particularly in crushed specimens. This is without introducing artifacts or removing intra-alveolar material or requiring additional equipment or resources. Our study does not control for underlying natural disease, regional differences in the lung, or sampling techniques. These limitations need to be taken into consideration. This represents initial data, and our study is ongoing with expansion to a busy medical examiner's office. Detailed statistical analysis is pending.

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**Lung; Insufflation; Histology**



**I51 Congenital Absence of the Left Circumflex Artery in Multiple Myocardial Ischemia: A Case Report**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of a rare coronary artery anomaly that may cause myocardial infarction, particularly in patients with multiple cardiovascular risk factors.

**Impact Statement:** This presentation will impact the forensic community by presenting clinicopathologic, autptic, and histological findings in a case of multiple myocardial ischemia events due to a Congenital Absence of Left Circumflex Artery (CALCx). The case will provide an important contribution to the differential diagnosis of myocardial infarction.

A 64-year-old man with a medical history of hypertension, diabetes, and obesity was admitted to the ER with an episode of oppressive retrosternal chest pain that started 30 hours before. He had a history of angina associated with palpitations in the previous two weeks.

On admission, he was conscious and reported a remission of the accused symptoms. An EKG showed sinus rhythm with a right bundle branch block, moderate ST elevation on inferior leads, and depression in right ventricular lead with associated Q and R waves, respectively. The findings were consistent with a subacute inferoposterior myocardial infarction. A transthoracic echocardiogram revealed a left ventricular ejection fraction of 40%, with akinesis of the septum and the inferior left ventricular wall. Laboratory tests showed high levels of troponin, with TnI value of 2336.4ng/l on admission (n.v.< 34,0ng/ml). The coronary angiography showed a dominant right coronary artery (RCA), several irregularities at the proximal end of the left main CA, and an occlusion at the middle end of the posterolateral branch. After ruling out anomalous origins of the LCx artery, a hypoplasia of the vessel was hypothesized by the cardiologist. Percutaneous coronary intervention was performed, obtaining a successful revascularization of the posterior interventricular artery. The PO course proceeded without complications and antiplatelet and lipid-lowering agents therapy was initiated. The patient was discharged with no symptoms and the indication for a regular cardiology follow-up. Three days after discharge, he was found suffering from severe dyspnea, loss of consciousness, and cyanotic. Despite resuscitation attempts, the patient died, and an autopsy was requested by a local prosecutor to determine the cause of death and to evaluate medical malpractice's hypothesis.

At autopsy, the heart weighed 650g in the fresh state. On the macroscopic examination, a widespread tissue discoloration with recent hemorrhagic infiltrates was observed on the posterior to the inferior wall of the left ventricle as well as on the adjacent papillary muscle.

Macroscopic view of the heart after formalin fixation showed degenerative atherosclerotic wall changes of the RCA and its main branches. Although narrowed by wall thickening in some sections, the arterial lumens were patent. The congenital absence of the LCx artery was confirmed after excluding anomalous vessel origins, thus supporting the angiographic findings.

Tissue specimens were prepared for microscopic examination and then studied using Hematoxylin and Eosin staining, CD15 and CD68 immunostaining methods, and Masson's trichrome staining. Histological investigations confirmed the exclusive presence of venous vascular components at the normal anatomical site of the LCx. In addition, a large transmural ischemic area with a heterogeneous morphology was observed in the left ventricle posterior wall and papillary muscle. Microscopic examination concluded for multiple and polychronous ischemic insults. The autptic and histological findings, integrated with the clinical records data, allowed reconduction of the cause of death to an acute cardiac ischemia in a patient with multiple comorbidities and a CALCx. CALCx is a rare coronary anomaly with an incidence of 0.0067%. The diagnosis requires the following criteria: absence of an anomalous LCx artery origin from the left coronary sinus or left coronary artery; absence of anomalous origin from the right coronary sinus, non-coronary sinus, RCA, or pulmonary artery; dominant (or superdominant) RCA. Although it is generally asymptomatic, CALCx may result in transient ischemia of the left ventricle posterolateral segments vascularized by the RCA. Circulating disorders of the RCA may indeed lead to more serious consequences when a collateral circulation normally provided by the Cx is absent. A better understanding of the natural history and clinical implications of CALCx may help improve the diagnosis, treatment, and hospital management of patients with this anomaly admitted for ACS.

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**Coronary Artery Anomaly; Congenital Absence of the Left Circumflex Artery; Myocardial Ischemia**

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**I52     Death From an Unexpected Superior Venous Laceration During the Implantation of a Leadless Intracardiac Pacemaker: The Role of the Forensic Pathologist**

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**Learning Objective:** After attending this presentation, attendees will be able to identify unpredictable adverse events of medical procedures such as the implantation of a leadless intracardiac pacemaker.

**Impact Statement:** This presentation will impact the forensic science community by providing relevant information to recognize iatrogenic lethal complications in the leadless pacemaker positioning procedure.

Each year, nearly one million people worldwide receive conventional cardiac pacemakers to treat bradycardia and heart block. Traditional pacemaker-related adverse events occur in 1 out of 10 patients; they are typically related to pulse-carrying transvenous leads that can dislodge, cause infection or venous occlusion.<sup>1</sup> To overcome these problems, leadless pacemakers were designed. These latest 1ml devices contain a battery, pulse generator, and electrodes and are delivered by femoral vein catheter with a non-surgical and mini-invasive implantation at the apex of the right ventricle through the inferior vena cava.<sup>2</sup> The indications for a leadless intracardiac pacemaker are predominantly permanent atrial tachyarrhythmia and atrioventricular block. According to the latest scientific literature data, the leadless pacemaker implantation has a low risk of adverse events such as cardiac wall perforation, pericardial effusion, and infections.<sup>3</sup>

A 79-year-old woman with a history of hypertension, diabetes, COPD, renal failure, and overweight was admitted to the hospital for multiple syncopal episodes with an accidental fall occurring the day before. Medical examinations resulted in a second-degree Type 2 atrio-ventricular block with severe aortic and mitral stenosis. The patient had a history of previous bacterial endocarditis and a right calcific fibrothorax with ipsilateral hemithorax retraction associated with pulmonary hypoventilation. Considering the symptomatic atrio-ventricular block and the previous endocarditis, six days after the admission she was submitted to the percutaneously implantation of a leadless intracardiac pacemaker. During the operation, the patient manifested unexpected cardiac tamponade. Despite medical assistance and the execution of pericardiocentesis, the patient was declared dead after a cardiorespiratory arrest unresponsive to advanced resuscitative support maneuvers. To figure out the cause of the sudden cardiac tamponade and in the suspicion of medical malpractice, the local prosecutor ordered an autopsy. The thanato-chronological parameters were coherent with the circumstantial historical data. The autopsy showed multiple rib fractures on both sides as results of resuscitation attempts, several calcific right pleural adhesions, and a focal blood clot in the pericardial cavity. The heart weighed 730g. The heart examination revealed a hematoma about 3cm in diameter surrounding a laceration of the intrapericardial superior vena cava, and a 4mm linear injury on the outer surface of the right ventricle not communicating with the cardiac cavity. The histopathological examination confirmed the presence of a full-thickness traumatic interruption of the superior vena cava with evidence of a de-lamination. The autopsic and histological findings, integrated with the clinical records data, reconducted the cause of death to a cardiac tamponade resulting from the iatrogenic laceration of the superior vena cava during the leadless pacemaker implantation.

In the present case, the superior vena cava was accidentally lacerated during the preliminary procedures of the catheter placement. Since there are no current guidelines or a practice advisory in the perioperative management of this atypical, injured site, it was classified as an unexpected adverse event.<sup>3</sup> It was also an unpredictable event because of the right calcific fibrothorax that caused a partial distortion of the superior vena cava root along with an increased vulnerability to traction. The matching between antemortem medical records and autopsy findings was crucial in the determination of the manner of the death. According to the results, the judge for preliminary investigations closed the case as accidental death that occurred due to complications of invasive and therapeutic procedures.

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**Leadless Intracardiac Pacemaker; Unexpected Iatrogenic Event; Forensic Pathology**

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**I53 A Fatal Massive Hemoperitoneum From a Ruptured Corpus Luteum Cyst**

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**Learning Objective:** After attending this presentation, attendees will recognize the ovary as a potential source of hemoperitoneum in women of reproductive age.

**Impact Statement:** This presentation will impact the forensic science community by documenting a rare case of fatal hemorrhage from a ruptured corpus luteum cyst, which is typically a benign entity. Other gynecologic sources of internal bleeding will be discussed, as well as risk factors for developing this condition.

The corpus luteum is a thin-walled, vascular, hormone-secreting body that normally develops within the ovary during the luteal phase of the menstrual cycle. Corpus lutea may form blood- or fluid-filled cysts that can rupture. In most instances, these ruptures are self-limited and cause temporary irritation of the peritoneum. More rarely, they can cause substantial blood loss that requires rapid diagnosis and medical intervention.<sup>1,2</sup>

Patients typically present with signs and symptoms of an acute abdomen, often with nausea and vomiting. In a woman of reproductive age, this has a broad differential, including ectopic pregnancy, ovarian torsion, appendicitis, acute peritonitis, intestinal ischemia, and splenic rupture. When a gynecologic source of abdominal hemorrhage is suspected, sonography is an effective and non-invasive modality for diagnosis.<sup>3</sup> Major risk factors for severe bleeding from a ruptured corpus luteum cyst are inherited bleeding disorders and anticoagulation.<sup>4</sup>

**Case Report:** A 16-year-old girl was witnessed to collapse outside her high school. She complained of severe abdominal pain and was taken to a nearby hospital, where she was admitted for exploratory laparotomy. Approximately 5 liters of blood were removed from her abdomen, although a clear source of hemorrhage was not found intraoperatively. She had no confirmed history of recent trauma, and splenectomy did not resolve the bleeding. After the patient developed signs of disseminated intravascular coagulation (DIC), she was taken to the intensive care unit, where she expired.

Autopsy identified a focally disrupted hemorrhagic corpus luteum cyst of the right ovary. No other possible sites of bleeding were found. The splenic artery was examined and found to be intact up to the point of ligation. Mediastinal soft tissue hemorrhage and bilateral hemothorax were also observed, consistent with the patient's clinical history of DIC.

In cases of unexplained massive hemoperitoneum, the forensic pathologist must perform a thorough examination for any potential source of bleeding that may have eluded earlier diagnosis. While not reported in this case, it may be useful to obtain information regarding anticoagulant use and personal/family history suggestive of an inherited bleeding disorder.

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**Corpus Luteum Cyst; Hemoperitoneum; Rupture**

### **I54     An Intimal Sarcoma of the Abdominal Aorta: An Unexpected Finding Only Possible by Autopsy!**

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**WITHDRAWN**

**I55 Looks Can Be Deceiving: Evaluating Beyond Hepatic Pathology in the Assessment of Chronic Alcoholism**

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**Learning Objective:** This presentation is a retrospective study of postmortem cases at the West Tennessee Regional Forensic Center evaluating the relative phenomenon of cases of chronic alcoholism with minimal hepatic pathology. It further investigates the correlation of hepatic pathology to the severity of chronic alcoholism.

**Impact Statement:** This presentation will impact the forensic science community by encouraging reevaluation of the postmortem assessment of cases of chronic alcoholism.

Alcohol abuse is a well-established cause of life-threatening pathologies of the liver, the primary metabolizer of alcohol. Less commonly, but notably, pathophysiologic changes to the heart, brain, and pancreas are known sequelae of chronic alcoholism. Paradoxically, liver pathologies have been observed to be absent in chronic alcoholics with extrahepatic, long-term consequences of alcohol abuse in postmortem evaluation.<sup>1-4</sup> While not the norm, there is little understanding among the forensic science community of these cases of chronic alcoholism with a normal/minimally affected liver (NMAL). With little existing research data available, this study questions the liver's centrality to predicting the severity of chronic alcoholism.

This retrospective study analyzes autopsy data from West Tennessee Regional Forensic Center (WTRFC) from 2006 to 2022 to elucidate the prevalence of chronic abusers of alcohol who paradoxically exhibit no or minimal pathologies of the liver. Data from natural and accidental deaths of chronic alcoholics were analyzed to identify trends in race, age, gender, and hepatic and extrahepatic complications. Cases of NMAL were identified as those with mild steatosis (< 30%), little to no inflammation, and no fibrosis or cirrhosis. Histology was reevaluated for cases with ambiguously stated findings. Review of investigation reports summarizing medical records, death scenes, and accounts of decedents' drinking behavior informed identification of chronic alcoholism. Cases of concurrent drug toxicities, suicide, homicide, or undetermined manner of death were excluded. Of the 455 cases that were evaluated, 416 comprised the research data. Thirty-three cases met the criteria of NMAL while 383 cases exhibited hepatic pathologies. Statistical analysis found a significant difference in the number of extrahepatic complications recorded in decedents with NMAL versus those with abnormal livers. Specifically, the decedents with NMAL had a greater number of extrahepatic complications on average.

The present study identified a relatively small number of cases of chronic alcoholism with NMAL. Thirty-nine cases with hepatic or extrahepatic complications associated with alcoholism were excluded because the investigative and autopsy report lacked a definitive statement characterizing the decedent as a chronic alcoholic. Social stigma associated with disordered alcohol use or insufficient investigation may have led to an inability to identify every case involving chronic alcoholism. Several factors affected selection of NMAL cases among documented chronic alcoholism. The study could not classify cases in which the postmortem state of the body (decomposition) hindered liver assessment. Discrepancies in reporting and limited examination hindered case selection.

Uniformity in diagnosis and reporting would aid in proper identification of cases with alcohol-related cause of death. Chronic alcoholism with extrahepatic complications but very limited hepatic complications might be due to epigenetic information not currently understood. This study could suggest that the gross and microscopic appearance of the liver may not be a true indicator of severity of chronic alcoholism. Investigation into the extrahepatic complications of chronic alcoholism may yield a better understanding of the disease. However, the gaps in the reporting, whether at the investigative or autopsy level, could be obscuring a complete assessment of this fact. Therefore, a prospective study into all complications of chronic alcoholism, with clearer investigative or diagnostic criteria, would be highly beneficial.

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**Alcoholism; Liver; Postmortem**

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**I56 The Body in the Mirror: An Autopsy in a Case of Situs Inversus Totalis**

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**Learning Objective:** After attending this presentation, attendees will better appreciate the features of a situs inversus totalis through the description of a case of sudden death that occurred after admission to the Emergency Department.

**Impact Statement:** The presentation will impact the forensic community through the description of anatomical characteristics related to a condition of prevalent radiological finding.

Situs inversus totalis is a rare genetic defect of malformative origin, caused by an anomaly of embryonic development, characterized by the complete specular transposition of the thoracic and abdominal viscera on the sagittal axis. The prevalence of situs inversus varies within different populations but is still less than 1 case per 10,000 people. It is often associated with congenital anomalies, such as Kartagener-type primary ciliary dyskinesia, polysplenia, biliary atresia, congenital heart disease, and malrotation of the middle intestine, as well as vascular anomalies (agenesis of the inferior retrohepatic vena cava, preduodenal portal vein, anatomical abnormalities of the hepatic artery) and malignant tumors. In the absence of congenital heart disease, subjects presenting with situs inversus are phenotypically healthy and live a normal life, with no consequences related to their anatomical condition. Among subjects with situs inversus totalis, the prevalence of congenital heart disease is 5–10%, with a greater frequency of transposition of the great vessels. In contrast, in subjects affected by situs inversus with levocardia, the prevalence of congenital heart disease reaches 95%.

The present report concerns the death of a 69-year-old man that occurred suddenly after being admitted to the Emergency Department for dyspnea. During hospitalization, the clinical conditions were immediately critical with an abnormality of vital signs. Despite medical intervention, the death was unexpectedly ascertained after a few minutes; for this reason, an autopsy was required to establish the cause of the death.

The autopsy examination allowed highlighting the complete inversion of thoracic and abdominal viscera; precisely, internal organs were flipped from the left to the right and vice versa. The brain was essentially normal. The heart was increased in volume and weight; coronary arteries were generally patent with wall thickening and sporadic calcification; left and right ventricles were increased in volume; myocardium was widely involved by the presence of whitish dyschromic areas; interventricular septum and left posterior ventricular wall were partially replaced by surgical patch. The lungs weighed 835g on the left and 723g on the right; the arterial branches were patent, while the airways were occupied by a small quantity of foamy material; at the cut, the release of abundant foamy material was appreciable. The kidneys were characterized by the poor distinction between the cortical and medullary components. The arteries were ubiquitously affected by wall thickening and moderate atherosclerosis. At the end of the investigation, the cause of death was identified as acute pulmonary edema due to acute heart failure in the context of chronic ischemic heart disease.

In conclusion, the presentation offers an extensive, anatomical, detailed description of a condition that presents mainly, if not solely, in the radiological literature. In addition, some autopsy procedural insights are provided for the diagnosis of pathological conditions possibly associated with situs inversus totalis.

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**Situs Inversus Totalis; Anatomical Abnormalities; Autopsy Findings**



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**I57 The Fulminant Fat Emboli Syndrome: Two Autopsy Case Reports and the Use of Platelet and Fibrinogen Markers**

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**Learning Objective:** The goal of this presentation is to review the three sub-entities of the fat embolism syndrome, in particular the fulminant one; discuss some of the ways of highlighting fat globules; discuss the presence of a probable vital reaction in the fat emboli syndrome; and take into consideration the value of anti-CD61 and anti-fibrinogen antibody staining in highlighting the fat vacuoles on processed tissue.

**Impact Statement:** Forensic pathologists will be able to highlight fat emboli, in the fat embolism syndrome, on formalin-fixed paraffin-embedded tissue, without the need of cryostat frozen sections. They will be able to test for fat embolism syndrome years after the autopsy was performed.

**Introduction/objectives:** After a review of the literature, we noticed that the FULMINANT traumatic fat embolism syndrome is a sub-entity of the fat embolism syndrome that has not been published much. Moreover, to our knowledge the use of immunohistochemical markers to highlight the fat globules has not been used routinely even if suggested and practiced by some authors as potentially useful.

**Methods:** We present two autopsy cases of fulminant traumatic fat embolism syndrome. We describe the clinical presentation of the two decedents, the autopsy findings, and the use of anti-CD61 and anti-fibrinogen antibody staining on formalin-fixed paraffin-embedded tissue to reveal the platelets and fibrinogen abutting the fat vacuoles in the blood vessels.

**Results:** At autopsy, one of the bodies presented the classic fine cutaneous petechiae on the axilla and the prominent petechiae in the lung parenchyma. With the use of anti-CD61 and anti-fibrinogen antibodies markers, we were able to reveal and support the presence of platelets and fibrinogen surrounding the empty spaces in the blood vessels in the lung in both cases and in the brain and glomeruli in one case.

**Conclusion:** As described in the literature, our two cases are in accordance with the fact that when traumatic fat emboli syndrome presents shortly: a few hours, after the trauma (FULMINANT sub-entity) the prognosis and outcome are more likely to be dismal. Another important fact is that one of the many requirements to make a diagnosis of fat embolism syndrome is the demonstration of fat globules in the lung on histology. The postmortem diagnosis of fat emboli is usually made by combining special fat stains with routine histological sections. However, special fat stains cannot be performed in all institutions because of the need of cryostat frozen sections (Oil red O or Sudan III) or the toxicity of the stain (Osmium tetroxide). Anti-CD61 and anti-fibrinogen immunohistochemical stains appear to be useful markers to make more visible the fat globules in processed tissue. A preliminary study is in progress in the Département de Médecine Légale, Montpellier, France.

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**Fat Emboli Syndrome; Fulminant; Immunohistochemistry Stains**

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**I58 Acute Hemorrhagic Leukoencephalitis Features Associated with COVID-19: A Case Report and Literature Review**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of hemorrhagic neuropathologic findings that may be seen during autopsies of decedents with COVID-19 infection.

**Impact Statement:** This presentation will impact the forensic science community by serving as a review and a resource for COVID-19 autopsy cases found to have hemorrhagic neuropathologic lesions.

Acute hemorrhagic leukoencephalitis (AHLE) is a rare form of acute disseminated encephalomyelitis (ADEM) characterized by rapid and severe inflammatory demyelination involving the brain and spinal cord leading to punctate hemorrhages within the white matter. It is considered a severe form of ADEM and is typically preceded by a viral infection. Histologic findings include fibrinoid necrosis of the blood vessels, “ring and ball shaped” perivascular hemorrhages, and often mixed inflammation. AHLE has variable presentations, including a progressive and prolonged course that may lead to findings of perivascular demyelination or a fulminant course that can lead to death within days from onset. The mechanism is not entirely understood; a prevailing theory suggests a post-viral autoimmune process with an inflammatory response leading to perivascular demyelination, edema, and intraparenchymal hemorrhage.

We present a case of a 58-year-old man who died unexpectedly at his residence after a brief period of altered mental status. He had reported upper respiratory symptoms as well as loss of taste and smell in the week before his death but had not sought medical care. His past medical history included illicit drug use and hepatitis C infection. He was not vaccinated against COVID-19. Postmortem computed tomography demonstrated bilateral pulmonary ground-glass opacities, suggestive of COVID-19 pneumonia; no distinct abnormalities were identified in the brain. Autopsy revealed evidence of gross and microscopic features of viral pneumonia. However, examination of the brain at autopsy revealed unexpected findings including diffuse punctate hemorrhages throughout the subcortical white matter of the cerebrum and cerebellum. Histologic examination of the brain demonstrated multifocal ring-shaped microhemorrhages within the white matter characterized by a central core of prominent axonal swellings. The lesions did not involve the cortex, and notably did not have a significant acute inflammatory component. A diagnosis of AHLE was rendered.

A literature review revealed a range of hemorrhagic complications associated with COVID-19 including AHLE, ADEM, and ADEM-like pathology.<sup>1,3,4,6-8</sup> Stroke and spontaneous hemorrhage have also been described.<sup>2</sup> AHLE can be differentiated from ADEM by hyper-acute onset, rapid progression, and often sparing of the cortex. Involvement of the basal ganglia is rare with AHLE and more common with ADEM. Other conditions to consider in this setting include vasculitis, lymphoma, and progressive multifocal leukoencephalopathy. Importantly, punctate hemorrhage may not be detected on postmortem imaging.

COVID-19 infection has been associated with a wide range of neuropathologic changes, and our understanding of the neuropathology associated with COVID-19 infection continues to evolve. It has been postulated that both direct infection and parainfectious mechanisms contribute to the findings.<sup>5</sup> Additional research is needed to determine the pathologic mechanisms that cause hemorrhagic neuropathologic complications in COVID-19 infection.

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**COVID-19; Autopsy; Acute Hemorrhagic Leukoencephalitis**

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**I59 An Overlook on the Contribution of Postmortem Molecular Biology for the Diagnosis of SARS-CoV-2-Related Systemic Thrombosis as Cause of Death**

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**Learning Objective:** After attending this presentation, attendees will acquire a better insight on the systemic involvement related to SARS-CoV-2 infection, due to the recognition of ACE-2 receptor on endothelial cells with subsequent activation of the coagulation cascade.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of biomolecular postmortem investigations, namely viral RNA extraction and sequencing, in the establishment of a causal relation between viral infection and subsequent clinical manifestations leading to death.

The systemic involvement related to SARS-CoV-2 infection, due to the recognition of ACE-2 receptor on endothelial cells with subsequent activation of the coagulation cascade, is a fundamental phenomenon for understanding the pathogenesis of the disease and being able to try to arrive at the most appropriate treatment. As widely known, COVID-19 is an infectious disease transmitted by SARS-CoV-2 virus through droplets, which mainly affect the lungs. Although the most frequent symptoms are represented by fever, cough, fatigue, taste and smell loss, the disease can also lead to death due to the onset not only of acute respiratory failure, but also of systemic thrombosis as a consequence of the endothelial tropism of the virus. Such a systemic involvement has also emerged from the autopsic and histologic investigations carried out on four subjects, all dead from SARS-CoV-2; histology in particular has evidenced the presence of fibrin-rich thrombi at the level of pulmonary, cardiac, renal, and cerebral vessels; the subsequent extraction of SARS-CoV-2 RNA from oropharyngeal swabs and samples of heart, lungs, and aortic walls, helped to confirm the causal relation between thrombosis and viral infection, while the additional viral RNA sequencing has allowed the identification of the molecular variants of SARS-CoV-2 responsible for the subjects' death (delta in three cases; omicron in one case).

Considered the capability of SARS-CoV-2 to determine the onset not just of interstitial pneumonia, but also of microvascular dysfunctions secondary to a massive activation of both the immune and coagulative systems following the recognition of ACE-2 receptor on endothelial cells, the present findings provide additional evidence of the systemic nature of the infection as cause of a fatal outcome, especially in patients with comorbidities. Biomolecular postmortem investigations, namely viral RNA extraction and sequencing, in the establishment of a causal relation assumed a fundamental and important role.

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**SARS-CoV-2 Infection; Systemic Thrombosis; Postmortem Sequencing**

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**I60 Lymphohistiocytic Myocarditis Following a Messenger RNA (mRNA) -Based COVID-19 Vaccine: A Case Report**

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**Learning Objective:** Attendees will review the post-vaccine myocarditis epidemiology, clinical presentation, and autopsy findings (including microscopic and gross). The pathophysiology will also be discussed and literature pertaining to the topic will be reviewed as well.

**Impact Statement:** Myocarditis is a rare but potentially fatal adverse event associated with COVID-19 vaccines. The gross findings can be subtle to non-existent, and the microscopic findings are non-specific, thus, awareness of this entity is crucial for the practicing forensic pathologist.

**Introduction:** Myocarditis is a rare but potentially fatal adverse event associated with the mRNA-1273 COVID-19 vaccine. Gross findings in these autopsy cases can be subtle to non-existent, and the microscopic findings are non-specific, thus awareness and familiarity of the entity are necessary for the practicing forensic pathologist to recognize potential cases.

**Case:** A 40-year-old obese White male with a family history of heart disease and a remote history of COVID-19 infection (60 days prior to death) for which he received monoclonal antibody therapy was discovered unresponsive in bed by a family member. The decedent had received the first dose of the mRNA-1273 COVID-19 vaccine four days prior to his death and experienced several days of fever and headache. Of note, there was no reported complaints of chest pain leading up to death. Findings at autopsy included cardiac hypertrophy and hepatosplenomegaly. No gross heart lesions were identified. Microscopic examination of the heart revealed lymphohistiocytic myocarditis with focal myocyte necrosis. Testing for inheritable cardiac diseases was negative. Toxicology was negative and a COVID-19 nasopharyngeal swab was negative by polymerase chain reaction.

**Discussion:** While the close temporal relationship of death to the decedent's mRNA-1273 COVID-19 vaccination aided in the diagnosis for this particular case, such information is not always available to the forensic pathologist at the time of autopsy. When myocarditis is seen at autopsy, post-vaccine myocarditis, along with other causes of myocarditis, including COVID-19 infection, should be considered in the differential diagnoses. A detailed medical history that includes vaccination records may be helpful in identifying the underlying myocarditis etiology. Interestingly, the majority of myocarditis cases occur after the second dose of the vaccine, suggesting some type of immunologic priming. While the decedent's myocarditis occurred after the first COVID-19 vaccine dose, a similar type of immunologic priming may have occurred during his COVID-19 infection.

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**Myocarditis; COVID-19; Vaccine**

## I61 A Strategy for Effective Collection and Communication of Preliminary Forensic Information

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**Learning Objective:** After attending this presentation, attendees will understand forensic death scene investigation, including the crucial information investigators must gather to determine jurisdiction, the reason this information is so crucial, and the duties of an investigator both on and off the scene.

**Impact Statement:** This presentation will impact the forensic science community by providing a framework for medical units in the collection of information necessary for the determination of jurisdiction in a death scene investigation. Through this framework, information can be collected and relayed more efficiently, saving time for both investigators and medical units.

The jurisdiction of a Medical Examiner's Office extends over all unnatural human deaths, as well as the natural deaths of individuals that do not have a primary care provider. Determination of this jurisdiction falls to the forensic death scene investigators acting under the authority of the Medical Examiner on duty for that particular day. Whenever there is a deceased individual found, or a death reported, they will take the call, record the information, and potentially report to the scene, in order to provide a comprehensive rundown of the death to the Medical Examiner, who will either confirm or deny jurisdiction.

The job of a forensic death scene investigator is a uniquely integral role in the flow of the Medical Examiner's Office and, depending on the day, they may receive anywhere from no death calls to a multitude. These calls each require the acquisition of specific information to help determine if the death is a Medical Examiner case or not. Details such as the surroundings, medical history, and physical state of the deceased all play a significant role. Problems can arise in obtaining this information particularly in the reports given by first responders on the scene. Whether it be due to the omission of key details, lack of essential information gathered, or misinformation, trying to extract pertinent information can be unnecessarily time consuming.

To alleviate these problems, the creation of a report sheet was proposed. This sheet would be provided to first responders for use on a death scene. In order to determine which details and information is crucial, several forensic death scene investigators from the Summit County Medical Examiner's Office in Akron, OH, were interviewed, and a basic outline for the worksheet was created. A comprehensive explanation of the purpose behind each question was written up so when the report sheet is presented to a medical unit, they can understand why each question is asked and how its answer is used to aid in a forensic death investigation.

The development of this worksheet will promote a better understanding of information necessary for forensic investigations, as well as improve communication between first responders and death investigators. It will increase efficiency and help promote a more detailed form of reporting, which, if adopted, can save both first responders and forensic death scene investigators time.

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### Death; Investigations; Report

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**I62 The Identification Process of an Unidentified Transgender Woman**

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**Learning Objective:** After attending this presentation, attendees will be aware of the potential challenges in identifying transgender decedents in states of advanced decomposition.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of a multidisciplinary approach for identification of transgender individuals.

**Introduction:** A transgender decedent, an individual whose gender identity or gender expression differs from their sex assigned at birth, may present a challenge to identification in cases of advanced decomposition. Transgender individuals are recognized by their gender identity in the medicolegal process, which in some circumstances fails to match the name, medical records, or physical evidence discovered during investigation. This case required multidisciplinary collaboration, including investigative efforts, a detailed pathology report, and an anthropological assessment of the skeleton to make a positive identification.

**Case details:** The San Francisco Office of the Chief Medical Examiner was presented with a presumptive adult female in moderate to advanced stages of decomposition found in a locked short-term-stay residence. The poor condition of the remains made a positive identification on scene impossible, further complicated by the decedents use of several aliases. A possible identity, associated with the address, had medical history significant for scoliosis, a healed tibial fracture, an orchiectomy, and breast augmentation. All medical history was circumstantially collected in the emergency department under the subjects legally changed name; all past surgical records associated with their birth name and sex were unattainable. Autopsy revealed the remains of an adult female noted to have breast implants, external female genitalia, and the complete absence of a cervix, uterus, fallopian tubes, and ovaries. Computed tomography imagery was significant for capturing the presence of a healed right tibial fracture, scoliosis of the thoracic vertebrae, and the morphological features on the pelvis and cranium estimated to be a biologically male skeleton. Prioritized pelvic features included the subpubic angle, greater sciatic notch, subpubic concavity, ventral arc, and the medial aspect of the ischiopubic ramus. The cranium had morphological features, including large mastoid processes, a projecting glabella, a blunt supraorbital margin, and substantial projection of the mental eminence. Identification of this woman was possible due to the combined investigative findings, pathology report compiled during autopsy and forensic anthropological assessment conducted using CT imagery.

**Discussion:** The process of identifying a decedent is fundamental in forensic casework. Forensic anthropological analyses yield: sex, age, population affinity, and underlying pathology. Transgender individuals hold a gender identity or expression that differs from their sex assigned at birth creating a categorical misalignment. Decomposition further distorts classic identification methods making fingerprinting and visual identification obsolete. Cases involving transgender individuals can be convoluted as medical and dental records are often associated with a name and biological sex that no longer represents the gender identity or expression utilized by the decedent. Physical measures, including breast augmentation, gender reassignment surgery, and facial-skeletal modifications, can impact the features used by anthropologists to estimate biological sex. Sex estimation methods in forensic sciences fall into a binary system centered around biological sex thus failing to capture the spectrum of gender expressions. This case captures the complexities of identifying transgender individuals in advanced stage decomposition. Multifield collaboration, and sensitivity during the identification process prompted the positive identification of the presented male-female transgender decedent.

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**Unidentified; Transgender; Computed Tomography**

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**I63 An Analysis of Changes in Protein Markers Since the Moment of Death (“Time 0”) for the Estimation of the Early Postmortem Interval (PMI): An Experimental Study**

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**Learning Objective:** After attending this presentation, attendees will understand the role of protein markers in estimating the PMI.

**Impact Statement:** This presentation will impact the forensic science community by showing the quantitative alterations of these proteins in early PMI from the exact time of death.

**Introduction:** The estimation of the postmortem interval (PMI) through the analysis of potential biomarkers is a topic of growing scientific interest. The analysis of the PMI is very complex in the so-called “early PMI,” that is, in the first hours after death, especially when the thanatochronological parameters are not useful and it is necessary to accurately evaluate the time of death for forensic purposes (investigations on suspects, analysis of alibis, evaluation of the veracity of testimonies). In recent years, various authors have suggested studying the protein degradation pattern by focusing research on muscle samples.<sup>1</sup> However, many of these studies were carried out on animal samples or on bodies found in different scenes and therefore already subjected to non-homogeneous extrinsic variables (different temperatures, different PMIs, different cold storage times). Therefore, the analysis of markers in human models was started after time 0, that is, several hours after death. This study proposes the analysis of a series of protein markers on a human experimental model made on plasma taken from the exact moment of death.

**Materials and methods:** The operating protocol was approved by the Ethics Committee. After signing the informed consent of the family members, peripheral blood samples were taken in seven predefined times (PMI range 0h–2h) from patients who had died in the ICU from the moment of death, verified by the absence of heartbeat through ECG monitoring. Twenty-four cases were collected and divided into two main groups by age (cut-off 69.6 years), sex, and cause of death. Of these, ten cases were selected in proportional numbers from each group to guarantee the homogeneity of the sample and reduce the role of intrinsic potential variables.

The markers examined were HMGB1, Troponin T (TnT), Na + -K + -ATPase, CK-MB, PDH (pyruvate dehydrogenase), caspase 9, LDH. The bodies were exposed to constant room temperature. The samples were taken at seven predefined time intervals, starting from the exact moment of death up to the next two hours. The samples were immediately centrifuged and stored at -80°C. Subsequently, the samples were analyzed by ELISA 96-wells to examine the quantitative changes of the selected markers. The kits used were MyBioSource® with human target. The mean of the values obtained from all patients for each marker was evaluated.

**Results and discussion:** The study showed a modest variability of the averages of the values of the markers examined in the two hours, with oscillations in the order of ng/mL. However, with respect to “time 0,” an increase at time 5 minutes from death was evaluated for the markers TnT, ATPase, Caspase 9, and HMGB1. An increase at 2h was evaluated for the TnT, ATPase, caspase 9, and CK-MB markers. PDH1 showed a progressive increase from 10 minutes to 2 hours. LDH showed stability of the values in the whole range examined.

The data collected suggest potential variability for most of the markers already in the early PMI. Although the sample examined is small, the study described has the advantage of proposing a rapid, repeatable method, with a reduction of extrinsic variables and analysis of easily withdrawable biological fluids. These data can be integrated with the analysis of other markers and enhanced with multiparametric methods useful for defining an increasingly precise algorithm for estimating the PMI.

**Reference:**

- <sup>1</sup>. Sacco MA, Cordasco F, Scalise C, Ricci P, Aquila I. Systematic Review on Post-Mortem Protein Alterations: Analysis of Experimental Models and Evaluation of Potential Biomarkers of Time of Death. *Diagnostics (Basel)*. 2022 Jun 17;12(6):1490. doi: 10.3390/diagnostics12061490.

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**Forensic Sciences; Postmortem Interval; Proteins**

**I64    A Comparative Transcriptomic Analysis of Failing Hearts in Cases of Hypertrophic and Dilated Cardiomyopathy Reveals Differential Gene Expression**

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**WITHDRAWN**



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**I65 A Comparative Use of Aqueous Humor <sup>1</sup>H Nuclear Magnetic Resonance (NMR) Metabolomics and Potassium Concentration for Postmortem Interval (PMI) Estimation in an Animal Model**

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**Learning Objective:** Attendees will benefit from a combined approach based on two very different experimental techniques investigating two time-dependent methods and comparing them with the aim of determining an accurate PMI estimation in an uncommonly investigated biological matrix (aqueous humour) during an early PMI window (up to 24 hours).

**Impact Statement:** This presentation will demonstrate a common biological origin of both metabolome and potassium in the aqueous humour, reflecting in the same statistical information with respect to PMI estimation. What is more, during this time window, metabolomic modifications have a greater predictive power than potassium in estimating PMI, as the combination of the two methods does not improve the error in prediction.

We present a paper recently published in *International Journal of Legal Medicine*.<sup>1</sup> Estimation of the postmortem interval (PMI) remains a matter of concern in the forensic pathologist routine. Traditional and novel approaches are not yet able to fully address this issue, which relies on a multitude of complex biological phenomena triggered by death. For this purpose, eye compartments may be chosen for experimental studies because they are more resistant to postmortem modifications compared to other biological matrices. Vitreous humour, in particular, has been extensively investigated, with potassium concentration ([K<sup>+</sup>]) being the marker that shows the most significant correlation with the estimation of the time since death. Recently, a <sup>1</sup>H nuclear magnetic resonance (NMR) metabolomic approach based on aqueous humour taken from an animal model was proposed for PMI estimation, resulting in a robust and independently validated regression model. As vitreous and aqueous humour have a deep anatomical and physiological relationship, we decided to challenge the variation in [K<sup>+</sup>] in the very same experimental setup. [K<sup>+</sup>] was determined through capillary ion analysis (CIA) and a regression analysis was then performed. Moreover, it was investigated whether the PMI information related to potassium could improve the metabolome high predictive power (error in prediction < 100 minutes on a 24-hour PMI window) in estimating the PMI. Interestingly, we found that a part of the metabolomic profile is able to explain most of the statistical information carried by potassium, suggesting that both the rise in potassium and the modifications in metabolite concentrations relies on a similar, and possibly shared, biological mechanism. In the first 24h PMI window, the aqueous humour metabolomic profile shows greater predictive power than [K<sup>+</sup>] behavior, suggesting its potential use as an additional tool for estimating the time since death in the early postmortem (up to 24h) consistently with its well-known time-dependent rise in vitreous humour at further PMI windows (24–96 hours).

**Reference:**

- <sup>1</sup>. *International Journal of Legal Medicine* [<https://doi.org/10.1007/s00414-020-02468-w>] following a previous paper investigating the sole metabolomic data [<https://doi.org/10.1007/s11306-019-1533-2>].

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**Metabolomics; Postmortem Interval; Potassium**

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**I66     Metabolomics Improves the Histopathological Diagnosis of Asphyxial Deaths: An Animal Proof-of-Concept Model**

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**Learning Objective:** Attendees will appreciate how plasma metabolomics minute-by-minute analysis during the asphyxial phase preceding cardiac arrest is able to intercept those animals who will eventually not respond successfully to CPR or develop severe heart and brain damage. Moreover, the proposed approach is suitable to differentiate between asphyxial cardiac arrest and cardiac arrest due to ventricular fibrillation.

**Impact Statement:** This presentation paves the way to the implementation of metabolomics analysis to be used as a diagnostic tool for both cause and mechanism of death.

This paper was recently published in *Scientific Reports*.<sup>1</sup> The diagnosis of mechanical asphyxia remains one of the most difficult issues in forensic pathology. Asphyxia ultimately results in cardiac arrest and, as there are no specific markers, the differential diagnosis of primitive cardiac arrest and cardiac arrest secondary to asphyxiation relies on circumstantial details and/or on the single pathologist experience more than objective evidence. Histopathological examination is currently considered the gold standard for cardiac arrest postmortem diagnosis, being performed worldwide in almost every judicial autopsy. Here we present the comparative results of histopathology and immunohistochemistry versus those previously obtained by 1H nuclear magnetic resonance (NMR) metabolomics in a swine model, originally designed for wider clinical purposes, exposed to two different cardiac arrest causes, namely ventricular fibrillation and asphyxia.

Briefly, 20 healthy female Landrace/Large-white pigs of the same age and weight were randomized into two groups and exposed to asphyxial cardiac arrest by clamping of the endotracheal tube ( $n = 10$ ) or to ventricular fibrillation cardiac arrest by a pacing wire ( $n = 10$ ). Blood samples were collected from the internal jugular vein at baseline (after stabilization), at 1min intervals until the return of spontaneous circulation. At the end of the experiment, animals were humanely euthanized, and necropsy was performed. Results demonstrated that, while heart and brain microscopical analysis could identify the damage induced by cardiac arrest without providing any additional information on the cause, metabolomics allowed the identification of clearly different profiles between the two groups and showed major differences between asphyxiated animals with good and poor outcomes. Minute-by-minute plasma sampling allowed associating these modifications to the pre-arrest asphyxial phase showing a clear correlation to the cellular effect of the mechanical asphyxia reproduced in the experiment. The results suggest that metabolomics provides additional evidence beyond that obtained by histology and immunohistochemistry in the differential diagnosis of cardiac arrest.

In conclusion, modifications in plasma metabolome seem to reflect molecular mechanisms related to the asphyxial insult. The proposed metabolomic approach appears suitable to be used together with histopathology and immunohistochemistry, improving their predictive ability in the differential diagnosis between asphyxial and primitive cardiac arrest.

**Reference:**

1. Locci, E., Chighine, A., Noto, A. et al. Metabolomics improves the histopathological diagnosis of asphyxial deaths: an animal proof-of-concept model. *Sci Rep* 11, 10102 (2021) <https://doi.org/10.1038/s41598-021-89570-0>.

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**Metabolomics; Asphyxia; Cardiac Arrest**

**I67 Delayed Colonization of Concealed Carrion in a Laboratory Study**

Jodie A. Warren, PhD\*, San Jose State University, San Jose, CA

**Learning Objective:** After attending this presentation, attendees will have a better understanding of the delays of oviposition for *Calliphora latifrons* Hough due to concealing carrion in a laboratory study.

**Impact Statement:** Victims are commonly killed in their own homes by an offender known to them and often the offender reacts by concealing and moving the remains.<sup>1</sup> The disposal of concealed remains at a crime scene was most probably done in an attempt to evade responsibility for the crime. Concealing the remains facilitates transport and clean up at the same time as allowing the perpetrator to move the body unnoticed from the initial crime scene to a disposal site.<sup>2</sup> Wrapping or concealing may delay carrion insects from reaching the remains and, therefore, lead to underestimated postmortem intervals. Much of our development data is collected under laboratory conditions with beef liver and other butcher meats as the rearing medium.<sup>3,4</sup>

The objective was to examine if *C. latifrons* would colonize or attempt to colonize concealed beef liver in a laboratory setting and if so, what delays would be observed relative to the control. Four experimental colonies of *C. latifrons* were examined and compared to a fifth control colony at 25°C for each experimental scenario. Experimental scenarios included concealing beef liver within knotted plastic waste bags, in taped cardboard boxes, wrapped in shower curtain material and duct taped, excessively wrapped in moving stretch wrap, and within lined cosmetic bags with double zipper openings representative of miniature soft suitcases.

The exposed controls under laboratory conditions were colonized comparatively immediately under experimental conditions. While delays of oviposition in the experimental treatments were observed under most of the experimental conditions (in some instances no oviposition), delays were not observed under all conditions. The stretch plastic that was wrapped around the meat ~15 times did not delay the flies from laying eggs under the plastic and directly on the liver. Although a full lifecycle occurred in the open control waste bag, there was no oviposition on the closed knotted experimental waste bags for the length of the experiment. This research supports Charabidze et al.'s hypothesis that colonization arises from both detection and accessibility.<sup>5</sup>

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1. Ferguson C, Pooley K. Australian no-body homicides: Exploring common features of solved cases. *Journal of Forensic Medicine*. 2019;66:70-78.
2. Anderson GS. Factors that influence insect succession on carrion. In: Byrd JH, Tomberlin JK, eds. *Forensic entomology The utility of arthropods in legal investigations*. 3rd ed. Boca Raton FL: CRC Press; 2020: 102-138.
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4. Warren J-A, Ratnasekera TDP, Campbell DA, Anderson GS. Hyperspectral measurements of immature *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) raised on different food substrates. *PLOS ONE*. 2018;13(2):e0192786.
5. Charabidze D, Hedouin V, Gosset D. An Experimental Investigation Into the Colonization of Concealed Cadavers by Necrophagous Blowflies. *Journal of Insect Science*. 2015;15(1):149.

**Forensic Entomology; Concealed; Delayed Oviposition**

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**I68 Evaluating Storage Solutions for Entomological Gene Expression Work to Better Coincide With Common Practices for Entomological Evidence**

*Joshua Smith, PhD\*, Texas Tech University, Topeka, KS*

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**Learning Objective:** This presentation will help attendees become aware of one of the steps needed in the field of forensic entomology to allow for gene expression to be more practically applied in a casework setting.

**Impact Statement:** This presentation continues the needed conversation on how to set more consistent standards within the field of forensic entomology. The specific problem being addressed will help both researchers and those involved in casework as gene expression work becomes more prominent in the field.

An estimate of the age of a blow fly larval specimen removed from a corpse can provide an estimate of the minimum time since death.<sup>1</sup> This age estimate is made by comparing a physical characteristic of a larva, such as length, to a known reference growth curve for that species under similar conditions. Commonly, larval specimens are extended through boiling in water or ethanol prior to length measurement. Outside of physical characteristics to estimate larval age, numerous studies have investigated how molecular characteristics, such as gene expression, could also serve as age estimation markers.<sup>2</sup> A challenge with this approach, however, is the recommended storage conditions for gene expression require very cold temperatures (generally -20°C to -80°C) and placing larval specimens live into storage solution, which can cause them to shrink.<sup>3</sup> Larval specimens can be measured months after collection in casework, whereas they are usually measured soon after collection in research. This difference, when the specimens are measured, means larval lengths taken from gene expression work are likely not consistent with those from reference growth curves focused on physical traits. Specifically, samples stored in RNAlater under cold conditions can be smaller after only a short storage period.<sup>4</sup> Understanding whether specimens that are boiled can still be used for gene expression would allow for broader application of molecular methods for age determination in casework.

Whether a boiled larval blow fly specimen could still produce viable RNA for gene expression work has not been evaluated. In this work, the quantity and quality of RNA from third instar blow fly larval specimens killed in boiling water and stored at one of three conditions (RNAlater at room temperature; RNAlater at 4°C, or ethanol at 4°C) was assessed. RNA quantity for each specimen is being evaluated through spectrophotometric quantification. Quality will be assessed through the visualization of either housekeeping or sex-determination genes on agarose gels after reverse transcription. Preliminary results show all three storage conditions provide some success in obtaining viable RNA; however, storage in ethanol is less consistent and results in lower yield than samples in RNAlater. More replicates are currently being completed to determine the robustness of these results. This work will help establish guidelines that will make the molecular evaluation of larval age more compatible with practical casework circumstances.

**References:**

1. Catts, E.P., and M.L. Goff. 1992. Forensic Entomology in criminal investigations. *Annu. Rev. Entomol.* 37: 253-72.
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**Forensic Entomology; Specimen Storage; Guidelines**

### **I69 First Survey of Forensically Relevant Diptera in the Republic of the Gambia**

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**WITHDRAWN**

## 170 Forensically Important Insects Collected From Human Bodies in New Jersey

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**Learning Objective:** Attendees of this presentation will learn about the entomofauna of forensic interest collected directly from death scenes and corpses during autopsy in New Jersey. They will also learn how these data compare to those obtained from baits and animal models.

**Impact Statement:** This work will enhance the visibility of forensic entomology among the forensic sciences and will highlight how important it is to create collaborations between the pathologist and other experts.

The importance of the entomological evidence recovered at a crime scene or in the autopsy room has been abundantly discussed in the literature. Medicolegal forensic entomology focuses on the study of arthropods, which are attracted for various reasons to decomposing matter. By analyzing the biology and behavior of these insect groups, it is in fact possible to obtain potentially useful inputs on the minimum postmortem interval, movements of the remains, toxicological information, and more. The distribution of insects differs among geographical areas and among seasons; this applies also to insects of forensic interest. Considering that different species may have different ethology, different nutritional requirements, and different developmental rates, it is imperative for forensic entomology to be successfully applied in a given area that the presence of forensically relevant insects in that area during all seasons is known. These insects are normally surveyed using either traps baited with beef liver or animal carcasses; this model has been widely used across the world and has provided very interesting information about the biodiversity of forensic insects. However, it is often advisable that these data are validated on human remains; indeed, it is possible that some species collected in traps or on animal carcasses will not be found on human cadavers, and, at the same time, that there are species which only colonize human bodies that will not be observed on other substrates. With a land area of about 19,000 Km<sup>2</sup>, the state of New Jersey is considered the fifth smallest state; despite this, within the state it is possible to distinguish five different ecoregions: Appalachian Valley and Ridge, Highlands, Newark Basin Piedmont, Inner Coastal Plain, and the Outer Coastal Plain. This variety of ecosystems may suggest a different distribution of insects of forensic importance across the state; surveys have been conducted in New Jersey using traps and animal carcasses, but there is little information on which insects actually colonize human remains. To fill this gap, in summer 2022, the Department of Biology of Kean University has established a collaboration with the Office of the Chief State Medical Examiner (OCSME) to collect and identify representative samples of insects collected at death scenes and during autopsies; the insect collections will be performed during all different seasons and the insects will be identified morphologically or molecularly (metabarcoding). The results of this survey will be presented showing potential differences among geographical areas, seasons, indoor and outdoor scenes, and will also be compared with the published results of surveys conducted using baits and pig carcasses. Here we present the preliminary results of this survey.

### Forensic Entomology; Calliphoridae; Colonization

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**I71 An Alleged Patricide by Americium-241 Poisoning**

*Wendy Gunther, MD\*, Office of the Chief Medical Examiner, Tidewater District, Norfolk, VA*

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**Learning Objective:** After attending this presentation, attendees will review the diagnosis and presentation of americium-241 and topical ricin cream poisoning and consider methods of detecting radioactive poisoning in an exhumed, embalmed body.

**Impact Statement:** This case report will impact the forensic community by reviewing the procedure for exhumation and analysis of an embalmed body after allegations of patricide by either americium-241 or topical ricin cream poisoning.

Five months after an 82-year-old man died from Alzheimer dementia, police investigating his son on unrelated allegations reviewed a video from an in-home security camera, which appeared to show that the son and a hospice nurse euthanized the father with a sublingual pill. During the investigation, officers found bomb-making materials, and invited the Federal Bureau of Investigation (FBI) to collaborate. A police interview with the hospice nurse did not support allegations of homicide, but while investigating, FBI experts found searches on the son's computers for "How to commit murder using americium from smoke detectors" and "How to kill with topical ricin cream."

Americium-241 in smoke detectors emits primarily alpha particles with low levels of beta and gamma radiation. The half-life is 470 years. Early smoke detectors contained up to 2mCi of americium; current detectors contain about 0.9µCi. Intestinal absorption leads to concentration in the liver and skeleton; 3–4mCi of ingested americium will cause death.<sup>1</sup>

Police noted that the son proffered a smoke detector casing to the investigating detective, volunteering that he gutted smoke detectors to convert the casings to nanny-cams for friends. Receipts and casings for over 60 smoke detectors were retrieved during the investigation, which could provide a lethal dose of americium. The FBI recommended precautions for the exhumation of a possibly radioactive body.

Radiation Health consultants recommended lead shielding for the autopsy pathologist; the requirement for lead shielding for personnel performing the exhumation was expected to depend on the level of radiation detected. Hazardous Materials (Hazmat) consultants brought Geiger counters to the exhumation. A consultant pre-tested his Geiger counter on his smoke detector at home, confirming modestly elevated levels at very close range.

Exhumation occurred nine months after death, following thunderstorms. The grave was waterlogged; the wooden coffin had cracked, resulting in water accumulation around the body. Removal of the coffin lid showed a wet, embalmed, elderly decedent. Only background radiation was detected by the consultants on examination of the grave site, grave dirt, coffin, and body. No lead shielding was required for funeral home personnel. The waterlogged coffin could not be elevated; the decedent was removed from the coffin on a cloth shroud, with gloves to prevent possible ricin cream transfer to handling personnel and placed in a body bag for autopsy at the OCME.

Hazmat consultation at autopsy confirmed no elevation of radiation levels in the intact body, the liver, the long bones, or the intestines. Wet skin precluded any search for traces of ricin cream; random skin samples were provided to the FBI. Additional fluid and tissue samples were supplied for FBI and toxicology lab analysis, including chest fluid, bile, urine, and visceral tissue for drug analyses, and long bone (humerus) for FBI americium detection. Brain autolysis precluded autopsy confirmation of Alzheimer disease. Significant atherosclerotic and hypertensive changes were detected in the heart, vascular tree, and kidneys. There was no diarrhea in the large intestine such as might be identified in ricin poisoning. Clots in the heart and lungs were indeterminate grossly for antemortem origin.

After extensive testing, the toxicology lab detected only methanol from the embalming procedure. No poisons were detected. Histology demonstrated bilateral antemortem pulmonary embolism from deep venous thrombosis of the lower extremities. These conditions are not associated with sublingual medication, americium, or ricin poisoning.

The case was finalized without FBI ricin or long bone americium test results (expected to take years). Death was attributed to pulmonary embolism with contributing disease processes. The manner of death was deemed natural. Investigation of the son on unrelated charges is ongoing; no charges of homicide are planned.

**Reference:**

- <sup>1</sup>. ASTDR (Agency For Toxic Substances And Disease Registry) *Toxicological Profile for Americium*. April, 2004.  
<https://www.atsdr.cdc.gov/ToxProfiles/tp156.pdf>.

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**Americium-241; Ricin; Embolism and Thrombosis**



**I72 Fatal Butane Acute Intoxication in Custody—Pitfalls and Recommendations: An Analysis of Six Cases**

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**Learning Objective:** It will be possible to point out the strengths and pitfalls in the management of fatal butane acute intoxication in custody. In these cases, crime scene investigation should be considered mandatory to determine whether it is an intentional or accidental event. Moreover, toxicological analysis is essential to support the circumstantial data. On the other hand, the difficulty of making targeted sampling as well as adequate storage should be taken into account.

**Impact Statement:** The authors want to highlight the importance of a complete methodological approach starting from an accurate crime scene investigation, and then focused on to the optimal sampling and toxicological analysis procedure, all corroborated by a complete postmortem examination by autopsy, histology, and immunohistochemistry.

Fatalities due to inhalation of volatile compounds include a wide range of substances, with both intentional and accidental deaths. Butane's acute intoxications are the most reported cases, second only to toluene ones. Butane is a colorless and flammable gas belonging to the class of aliphatic hydrocarbons, commonly contained in lighter fluid, fuel, hair spray, deodorants, etc. Butane-related deaths are described as a growing occurrence, especially among young people because of the easy availability on the market of these products, but forensic literature provided only a few reports. In Italy, only a few fatalities have been described, most of which occurred in custody. In this country, prison cells are equipped with combustible gas cylinders and, considering the hallucinogenic and euphoric properties of butane, it is not infrequently used by drug addicts as an alternative practice with fatal outcomes. On the other hand, for example, an Italian two-decades-long study about suicide in the detention regime pointed out that butane-related deaths were approximately 6%. In addition to this, eight other similar events are described in the literature. The authors analyzed six fatal butane intoxications that occurred in custody between 2016 and 2022, proposing a shared methodology for a broad-spectrum approach to similar cases.

In only three of them, a crime scene investigation was carried out by a coroner. In all cases, external examination, autopsy, and histological examination were performed. General signs of asphyxia—such as abundant foam to the bronchi and trachea, and subpleural and subpericardial hemorrhagic petechiae—were a common finding. Histological studies highlighted massive intralveolar edema and polyvisceral congestion; foci of contraction band necrosis were found one time. In one case, the toxicological investigation was not performed: otherwise, the qualitative analysis for the butane test, in the sample supplied, gave positive results in four out of five events. Immunohistochemical investigation was also carried out on paraffin-embedded brain samples to detect early anoxia markers.

Comparing the approach used in these six cases with the literature, it was possible to point out the strengths, but also pitfalls, in the management of this type of death in prison. In these cases, crime scene investigation should be considered mandatory to determine whether it is an intentional or accidental event. Moreover, toxicological analysis is essential to support the circumstantial data. On the other hand, for example, the difficulty of making targeted sampling as well as adequate storage should be taken into account.

In conclusion, the authors want to highlight the importance of a complete methodological approach starting from an accurate crime scene investigation, and then focused on to the optimal sampling and toxicological analysis procedure, all corroborated by a complete postmortem examination by autopsy, histology, and immunohistochemistry.

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1. "Two Cases of Acute Propane/Butane Poisoning in Prison", Riccardo Rossi et al.; doi: 10.1111/j.1556-4029.2011.02003.x.
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**Butane-Related Death; Death in Custody; Forensic Toxicology**

## 173 Death as a Consequence of Cardiorespiratory Arrest Caused by a Cocaine-Induced Generalized Seizure: Toxicological Data in a Case Report

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**Learning Objective:** A manifestation of cocaine-induced toxicity is the onset of epileptic crises.<sup>1-5</sup> Generally, the diagnosis of seizures caused by cocaine consumption is made by excluding other possible causes and toxicological analysis confirms the diagnosis. However, studies dealing with cocaine and its metabolites concentration in blood capable of inducing seizures are rare. We present the case of the death of a 26-year-old subject due to cardiorespiratory arrest, resulting from a cocaine-induced seizure, providing toxicological data about the doses at which the crisis developed.

**Impact Statement:** To our knowledge, there are no studies reporting concentrations of cocaine and its metabolites in organs in cases of death resulting from cardio-respiratory arrest during epileptic seizures induced by cocaine. The purpose of the present study is to present the case of the death of the above-mentioned 26-year-old subject.

According to the literature, seizures caused by cocaine consist of generalized tonic-clonic crises, which generally resolve spontaneously.<sup>2-7</sup> These episodes can equally manifest in subjects without previous epileptic crises in their medical history and in individuals affected by epilepsy.<sup>3-5</sup> In literature, cases of death occurred as result of epileptic crises induced by cocaine intake have been described.<sup>1,8</sup> However, studies dealing with cocaine and its metabolites concentration in blood capable of inducing seizures are rare.<sup>3</sup>

**Case Report:** As stated by some friends, at around 10:00 p.m., a 26-year-old boy, after having dinner, drank alcohol and snorted cocaine (6 or 7 strips). The day after, at around 12:00 a.m., he woke up complaining about an intense headache and nausea. After 1:00 p.m., he developed tonic-clonic seizure, as inferred by the pathologist who subsequently performed the autopsy from the description provided by witnesses. Rescuers arrived on site at 1:35 p.m. and found the subject in cardiorespiratory arrest. Despite the RCP maneuvers, the subject was declared dead. Autopsy was performed three days after the death. Organs and biological fluids samples were then taken for subsequent histological and toxicological analysis.<sup>16</sup> No pathological signs were detected during macroscopic and microscopic heart examination. Toxicological analysis of samples collected during autopsy, revealed concentration of 30.43ng/ml of cocaine and 222.44ng/ml of benzoylecgonine in the blood, less than the amount at which an overdose occurs, and 487.90ng/ml of cocaine and 16,285.28ng/ml of benzoylecgonine in urinary samples, that allowed the pathologist to affirm that the subject at the time of development of the crisis had already metabolized a quantity of the drug. No other drug was detected. The cause of death was attributed to cardiorespiratory arrest resulting from cocaine-induced seizure.

**Discussion:** Seizures are a possible side effect of cocaine use.<sup>2-5</sup> Different mechanisms for their development have been hypothesized: repression of central nervous systems inhibitors; increase in serum serotonin levels; cocaine sensitization defined as kindling.<sup>9</sup> According to this phenomenon (repeated intake of cocaine at “sub-convulsive” constant doses), a subject has a greater predisposition to develop epileptic seizures even at doses that, if taken sporadically, would not induce this side effect.<sup>4,5,10-12</sup> Dhuna affirmed that intravenous administration of cocaine is more related to the development of seizures than smoking and snorting the drug.<sup>5</sup> Most of cocaine-induced epileptic seizures resolve spontaneously without neurological disease. Nevertheless, seizures that caused death have also been described in literature, for respiratory paralysis or following the development of arrhythmias, including atrial or ventricular tachyarrhythmia, asystole, bradycardia or atrium-ventricular blocks.<sup>1,4,8,13</sup> In our case, toxicological analysis performed on samples collected during autopsy revealed a concentration of 30.43ng/ml of cocaine and 222.44ng/ml of benzoylecgonine in the blood, less than those in which an overdose occurs and 487.90ng/ml of cocaine and 16,285.28ng/ml of benzoylecgonine in urinary samples, that allowed the pathologist to affirm that the subject at the time of the crisis had already metabolized a quantity of the drug.<sup>14,15</sup>

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**Cocaine; Seizure; Toxicology**

## 174 Sodium Nitrite Poisoning: A Case Series of an Emerging Suicide Mechanism

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**Learning Objective:** This presentation looks at four separate cases of sodium nitrite poisoning, three as known suicides. This uncommon mechanism of suicide will be discussed in detail, including toxicology, pathology, findings on postmortem exam, and common co-ingested substances. This will also be accompanied by a comprehensive review of current literature on sodium nitrite in the forensic setting.

**Impact Statement:** Sodium nitrite provides a challenge to forensic providers, as it is readily available, rapidly metabolized, and sometimes only identifiable from its physiologic effects. The frequency of its use in fatal poisoning, especially in suicides, has significantly increased in recent years. This detailed review and four cases will aid AAFS members in properly differentiating this cause of death, as well as identifying decedents in which it should be considered.

Sodium nitrite is a readily available substance commonly used for food preservation, antimicrobial defenses, and industrial chemistry.<sup>1-3</sup> However, in recent years, this simple substance has shown increasing use as a suicide agent.<sup>4-6</sup> While the median LD50 for the compound itself is 180mg/kg, corresponding to a lethal dose around 15g for an adult male, as little as 1–3g can be sufficiently lethal due to rapid progression of symptoms.<sup>7-9</sup> Currently, 50g of this substance are available without restriction from online marketers for less than \$10.<sup>1,2</sup>

Sodium nitrite is tasteless, rapidly dissolvable in water, and easily absorbed. It rapidly converts to nitrate once absorbed, in the process reducing the iron in hemoglobin and resulting in methemoglobinemia and rapidly progressive hypoxemia.<sup>3</sup> At the same time, the resulting nitrate results in vascular dilation via the cGMP system, only worsening oxygen delivery.<sup>10</sup> As the hypotension and hypoxia progress, significant nausea ensues, resulting in common co-ingestion of antiemetics, along with use of other substances to aid in sedation while the toxicity develops, including reported cases of ranitidine, doxylamine, and opioids.<sup>12,13</sup> This is also frequently accompanied by ingestion of any other medications available, commonly including benzodiazepines, antipsychotics, SSRIs, and acetaminophen. Depending on underlying comorbidities this then typically results in death via bradycardic shock.

Care must be taken in differentiation, especially in pediatric decedents, as methemoglobinemia can occur with congenital conditions, as well as from benzocaine products, high-nitrate well water (especially if boiled), and high doses of home-made baby food from beets, carrots, or spinach before around four months of age.<sup>14,15</sup> Should resuscitation have been attempted, decedents may have received the rescue medication methylene blue in an attempt to reverse the reaction. Since this product is rapidly broken down, the chemical itself is often undetectable, though findings of severely elevated methemoglobinemia with elevated urine nitrite are reliable for diagnosis. High levels can also sometimes be seen from incomplete absorption from gastric contents or in vitreous fluid.

As an illustration of this unique poisoning agent, four decedents are presented, all of whom were found to have lab results consistent with sodium nitrite ingestion. The first, a 17-year-old female, had purchased a kit containing sodium nitrite with Primeperan and Tagamet with detailed instructions which she had followed. The second, a 51-year-old male, had purchased a package of the sodium nitrite powder and ingested it along with his prescribed trazodone and hydroxyzine after contracting COVID-19 earlier in the year and feeling he could not fully recover. The third, a 27-year-old male, was found dead after intentional ingestion of “seeds” for suicide, later found to contain high doses of sodium nitrite. And finally, the fourth, a 33-year-old male, was found dead on a welfare check with no clear signs of suicide. He was found to have a genetic cardiomyopathy variant which had resulted in chronic health issues not elucidated prior to his death along with high levels of sodium nitrite, but without a clear source of the poison. These four cases provide a framework for recognizing decedents in which this cause should be considered, along with review of their autopsy findings and toxicology.

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**Sodium Nitrite; Poisoning; Suicide**

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**I75 The Use of Over-the-Counter Tests to Detect Pregnancy in Postmortem Urine and Vitreous Fluid**

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**Learning Objective:** Attendees will learn the utility of over-the-counter pregnancy tests in determining the gestational status of decedents of child-bearing age using postmortem urine, vitreous fluid, and decompositional fluid.

**Impact Statement:** This presentation outlines a method to address the historic underreporting of pregnancy-associated deaths using an easily accessed, economical, and extremely reliable method of detection. This research seeks to encourage forensic pathologists to test female decedents of child-bearing age to unmask and address the frequency of pregnancy-associated deaths. Although this project was initially conceived as a method to detect pregnancy in deaths due to violence, the recent Supreme Court decision to overturn *Roe v. Wade* makes it likely that maternal mortality due to all causes, including violence, are likely to increase, making the findings applicable to a potentially greater number of cases.

Pregnancy-associated deaths—deaths caused not by the pathologies of gestation, but instead due to violence temporally associated with pregnancy—are a significant source of maternal mortality. Research has found that as many as 20 percent of deaths during pregnancy were at the hands of another, making homicide the leading manner of death for expectant mothers.<sup>1</sup> While common sense would suggest that the identification of products of conception would be evident during the postmortem examination, in fact, pregnancies of fewer than 4 to 6 weeks after conception are unlikely to be anatomically identified at autopsy without microscopic examination of the uterus.

The difficulty in identifying early pregnancies likely contributes to the issue of underreporting, as this time period may represent the earliest point at which a woman realizes she is expecting and disclosure of such information may elicit a homicidal response from her partner, often because the purported father is unwilling to support a child or believes he is not the biological father. We purport that low-cost urine pregnancy test sticks are an effective instrument to identify early gestation in homicide victims. Urine pregnancy test sticks can detect minute quantities of human chorionic gonadotropin (hCG), the hormone that facilitates the production of progesterone and the continued viability of the implanted egg, as soon as six days after the implantation of a fertilized egg into the uterine wall.

This study employed toxicological samples derived from autopsy to examine the validity of postmortem pregnancy testing using over-the-counter test sticks. The study found that postmortem urine will trigger a positive pregnancy test throughout gestation. Corresponding samples of vitreous fluid were similarly effective in detecting pregnancy, as was decompositional fluid. Testing of refrigerated specimens that were up to three years old also resulted in positive pregnancy tests, indicating that B-hCG is stable over time.

This pilot study shows the value and validity of postmortem pregnancy testing using commercially available test sticks on urine, vitreous fluid, and decompositional fluid. The method is economical, easily interpretable, quick, and technically simple to perform.

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**Pregnancy; Homicide; Postmortem Testing**



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**I76 Jay Dix Memorial Bonus Day**

*Michael A. Graham, MD, Saint Louis University School of Medicine, Saint Louis, MO; Joseph A. Prahlow, MD\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI; Andrew Baker, MD\*, Hennepin County Medical Examiner's Office, Minneapolis, MN; Susan F. Ely, MD\*, Office of the Chief Medical Examiner for the City of New York, New York, NY; Joyce L. deJong, MD\*, Western Michigan University Homer Stryker MD School of Medicine, Kalamazoo, MI; James R. Gill, MD\*, Office of the Chief Medical Examiner, Denver, CO*

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**Learning Objective:** This year's lecturers will discuss the medicolegal investigation of deaths related to the environment; deaths due to blunt force injuries; deaths due to firearm injuries; deaths due to natural disease; and deaths temporally associated with sports and recreation. 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 can expect to learn how and why deaths related to the previously specified topics occur. This presentation will impact the forensic science community by informing attendees of a systematic approach to the evaluation of such deaths that can easily be implemented in their daily practices.

There are multiple factors that can play a role in deaths that are related to the environment. This lecture will review human and environmental factors that potentially affect a person's ability to survive under a variety of environmental conditions. What constitutes "hostile" environmental conditions, how humans attempt to adapt to changes in the environment, and what prevents successful adaptation to changing environmental conditions will be discussed. There will be discussion of how humans get into hostile environments, how they attempt to adapt to these conditions, and what factors prevent adaptation and/or escape from inhospitable environments. Deaths caused or contributed to by cold, heat, altitude, drowning, animals, and lightning will be among the topics discussed. (Presenter—Joseph Prahlow, MD)

Firearm fatalities are a major cause of non-accidental morbidity and mortality in the United States. Multiple factors and mechanisms are involved in producing firearm injuries. Understanding and evaluating firearm injuries requires basic understanding of how these injuries are produced and how to distinguish them from other types of traumas. This lecture will provide a comprehensive review of these issues. (Presenter—Andrew Baker, MD)

Blunt force injury is one of the major categories of mechanical injury. Blunt force injuries are among the most common injuries sustained by persons. These injuries include abrasions (scrapes), contusions (bruises), and lacerations (tears). Blunt force is also a substantial component of chop wounds, injuries caused by relatively heavy-edged objects such as a machete or axe. Multiple factors and mechanisms are involved in injuries and deaths involving blunt forces. Understanding and evaluating injuries and deaths in which blunt force injuries may have played a role requires a basic knowledge of injuries caused by blunt forces and how to distinguish them from other types of traumas; recognition of patterned injuries; and recognition of injury patterns (e.g., pattern of falling versus pattern of being struck by an object). This lecture will provide a comprehensive review of these issues. (James Gill, MD)

Natural diseases that result in the sudden unexpected deaths of adults constitute a significant number of cases evaluated by the medical examiner. These deaths may involve previously undiagnosed common conditions well-known to cause sudden death, conditions that cause sudden death under characteristic circumstances, common conditions that rarely cause death, rare conditions, and lethal conditions lacking anatomic manifestations. Death can involve a variety of lethal mechanisms. In some cases, natural diseases can precipitate a traumatic incident. Although the manner of death in these cases is typically "natural," in some cases the circumstances precipitating death can warrant a non-natural manner such as homicide. This lecture will provide a comprehensive review of these issues. (Susan Ely, MD)

There are multiple factors that can play a role in deaths that are temporally related to participating in and, occasionally, while being a spectator at sporting or other recreational activities. This lecture will review several issues that arise in the context of investigating deaths that occur in relation to sports/recreational events. Understanding factors that are involved in these deaths also helps in instituting safety measures to protect participants and spectators. (Presenter—Joyce deJong, DO)

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**Pathology; Trauma; Natural Death**



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**I77     A Non-Destructive DNA Extraction Technique for Application on Insect Puparia (Diptera: Calliphoridae)**

*Sydnee Wedel, BSc\*, Arizona State University, Glendale, AZ; Jonathan Parrott, PhD\*, Arizona State University, Glendale, AZ*

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**Learning Objective:** Attendees will learn the importance of insect evidence, in particular those pertaining to insect puparia. This presentation will detail the usefulness of DNA identification of insect evidence and how this can be used to both genetically identify while maintaining the external characteristics used in traditional taxonomic techniques.

**Impact Statement:** DNA extraction from entomological evidence is traditionally a destructive technique, especially with insect puparia. This presentation will detail a non-destructive technique that allows extraction of DNA of up to 400 base pairs from puparia, while maintaining external characteristics used for morphological identification.

Correct species identification is a fundamental prerequisite of any minimum time-of-death investigation using insect evidence. Traditional methods use taxonomic keys that can identify species. However, this technique is reliant on non-damaged specimens, species being included in the taxonomic key, and availability of keys to the developmental stage. One particular immature stage that is difficult to identify using traditional methods is the pupal stage. The puparium, which is the “casing” that the insect is developing in, is primarily made of chitin and is durable. This pupal stage is typically the longest of all immature stages, retains few taxonomic features to identify, and has few keys available to use. DNA extraction methods can be used to facilitate this issue; however, most methods are destructive, leaving little to none of the original samples for future use.

This project aimed to create a non-destructive DNA technique that could be applied to insect puparia evidence and extraction/amplification of mitochondrial DNA while retaining the external features of the evidence, allowing future use of any available taxonomic keys. Two treatments, sterile scrapping ( $n=30$ ) and swabbing ( $n=30$ ), were applied to the internal structure of the puparium while the outside was kept untouched. The internal structure contains no diagnostic features that are used for any identification purposes. This was performed on *Calliphora latifrons* (Diptera: Calliphoridae). Using a modified QIAGEN® DNeasy® method, DNA was extracted. An approximate 350 base pair region of the cytochrome oxidase I was amplified. Successful amplicons were sequenced using Sanger sequencing at Arizona State University and inputted onto Blastn for species assignment. Results showed that both methods produced successful DNA extraction and amplicons. Future work will examine methods to extract higher molecular weight DNA for species that require longer sequence reads. This method can be applied to entomological evidence to extract DNA while retaining external features traditionally used in taxonomic keys.

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**Entomology; DNA; Calliphoridae**

## 178 The American Board of Forensic Entomology-Certified Workshops: How Collaborative Efforts Between Researchers, Practitioners, and Professionals Continue to Refine and Strengthen the Discipline

*Jennifer Rhinesmith-Carranza, DPhil\*, Texas A&M University, Bryan, TX; Casey Flint, DPhil, Texas A&M University, Bryan, TX; Jeffery Tomberlin, PhD, Texas A&M University, College Station, TX*

**Learning Objective:** This presentation will explore a brief history of the use of entomology in legal investigations, including a review of recent developments and standardizations accepted by the discipline. It will then recount the recent approval of a standardized forensic entomology workshop and provide an example of how such workshops can provide critical skills and knowledge to forensic professionals and the potential for these workshops to generate critical data needed for applications of forensic entomology.

**Impact Statement:** This presentation will impact the forensic science community by recounting recent milestones in the field of forensic entomology and highlighting the importance of the best practices and knowledge communicated during an American Board of Forensic Entomology (ABFE) - certified forensic entomology workshop. It will also demonstrate how data generated from collaborative efforts resulting from these workshops can fill gaps of knowledge in forensic entomology.

The forensic applications of entomological science are diverse and include insights into investigations of death, abuse, neglect, food contamination, public safety, and much more. The use of insects to assist in homicide investigations dates back to Sung Tz'u in the 13<sup>th</sup> century, and the discipline has come a long way from trace evidence on sickles in rice fields while continuing to refine, advance, and further strengthen the foundation of its forensic applications in peer-reviewed science.

One of the major milestones in the standardization of forensic entomology was the formation of ABFE, the certifying body for experts in North America, in 1996. Since then, the ABFE has established three levels of certification, each with their own requirements and privileges. Member and Diplomate status are intended for those analyzing evidence and providing expert opinions, while the Technician certification, established in 2017, is designed to certify qualifying professionals in the proper handling of entomological evidence. These certifications, along with (1) the discipline's proactive response to the National Research Council's 2009 call for increased standardization and (2) the publication of entomological evidence collection standards through the American Academy of Forensic Science's (OSAC) Crime Scene Investigation and Reconstruction subcommittee, have served as several critical steps in strengthening and standardizing the scientific foundation upon which entomology is applied in forensic contexts.

In 2021, the ABFE certified a standardized forensic entomology workshop designed to educate professionals on the best practices regarding entomological evidence. One of the first of these workshops was conducted in July 2022 by the Forensic Laboratory for Investigative Entomological Sciences (F.L.I.E.S.) Facility in conjunction with the Federal Bureau of Investigation (FBI) Denver Division Evidence Response Team and served to train forensic professionals from the FBI, Naval Criminal Investigative Service, Air Force Office of Special Investigations, United States Fish and Wildlife Service, and more. Not only did this workshop serve to educate federal, state, and local forensic professionals in best practices, but its design, preparation, and execution allowed for the generation of local data for multiple forensically relevant species in the Colorado area as well as provided a much-needed validation study for existing development data for *Phormia regina* (Diptera: Calliphoridae), a species commonly used in time of colonization calculations in forensic entomology.

This presentation will cover these recent milestones and will recount with specific examples how the development data and validation work generated by this workshop provides critical data missing in forensic entomology at this time, and how the applications of this data will help refine the use of entomology in forensic investigations.

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### Entomology; Time of Colonization; Postmortem Interval

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**I79     An Overview of the Decomposition Process During North Dakota Extreme Winter Temperatures**

*Lavinia Iancu, PhD\*, University of North Dakota, Grand Forks, ND*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the decomposition process dynamics during extreme winter temperatures in North Dakota, crucially important data for the postmortem interval (PMI) estimation in an environment dominated by negative temperatures and heavy snow.

**Impact Statement:** This presentation will impact the forensic science community by providing one of the most complete overviews of decomposition during winter, serving at the same time as a means of reference for forensic cases from cold environments.

The postmortem interval (PMI) is the time elapsed between death and the body discovery, with its estimation being extremely important in the court of law in cases of homicide and suspicious deaths. The decomposition process of an animal or a human carcass can be divided into five stages of decay based on physical and chemical changes. The physical changes commonly documented are temperature changes (algor mortis), muscular contraction/relaxation (rigor mortis), and the pooling of blood by gravity after blood circulation stops (livor mortis). The tissues progress through these stages faster during higher temperatures, while the lower temperatures can cause a delay or even can halt decomposition, making the PMI estimation challenging. At the same time, the decomposing tissues attract both vertebrate and invertebrate scavengers and host a diverse microbial community.

Studies have primarily focused on decomposition during elevated temperatures, using outdoor locations or controlled laboratory conditions. However, scarce information exists on decomposition during negative temperatures. It is well known that the decay rate is influenced by many factors, including the cause of death, environment, season, vertebrate and invertebrate scavengers, humidity, oxygen content, precipitation, and temperature. Among all factors, environmental temperature plays a crucial role for insect activity, microbial content, and rate of the decomposition process.

Consequently, the current research aimed to provide data related to the decomposition process of swine carcasses along six months of extreme winter temperatures, in a North Dakota outdoor location, analyzing the evolution of the stages of decomposition, the climatic conditions, and the tissue necrobiome structural patterns during carcass breakdown. The results that will be presented during this meeting will provide much needed information related to the pattern of the decomposition process during temperatures as low as -40°C and with heavy snow conditions. This data is part of a broader study that includes microbial spatial and temporal evidence currently under investigation.

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**Decomposition Process; Winter; North Dakota**

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**180     An Analysis of the Decomposition Process in Two Restricted-Access Environments in a Temperate Climate: Suitcases Versus Wheeled Bins**

*Hannah Andrews, MSc, Discipline of Medical, Molecular & Forensic Sciences, Murdoch University, Perth, Western Australia, AUSTRALIA; Ian Dadour, PhD, Source Certain, Wangara, Western Australia, AUSTRALIA; Paola Magni, PhD\*, Discipline of Medical, Molecular & Forensic Sciences, Murdoch University, Murdoch, Western Australia, AUSTRALIA*

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**Learning Objective:** Following this presentation, attendees will have a better insight into the variations of decomposition in restricted access environments and the application of estimating the PMI in such cases.

**Impact Statement:** This study will impact the forensic community by comparing different methods of scoring decomposition and using both the taphonomic process and forensic entomology to estimate the postmortem interval of remains in full access, relative access, and restricted access environments.

Decomposing remains, human and/or non-human, are regularly recovered from concealed environments, such as burial, wrapping, suitcases, garbage bins. In such locations, these remains are protected from abiotic and biotic factors, including any carrion fauna that has little to no access to them. Many of these factors influence the rate and pattern of decomposition and, hence, the accuracy and reliability of the postmortem (PMI) estimation. The PMI is of vital importance in a forensic investigation as it can be used by law enforcement to include and exclude potential perpetrators of a crime. The different methods of concealment, the location, and the season of concealment affect the progression of decomposition and the associated insect activity. Consequently, it is necessary to understand the unique decomposition processes involved based on when/where/how/how long remains are concealed.

This study extends the current research on decomposition and insect activity in concealed environments. Swine carcasses concealed in hard-covered suitcases and wheeled bins with hinged lids were compared with each other and with control carcasses placed on the surface with full exposure during the months of June to November (winter and spring) in Western Australia. The hard-covered suitcases were classified as “restricted access,” the wheeled bins as “relative access,” and the exposed carcasses as “full access.” Temperature and humidity data loggers were placed in the field as well as inside the suitcases and the wheeled bins. External examination was recorded using photography, which was performed on a daily basis from the beginning to the end of the experiment, while collection and laboratory analyses were performed monthly. The analysis of the three treatments was performed comparing the decomposition process of the controls with that in the suitcases and wheeled bins, followed by a complete analysis of the insect assemblages colonizing the carcasses of both control and treatments. The rate and pattern of decomposition were analyzed using the Total Body Score method, in conjunction with accumulated degree days. Entomological analyses followed the best practice and guidelines for forensic entomology. From this study, more accurate PMI estimations will be readily relevant to cases of concealment.

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**Taphonomy; Entomology; Limited-Access Environment**

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**181     Decomposition in the Desert: An Analysis of Initial Insect Activity on Swine Remains**

*Lauren Weidner, PhD\*, Arizona State University, Glendale, AZ*

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**Learning Objective:** Attendees will learn about the initial insect arrival and activity on decomposing swine remains in the Sonoran Desert, specifically in Phoenix, AZ. Attendees will see a comparison of insect activity across all four seasons and throughout three weeks of decomposition.

**Impact Statement:** This presentation is the first to catalog and discuss insect interactions in-depth on decomposing remains in the Phoenix area. The forensic science community will receive an updated list of forensically relevant insects, arrival times, and insights into how the environment of a desert landscape (hot and arid) can impact forensically relevant insects' behavior.

The field of forensic entomology is rapidly growing due to the valuable information that entomological evidence can provide, including insights into how long remains have been present, through the determination of a time of colonization (TOC), the presence of drugs or toxins, and if remains have been relocated. A recent review of the literature has shown that studies examining insect arrival and colonization of decomposing remains in Arizona are nearly non-existent, with no information available in over 40 years and none ever conducted in the Phoenix area.<sup>1</sup>

Phoenix is located in the Sonoran Desert, an area that can be extremely arid and hot, creating an environment not commonly encountered throughout the United States. In addition, it is one of the fastest-growing heat islands in the country. These environmental conditions make comparing other entomological decomposition studies unreliable. To remedy this knowledge gap, we conducted four decomposition studies across one year in Phoenix using two swine carcasses per season (Jan, April, July, and October), for a total of eight carcasses. Decomposition trials ran for a total of three weeks, and each carcass check consisted of temperature collections, regular and thermal photographs, insect sweeps, hand collections, and pitfall collections. Four families of flies (blow flies, flesh flies, house flies, and cheese skippers) were present at every carcass in every season. Six of the eight swine carcasses were initially colonized by blow flies with the remaining two carcasses colonized by flesh flies. Blow flies were the predominant family that colonized all remains across the year and included eight species. In the spring and winter all remains were colonized within six hours of placement, while carcasses in the fall varied with colonization occurring within four hours for one carcass and 24 hours for the second. Carcasses in the summer experienced a severe delay in colonization, with initial colonization occurring between 42–50 hours after exposure. These findings show the potential impacts that high temperatures and arid environments have on initial insect colonization and the negative effect this delay can have on the accuracy of the TOC if environmental variables are not considered when analyzing entomological evidence.

**Reference:**

- <sup>1</sup>. Weidner, L. M., Meeds, A. W., Noblesse, A. P., & Hans, K. R. (2021). A review of Forensic Entomology literature in the southwestern United States. *Wiley Interdisciplinary Reviews: Forensic Science*, 3(5), e1421.

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**Forensic Entomology; Decomposition; Initial Insect Arrival**

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**182 Impacts of Climate Change in Forensic Entomology Casework**

*Michelle Sanford, MS, PhD\*, Harris County Institute of Forensic Sciences, Houston, TX*

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**Learning Objective:** After attending this presentation, attendees will be aware of the current and potential future impacts of climate change on forensic entomology casework.

**Impact Statement:** This presentation will impact the forensic community by illustrating the needs for future research on the development of forensically important insects and considerations for existing data application to casework. This presentation will also highlight the potential for shifts in the forensic insect community that may alter succession-based postmortem interval estimates.

As global temperatures rise, so does our awareness of the impacts and potential future impacts on our work and way of life. One of the lesser appreciated aspects of climate change is the potential for its impact on forensic entomology casework in both developmental estimates based on temperature and insect communities, due to temperature change and extreme weather events. As poikilothermic animals, insects have a close relationship to environmental temperatures, and this relationship forms much of the basis for their use in estimation of time of colonization (TOC) and minimum postmortem interval (mPMI) in casework. Insects also have rapid generation times and many species with close associations to humans have the ability to adapt to local climatic conditions.<sup>1,2</sup>

Harris County, TX, located along the upper Gulf Coast, is the fourth-largest county in the United States and a hub for commerce and human migration. For these reasons, several introduced fly species with origins in subtropical and tropical locales have established populations. In the ten years since the Harris County Institute of Forensic Sciences integrated forensic entomology into their existing services, temperatures in the Houston area have increased on average 0.5°F (0.3°C), based on data from the two biggest airports: HOU and IAH (as compared to previous the ten-year period). The city of Houston is also considered a significant urban heat island with an estimated effect of 7.46°F (4.14°C) and observed maximum temperature difference within the city in the summer of 2020, of 17.1°F (9.5°C). In addition, several major extreme weather events have recently occurred in Harris County. Hurricane Harvey in 2017 was considered a 1,000+-year flood event that affected all of southeast Texas. Winter Storm Uri in 2021 deposited highly unusual snow and below-freezing temperatures throughout the state and all the way to the Gulf Coast, with a record low temperature of 13°F (-11°C) recorded in the City of Houston. Temperature changes have the potential to affect local insect populations' response to temperature through selection over time while extreme events may cause more dramatic shifts in insect community composition.

In this study, the impact of temperature on several casework estimates was applied to explore how much the TOC estimate changed with increasing temperatures for each respective case. This was accomplished by incrementally increasing the hourly weather station temperature data based on the observed 0.3°C increase up to and including the global goal to increase only 1.5°C. Additionally, casework was used to explore how the forensic fly community changed with respect to both Hurricane Harvey and Winter Storm Uri, particularly with respect to introduced species. These results suggest that climate change has the potential to influence both temperature-based estimates of TOC and succession-based estimates. They also suggest that there may be a temporally limited accuracy to published development data in addition to its geographical variability, highlighting the need for standardized development data collection and reporting combined with repetition geographically and temporally in order to provide the most accurate data for use in casework.

**References:**

1. Owings CG, Spiegelman C, Tarone AM, Tomberlin JK. Developmental variation among *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) populations from three ecoregions of Texas, USA. *Int J Legal Med* 2014;128(4):709–17. <https://doi.org/10.1007/s00414-014-1014-0>.
2. Gallagher MB, Sandhu S, Kimsey R. Variation in developmental time for geographically distinct populations of the common green bottle fly, *Lucilia sericata* (Meigen). *J Forensic Sci* 2010;55(2):438–42. <https://doi.org/10.1111/j.1556-4029.2009.01285.x>.
3. Climate Central. *Hot Zones: Urban heat islands*. 2021.
4. *Heat Watch. Houston & Harris County*. Houston, TX, 2020.

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**Medicolegal Death Investigation; Temperature; Forensic Entomology**

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**I83      Generating Fluorescent Blow Fly Strains to Aid in the Validation of Postmortem Interval Estimates**

*Amber MacInnis, MS\*, Florida International University, Miami, FL; Andre Luis da Costa da Silva, PhD, Florida International University, Miami, FL; Anthony Bellantuono, PhD, Florida International University, Miami, FL; Matthew DeGennaro, PhD, Florida International University, Miami, FL; Jeffrey Wells, PhD, Florida International University, Miami, FL*

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**Learning Objective:** Attendees will learn of the substantial current difficulties of designing forensic entomology validation experiments for crime scene conditions and how these may be overcome by genetically engineering carrion insects. Attendees will be introduced to the technology of creating genetically modified insects.

**Impact Statement:** This presentation will impact the forensic entomology community by showing the path to an objective measure of uncertainty associated with an estimate of postmortem interval using insect evidence.

The age of a blow fly larva may be used in forensic investigations to estimate the minimum postmortem interval (PMI<sub>min</sub>). In forensic entomology, a maggot of unknown age is compared to experimental reference data to predict its age adjusted for temperature. Typical reference data illustrate development rate in a setting very different from a corpse subject to natural insect colonization. However, empirical validation of maggot age prediction under crime scene conditions presents major logistical hurdles. This would require knowing the true age of the maggot, but in a large corpse or carcass subject to wild insect colonization, all that can be known is that no larva is older than the time since corpse exposure. If a larva could be persistently marked, eggs of known age could be added to the corpse population, would develop under casework conditions, and, if recaptured, would be of known age.

We produced such recognizable larvae by genetically engineering them to produce a fluorescent protein; 1,391 pre-blastoderm *Phormia regina* embryos were injected with a piggyBac donor plasmid containing a marker gene, DsRed-Express2, driven by a heterologous constitutive promoter. The survival rate post-injection was 33%. Surviving adults were outcrossed to wild type (non-injected) and the G1 offspring were screened for fluorescence. We found eight positive uniformly fluorescent larvae from one injected cross and the estimated transformation rate was 1.8%. The stable transgenic line expresses DsRed across all life stages and homozygotes fluoresce brighter than heterozygotes. The insertions were characterized by next generation short read sequencing and genome assembly. A blast search of the transgene against the genomes showed the same single insertion location in all six fluorescent G1 individuals, which appears to be intergenic. We are currently assessing the effect of transformation on the development rate. If development is indistinguishable from wild-type flies, we believe that such transformed strains can be used in age-prediction validation studies.

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**Forensic Entomology; Transgenic; Calliphoridae**



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**184     The Impact of Temperature and Diet on the Development of the House Fly, *Musca Domestica* (Linnaeus) (Diptera: Muscidae)**

*Casey Flint, DPhil\*, Texas A&M University, Bryan, TX; Jennifer Rhinesmith-Carranza, DPhil, Texas A&M University, Bryan, TX; Riley Bell, BS, Texas A&M University, San Antonio, TX; Jeffery Tomberlin, PhD, Texas A&M University, College Station, TX*

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**Learning Objective:** After attending this presentation, attendees will understand how temperature and diet affects the development of a forensically relevant insect species commonly associated with abuse and neglect and the application of these data to a time of colonization estimation.

**Impact Statement:** This presentation will impact the forensic science community by providing the first forensically relevant development study of the house fly, *Musca domestica* L. (Diptera: Muscidae) in the United States. Conclusions from forensic case work are limited as there are no known development data on this species in the United States, and this study provides key missing information in the discipline of forensic entomology, especially in application to forensic entomology casework involving investigations of abuse, neglect, and homicide.

The application of entomology to the law frequently involves insects as pests or consuming the tissue of live or dead animals and humans. The larval stage of most Diptera is focused on consuming the resource available and accumulating enough nutrition to support the pupal transition to the adult life stage. The biology of the insect is important to forensic investigations because the larvae are largely immobile due to a lack of legs and wings; therefore, they will often feed only on the resource on which they are deposited as either eggs or larvae. If an immature Diptera is collected during a forensic investigation, an entomologist can identify the age and the species to calculate a time of colonization based on the development rate and temperatures experienced by the insects in question.

One species of forensic relevance that has not received much attention by the research community specific to forensic entomology is the house fly, *Musca domestica*. This species is synanthropic and commonly associated with food waste, manure, and other organic waste. They can be found associated with remains or in circumstances of abuse or neglect, especially if fecal matter or urine are present, but are not commonly used in medicolegal time of colonization estimates. This limitation is due, in part, to a marked lack of published development data for this species. Only one development study using the commonly accepted by-stage duration breakdown for use in degree day calculations is currently published for this species, and it is from a fly population in China. Population differences in genetics and development time, along with the temperature range studied, impact the ability to accurately use this study in the United States, especially in cases of myiasis.

In the current study, the impact of temperature and diet on immature house fly development was studied. Clutches of ~250 *M. domestica* eggs were placed on one of two different diets, pork chops or beef cattle manure, and placed in a Percival incubator at either 24°C or 37°C with 60% RH. These two temperatures represent an average indoor temperature and an average body temperature, both of which are relevant to investigations of abuse, neglect, or homicide. Hourly checks were conducted to monitor egg hatch, followed by 4-hour checks through the end of the second instar stage, and concluding with 6-hour checks until adult emergence. Three larvae were sampled at each time point to verify life stage. Results indicate that temperature as well as diet affect house fly development. Beef cattle manure did not prove to be a suitable diet for complete development of house fly larvae, likely due to its inability to retain moisture, even if additional moisture was added throughout the development duration. Individuals developing at 37°C developed faster than those at 24°C, resulting in a difference of more than 24-hours in complete development time. This study is the first in the United States to investigate *M. domestica* development in a forensic context. This information can provide crucial information to forensic investigations, particularly in cases where *M. domestica* larvae are the only entomological evidence collected.

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**Forensic Entomology; Time of Colonization; House Fly**

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**185 The Influence of Biomass and Bisection of Remains on the Production of Necrophagous Flies**

*Samantha Sawyer, PhD\*, Curry College, Milton, MA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how manipulation of remains (i.e., vertebrate scavenging) influence arthropod colonization patterns.

**Impact Statement:** This presentation will impact the forensic science community by discussing the impact of body manipulation on the quantity of entomological evidence in casework involving medicolegal and urban forensic entomology.

The presence of necrophagous insects can be utilized to provide forensically important timelines through the time of colonization interval and may provide context in relation to the condition and treatment of decedents before, during, or after death (i.e., neglect and abuse). Previous research has indicated that bodily openings generated by scavengers have the potential to increase the abundance of necrophagous insects at remains with preliminary evidence indicating carrion biomass has an additional effect. Furthermore, it has been established that remains with a high surface area-to-volume ratio decompose faster than those with relatively smaller ratios. However, no research has conducted a controlled experiment to quantify insect production from remains by artificially increasing surface area through disarticulation.

This research aims to better understand the role surface area and biomass play on the production of insects at remains. It is hypothesized that artificially increased surface area through bisection will allow for the greatest abundance of insects emerging from remains, with larger biomasses having the greatest effect. To test this hypothesis, 24 adult feeder rats equally representing three size classes (eight small (3–45g), medium (85–175g), and extra-large (275–375g) rats) were purchased frozen. Four rats from each size class were bisected along the transverse plane, where both halves of a body were considered one replicate. The biomass, diameter, and circumference of the torso were measured from all rats and placed under vertebrate exclusion cages on top of sand in a plastic tub and placed outdoors with at least 30m from the next set of remains on the Curry College Campus in Milton, MA. Remains were left to be colonized for 48 hours before being placed in individual rearing chambers, allowing larvae to develop to adulthood. The remaining biomass was measured after larvae left remains to pupate. Adults were identified to the lowest taxonomic resolution and quantified before calculating successful pupations and emergence. Influence of biomass and surface area on adult population metrics and the residual carrion biomass at the conclusion of the study will be discussed.

This presentation will highlight the need to further understand the surface area to volume ratio of remains, its influence on insect colonization, and its utility in criminal and civil litigation involving insect evidence. Additionally, the need for more interdisciplinary research among entomologists, anthropologists, and wildlife biologists in decomposition ecology will be discussed.

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**Dismemberment; Entomology; Decomposition**

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**186     The Effect of Plastic Bags on Decomposition and Necrophagous Fly Composition**

*Kristi Bugajski, PhD\*, Valparaiso University, Valparaiso, IN*

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**Learning Objective:** Attendees will learn about the effect of plastic bags on the necrophagous fly composition. This presentation will detail how the plastic bags affected the fly composition and the differences between a thick and thin plastic bag.

**Impact Statement:** Plastic bags can be found in homicide, suicide, and accidental death investigation cases involving asphyxiation. The effect that they have on decomposition and the fly community is important for forensic scientists to know for case work. Accurate PMI estimations are crucial to forensic investigations, and any factors that influence blow fly colonization are important for entomologists to consider.

Forensic entomology uses insects to help estimate the minimum postmortem interval (min-PMI) based on blow fly colonization. Accurate PMI estimations are crucial to forensic investigations, and any factors that influence blow fly colonization are important for entomologists to consider. This study focused on placing plastic bags of different thicknesses over the head of pigs. Flies typically lay their eggs in the mucous membranes, and researchers wanted to see the impact of blocking their preferred oviposition sites. Asphyxia via plastic bag has been documented in case studies in the literature as a means of suicide, homicide, and accidental death. Researchers were interested in the effect of plastic bags on decomposition and Dipteran behavior.

Nine fetal pigs were thawed from frozen 24 hours before the start of the experiment. Six fetal pigs were covered with plastic bags: three with thin plastic bags (average bag weight of 5.43g) and three with thick plastic bags (average bag weight of 11.9g). The remaining three pigs served as the control group. These pigs were placed outside, monitored for six days and checked three times daily to look for the presence of blow fly eggs, larvae, and adults. Two trials were done; one commenced on September 27, 2021, and the second on October 19, 2021. Third instar maggots were collected off each pig with the date of collection recorded, pig number, and type of plastic bag (thick, thin, or none). Data analysis included species identification of each maggot collected and statistical analysis of the stages found on the pigs. ANOVA with Tukey post hoc tests were conducted to look for significant differences in the timing of blow fly oviposition and life stages between treatments.

The plastic bags caused no significant differences in the timing of blow fly life events between treatments. However, the presence of plastic bags did affect the species composition of necrophagous flies between different treatments. Among all the treatments, *Lucilia coeruleiviridis* (Macquart) was the dominant species found. *Sarcophagidae* sp. were not found on controls but were found on the thin and thick bag treatments, whereas *Phormia regina* (Meigen) and *Calliphora vomitoria* (L.) were only found on controls. The two dominant flies found (*L. coeruleiviridis* and *Sarcophagidae* sp.) both present problems for PMI calculations. *Lucilia coeruleiviridis* is extremely difficult to rear in a laboratory setting, so the data sets that exist for other species are not available for this fly, and it is not possible to identify *Sarcophagidae* larvae to species. Proper growth tables are essential to accurately estimate the PMI. Scavenging and rain were problematic in this research, especially in the second trial. Future studies will examine ways to discourage vertebrate scavenging, which impacted the results by displacing pigs during the second trial.

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**Forensic Entomology; Necrophagous Fly Composition; Plastic Bags**

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**I87 Cadaver Microbial Signatures of the Submandibular Glands and Thyroid for Forensic Investigations**

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**Learning Objective:** After attending this presentation, attendees will be informed about postmortem microorganisms associated with the submandibular glands and thyroid for use in forensic casework for microbial fingerprinting.

**Impact Statement:** This presentation will impact the forensic science community by providing information on the role of the submandibular glands and thyroid on their potential use as biomarkers for postmortem interval determination.

The study of the thyroid is an emerging topic, particularly in postmortem microbiome studies, due to the organ's ability to affect the endocrine system. Also, the submandibular gland is a promising, emerging gland of study due to its position relative to the oral cavity. Previous thanatomiobiome studies have demonstrated that bacteria belonging to the phyla Firmicutes, Proteobacteria, Bacteroides, and Pseudomonadota predominate internal organs and have been considered an important biomarker for postmortem interval. Further, *Clostridium* species that dominate in internal organs are linked to the hypoxic change that occurs after death, which leads to the switch of bacteria to become obligate anaerobes. Therefore, obligate anaerobes dominate the body after death due to their ability to thrive off fermentation products. 16S rRNA gene sequencing has been critical in thanatomiobiome studies, which refers to the human microbiome (microorganisms within the body) after death. Currently, it has not been elucidated regarding the microorganisms that are associated with the decay of submandibular and thyroid glands.

We hypothesized that through sequencing of the 16S rRNA gene of the submandibular and thyroid glands, the presence of Firmicutes and Proteobacteria will indicate potential biomarkers for postmortem interval. The present study revealed the postmortem microbial signatures of the submandibular and thyroid glands using the 16S rRNA gene, specifically the V3-V4 hypervariable regions, using universal primers 341F and 805R. We investigated a total of 37 cadavers obtained from ongoing criminal casework, 17 submandibular samples and 20 thyroid samples, and found that there is a correlation between microbial abundance in these postmortem glands. The predominating phyla of interest found in both glands were Firmicutes and Proteobacteria. The predominating genera were *Paenibacillus* and *Streptococcus* in both glands, respectively. Further experimentation of the submandibular and thyroid glands will help to link oral thanatomiobiome communities to "microbial clock" determinations, thus enhancing postmortem interval estimation.

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**Cadaver; Microbial Signatures; Forensic Investigation**

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**188 Current Challenges in Protein Degradation-Based Postmortem Interval (PMI) Estimation**

*Stefan Pittner, PhD\*, Paris-Lodron University of Salzburg, Salzburg, AUSTRIA; Fabio Monticelli, PhD, Paris-Lodron University of Salzburg, Salzburg, AUSTRIA*

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**Learning Objective:** This presentation discusses the current challenges to determine the time since death based on postmortem protein degradation, an innovative method that is, however, rarely routinely applied.

**Impact Statement:** A precise determination of the time since death is of high importance in forensic routine. Further research in this field is required to expand the prevailing range of applications in forensic PMI estimation. This presentation will contribute to understanding the current and future challenges of this method.

In the past two decades, several “novel approaches for PMI estimation” based on the analysis of postmortem protein degradation have been published. Methods such as immunohistochemistry, western blotting, and lately especially mass spectrometry (=proteomic analysis) were proposed to analyze the decomposition of protein molecules in several different body tissues for early (e.g., liver, heart, kidney, brain), intermediate (e.g., lung, heart, skeletal muscle), and long PMI ranges (e.g., cartilage, bone). However, there is currently no single technique that is applied in routine forensic investigation. The reasons include: (1) impractical application protocols, (2) heterogeneity of reference literature on behalf of techniques and protocols and thus, restricted comparability of results; as well as (3) limited understanding of the methodic boundaries, such as influencing factors and exclusion criteria. (1) A critical methodic issue in the analysis of protein degradation-based PMI estimation is bringing degradation processes to an end at the time of sampling. This is often achieved by snap freezing in liquid nitrogen and storage in freezer compartments (this technique must be disregarded due to possible freezing artifacts if structural integrity should be preserved (e.g., histology)). However, because liquid nitrogen is impractical for routine application (availability, high acquisition costs, complicated transport, etc.), alternatives are often required. (2) Data transfer from animal models to human application is often viewed critically. However, there can even be differences within, or between, organs, tissues, or body parts to another. Also, postmortem protein degradation patterns detected with one analysis method do not necessarily occur with another, eventually hindering variations and adaptations of protocols. Additionally, complex and time-consuming sample preparations can be inconvertible in the morgue and the field. (3) Proteolysis, as a metabolic process, underlies several influencing factors, including individual and environmental properties and circumstances of death. While (basic) research can be carried out under standardized conditions, these exact conditions rarely occur in routine practice. Additionally, the effects are not limited to *in situ* tissue, but likewise affect a sample once it has been collected, potentially biasing the interpretation of the analysis.

We provide an overview of the current research status, with special attention to the (lack of) applicability of the proposed approaches/methods in forensic practice and discuss the difficulties and challenges, in order to advance existing approaches for reliable routine application. Additionally, we propose a standard procedure for sample collection and preparation and research strategies for actual progress in the field of protein degradation-based PMI estimation.

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**Protein Degradation; Time Since Death; Applicability**

## I89 First Observations on the Environmental Impact of Terrestrial Body Decomposition Within a Canadian Continental, Humid (Dfb) Climate

Emily Pecci, BSc\*, Université du Québec à Trois-Rivières, Trois-Rivières, QC, CANADA; Shari Forbes, PhD, Université du Québec à Trois-Rivières, Trois-Rivières, QC, CANADA; Francois Guillemette, PhD, Université du Québec à Trois-Rivières, Trois-Rivières, QC, CANADA

**Learning Objective:** Attendees will be presented with the first Canadian study on the environmental impact of body decomposition. They will learn about key trends observed in soil carbon chemistry and bacterial function, and how these changes are relevant in ecological and forensic contexts.

**Impact Statement:** The concepts and findings outlined in this presentation will help deepen our understanding of the impact of body decomposition on soil chemical and bacterial dynamics. This can aid in the development of decomposition indicators for postmortem interval (PMI) estimation, grave detection, and the management of decomposition research facilities.

Bodies decomposing within terrestrial settings can generate and release significant amounts of fluid and biological by-products into the surrounding soil.<sup>1</sup> The affected area, known as the Cadaver Decomposition Island (CDI), is considered an environmental perturbation with ephemeral or long-lasting effects.<sup>2</sup> It is well known that bacteria are sensitive to shifts in the amount and lability of energy substrates, such as carbon.<sup>3</sup> Their response to such changes can influence the role of soil bacteria in the cycling of nutrients and energy within an ecosystem.<sup>4</sup> It is, however, unknown how soil bacteria respond to potential changes in organic carbon within a CDI. This presentation aims to address this gap in knowledge by examining the spatial and temporal impact that body decomposition has on soil carbon chemistry and bacterial function.

A human-analog study was conducted using three pig carcasses (*Sus scrofa domesticus*) that were surface-deposited in a temperate mixed forest (Trois-Rivières, QC, CANADA). The A and B horizons of the loamy-sandy soil were sampled at incremental distances (0-200cm) from each pig carcass during the summer, fall, and spring of 2019–20. The chemical characteristics of the soil's carbon pool were evaluated through total organic carbon (TOC) levels and parallel-factor analysis (PARAFAC) of excitation-emission fluorescence spectra. Microbial responses were additionally examined based on bacterial respiration, cell abundance, and carbon substrate utilization capacity.

Changes in both soil carbon chemistry and bacterial function were observed within the A-horizon of soils underneath and up to 20cm from the carcasses. The carbon pool within these soils demonstrated differences in TOC levels and in the composition of protein-like, humic-like, and polyphenolic compounds. The same soils also displayed peaks in bacterial respiration and cell abundance, along with a shift in carbohydrate metabolism. All measures displayed the greatest change in the summer, with a gradual dissipation with each subsequent season. However, carbon composition remained altered into the spring. The trends in this study demonstrate that body decomposition can noticeably impact the environment, both chemically and biologically. Most of these changes appear to be transient and spatially constrained to the topsoil closest to a body, except for alterations in soil carbon composition, which may persist for an extended period. Although the present results are from non-human models, they provide valuable insight into the detectable environmental disturbance of body decomposition. This can contribute to the development of techniques for PMI estimation and grave detection as well as for the monitoring and mitigation of cross-contamination between bodies and deposition sites within a decomposition research facility.

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### Decomposition; Soil Chemistry; Microbiology

## 190 Metabolite Profiles and Expressed Microbial Metabolic Pathways in Human Decomposition-Impacted Soils

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**Learning Objective:** After attending this presentation, attendees will better understand changes in soil metabolite patterns during human decomposition and their collective importance for time-since-death estimations.

**Impact Statement:** This work improves our understanding of expressed microbial metabolic activity observed in soil during human decomposition and has implications for postmortem interval (PMI) estimations that are based upon microbial models.

Human decomposition in terrestrial ecosystems is a dynamic process that creates localized hotspots of increased biogeochemical and microbial nutrient cycling in the soil.<sup>1,2</sup> Biogeochemical changes in these hotspots have been well-characterized across multiple time scales and seasons. In contrast, microbial successional changes (bacterial and fungal) have been characterized to some extent; however, this focus has primarily been restricted to surveys of taxon changes in terms of relative rather than absolute abundance and by using extraction methods that do not distinguish between the presence of living and dead microbes. By comparison, there has been little focus on changes in the products of microbial metabolism directly attributable to living organisms that are actively responding to nutrient and abiotic changes in their environment.

The goal of our study was to gain further insight into microbial nutrient cycling during human decomposition by exploring changes in soil metabolite concentrations and expressed metabolic pathways over time. Three human decomposition trials were conducted at the University of Tennessee Anthropology Research Facility, Knoxville, TN. A total of nine ( $n=3$  per trial) donated human subjects (donors) were placed at the facility unclothed and in direct contact with soil and were allowed to decompose naturally. Impacted soils immediately proximal to donors were sampled to a depth of 15cm; pH, electrical conductivity (EC), and soil metabolites were measured over a period of four months. Soils impacted by decomposition products exhibited significant decreases in pH and increases in EC (ANOVA,  $p < 0.05$ ) during the period of greatest tissue loss (active decomposition). Soils remained significantly impacted by the end of each trial.

Metabolomic profiles also showed significant differences between decomposition-impacted and control soils primarily attributable to changes in concentrations of the metabolites: indoleacrylate, xanthine, xanthurenic acid, kynurenic acid, and taurine (ANOVA,  $p < 0.05$ ). Pathway analysis based upon Partial Least-Squares Discriminant Analysis (PLSDA) revealed that the greatest contributors to changes in metabolic pathways included amino acid (phenylalanine, alanine, aspartate, glutamate) and sulfur (sulfur, taurine, and hypotaurine) metabolism, and amino acid (phenylalanine, tyrosine, and tryptophan) biosynthesis. From these results, four unique metabolite groupings were identified. Two groups increased in early decomposition during the period of rapid tissue loss and were primarily associated with amino acid metabolism, indicating the presence of muscle tissue and protein catabolism. The second pair of metabolite groups increased at later time periods during which decomposition rates typically slow and soil oxygen is reduced; these groups were associated with sulfur, methane, and nitrogen metabolism, and C fixation. This work demonstrates a progression of changes in microbial metabolic pathways and associated metabolite products in soil during decomposition that further informs our understanding of the role of soil microbes in these processes and refines their potential utility for use in time since death estimates.

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### Human Decomposition; Metabolomics; Soil Microbiology



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**I91 COVID-19 and Brain-Heart-Lung Crosstalk in the Cadaver Microbiome**

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**Learning Objective:** After attending this presentation, attendees will understand how to use 16S rRNA amplicon sequencing analyses to characterize the postmortem microbiome of brain, heart, and lung cadaver tissues from COVID-19 cases vs. non-COVID.

**Impact Statement:** This presentation will impact the forensic science community by revealing the specific bacterial signatures associated with the brain, heart, and lung of cadavers with COVID-19 vs. non-COVID cadavers. These signatures could help to improve trace evidence regarding characteristics of manner of death for criminal cases.

The fact that SARS-CoV-2, the coronavirus that causes COVID-19, can translocate within days of infection to the brain and heart and that the virus can survive for months is well characterized. However, studies have not investigated the crosstalk between the brain, heart, and lungs regarding microbiota that simultaneously co-inhabit these organs during COVID-19 illness and subsequent death.

Given the significant overlap of cause of death from or with SARS-CoV-2, we investigated the possibility of a microbial fingerprint from SARS-CoV-2 death. The 16S rRNA V4 region was amplified and sequenced from each sample, and non-parametric statistics were used to determine the resulting microbiota profile and its association with cadaver characteristics. We also assessed respiratory viral co-infections among SARS-CoV-2 lung tissue samples through respiratory virome characterization using the Twist Respiratory Virus Panel. The results demonstrated that microbial communities vary significantly during human decomposition due to COVID-19 infection. When comparing non-COVID-19 infected tissues versus COVID-19, there is statistical difference ( $p < 0.05$ ) between organs from the infected group only. When comparing the three organs, microbial richness was significantly higher in non-COVID-19-infected tissues than infected. Unifrac distance metrics showed more variance between control and COVID-19 groups in weighted analysis than unweighted; both were statistically different. Unweighted Bray-Curtis principal coordinate analyses revealed a near-distinct two-community structure: one for the control and the other for the infected group. Both unweighted and weighted Bray-Curtis showed statistical differences. Twist Respiratory Virus Panel analysis demonstrated that lung tissues from COVID-19-positive cases showed one subject that was co-infected with Human betaherpesvirus 5. Further, a bacteriophage virus closely associated with *Escherichia coli* was detected in the negative SARS-CoV-2 cases.

Data obtained from these studies facilitated the defining of microbiome signatures in COVID-19 decedents that could be identified as taxonomic biomarkers effective for predicting the occurrence, co-infections involved in its dysbiosis, and the evolution of the virus.

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**COVID-19; Cadaver; Postmortem Microbiome**

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**I92 Forensic Imaging Curriculum at a State-Wide Medical Investigator's Office: The New Mexico Experience**

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**Learning Objective:** Attendees will learn about the development, implementation, and benefits of a formal Forensic Imaging curriculum for Forensic Pathology Fellowship.

**Impact Statement:** This presentation will describe emerging techniques in Forensic Imaging on a global scale and will demonstrate a formal curriculum for Forensic Imaging. In doing so, we intend to open the dialogue for the need for development of standards in training for this developing field in forensic science.

Medicolegal death investigation is a dynamic field that continues to evolve over time as new tools emerge. Forensic Imaging has a wide range of applicability to medicolegal death investigations and access to advanced imaging techniques is expanding. As this is an evolving field, there is no standard curriculum for Forensic Imaging.

The Office of the Medical Examiner (OMI) in Albuquerque, NM, has developed a curriculum that can be used as a framework for other programs. The OMI is fortunate to have a unique collaboration with the University of New Mexico Department of Radiology. Over a decade ago, our institution acquired both Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) equipment. Currently, all decedents are imaged with whole body CT (including scout views) at intake. All available information, including the investigator's report, scene photos, and CT images are used to triage decedents for autopsy, partial autopsy, or external examination. This format allows our fellows to immerse themselves in "on-the-job training" with Forensic Pathology and Radiology faculty in addition to formal conferences.

The Chief of Forensic Imaging, a Fellowship-trained Forensic Radiologist, developed a didactic lecture series, a Forensic Imaging Journal Club, and an Interesting Case Conference for the Forensic Pathology Fellowship. The didactic lectures include introductory lectures on Forensic Imaging, with emphasis on postmortem CT (PMCT), forensic pediatric presentations (with collaboration between clinical and imaging subspecialized pediatric trained physicians), a Neuroradiology subspecialty lecture, and a Radiologic Identification lecture. The Journal Club explores a variety of topics ranging from decomposition changes on imaging to forensic imaging in mass casualty events. Interesting case conferences are utilized to reinforce fundamental knowledge and to review uncommon and difficult cases. At the completion of the academic year, the faculty and attendings are surveyed about the strengths and limitations of the formal and informal educational format. The information from this review is utilized to improve the Forensic Imaging program for future Forensic Pathology Fellows at our institution. Our experience can be used for other training programs to continually improve educational offerings of Forensic Imaging curriculum for Forensic Pathology Fellows.

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**Forensic; Imaging; Education**

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**193 Micro-Computed Radiological Analysis of Traumatic vs. Iatrogenic Rib Fractures**

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**Learning Objective:** In forensic traumatology, the identification of specific parameters potentially useful for differentiating traumatic from iatrogenic injuries could contribute to the trauma reconstruction. In the present study, rib fractures secondary to direct thoracic impact or cardiopulmonary resuscitation are evaluated through an emerging technology in the forensic radiology field (Micro-Computed tomography) in order to identify morphological characteristics useful for the differentiation of the traumatic mechanism causing the fracture.

**Impact Statement:** The results obtained in the present study showed that Micro-Computed tomography is a feasible method for the morphological description of rib fractures, allowing the identification of a specific morphological parameter that could be useful for differentiating if a blunt thoracic trauma is secondary to a traumatic event or cardiopulmonary resuscitation.

**Introduction:** Rib fractures are a common finding during postmortem examination in victims of blunt thoracic trauma or as a consequence of cardiopulmonary resuscitation (CPR). The interpretation of the detected injuries in order to differentiate whether they derive from a direct thoracic impact or from a thoracic compression during iatrogenic maneuvers is crucial for the reconstruction of the blunt thoracic trauma. Although the literature provides evidence concerning the relationship between the topographical distribution of rib fractures and the traumatic mechanism involved in their production, the morphological aspect is not thoroughly described. Furthermore, to the best of our knowledge, the use of micro-computed tomography (micro-CT) for this purpose has not yet been investigated. Therefore, the present study aims at identifying micro-radiological parameters potentially useful for differentiating traumatic from iatrogenic rib fractures.

**Materials and methods:** The study sample consists of autopsy cases in which rib fractures were detected during postmortem examination. Rib fractures secondary to traumatic events (i.e., “Traumatic Group”) or resuscitation maneuvers (i.e., “Iatrogenic Group”) were included. For each case, epidemiological, circumstantial, and postmortem data were evaluated. Moreover, morphological parameters (Morphology, Orientation, Inner Edges, Outer Edges, Lateral Offset, Peeling) were investigated through micro-CT scans of the collected rib fractures. For each morphological parameter, a Fisher’s exact test was performed for the evaluation of the Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), and Negative Predictive Value (NPV).

**Results:** Micro-radiological analysis of 25 rib fractures pointed out a statistically significant association between Smooth Outer Edges and the “Traumatic Group” and between Rough Outer Edges and the “Iatrogenic Group.” No statistically significant associations were found for the other investigated parameters.

**Conclusions:** Micro-CT evaluation could become a valid technique for the description of morphological characteristics of rib fractures secondary to different injury mechanisms. This study allowed the identification of a morphological parameter (Outer Edges), which could be useful for differentiating traumatic from iatrogenic rib fractures.

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**Micro-CT; Rib Fractures; Iatrogenic and Traumatic Injuries**

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**I94     Playing With Fire: The Value and Pitfalls of Postmortem Computed Tomography (PMCT) for the Forensic Investigation of Burnt Bodies**

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**Learning Objective:** After attending this presentation, attendees will appreciate the usefulness of Postmortem Computed Tomography (PMCT) in burnt bodies. The purpose of this presentation is to provide an overview of all the advantages of the application of PMCT and 3D PMCT in cases of charred bodies.

**Impact Statement:** Through the explanation of the main radiological signs of forensic interest in cases of burnt bodies, this presentation will impact the forensic science community by showing how this radiological technique can help the forensic pathologist by providing complementary information to the full autopsy.

Postmortem computed tomography (PMCT) is currently an accessible and increasingly used tool for forensic investigations. This radiological technique offers a detailed visualization of the main anatomical structures, allowing for the observation and description of skeletal lesions or foreign bodies (e.g., bullet, prosthesis) before the dissection and thus can help the forensic pathologist by providing complementary information to the full autopsy. Therefore, PMCT represents a useful procedure in many forensic cases that are often very difficult to explain with only a traditional autopsy, as in the case of burnt bodies. In the forensic field, burnt bodies represent a significant challenge to the forensic pathologist. In these cases, the most important issues are to identify the corpse and to determine whether the exposure to heat is the cause of the death or has occurred after the death. The latter task has traditionally been pursued relying on autopsy, even if in some cases it can be particularly difficult from a technical point of view and of limited informativeness. PMCT may help to overcome these limitations, especially by helping to identify the victims and infer the cause of death.

In our bicentric study, we performed PMCT on nine burnt bodies before full forensic autopsy at the Catholic University of the Sacred Heart in Rome, Italy, and at the University of Florence in Florence, Italy.

During the presentation, the (full) concordance of PMCT and autopsy findings will be discussed and imaged regarding the signs of vitality at the time of carbonization (e.g., heat hematoma; thermal damage in the airways; presence of soot inside the upper respiratory tract; pulmonary edema), and the differences between traumatic signs (e.g., traumatic fractures) and heat-related changes (e.g., heat fractures, mottled lucencies) will be presented. Finally, 3D PMCT images will be shown to discuss how these images, combined with other radiological and autopsy findings, can help identify burnt bodies.

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**PMCT; Burnt Bodies; Heat Lesions**

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### **I95 Postmortem Computed Tomography (PMCT) in Diagnosing Deaths Due to Hemopericardium**

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**WITHDRAWN**

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**I96 A Pilot Study to Investigate If and How Postmortem Micro-Computed Tomography (Micro-CT) Can Be Used to Differentiate Stillbirths From Livebirths**

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**Learning Objective:** After attending this presentation, attendees will better understand the importance of postmortem data to differentiate stillbirths from livebirths.

**Impact Statement:** This presentation will impact the forensic science community by reporting the first study that deals with the use of a novel postmortem technique (micro-CT) to differentiate stillbirths from livebirths.

Differentiating stillbirths from livebirths is one of the most challenging tasks for forensic pathologists. Historically, the main method used in determination of live birth was the flotation test, also known as hydrostatic test. The test is based on the premise that if an infant has breathed before dying, the lungs will be inflated. Therefore, if the lungs float when immersed in water, this indicates that they are inflated, and the test is considered positive. If the infant has not breathed, the lung sinks, and the test is considered negative. However, there are arguments about the reliability of the flotation test: in fact, it can be considered at best as a suggestive pointer, but never a definitive test itself. Sometimes this method can be helped by performing microscopic examination of the lungs. The latter is based on the evaluation of alveoli's aeration—that supposes live birth—while uniformly unaerated is indicative of stillbirth. Nevertheless, according to literature, histological studies are more indicative of fetal lung maturity than the presence or respiration. In past years, a great contribution was made by postmortem imaging. The utility of postmortem imaging is widely acknowledged, with the major advantage being that it can occur before the autopsy without disruption of the body. The postmortem Computed Tomography (PMCT) has been used to investigate the presence and the distribution of air in lungs or in the gastrointestinal tract in infants. Gas distribution may be regarded as indicating inhaled and swallowed air in the lungs and in the gastrointestinal tract, and is, therefore, used as a proof of live birth.

Recently, higher resolution than PMCT has been reached through Micro-Computed Tomography (micro-CT). Indeed, common CT scans are limited to a resolution of one millimeter. Instead, micro-CT scanners can work at the level of one micron, which is a thousandth of a millimeter. However, until now, no studies in which micro-CT is used to differentiate stillbirths from livebirths are available in the scientific literature. For this reason, the authors investigated two samples by micro-CT (one from the upper lobe and one from the lower lobe of the right lungs) of two groups: (1) group A, consisting of 14 stillbirths of  $\geq 24$  weeks of gestational age; (2) group B, consisting of 4 livebirths and 10 adults. The samples were scanned by an X-Ray microtomography (SkyScan1172, Bruker). For each case, micro-CT images were analyzed by the CTvox software. In all cases of group A, the authors demonstrated the absence of areas with attenuation suggestive for the presence of air/gas. On the contrary, in group B, air/gas was identified in all fields.

In light of the above, the present study allows us to suggest the micro-CT as a powerful tool to clearly distinguish between aerated and non-aerated lungs. However, further studies on broader samples are necessary to standardize techniques and procedures.

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**Stillbirth; Postmortem Imaging; Micro-CT**

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**I97 Cold Eyes: Postmortem Iris Recognition Under Autopsy Procedures**

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**Learning Objective:** This presentation will inform attendees on the background of postmortem iris recognition, as well as the results of our study conducted under autopsy procedures. We will present data showing that recognition was still successful in these conditions, and even improved when saline was added to the eye following the toxicology vitreous humor withdrawal. The future implications of this technique for law enforcement and forensics will also be addressed.

**Impact Statement:** This presentation will present findings suggesting the success of iris recognition while under autopsy procedures. We intend to encourage further research and the future implementation of this technique as a reliable form of recognition for law enforcement and forensics that will aid in identification.

Biometrics is the science of identifying individuals based on physical characteristics, such as facial, fingerprints, and iris.<sup>1</sup> Although live iris scans are a common biometric, postmortem iris recognition is a newly researched form of identification. In recent years, researchers have shown iris recognition is possible and successful even after death in hospital mortuary conditions. Trokielewicz et al. showed promising results that recognition was possible up to 503 hours after death.<sup>2</sup> Thus, postmortem iris recognition is possible, but limited research exists outlining its feasibility, limitations, and/or applicability within the criminal justice system.

To better understand this technique, this research project examined how autopsy procedures may affect the quality and matching success of postmortem iris recognition. Images of 73 eyes from 36 deceased individuals were collected using a commercially available iris camera. Iris images were collected prior to autopsy procedures when the eyes were unaltered (original), after the vitreous humor was removed (which resulted in the eyes deflating; deflated), and finally when saline was injected to reinflate the eyes (saline). The same volume of saline was injected into the eye to replace the volume of vitreous humor removed. The quality metrics of the images were then compared between the three treatments (original, deflated, saline) to determine any significant difference.

Quality score results suggested that adding saline to the eye during reinflation improves the initial image quality of the iris scan. In 27.7% of cases, there was an increase in saline quality of over 10 compared to that of the original quality. Additionally, Post hoc (Games-Howell) tests revealed a significant difference between match scores of Original – Saline vs. Original – Deflated ( $p < 0.01$ ) and Original – Saline vs. Deflated – Saline ( $p < 0.01$ ). These results support the finding that treating the eye with saline improves quality and matching scores. In addition, the scans were successful before and after the autopsy procedure, suggesting that postmortem iris recognition may be a feasible and applicable form of biometrics. This research hopes to aid further investigation in postmortem iris recognition and its implementation as a reliable form of identification within the criminal justice system.

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**Iris Recognition; Autopsy; Biometrics**



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**I98     Cardiac Genetic Background in Sudden Infant Death Syndrome (SIDS): Is It a Matter of Structure or Electrical Conduction?**

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**Learning Objective:** This presentation provides an overview of sudden infant death (SIDS) and analyses the main forensic issues associated with the cardiac genetic background and with the interpretation of variants in these fatal events. After attending this presentation, attendees will understand some principles of molecular autopsy applications in SIDS cases.

**Impact Statement:** This presentation will have an impact on the forensic science community as postmortem genetic testing is a widely accepted diagnostic tool and could provide a monogenic cause for SIDS, resulting in prediction and prevention of the risk of sudden cardiac death (SCD) in the family members.

Sudden infant death syndrome (SIDS) is still the leading cause of death for newborns in developed countries. The pathophysiological mechanisms underlying SIDS have not been fully clarified, but in some of these cases, variants of genes associated with inherited cardiac diseases are found. In this research, an analysis of SCD-related genes was performed to determine the prevalence of rare pathogenic (P) or likely pathogenic (LP) variants that could provide an unambiguous explanation for the unexplained sudden death of the infant. The purpose was also to identify the type of genes involved to clarify the prevalent underlying mechanisms of sudden cardiac death (SCD) in SIDS.

In our study, 76 SIDS cases underwent Next Generation Sequencing (NGS) analysis with a custom panel of SCD-related genes. Rare variants were classified according to the guidelines provided by the American College of Medical Genetics and Genomics (ACMG). Postmortem genetic testing found that more than half of the newborns carried at least one variant in SCD genes. Most genetic variants were in genes encoding structural proteins. According to ACMG guidelines, only a minority of genes hosted at least a P or LP variant mainly found in genes with structural or structural/arrhythmogenic functions. Several variants were classified as of uncertain significance (VUS). The results reported in our study support the utility of molecular autopsy in SIDS. Only a minority of SIDS cases carried a variant reportedly associated with arrhythmogenic syndromes. Most of the variants concerned structural genes that were supposed to have a close interaction with ion channels, thus providing an explanation for the arrhythmic event. The identification of a variant as pathogenic has important implications for the relatives, so the clinical translation should be carried out carefully. Therefore, segregation analysis, reclassification of VUS variants, and identification of new associated genes could clarify the implications of the current findings.

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**SIDS; Molecular Autopsy; Genetic Variants**

**I99 A Case of Renal Failure Secondary to Rhabdomyolysis in an Infant**

*Amanda Saunders, BS\*, Howard University, Clinton, MD; Sarah Avedschmidt, MD, Independent Contractor, Sacramento, CA*

**Learning Objective:** Attendees will learn about a case of an infant with rhabdomyolysis and how it was diagnosed through an autopsy.

**Impact Statement:** This presentation will impact the forensic science community by adding information regarding how to diagnose rhabdomyolysis and what to look for if this is the suspected diagnosis.

Rhabdomyolysis is a common diagnosis among the pediatric population. The condition is most often precipitated by viral infections in children under ten years old, but other causes include medications or trauma.<sup>1</sup> While rhabdomyolysis is often simple to diagnose clinically by testing the serum and/or urine levels of myoglobin and creatine kinase, during a forensic autopsy, the clinical findings may not be as apparent and postmortem testing options are more limited. The purpose of this case study is to educate students and physicians about the determination of cause of death in pediatric cases with suspected rhabdomyolysis with the utilization of myoglobin immunohistochemistry.

We present a case of a 9-month-old male who was reported to have vomiting, diarrhea, fever, lethargy, and red-tinged diapers approximately 48 hours before death. The child's activity level was noted to have decreased, then he was witnessed to go unresponsive, prompting the mother to call 911, but efforts to revive the infant were unsuccessful. The decedent had no significant medical history but was noted to have been behind on his routine pediatric vaccinations.

At autopsy, the external examination was unremarkable. Internal examination revealed congested pulmonary parenchyma and a scant amount of dark, tea colored urine was present in the urinary bladder. All other organ systems were unremarkable. Microscopic analysis of tissue sections showed acute tubular necrosis of the kidneys as well as myoglobin accumulation with myoglobin immunohistochemistry. Muscle sections of the psoas and diaphragm demonstrated extensive degeneration of the myocytes; however, myocyte shape and size were uniform. Toxicology showed elevated potassium, creatine, and urea nitrogen levels, indicative of acute renal failure. Finally, a postmortem nasopharyngeal swab was positive for human rhinovirus and parainfluenza virus 3. Cause of death was determined to be acute renal failure, due to rhabdomyolysis secondary to an acute viral infection.

In rhabdomyolysis, intracellular proteins are released into the blood stream after skeletal muscle breakdown, causing acute kidney injury.<sup>2</sup> The most common viral causes of rhabdomyolysis are influenza A/B, parainfluenza, coxsackie, Epstein-Barr, herpes simplex, adenovirus, and cytomegalovirus and up to 40% of children with this condition develop renal failure. Patients often present with muscle pain, weakness, and dark-colored urine, but these symptoms may be less evident in infants.<sup>3</sup> Antemortem, serum creatine kinase, and myoglobin can be used to diagnose rhabdomyolysis; however, these are not as helpful postmortem. A case series of 52 autopsies found that 96% of cases showed microscopic acute tubular necrosis of the kidney. Moreover, 95% of patients had positive myoglobin immunohistochemistry (IHC) in formalin-fixed kidney tissue.<sup>2</sup> In cases where kidney samples are negative, the authors suggest that myoglobin IHC can be performed on urine and/or blood cell blocks to aid in diagnosis.<sup>2</sup> Often, rhabdomyolysis does not have a clear clinical presentation and can be difficult to diagnose at autopsy, especially in the absence of biochemical data. Due to the mostly insignificant findings upon autopsy, forensic pathologists must rely on a combination of case history, vitreous electrolytes, and microscopic analysis of tissue samples for an accurate diagnosis. Furthermore, in cases where rhabdomyolysis is suspected, myoglobin IHC of the kidney and urine and/or blood cell blocks can aid in the diagnosis.

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**Rhabdomyolysis; Pediatrics; Autopsy**

## I100 An Infant Death Due to COVID-19 Infection With Recovery and Survival of a Twin Sibling: A Comparison of Autopsy and Histologic Findings in Pediatric and Adult Cases and a Review of the Surviving Twin's Course

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**Learning Objective:** After attending this presentation, attendees will understand how there may be differences in clinical presentation and autopsy and histologic findings between infants and adults who die due to coronavirus disease 19 (COVID-19) infection caused by a novel coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

**Impact Statement:** This presentation will impact the forensic science community by detailing how infant and adult cases of COVID-19 infection may appear differently by gross and microscopic examination in order to avoid misdiagnosis.

Pediatric disease due to COVID-19 infection tends to be milder than the disease presentation in adults, and the symptoms in childhood infection are commonly similar to those of other acute viral respiratory infection with cough and fever being the most common symptoms.<sup>1-5</sup> The clinical presentation in children may include an excessive immune response to COVID-19 infection called severe multisystem inflammatory syndrome (MIS-C), similar to Kawasaki disease and toxic shock syndrome, that may result in multisystem organ failure and shock.<sup>6-8</sup> Adults with COVID-19 infection usually have symptoms of fever and cough and may have dyspnea and radiographic findings of pneumonia.<sup>9-11</sup> More severe infection in adults may lead to hypoxemia due to pneumonia with ventilation/perfusion mismatch (pulmonary vasoconstriction) and acute severe respiratory distress syndrome (ARDS).<sup>12</sup> Therefore, comparing the infection behavior in children versus adults, the infection may be generalized in a multisystem syndrome in the pediatric population and tends to be localized to the lungs in the adult population. It has been documented that Hispanic/Latino children and non-Hispanic Black children have a higher incidence of infection than non-Hispanic White children, and those children with underlying medical conditions and infants (less than 1 year old) may be at an increased risk for severe disease.<sup>14,13</sup> A higher mortality rate has been noted in children less than 2 years of age who tend to have a respiratory phenotype of disease, while lower mortality rates have been seen in children greater than 2 years of age who tend to have the MIS-C phenotype of disease.<sup>14</sup>

A 7-week-old male infant, former 34-1/7 gestational weeks preemie, was found dead in his crib next to his twin sibling. Reportedly, the 1-year-old sibling had symptoms of a runny nose and mild cough, and a day later the twins had similar symptoms. This twin was pronounced dead at the hospital after he was found unresponsive and the other twin was brought in for difficulty breathing the same day and was intubated, diagnosed with COVID-19 infection, and remained hospitalized for almost a month before he was discharged in good condition. The decedent's autopsy and histologic findings and the hospitalized twin's hospital course will be discussed. The older sibling and mother reportedly also tested positive for COVID-19 infection at the time of hospital presentation. This report will detail the differences noted between infant/pediatric and adult deaths due to COVID-19 infection at autopsy, both grossly and histologically. Forensic pathologists should be aware that COVID-19 deaths may present differently in pediatric and adult cases.

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### COVID-19; SARS-CoV-2; Infants

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**I101 Postmortem Genetic Testing in Sudden Unexplained Death: A Public Health Laboratory Experience**

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**Learning Objective:** After attending this presentation, attendees will understand the indications and options for postmortem genetic testing in cases of sudden unexplained death in young individuals.

**Impact Statement:** This presentation will impact the forensic science community by introducing forensic pathologists to advancing technologies, thus providing an avenue to report an accurate cause and manner of death in autopsy negative decedents with sudden unexplained death.

Sudden unexplained death in the young (SUDY) poses a diagnostically challenging situation for practicing autopsy pathologists, especially in the absence of anatomic and toxicological findings. Recent developments in postmortem genetic testing offer a modality that may provide crucial information to establish cause and manner of death. Next generation sequencing (NGS) -based panels using postmortem tissue or fluid samples can identify pathogenic variants associated with SUDY, including arrhythmogenic channelopathies and cardiomyopathies. Whole exome sequencing offers an even broader array of identification of molecular variants. The Wisconsin State Laboratory of Hygiene (WSLH) is a state-run public health laboratory that performs postmortem genetic testing in challenging autopsies, a service provided at no cost to Wisconsin medical examiners and coroners as part of their public health mission. Starting in 2016, WSLH began testing using a panel-based detection system and more recently has expanded to whole exome sequencing.

The current study examines sequencing data from 18 deceased patients (ages 2 months to 49 years, 5 female) submitted to WSLH, from 2016–2021. Panel-based NGS was performed on 15 cases, and whole exome sequencing was performed on the most recent 3 cases. Identified sequence variants of interest were confirmed using Sanger sequencing. Six cases revealed pathogenic or likely pathogenic genetic variants known to be associated with arrhythmogenic right ventricular cardiomyopathy, dilated cardiomyopathy, Brugada syndrome, mitochondrial deficiency, and pseudoxanthoma elasticum. Nine cases yielded variants of unknown significance. The remaining three cases contained no significant variants.

Postmortem genetic investigation can provide important insight for family members of decedents. A case example in the current study identified a pathogenic variant in a 4-year-old boy, associated with Brugada syndrome, an arrhythmogenic disorder due to a defective cardiac sodium channel, encoded by the gene SCN5A. Further investigation of this case included genetic testing of living family members, identifying several with the pathogenic variant. This information can be invaluable to family members harboring the pathogenic variant, such as planning early management options before onset of deadly arrhythmia.

With many genetic conditions, the phenomenon of incomplete penetrance must be considered; genotype does not necessarily dictate phenotype. A case example includes a 35-year-old female decedent with an identified likely pathogenic variant in the TTN gene, encoding the giant protein, titin, which plays key sarcomeric structural roles and has been associated with dilated cardiomyopathies. Interestingly, the decedent had no gross or histopathological findings of this disease at autopsy. Accordingly, as with any test result, interpretation must be done with great care, and genetic counselors are essential in communicating these complex topics with families of decedents.

Weighing the costs and benefits of different methodologies of postmortem genetic testing is an important consideration for autopsy pathologists. While panel-based testing may offer definitive pathogenic variants in some cases, less frequent variants may be excluded, which can lead to false negative results. Whole exome testing provides a vast amount of data, and rare variants may be identified. However, it may be difficult to determine the pathogenicity of these findings if they have not been previously reported and reproduced.

In summary, postmortem genetic testing in young decedents of sudden unexplained death can provide invaluable information to autopsy pathologists to establish accurate cause and manner of death and to family members of decedents to identify relatives with the same genetic variant to allow appropriate medical management. Advancing technologies and testing options, including NGS panels and whole exome sequencing, are increasingly available for pathologists faced with such diagnostic challenges.

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**Sudden Unexplained Death in the Young; Postmortem Genetic Testing; Next Generation Sequencing and Whole Exome Sequencing**

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**I102 The Role of Myocardial Biomarkers in Sudden Cardiac Death: Forensic Utility**

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**Learning Objective:** After attending this presentation, attendees will show the potential role of biomarkers of myocardial injury in sudden cardiac death (SCD).

**Impact Statement:** This presentation will impact the forensic science community by proposing the use of these markers in sudden cardiac death, especially when macroscopic findings are low evident or not pathognomonic.

**Introduction:** Cardiovascular diseases, especially myocardial infarction (MI), are a global public health problem, and represent the main cause of sudden death in the Western World.<sup>1</sup> In clinical practice, myocardial damage markers, especially high sensitivity markers, are used more with new algorithms (rule in-rule out) for early diagnosis of myocardial infarction.<sup>2</sup> In forensic practice, instead, there are no defined protocols that include the postmortem analysis of laboratory data for diagnostic purposes. In sudden cardiac death (SCD), it is often complicated to find evidence at autopsy or histopathological investigation, especially when the cause of death is due to arrhythmia or myocardial ischemia occurred in a few hours. In these cases, it is difficult to find pathognomonic signs. Today, there are few studies about the role of biomarkers in postmortem diagnostics. This study is focused on the role of myocardial damage markers in SCD deaths, due to arrhythmic or ischemic causes.

**Materials and methods:** The examined cases were divided into two groups (sudden cardiac deaths; deaths from other causes). An autopsy was performed in all cases, with peripheral venous blood sampling. The whole heart was taken for histopathological examination. The blood sample was taken in an EDTA tube and immediately centrifuged at 4,000rpm for about five minutes in order to extract plasma. The method involved the use of fluorescence immunoassay by placing drops of sample on a disposable well containing absorbent filter. The analysis was based on the amount of fluorescence documented through murine and goat monoclonal and polyclonal antibodies. The molecules investigated were Troponin I (TnI), Creatin Phospho Kinase-MB (CKMB), myoglobin (MYO), B-type natriuretic peptide (BNP), and D-Dimer (DDim). The device provided a quantitative result with reference ranges. The reading of the same sample was taken in duplicate, and the mean of the values was calculated. The data obtained were compared with the autopsy findings, histological findings, and clinical and laboratory data available before death on admission.

**Results:** In the SCD Group, the analysis showed a pathological increase in CK-MB, TnI, and MYO indices. These values were found to be with higher peaks for CK-MB (up to 80ng/mL), TnI (up to 30ng/mL), MYO (up to 260ng/mL) in SCD cases with acute myocardial infarction with coronary stenosis found. In the group of deaths not associated with SCD, only an increase in CK-MB value (up to 26ng/mL) was found, while the other values were in the normal range. The lowest value of CK-MB was found in hanging (18ng/mL). In none of the cases was there an absolute negativity of the values, even when using vitreous humor as an alternative sample.

**Discussion and conclusions:** Postmortem quantification of cardiac markers confirms the role of these investigations as potential support for the diagnosis of SCD. The proposed method has the advantages of being rapid (about 20 minutes), inexpensive, and allowing simultaneous measurement of multiple markers. We therefore propose the inclusion of the laboratory data in defined postmortem diagnostic protocols, comparing the clinical and autopsy evidence. The study demonstrates that in no case were the values of the five markers negative, reflecting the persistence and measurability of the markers after death. The analysis performed also suggests a potential applicability of this method even on alternative biological matrices that can be easily taken, such as vitreous humor, opening a new research frontier in postmortem laboratory diagnostics.

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**Forensic Sciences; Biomarkers; Sudden Cardiac Death**



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**I103 An Unusual Case of Self-Poisoning With Sodium Nitrite in an Asthmatic Patient**

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**Learning Objective:** This presentation presents an unusual case of a rare type of suicide: sodium nitrite poisoning. This case deals with an asthmatic patient who had methaemoglobin (MetHb) blood values lower than those usually considered fatal. Therefore, it is intended to draw the attendees' attention to the individual variability of lethal MetHb values from subject to subject and, in particular, to emphasize the importance of any pre-existing pathologies that may act as accelerating factors in death.

**Impact Statement:** By drawing attention to an infrequent topic, this presentation is a cue to start collaborations between various forensic pathology centers, also at international level, in order to collect a numerically significant case history of suicides with sodium nitrite poisoning in subjects with pre-existing pathological conditions, particularly cardiopulmonary. This could allow the verification of the validity of this presentation's thesis.

Until recently, suicide by ingestion of sodium nitrite was an extremely rare event. However, since 2020, there has been an increase in the number of cases, particularly among young people. This is directly related to the fact that the substance itself is now easier to find: sodium nitrite is readily available on various online sites. Sodium nitrite is an inorganic compound usually found as a crystalline powder and is highly soluble in water. It is mainly used in the food industry as a preservative and in the medical field as an antidote to cyanide poisoning. When ingested in sufficient quantities, it can be fatal. The main toxic effect caused by nitrite intake is an increase in methemoglobinemia (MetHb), a life-threatening condition in which hemoglobin is oxidized to methemoglobin, resulting in hypoxia. This triggers the onset of acute symptoms, and severity increases with increasing MetHb values (%): from 15% to 20% cyanosis; from 30% to 40% headaches, tachycardia, fatigue, and dizziness; for values > 70%, it is typically lethal.<sup>1-3</sup> However, pre-existing cardiopulmonary diseases may aggravate these clinical effects.<sup>4</sup>

We describe an unusual case of self-poisoning with sodium nitrite in an asthmatic 30-year-old patient. The woman was found in her home and, next to the body, was an open package of sodium nitrite. An autopsy was performed to determine the cause of death, during which a measurement of the levels of methemoglobinemia in blood was carried out: this analysis revealed a MetHb concentration of 17.9% [n. v. 0.2–0.6%], consistent with an acute intake of sodium nitrite. However, this MetHb value is not usually fatal. In view of the patient's asthmatic condition, it is possible to hypothesize that the association of this pathological condition with the ingestion of sodium nitrite acted as an accelerating factor that could have led to the woman's death, with testable MetHb values lower than those normally regarded as lethal (>70%). As no similar cases were accounted for in the literature, collecting a case history of similar deaths would be desirable and would allow the scientific community to assess the actual impact of the co-existing pathologic contributing causes in the amplification of lethal effects associated with sodium nitrite intoxication.

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**Sodium Nitrite Self-Poisoning; Asthma; Methemoglobinemia**

## I104 Dying From an Antidote: A Case of Intravenous Lipid Emulsion Overdose in a Suspected Anesthetic Intoxication

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**Learning Objective:** The Food and Drug Administration approved intravenous lipid emulsion 20% (IVLE) as a source of calories and essential fatty acids for patients requiring parenteral nutrition. This product is composed of 20% soybean oil, 1.2% egg yolk phospholipids, 2.25% glycerin, and water for injection.<sup>1</sup> IVLE therapy is now also widely accepted as a standard therapeutic intervention following local anesthetic systemic toxicity.<sup>2</sup> The mechanism of action of IVLE is not completely understood, but it is believed that IVLE provides an intravascular compartment for lipid soluble drugs.<sup>3</sup> The present case highlights possible fatal consequences of overdose of intravenous lipid emulsion and how antidotes may represent a suspected cause of death.

**Impact Statement:** In literature, several case reports about rapid infusion of intravenous lipid emulsion are limited to pediatric patients, whereas only few case reports recorded fat overload syndrome after infusion of IVLE in an adult patient.<sup>6</sup> Even if still unknown, being aware of fat overload syndrome may be helpful for health care professionals in order to be sensitized on possible risks of intravenous lipid emulsion's overdose and, consequently, on the correct administration and dosage of antidotes.

A 28-year-old woman underwent left hip arthroscopic surgery for femoral-acetabular conflict syndrome. She did not suffer from other major pathologies. During surgery she experienced chest pain and desaturation, followed by cardiovascular arrest. She was then resuscitated and transferred to the emergency department in a comatose state (GCS 4) and admitted to the intensive care unit. The same evening, she had a hypotensive episode with bradycardia; after the suspicion of local anesthetic toxicity, adrenaline and IVLE 20% bolus + continuous infusion were administered. In the morning, she presented with a new hypotensive episode with bradycardia to asystole and died.

On autopsy examination, a ubiquitous presence of whitish, dense, oily material within the vascular system was found. Histological analyses revealed the presence of abundant optically hollow halos within the vessels and capillaries in all organs examined: in the larger caliber vessels such halos were mixed within the blood column and in the smaller caliber vessels, replaced it. That result was compatible with the intravenous administration of substance of adipose nature. Whereas the correct infusion dose of IVLE 20% is 1.5ml/h, in this case, the dosage prescribed was much higher, amounting to 1,500ml/h, revealing an obvious posological error. It is believed that the administration of intravenous lipid emulsion led to further hypoxic insult from deficient oxygen supply, resulting in the woman's death.

The potential risks of administering relatively high doses of IVLE are uncertain, and the optimal dose has not been established yet.<sup>4</sup> Fat overload syndrome is a well-known complication of intravenous lipid emulsion therapy. It has been reported in patients receiving high dosage of IVLE and/or for an extended period of time. Another cause is accidental lipid overdose due to an excessive infusion rate.<sup>4</sup> It is characterized by headaches, fever, jaundice, hepatosplenomegaly, respiratory distress, and spontaneous hemorrhage; other symptoms include anemia, leukopenia, thrombocytopenia, low fibrinogen levels, and coagulopathy.<sup>1</sup> One of the most consistently observed effects in IVLE overdose is respiratory distress, which can be led back to multifactorial origin.<sup>6</sup> Postmortem findings displayed a pattern of fat deposition, including large deposits in the heart, kidneys, and small blood vessels.<sup>5</sup>

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### Intravenous Lipid Emulsion; Overdose; Fat Overload Syndrome



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**I105    Herbicide, Fungicide, Suicide: A Case of Glyphosate and Lime Sulfur (Calcium Polysulfide) Poisoning**

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**Learning Objective:** After attending this presentation, attendees will better recognize the toxicology and pathologic effects of glyphosate and lime sulfur.

**Impact Statement:** This presentation will impact the forensic science community by documenting a rare case of co-ingestion of readily available lawn care chemicals. Forensic pathologists and toxicologists in particular should be aware of the manifestations of fatal poisonings.

Glyphosate is the most widely used herbicide in the world. It is available in a variety of different formulations, with concentrations ranging from 1–41%. One of its purported benefits is low toxicity in humans, and reports of fatal acute poisoning with glyphosate are rare.<sup>1</sup> Lime sulfur, which is a mixture of calcium polysulfides and thiosulfate, has been used as an agricultural fungicide since the 19<sup>th</sup> century. When mixed with acid (such as stomach acid), lime sulfur generates toxic hydrogen sulfide gas. Suicide by direct ingestion of lime sulfur has been occasionally reported.<sup>2,3</sup> Lime sulfur has also been mixed with acidic household products, such as toilet bowl cleaners, to synthesize inhalable hydrogen sulfide.

**Case report:** The decedent is a 26-year-old man who was arguing with his wife over accusations of infidelity. During a brief period when his wife had left the house, he retrieved two bottles from the garage: Hi-Yield Killzall®, an herbicide concentrate containing 41% glyphosate, and ORTHO® Dormant Disease Control, a lime sulfur fungicide containing 26% calcium polysulfide. After consuming an unknown quantity of the two liquids in his kitchen, he went downstairs and collapsed. His wife returned after a short interval and found the decedent unresponsive and with foam extruding from his mouth. She summoned emergency medical services, who arrived and transported the decedent to a nearby hospital. He was found to be profoundly acidotic and was admitted to the intensive care unit following cardiac arrest. His clinical condition deteriorated, and care was withdrawn. He expired approximately four hours after the fatal ingestions.

Autopsy revealed diffuse edema, hemorrhage, and necrosis of the gastrointestinal tract. The lungs showed edema and congestion, with a combined weight of 1,210 grams. Hemorrhage of the anterior tongue was also noted. The rest of the autopsy was non-contributory. Toxicology studies performed on hospital blood were positive for thiosulfate (160mcg/mL, reference range < 2mcg/mL) and negative for common drugs of abuse.

To our knowledge, this is the first reported case of fatal co-ingestion of glyphosate and lime sulfur. A thiosulfate assay performed on hospital blood was helpful to confirm the exposure.

**References:**

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**Poisoning; Glyphosate; Lime Sulfur**

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**I106 A Homicidal Poisoning Disguised by Drowning: How Stomach Content Analysis Helped to Solve the Mystery**

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**Learning Objective:** After attending this presentation, attendees will recognize the importance of “outdated” samples for toxicological analysis, such as the stomach and its content, in cases with limited history and unknown circumstances surrounding death.

**Impact Statement:** New technologies and the evolution of the medical forensic practice in many countries such as Portugal led to undervaluing, and even abandoning, the collection of classic forensic samples for ancillary examinations, particularly for Toxicology. This presentation will impact the forensic science community by remembering the invaluable role of these samples in solving many cases, including homicides.

**Background and case report:** Pesticides, widely used in agriculture, make pesticide poisoning an important cause of morbidity and mortality, accidentally, to commit suicide or for homicidal purposes. Although in Portugal suicidal toxicological cases are common, homicides are quite rare.

The authors report a case of a 46-year-old unidentified male who was found dead floating at a river mouth in Portugal, with nothing else than his clothes. A case of drowning was presumed, and the corpse was brought for autopsy to the National Institute of Legal Medicine and Forensic Sciences in Coimbra, Portugal.

The external examination showed abrasions and lacerations mainly on the head and limbs. Internally, red-tinged froth and fluid exuding from the lumen of the sectioned larynx and trachea were found. The lungs appeared voluminous and crepitant and presented blotchy areas of hemorrhage (Paltauf's spots) visible on the pleural surface. The stomach contained 900mL of a brown mud-like substance with semi-digested food material, without any particular odor. The gastric mucosa showed several subepithelial hemorrhagic suffusions.

Classic samples for toxicology analysis were taken, namely blood, urine, stomach and content, liver, and kidney, as well as heart and lungs for histology. Toxicological studies revealed sertraline and alprazolam in blood, both in therapeutic concentrations and the pesticide cyhalothrin in blood and a large quantity in the stomach. Alcohol and drugs were not detected. Histology revealed emphysema aquosum, congestion, microhemorrhages, and signs of gastric content aspiration in the lungs, all non-specific but consistent findings with drowning.

Identification was later achieved through a serial number located on a nicotine spray found in his clothes. Further investigation by the criminal police that included wiretapping, together with autopsy and toxicological analysis, led to the conclusion that it was a homicide by drowning after poisoning with the pesticide cyhalothrin.

**Discussion and conclusion:** The authors will analyze the characteristics of the pesticide detected, its effects on humans, and will review the intoxications worldwide. Moreover, they will discuss how this finding can be harmonized with the drowning signs also observed. Explanation for traumatic injuries will also be provided. The conclusion of the autopsy report and the mechanism of death will be fully interpreted. The final puzzle of this crime will be shared with the attendees.

The authors want to emphasize the importance of toxicological studies, in this case the gastric content, which sometimes takes a back seat. Stomach content is a matrix, together with urine, liver, and kidney, among others, that should continue to be taken especially in cases with a poor history or unknown circumstances of death. Finally, forensic pathologists should never forget that a body recovered from the water may have died of everything, even drowning.

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**Homicide; Cyhalothrin; Drowning**

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**I107 A Homicidal Poisoning With Amlodipine**

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**Learning Objective:** Amlodipine is an unusual drug for poisoning, but potentially lethal and dangerous, since if dissolved in a liquid, it is odorless and tasteless. From this case, attendees can understand how, in certain concentrations and considering the pathological history of the subject, it can determine cardiocirculatory outcomes up to shock and death.

**Impact Statement:** This work will allow attendees to understand the importance of a multidisciplinary approach in forensic medicine. Indeed, the toxicological analysis was carried out with success even in samples taken from a putrefied body thanks to the circumstantial and anamnestic data, without which there would have been no cause of death and no solution to the case.

Amlodipine is a long-acting calcium channel blocker (CCB) of the dihydropyridine class used in the treatment of essential hypertension and angina pectoris. According to data from the poison centers in the United States in 2002, the percentage of CCB intoxications stands around 16% of all cardiovascular drug exposures, while related deaths are around 38%.<sup>1</sup> As known, amlodipine toxicity results in bradycardia, hypotension, atrioventricular and bundle branch block, decreased cardiac inotropy, and profound vasodilation, which could lead to acute heart failure.<sup>2-3</sup>

This work presents the case of a 76-year-old woman found in summer in an advanced state of decomposition, inside a sleeping bag placed on the edge of the road. During the forensic inspection, there was no evidence of traumatic injury, and a temperature of 51°C was found inside the sleeping bag. The autopsy results and the total body CT examination made it possible to exclude with certainty the presence of traumatic (contusive) or specific injury (firearm or weapon). Histologically, a very serious coronary and myocardium sclerosis from probable chronic cardiocirculatory insufficiency was found. On the other hand, toxicological screening, performed by liquid chromatography combined with high-resolution mass spectrometry (LC/HRMS) on the blood sample taken from the femoral artery, revealed the presence of alprazolam and amlodipine; in particular, the concentration of amlodipine was 130ng/ml, corresponding to the ingestion of about 9-10 capsules (amlodipine 5mg), falling within the lethality range (100-200ng/ml). Since no capsules had been found inside the gastric contents and due to the rapidity of action of this drug, it is compatible with the hypothesis of ingestion of granular content. These data were fundamental to the trial, as it later turns out that the woman was the victim of a homicide by her daughter-in-law, who dissolved the granular equivalent of 9-10 capsules of amlodipine in a cup of coffee, causing her death by overdose and subsequent heart failure, even considering her pre-existing heart condition.

**References:**

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**Amlodipine; Intoxication; Vasodilation**

### **I108    Lithium Urinary Levels and the Suicide Mortality Rate in Portugal**

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**WITHDRAWN**

## I109 Metformin Fatal Intoxication: Suicide or Accident?

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**Learning Objective:** Metformin overdose is life-threatening and has an extremely high death rate. The aim of this work is to describe metformin intoxication, the clinical consequences, the severity of accidental or intentional exposure, and the need for a suspect, especially in cases where there is inexplicable hypoglycemia and lactic acidosis.

**Impact Statement:** When a patient with severe lactic acidosis of unknown origin shows up in the emergency department, it is important to consider the possibility of metformin intoxication. This presentation will impact the forensic science community by focusing on the need to perform an early diagnosis of metformin intoxication in order to reduce the mortality associated with the complications of lactic acidosis.

Metformin is a drug belonging to biguanides, used as first-line in treating type 2 diabetes, especially in overweight patients. Literature rarely reports its improper use for weight loss in patients with an eating disorder, such as purging.<sup>1,2</sup> Lactic acidosis is a well-known complication in metformin treatment, especially in intentional overdose or in a patient with renal insufficiency.<sup>3</sup> Presented here is a fatal case of metformin intoxication of a non-diabetic 60-year-old woman employed in a pharmacy with no remote pathological history, and who was not overweight. Returning home from work, she experienced severe abdominal pain with associated constipation, general malaise, and excessive thirst. For these reasons, she went to the emergency department. Presenting a regular blood sugar level (75mg/dL), the woman voluntarily decided to leave the hospital. The next day, due to worsening health conditions, she was admitted to the Intensive Care Unit in a state of unconsciousness. Her analyses showed severe hypoglycemia (27mg/dL), hyperkalemia (6.1mEq/L), severe lactic metabolic acidosis, creatinine of 7.44mg/dL, and glomerular filtration rate of 5 and a troponin level of 1,031pg/mL. She was promptly treated with hemodynamic and respiratory support, continuous venous hemofiltration (CVVH), and bicarbonate infusions. Meanwhile, her daughter found a box of half-consumed metformin pills in the house. Excluding all other possible causes, physicians thought of possible metformin intoxication. Despite treatment, the woman died because of multiple organ failures caused by persistent hypoglycemia. Nothing relevant was found at the autopsy and histology exams. Instead, the toxicological examination was fundamental: the blood concentration of metformin was 49,869ng/ml, much above the upper therapeutic limit (1,000ng/ml), leading to the conclusion that an excessive intake of metformin caused the death. Investigation into the patient's private life did not reveal any psychiatric disorders or previous suicide attempts. Therefore, it is more likely that the death was caused by accidental intoxication rather than a suicidal purpose, probably due to the improper use of metformin to lose weight.

### References:

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3. Stevens A, Hamel JF, Toure A, Hadjadj S, Boels D. Metformin overdose: A serious iatrogenic complication-Western France Poison Control Centre Data Analysis. *Basic Clin Pharmacol Toxicol.* 2019 Nov;125(5):466-473. doi: 10.1111/bcpt.13273. Epub 2019 Jul 4. PMID: 31215744.

### Metformin; Intoxication; Weight Loss

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**I110 Morphine Toxicity on Endothelial Differentiation of Vascular Mesenchymal Stromal Cells**

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**Learning Objective:** After attending this presentation, attendees will be knowledgeable about the molecular mechanism of morphine-mediated damage to the endothelial cells.

**Impact Statement:** The presentation will impact the forensic science community by showing that morphological and immunofluorescence methodologies may lead to the identification of new markers of endothelial cell damage that could become complementary investigations to those traditionally used in Forensic Pathology.

The most common pulmonary findings in opiate-related fatalities are pulmonary congestion and edema, as well as acute/chronic alveolar hemorrhage. The cause is thought to be damage to the capillary endothelium due to hypoperfusion-related ischemia or to the direct toxic effect on endothelial cells.<sup>1</sup> Previous studies demonstrated the expression of opioid receptors on human vascular mesenchymal stromal cells (vMSCs) and observed that vMSCs showed functional characteristics impairment following acute morphine exposure.<sup>2</sup> However, data is still scarce regarding the contribution of this impairment to the opiate-related pathophysiology of endothelial cell injury.

In the present study, the effects of an *in vitro* morphine exposure on the physiological activity and maintenance of human vMSCs were assessed. Cells were exposed to 0.1mM, 0.4mM, 0.8mM, and 1mM morphine sulphate for seven days. Afterward, cell viability, proliferative potential, oxidative stress, and pro-senescence effect were evaluated, together with an ultrastructural analysis by transmission electron microscopy (TEM). Finally, *in vitro* vascular differentiation was analyzed.

A decrease of cell viability, a significant growth inhibition, and a pro-oxidant and pro-senescence effect were seen after treatment with the upper doses (0.8mM and 1mM). In our system, morphine exposure induced ROS production, which was balanced by an upregulation of the autophagic process. Ultrastructural TEM analysis confirmed the results, showing numerous long-shaped mitochondria and several lysosomes, together with autophagic vesicles. The functional decline and the reduced proliferation capacity of the structures responsible for regeneration when the endothelium was damaged, as noted in the present study, may contribute to the opiate-related pathophysiology of endothelial cell injury. Although confirmation studies are required, the results of this study seem to be encouraging and demonstrate that the approach based on morphological and immunofluorescence methodologies may have a high potential for being a useful tool or as an addition to other methods used in forensic pathology.

**References:**

- <sup>1.</sup> Dettmeyer RB. Histopathology and Drug Abuse. *Forensic Histopathology*, 2018.
- <sup>2.</sup> Holan V et al. The Impact of Morphine on the Characteristics and Function Properties of Human Mesenchymal Stem Cells. *Stem Cell Rev*, 2018.

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**Forensic Pathology; Morphine Toxicity; *In Vitro* Endothelial Damage**

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**I111 Sudden Death Due to Methamphetamine-Associated Pulmonary Hypertension: A Case Report**

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**Learning Objective:** After attending this presentation, attendees will have a better knowledge of the relationship between chronic methamphetamine use and the development of pulmonary arterial hypertension.

**Impact Statement:** This presentation will impact the forensic science community by presenting a case report in which the clinical history and autopsy findings helped clarify the progressive cardiac remodeling secondary to chronic methamphetamine use that lead to a sudden unexpected death.

A link between the chronic use of methamphetamine and the development of pulmonary arterial hypertension (PAH) has been suggested by previous research. A 45-year-old White male with a past medical history of methamphetamine use was swimming in a lake. He was witnessed collapsing while standing in the water close to the shore. When the Emergency Medical Service providers arrived on the scene, the subject was already deceased. The autopsy revealed severe tooth decay, bi-ventricular cardiac hypertrophy, enlargement and atherosclerosis of the pulmonary artery, and microscopic findings consistent with pulmonary hypertension. Toxicological analyses revealed amphetamine and methamphetamine in the peripheral blood but in non-toxic levels. No evidence of drowning was present. The sudden death was due to severe pulmonary hypertension in a chronic methamphetamine user.

Amphetamine and methamphetamine increase catecholamine concentration in the central and peripheral nervous systems, causing neurological dysfunction. However, limited information is available regarding their effect on the cardiovascular and respiratory systems. Chronic methamphetamine use leads to an increased release of serotonin, mitochondrial dysfunction, oxidative stress, and progressive DNA damage to the pulmonary endothelium, resulting in the proliferation of pulmonary artery smooth muscle cells and progressive remodeling of the pulmonary arteries. Long-term severe PAH eventually leads to progressive cardiac failure, with potentially fatal outcomes, including sudden deaths. Interestingly, a recent study reported that a patient obtained a resolution of PAH possibly after the cessation of methamphetamine use. In the presented case, the undiagnosed PAH led to severe cardiac remodeling, with progressive hypertrophy and terminal right heart failure, leading to sudden death. Subjects with a history of chronic amphetamine (or its derivatives) use, both for recreational and therapeutic purposes, should be closely monitored to detect early signs of possible PAH. In these cases, cessation of offending agent may result in the resolution or improvements of PAH symptoms, and a preventive approach may help avoid severe PAH development and fatal outcomes.

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**Methamphetamine; Pulmonary Hypertension; Sudden Death**

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**I112 The Role of Carbon Dioxide in Death Related to Must Exhalations: Case Scenarios and Toxicological Results in Comparison**

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**Learning Objective:** After attending this presentation, attendees will understand the toxic effects of carbon dioxide in deaths due to must exhalations in closed environments.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the utility of the autopsy and toxicological investigations through the analysis of carbaminohemoglobin to demonstrate exposure to carbon dioxide.

**Introduction:** Alcoholic fermentation is a chemical process carried out during winemaking after the pressing of the grape must. This phase lasts up to 15 days, and it is carried out by the yeasts of the *Saccharomyces cerevisiae* species which, through an anaerobic metabolism, oxidize sugars and convert pyruvic acid into CO<sub>2</sub> and ethyl alcohol. CO<sub>2</sub> is an odorless and colorless gas. These characteristics make the identification of toxic CO<sub>2</sub> levels very complex, and its high density facilitates its saturation in a closed environment with lethal effects. In forensic literature, accidental CO<sub>2</sub> intoxication is a very rare event. Unlike carbon monoxide (CO), there are no known methods of pCO<sub>2</sub> investigation in postmortem fluids. In this work we describe the forensic and toxicological investigations carried out in four cases of CO<sub>2</sub> intoxication with an innovative experimental analysis approach.

**Case report:** Investigators intervened in the death of four people who entered a tank where the fermentation of wine was taking place.

**Material and methods:** An inspection was carried out on the scene with the help of the fire fighters, using suitable respirators. A measurement of environmental gases and fumes was performed on site. An autopsy was performed on all cadavers with histological investigations. A screening toxicological analysis was then performed on blood and vitreous humor with an immunoenzymatic method using ILAB 600. Subsequently, the confirmation analysis of the blood concentration of ethanol was carried out after solid phase microextraction (SPME) with gas chromatography combined with mass spectrometry (GC/MS). The determination of carboxyhemoglobin was carried out by a spectrophotometer and finally by the spectrometric scan for carbaminohemoglobin (Hb CO<sub>2</sub>) that is an analysis of the hemoglobin bound to CO<sub>2</sub> on the terminal iron and lysine. The data obtained were compared with the results of a literature review about deaths from CO<sub>2</sub> inhalation carried out on PubMed® NCBI and Scopus® engines.

**Results:** Environmental scene analyses showed toxic levels of CO<sub>2</sub> and sulfuric acid. The toxicological analysis showed that in all four cases, the presence of alcohol in the blood was between 1.37g/L and 2.23g/L. The concentration of CO was between 6.5% and 9.9%. The spectrometric analysis of Hb CO<sub>2</sub> showed in all four cases an abnormally high peak compared to oxyhemoglobin. Literature research showed only two cases of CO<sub>2</sub> poisoning due to wine fermentation.<sup>1,2</sup>

**Discussion:** CO<sub>2</sub> is an inert gas that can cause variable clinical effects (i.e., headache, tachycardia, tachypnea up to coma and death). Such deadly effects occur quickly when the gas reaches a saturation between 20% and 30%. In the reported cases, death occurred from asphyxia in a confined environment due to oxygen deficiency and overproduction of CO<sub>2</sub> in a vitiated atmosphere. In our cases, we report for the first time in the literature the postmortem identification of a Hb CO<sub>2</sub> peak in the blood of the four cadavers as a potential index of prolonged exposure to CO<sub>2</sub>. We also highlight the presence of blood ethanol in all subjects, probably attributable to the inhalation or concomitant ingestion of alcohol as well as a rapid putrefaction despite regular storage in the mortuary refrigerator, probably related to the possible accelerating role of inhaled gases.

**Conclusions:** The cases evidence the need for maximum caution during the vinification process, in closed, unventilated, and unsafe environments. In these cases, we propose the evaluation of Hb CO<sub>2</sub> as a potential marker of exposure to CO<sub>2</sub> as well as the measurement of the gas levels on the scene through special environmental detectors, with the help of experts, and comparison with autopsy evidence.

**References:**

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2. La Harpe R, Shiferaw K, Mangin P, Burkhardt S. Fatality in a wine vat. *Am J Forensic Med Pathol.* 2013 Jun;34(2):119-21.

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**Forensic Sciences; Carbon Dioxide; Carbaminohemoglobin**

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**I113 Unravelling the Mechanism of Death Through Urine Metabolome Analysis: An Infant Methadone Intoxication**

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**Learning Objective:** The attendees will learn how metabolomics and multivariate statistical analysis may represent a potentially powerful tool in forensic pathology routine, not only in postmortem interval estimation but also in the identification of the mechanism of death.

**Impact Statement:** To the best of our knowledge, this is the first published report in which metabolomics has been used to help addressing the mechanism of death in a real forensic pathology case. This is a practical example of how metabolomics may be used not only for basic science purposes but also for applied fields as forensic pathology.

The case presented was recently published in the *International Journal of Legal Medicine*.<sup>1</sup> Briefly, a 2-month-old infant with no significant medical history was found dead in his bed. The case was initially suspicious for a Sudden Infant Death Syndrome due to epidemiological and circumstantial features. Postmortem computed tomography ruled out traumatic lesions. Pathological findings excluded malformations and were consistent with an acute respiratory failure (later confirmed with histopathological analysis), while toxicological analysis revealed an elevated blood methadone concentration (570ng/mL), which is in the toxic and/or fatal concentration range for adults. The cause of death was then ascribed to an acute methadone intoxication.

In addition to the routine approach, the urinary sample collected at autopsy was further investigated with a <sup>1</sup>H NMR metabolomic approach and the identified metabolomic profile was challenged with the urinary metabolomic profiles previously obtained from 10 newborns who experienced perinatal asphyxia and 16 healthy control newborns. At the principal component analysis, the urinary metabolomic profile of the methadone intoxicated infant was well distinguishable from healthy controls and, intriguingly, very similar to the ones belonging to the perinatal asphyxia newborns, especially to those belonging to the newborns characterized by the worst outcome, namely death. Although separation is driven by modifications occurring in the entire urinary metabolome, the increase in lactate and the decrease in myo-inositol, betaine and taurine seems to play a key role. The results, on one hand, support the cause of death through an inhibition of the respiratory drive (hence excluding the alternative mechanism though a longQT arrhythmogenic death); on the other hand, results offer several hints on a shared derangement in metabolic pathways between different mechanisms of asphyxia/hypoxia. To the best of the authors' knowledge, this is the first published report of the use of a metabolomic approach in a pathological case, in which metabolomics offers useful and additional information regarding the mechanism and the cause of death.

**Reference:**

- <sup>1</sup>. *Journal of Legal Medicine* [<https://doi.org/10.1007/s00414-021-02772-z>]. Control group was obtained by a previously published paper investigating perinatal asphyxia through metabolomics [<https://doi.org/10.1371/journal.pone.0194267>].

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**Metabolomics; Toxicology; Pathology**

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**I114 A Hepatic Hemangiosarcoma Presumed by Imaging as a Hemangioma: A Case Report and Literature Review**

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**Learning Objective:** After attending this presentation, attendees will understand the subtle clinical presentation of hemangiosarcoma, its imaging findings, and its clinicopathologic correlates.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating an unusual and benign mimicker presentation of an overtly malignant tumor and the associated diagnostic challenges posed.

Hemangiosarcomas of the liver are rare malignant neoplasms accounting for a small fraction (approximately 2%) of all primary liver malignancies and are associated with male preponderance, occurrence most commonly past the fifth decade, environmental chemical exposure, and poor prognosis. Conversely, hemangiomas of the liver are the most common benign primary tumor of the liver, show female preponderance, occurrence most commonly between the third and fifth decade, and are mostly found incidentally (on imaging or at autopsy), and remain asymptomatic. Here we describe a case of a 96-year-old female with a history of dysphagia, night sweats, and unintentional weight loss within the past three months (9.6% of body weight) who presented with a liver containing multiple indeterminate lesions of various sizes (up to 4.8cm greatest dimension) presumed to be a hemangioma by multiple imaging modalities, including ultrasound, CT, and MRI. Due to the periarterial location of the dominant hepatic nodule and procedural risks, antemortem biopsy was not performed. Autopsy done several months later revealed hemangiosarcoma of the liver with nodular tumor replacement of the entire hepatic parenchyma.

Hemangiosarcoma of the liver is highly variable in radiologic appearance and presents a diagnostic and treatment challenge as these lesions are typically unfavorable for biopsy. Thus, definitive pathologic diagnosis is technically limited due to procedural hemorrhage risk of a vascular tumor, low diagnostic yield (often mainly blood), and high-risk anatomical location. As typical for a high-grade sarcoma, it is fast-growing, aggressive, and often unresectable at time of diagnosis, conferring a poor prognosis. Hemangiomas are typically diagnosed with high sensitivity and specificity via contrast-enhanced CT or MRI imaging. But, like hemangiomas, hemangiosarcoma may present as single or multiple masses, with nodular enhancements and architecture ranging from sinusoidal or cavernous spaces to honeycomb structure resembling hemangiomas to a mimicker of a hypervascular hepatocellular carcinoma by imaging. The mimicry of this aggressive tumor for its much more common benign counterpart is both clinically relevant and of diagnostic significance in the postmortem setting. The fragile nature of the vascular tumor can present itself as a lethal source of bleeding, even without trauma. It is recommended that clinicians, including radiologists and autopsy pathologists, become familiar with this entity due to its close mimicry of a benign lesion. The combination of the clinical narrative with gross examination and histological diagnosis is crucial to develop a definitive final anatomical diagnosis. Autopsy pathology was critical in this case to make the diagnosis that was missed on repeated antemortem imaging studies.<sup>1-5</sup>

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**Hemangiosarcoma; Hemangioma; Imaging**

## I115 Postmortem Magnetic Resonance Imaging and Forensic Pathology: An Ever-Closer Relationship

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**Learning Objective:** The goal of this study is to investigate the potential role of *ex situ* MRI of different organs after formalin fixation, evaluating benefits and limitations when approaching these issues.

**Impact Statement:** This presentation will impact forensic science by demonstrating the possibility to study *ex situ* fixed organs for forensic purposes with an MRI protocol, reducing the execution times of the examination compared to the entire corpse, and orienting subsequent histological investigations.

**Background:** For decades, postmortem magnetic resonance imaging (PMMRI) has been recognized as a last potential useful adjunct to the autopsy, particularly in natural causes of death.<sup>1</sup> Currently, postmortem computed tomography and PMMR cannot normally replace traditional autopsy, but they can provide an alternative to autopsy in specific cases.<sup>2</sup> Therefore, in a study by Femia et al. on the comparison between conventional autopsy and PMMR, the latter was able to identify the cause of death in 50% of cases; in detail it diagnosed 56% of cardiovascular causes of death, 29% of pulmonary causes, and 100% of intracranial causes, resulting in a sensitivity and specificity both estimated at 57%.<sup>3</sup> In the forensic field, PMMR research has mainly focused on the brain and heart, since both organs often show findings of forensic relevance.<sup>4,5</sup> However, these investigations, also carried out *ex situ*, require particular precautions concerning the temperature bias formalin fixation shortened both T1- and T2-values as a consequence of the reduction in the water content of the subjected organs.<sup>6</sup> This study aims to present three target organs of study at the Forensic Pathology Section of the University of Foggia: heart, brain, and skin.

**Case series:** The heart came from a 47-year-old factory worker who, during working hours, had felt sick and, after a few minutes, died in front of his colleagues. The personal medical history was negative for cardiovascular diseases, while the family history showed that the father had died of sudden death at an age of less than 65 years old. Hyperintense sub-endocardial area in STIR sequence at the mid-cardiac area at the LV septum and the corresponding histological H&E preparation in which foci of myocells characterized by contraction band necrosis, and the presence of amorphous eosinophilic material were visible.

**T2-FFE sequences:** In this latter, a hemi-circumferential in-homogeneously hypointense area of the altered signal at the mid-cardiac level is more evident along the LV lateral and posterior wall and the corresponding histological samples. The skin samples, on the other hand, came from single ammunition firearm injuries taken during an autopsy examination to identify signal alterations useful for the characterization of the lesions themselves. In this regard, the SWI (Susceptibility Weighted Imaging) sequence, commonly used to highlight venous blood, hemorrhages, and iron deposits, was selected to identify the presence of heavy metals. The observers agreed on the estimate of the size of the hole, as well as on the presence of metal artifacts and hemorrhagic infiltrates in the vicinity of the lesions macroscopically identified as entrance holes. This aspect was subsequently confirmed by histological investigations. Finally, the brain came from a young adult who had suffered a head injury. Even in this case, the observers (after randomization and blinded for the macroscopic characteristics highlighted during the autopsy examination) agreed on the characteristics of the alterations in the images viewed. Therefore, following the histological confirmation of the data, the cause of death was attributed to the TBI.

**Discussion and conclusions:** Published evidence and the case series provided suggested that PMMRI has demonstrated its wide functionality and its high diagnostic power in all fields of the postmortem examination, from the investigation around the cause of death such as non-traumatic in both postmortem investigations, forensic and clinical. On the other hand, the recent advances in this area and the potential endless evolution of technologies and radiological equipment cannot be ignored and little time will be needed for these techniques to be included in the guidelines for postmortem protocol diagnosis. However, it cannot be forgotten that it can be considered able to make refined, but is not a substitute for, the conventional autopsy.

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### PMMRI; Sudden Death; Traumatology

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**I116 The Use of Hand-Held Scanners in Forensic Traumatology**

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**Learning Objective:** After attending this presentation, attendees will gain information on the existence of everyday electronic devices equipped with 3D detection systems, and their potential utility for the evaluation of traumatic injuries.

**Impact Statement:** This presentation will impact the forensic scientific community as it foresees a complementary utilization of hand-held scanners at death scene investigation and at autopsy.

Although in forensic practice the flexible meter is considered the gold standard for measuring injuries, a number of alternatives have recently been proposed even if none of them seem to surpass the traditional method. Everyday electronic devices equipped with 3D detection systems could be particularly useful in the morphometric assessment of body lesions at death scene investigations and at autopsies and yet to date in the forensic field, there are no studies that have tested these technologies for image acquisition and measurements of injuries.

This study aims to measure lesions experimentally produced on human skin using two hand-held scanners, equipped with different technologies for acquiring images (laser or infrared), in order to verify their precision and accuracy.

Twenty lesions of four different types (bruises, abrasions, cuts, and stab wounds) were produced on surgically amputated lower limbs. Each lesion was manually measured by an operator using a meter. The limbs were subsequently scanned by an iPad® Pro with a LiDAR system (laser) and an Asus® Zenfone with a Google® Tango system (infrared). On the acquired images, three different operators measured each lesion three times in three different moments using a computer with a desktop software called MeshLab. For each hand-held scanner, accuracy and precision within and among different operators were evaluated and statistically compared to manual measurements (i.e., the “gold standard”).

Statistical analyses showed that only LiDAR technology equipped on the iPad® PRO provided the diagnostic efficiency (in terms of precision and accuracy) required for forensic applications. Preliminary results look promising. Hand-held scanners are cheap, simple to use, and, above all, portable, being functional also for death scene investigations to supplement or even replace the tools currently used. However, prior to their use in the forensic field, an extensive validation study needs to be performed for testing “real life” conditions, which could negatively influence their diagnostic efficiency.

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**Injury; Hand-Held Scanner; Measure**



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**I117 Coronaric Myointimal Hyperplasia: A Rare Cause of Sudden Young Death**

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the importance of histological examination and specific histological stains of heart fragments in sudden young death cases.

**Impact Statement:** This presentation will impact the forensic science community by presenting a rare case in Italy of sudden young death with coronary myointimal hyperplasia.

Myointimal hyperplasia is an alteration of the arterial wall that consists of an intimal smooth muscle proliferation, resulting in the formation of non-thrombotic, non-inflammatory wall thickenings that reduce the blood vessel lumen and obstruct normal blood flow. It is an infrequent event, generally secondary to vascular wall trauma, such as those occurring during angioplasty or other endovascular procedures, and mainly affects the carotids.

The authors present a case of sudden death of a 19-year-old boy with no history of cardiovascular disease and apparent good health who, during a football match with his friends, got sick and collapsed on the field. Despite the immediate emergency rescue, the boy presented a cardiovascular arrest. Electrocardiographic tracks performed for the duration of the rescue showed an undetectable rhythm. Autopsy excluded hypertrophic cardiomyopathy and other macroscopic heart anomalies as possible causes of death. On cut-section, the myocardial muscle had no dyschromic areas and increased thickness.

The heart fragments were fixed and dehydrated in the descending series of alcohols, cleared in xylene, and paraffin embedded. Successively, paraffin-embedded tissue sections were cut using a microtome and stained with hematoxylin and eosin. Microscopic observation showed myocardium disarray, fibrosis affecting  $\geq 2$  segments in the subendocardial-midmural layer of the left ventricle and interstitial inflammatory infiltrate.

Histological examination performed with specific stains (Azan's trichromica) on the boy's heart showed diffuse myointimal hyperplasia of the coronaries with presence of focal fibrous intimal plaques which occluded the coronary lumen for more than 70%, in particular on the right coronary artery and the interventricular artery at half of its course. Chronic blood hypoperfusion to myocardial tissue, worsened by stress tachycardia and increased heart contraction in response to physical activity, resulted in acute myocardial ischemia (anterior STEMI detected to the ECG), followed by the lipothymic episode and irreversible cardiocirculatory arrest.

Myointimal hyperplasia, a rare disease in the general population and especially in the young, is responsible for chronic hypoperfusion, and may represent a precipitating factor in acute coronary thrombosis. The absence of symptoms and the difficulty in diagnosis represent serious obstacles in the prevention of sudden deaths at a young age.

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**Sudden Young Death; Myointimal Hyperplasia; Acute Coronary Syndrome**

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**I118 Elder Abuse in Europe's "Most Elderly City": A Ten-Year Report (2010–2019)**

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**Learning Objective:** After attending this presentation, attendees will have learned about the spread of the phenomenon of elder abuse in Genova, Italy, the oldest city in Europe (27.8% of the population is over 65 years of age).

**Impact Statement:** This presentation aims to bring the attention of the forensic science community to the crucial features of elder abuse in order to assess the trend of the phenomenon and to propose preventive strategies.

The World Health Organization (WHO) defines elder abuse as “a single or repeated act, or the lack of appropriate action, occurring within any relationship where there is an expectation of trust which causes harm or distress to an older person.” Thanks to the Penal Court of Genova, with the help of the Public Prosecutor's Office, we have analyzed the cases of elder abuse reported to the judicial authorities in the territory of Genova from 2010–2019. In this way, we were able to depict the state of the phenomenon and to trace its evolution over the ten years considered.

A total of 1,237 offences were reported in the period of 2010 to 2019. Fourteen cases occurred in residential facilities for the elderly, the maltreating subjects being nurses or care workers. The unlawful behavior involved the imposition of uncomfortable positions for long periods, forced immobilization, and abuse of the means of constraint.

Maltreatment in the family setting accounted for 239 reports. This type of abuse threatens the person's physical safety, mental salubrity, and dignity, and refers to conduct that is enacted with the intention of engendering a state of permanent oppression. In 84% of cases, the perpetrator was a son/daughter of the victim; most perpetrators (63%) were affected by problems of alcoholism, substance abuse, or psychiatric disorders.

There were 581 cases referring to a situation in which the abuser exploited the elderly person's frailty to commit physical abuse. Victims' children proved to be the main offenders, with the home being the most frequent setting of maltreatment. Situations of ongoing daily conflict may explode in physical violence that causes fractures, bruises, or lacerations.

The abandonment of an incapable person (86 reports) occurs among the children of an infirm elderly subject, each of whom may, for various reasons, refuse to take on the responsibility of caring for a parent who is no longer self-sufficient. Financial exploitation (317 reports) proved to be the second most-frequent type of abuse: solitude, isolation and the presence of organic diseases are the main risk factors. The victims suffered from mental deficiency (Parkinson's disease, dementia, depression) and had a poor social network. The oldest (> 85 years) proved to be at the greatest risk of financial abuse, often being isolated and dependent on others. The majority of the offenders (75%) did not have a daily relationship with their victims, but gained their trust to defraud them (caregivers, the staff of residential facilities, etc). No cases of sexual abuse were found.

Regarding all cases, the most common setting of abuse (47%) was seen to be the elderly person's own home. Regarding the sex of the victims, 54% of the elderly subjects who had suffered maltreatment were women. Regarding the sex of abusers, the majority of cases of abuse are perpetrated by men (65%): maltreatment perpetrated by males tended to be physical and violent (personal injury, abuse in the family), while females were more often involved in psychological and financial abuse.

Given the progressive aging of the global population, there is a need to increase our knowledge of the characteristics of elder abuse and its related mechanisms, to implement adequate prevention, and to ensure prompt identification and effective management of the phenomenon. Doctors and the staff of emergency departments, hospitals, and residential facilities play a fundamental role in the screening of cases of abuse. Health care professionals should receive adequate clinical-forensic training in order to recognize cases of abuse and to gather evidence that may be useful in criminal proceedings.

Further studies will investigate how the COVID-19 pandemic situation has affected the phenomenon of elder abuse in our city.

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**Elder Abuse; Ageism; Domestic Violence**



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**I119 An Epidemiology Analysis of a Medical-Legal Investigation of Child and Adolescent Fatalities in Taiwan**

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**Learning Objective:** After attending this presentation, attendees will be better able to assist frontline medical personnel in the protection of children, promote child abuse prevention or related policy formulation, and protect the well-being of children and adolescents.

**Impact Statement:** This presentation will impact the forensic science community by providing reference data about the epidemiological analysis of deaths in children under the age of 6 years.

While overall the population of Taiwan is aging due to the low birth rate, the numbers of child abuse and child death is surging even with the historically lower youth population. Protecting children against abuse and neglect is an increasingly important challenge in Taiwan. The purpose of this study is to create a database to allow us to better understand and analyze child abuse morbidity and mortality data from Taiwan.

In this study, a database has been established for “Forensic Autopsy of Child and Adolescent Deaths” and used to analyze trends in child abuse deaths and correlated risk factors. We hope our results can both assist in the creation of more effective child abuse prevention programs and improve the quality of forensic investigation of child and adolescent fatalities in Taiwan.

To work toward a solution, it is necessary to examine the physical and mental extent of drug abuse and its relation to child abuse, and investigate the pattern of death, pattern of injuries of children and infants, and the risk factors of abusers. The database documenting the death patterns of different age groups has been updated annually and the mortality risk factors were analyzed. We studied the statistical correlations between the risk factors of guardians or caregivers such as psychological disorders, alcoholism, substance abuse, and mental retardation in addition to cases of child abuse that include domestic violence, care negligence, and suicide-homicide. Out of 19,431 medicolegal autopsy fatalities from the Institute of Forensic Medicine, Ministry of Justice in Taiwan, 1,054 (5.4%) involved children under 6 years of age. The data was collected and analyzed by age groups during 2000–2019. The accidental risk factors of different age groups were significantly different. It is worth noting that falling from height is the highest-ranked pattern of death for all age groups. In more than half of the 128 forensic autopsy deaths of homicides under the age of 6, most perpetrators were parents (75 cases, 58.6%), showing that the deaths of pre-school childcare deaths were significantly related to the main caregivers such as parents. The epidemiological analysis reveals that child fatalities under 6 years of age have large differences of mortality statistics between the age groups compared to the general population.

In future work, the important areas for further statistical analysis should include the cases of perpetrators of other homicide deaths, the infant and young child deaths involving substance abuse, the types of illicit drugs-related, and the abuse patterns. These results can be used to establish strategies to help the frontline medical personnel, such as pediatricians, to identify child abuse, better formulate policies for child abuse prevention, protect the well-being of children and infants, and to improve judicial human rights.

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**Forensic Autopsy; Child Maltreatment; Patterns of Death**

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**I120 Extreme Shortness of the Umbilical Cord: A Case Report**

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**Learning Objective:** After attending this presentation, attendees will be aware of the importance of forensic investigations for the estimation of causes of perinatal death in cases of difficult interpretation.

**Impact Statement:** This presentation will impact the forensic community as the publication in the literature of cases of intrauterine mortality from rare causes, such as placental insufficiency given by an extremely short funiculus, will support the forensic pathologist in defining the cause of death.<sup>1-3</sup>

The chapter on the causes of perinatal deaths is extremely broad and can include problems related to childbirth, but also pathological pictures related to the mother or fetus. In these cases, the intervention of the forensic pathologist and postmortem studies are crucial in identifying the cause and timing of death. The case reported here provides an example of how postmortem examination can detect macro and microscopic aspects essential to identify the causes of fetal death.

A nulliparous mother was 27 years old and had experienced blood loss during the eighth week of pregnancy. The pregnancy continued regularly up to the 26<sup>th</sup> week, during which the mother went to the emergency room for abdominal pain. The doctors hospitalized the woman with the diagnosis of “patient in labor and threatened with preterm birth.” At admission, the presence of the fetal heartbeat was detected, and about two hours later the labor began. The child was born by vaginal delivery and appeared pale, atonic, in the absence of spontaneous heartbeat and breathing, and with Apgar index of “0.” After the resuscitation maneuvers, death was declared. From the medical-legal investigations, a picture of placental insufficiency with consequent malperfusion of the fetal-placental unit, associated with an extremely short umbilical cord (18cm overall) emerged.

The length of the umbilical cord is generally between 50 and 60cm. Rarely, the umbilical cord can be too short or too long. The umbilical cord is defined as short when its length is less than 35cm. Extreme lengths are associated with a further increased risk of adverse outcomes, including a more-than-doubled risk of perinatal death. No modifiable risk factors associated with the development of a short cord have been identified. Diagnosis is possible through ultrasound, although it is not always possible to visualize the actual length of the cord, as is often the case when complications occur. In the presented case, the forensic investigations and, in particular, the histological investigations proved to be fundamental for the diagnosis, highlighting a picture of placental insufficiency with consequent malperfusion of the fetal-placental unit due to an extremely short umbilical cord (18cm in all). The samples obtained for the histopathological study showed post-placental hypoxia with morphological abnormalities of villous development, intervillous hemorrhages, and microhemorrhages of the maternal plate. The funiculus did not show structural anomalies, except for the extreme brevity.

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**Short Umbilical Cord; Intrauterine Fetal Death; Forensic Investigation**

**I121 Fatal Fistulas: A Case Report of Fatal Button Battery Ingestion in a 13-Month-Old With a Literature Review**

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**Learning Objective:** After attending this presentation, attendees will understand the public health crisis surrounding the fatal consequences of button battery ingestion in the pediatric population. Attendees will learn the types of batteries that pose the greatest risk, the possible lethal injuries, and which population is at greatest risk.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of button battery ingestion as an immediate and delayed cause of death. Most cases of foreign body ingestion are unwitnessed and may not be known before fatal consequences occur. Diligent examination is needed to find and diagnose a fatal case due to foreign body ingestion.

The case is that of a previously healthy 13-month-old female with sudden hematemesis who became unresponsive in her car seat. Despite resuscitative efforts, she was pronounced dead at the hospital three hours later. No history of foreign body ingestion or injury was reported. At autopsy, the proximal esophagus exhibited a 2.5cm rounded erosive lesion with a 0.5cm communicating erosion into the aortic lumen just below the arch. Histologic examination revealed erosion of the esophageal mucosa with hemorrhage, acute and chronic inflammation, gray-purple and transparent refractile materials with multi focal foreign body giant cell reaction amid liquefactive necrosis and saponification of underlying esophageal tissues. The stomach contained 120mL of partially clotted blood. The pre-autopsy radiograph revealed a radiopaque elliptical object in the child's fecal-soiled diaper, which had been taken off in the emergency department and received separately in the body bag. A 20mm lithium 3-volt battery was recovered from the diaper. The cause of death was complications of aortoesophageal fistula due to lithium battery consumption and manner of death was accident.

A button cell or coin battery is a thin cylindrical battery often used to power small toys, watches, and hearing aids. Not long after its invention, button battery (BB) ingestions by children were reported, sometimes with fatal consequences. The national button battery ingestion hotline was started in 1982 and has since documented reported cases; 3,500 of the 70,000 foreign body ingestions by children reported annually are due to BBs. Over the past 20 years, while the number of overall reported BB ingestions has not dramatically increased, the number of serious or fatal consequences has quadrupled.

Button batteries pose a serious threat when ingested due to multiple mechanisms. The main mechanism of injury is through electrolytic current generated by the battery that hydrolyzes the surrounding tissue causing an increase in pH (an alkaline burn). The small diameter of a child's esophagus/trachea allows tissues to touch both the positive and negative poles of the battery, thus discharging it. Proposed reasons for the recent increase in reported injuries include the introduction and use of the 20mm battery (larger than previous), increased voltage to 3v (from 1.2v), and the use of lithium over mercury. Up to 13% of children under 6 years old will develop serious or fatal consequences after BB ingestion; 92% of involved BBs are 20mm lithium batteries. Tissue damage from BBs can occur in under two hours and perforation can happen within 6-12 hours of ingestion. The most common place for initial injury is the proximal esophagus (up to 60%). The most common fatal injury is hemorrhage (80%), with most due to aortoesophageal fistula (60%).<sup>1-7</sup>

Forensic investigators need to be diligent when examining these types of cases since pertinent history and the culprit object may be missing. Some 56% of BB ingestions are unwitnessed, and 46% will have already eliminated the battery. Associated symptoms may develop hours to days after ingestion and can be vague or entirely overlooked.

X-ray examination at autopsy for a double-ringed circular or elliptical object, with the "halo sign" seen with BBs, may reveal an extracorporeal BB, as in our case. In most cases of foreign body ingestion, once an object is removed, the danger is gone. However, with BB injuries, delayed injury can occur one month after elimination. It is essential that medical examiners remember to think of BB ingestion as a cause of pediatric aortoesophageal injury, even after the offending foreign body has passed. Histologic findings of liquefactive necrosis, saponification, and mixed inflammation (alkaline burns) may also support diagnosis of a BB injury in the absence of a retained foreign body.

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**Button Battery; Foreign Body Ingestion; Aortoesophageal Fistula**

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**I122 Juvenile Suicide Trends: A Comparative Analysis of Factors Surrounding Georgia's Child and Teen Deaths**

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**Learning Objective:** This presentation will analyze demographic, spatial, and modality patterns found within juvenile suicide cases reported to the Georgia Bureau of Investigation (GBI) Medical Examiner's Office (MEO) over a five-year period. Through this analysis, correlations between completed suicidal acts, extraneous variables, and predispositional factors will be reviewed.

**Impact Statement:** Trends found within this analysis will allow those serving in the public sectors to outline areas of concern leading to increased suicidal behaviors and aid in the prevention of future youth suicides.

**Introduction:** According to data published by the Centers for Disease Control (CDC), the suicide crisis among the American population continues to represent one of the highest causes of death with a 16.2% increase in Georgia juvenile suicides since 1999.

**Materials and methods:** From 2016–2020, 194 juvenile suicides were reported to the GBI MEO for review. The GBI MEO has jurisdiction in 155 out of 159 counties across the state of Georgia; thus, minor discrepancies in final sample sizes are plausible, though the overarching principles and trends are still applicable. Multiple data points, including predispositional factors (race and sex), geographic incident location, pre-existing Division of Family and Child Services (DFCS) history, and modality, were recorded for each suicide. These data points acted as dependent variables that were tracked across varying ages of the reported deaths, allowing for multiple factors and potential trends to be viewed on a macroscopic level.

**Results:** Juvenile suicides reported to the GBI MEO peaked in 2020 with 41 total cases reported, an 8.5% increase from the 35 cases reported in 2019. Of the 194 reported cases, 68.0% of deaths involved a White decedent, compared to 22.7% involving a Black decedent, and the remaining 9.3% being comprised of varying minority groups. Furthermore, males accounted for a significantly higher number of deaths overall compared to females (69.5% versus 30.5%, respectively), and previous DFCS involvement accounted for only 35% of juvenile deaths. No significant trends were found between data sets showing that one regional suicide typically induces an immediate suicide pandemic; however, several instances of possible suicide pacts or related suicides were noted. Firearm-related deaths significantly increased during the reviewed period from 35.9% in 2016 to 53.7% in 2020.

**Conclusions:** The analysis performed for these untimely deaths shows that the suicide crisis is actively progressing within the juvenile population, though the details surrounding each case have evolved. It is evident that some population groups, specifically White males within this study sample, do have heightened associations with suicidal behaviors. These findings show that in order to provide adequate preventative measures, the individuals responsible for monitoring the nation's youth and those assigned to investigate their tragic deaths must work alongside one another to determine what variables can be altered to avoid another juvenile falling into the suicide pattern.

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**Suicide; Youth; Demographics**

**I123 Kennel Cough: A Case of a Lethal *Bordetella Bronchiseptica* Infection in an Infant**

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**Learning Objective:** The objective of this presentation is to illustrate a novel case of a natural death due to a rare infectious etiology.

**Impact Statement:** This presentation will impact the forensic community by emphasizing the need for thoughtful and thorough investigative and infectious disease work up.

**Introduction:** *Bordetella bronchiseptica* is a gram-negative coccobacillus that causes kennel cough and is commensal in the airways of pets, especially dogs, cats, and rabbits.<sup>1</sup> Infection of *B. bronchiseptica* is rare in immunocompetent humans; however, the pathogen has been reported to cause respiratory diseases in immunosuppressed humans.<sup>2</sup> Very few cases of *B. bronchiseptica* infection are reported in immunocompetent humans in the literature. Here we report a unique and rare case of *B. bronchiseptica* in a 9-month-old infant.

**Materials and methods:** The decedent was a 9-month-old African American male who was found face down and unresponsive while co-sleeping with his mother in an adult bed. The decedent had eczema as his only significant medical history and was up to date with all pediatrician appointments and vaccinations. In the 72 hours leading up to his death, his mother described him as congested, with a cough and mostly normal breathing. Of note, the patient was in contact with, and as his mother described it, inseparable with a blue-nosed pit bull puppy. The decedent's mother did not know the dog's health history or if its vaccinations were up to date.

**Results:** At autopsy, the internal examination showed heavy, congested, and edematous lungs. Of note, the trachea was grossly narrowed and constricted. There was tan-white frothy thick gastric material in the upper airway. The remaining examination was unremarkable. Microscopic sections of the trachea showed acute and chronic inflammation consistent with bacterial tracheitis. The following postmortem labs were negative: metabolic screening, bacterial cultures of the heart and blood, viral cultures, and toxicology. Postmortem bacterial cultures of the lungs grew *Bordetella*. The Center for Disease Control (CDC) identified the species from the lung cultures as: *Bordetella bronchiseptica*. The cause of death was determined to be bacterial tracheitis due to *Bordetella bronchiseptica*. The manner of death was certified as natural.

**Discussion:** *B. bronchiseptica* is an obligate aerobe, which is found in the airways of domesticated pets such as dogs, cats, and rabbits. The infection may be *B. bronchiseptica* symptomatic in animals, often referred to as "kennel cough." For immunocompetent humans, transmission is rare, but can occur through direct contact with respiratory secretions of animals.<sup>3</sup> A literature review demonstrated that *B. bronchiseptica* infections in immunocompetent humans are extremely rare. It is not well documented and thus far, less than 1% of these infections exist in immunocompetent people.<sup>4,5</sup> Here we document a case of kennel cough causing death.

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**Natural; Bacterial Tracheitis; *Bordetella Bronchiseptica***

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**I124 Neonatal Pulmonary Hemorrhage: A Hazardous Disease**

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**Learning Objective:** This work aims to present a rare fatal case of neonatal pulmonary hemorrhage in a completely healthy newborn. Since such disease can lead to threatening complications and consequently to potential civil claims against physicians, forensic studies can provide useful data to better understand its still undefined pathophysiology.

**Impact Statement:** This case shows how neonatal pulmonary hemorrhage can also affect perfectly healthy newborns. Forensic analysis, both macroscopic and histological, can provide the scientific community useful data to better understand pulmonary hemorrhage physiopathological mechanisms; such information will be pivotal to detecting potential diagnostic tools and therapeutic protocols. Furthermore, new frontiers on such diseases can impact medical liability, helping reduce claims in the pediatric setting.

Pulmonary hemorrhage (PH) is a fatal event that can be encountered in a neonatal intensive care unit and occurs at a rate of 1–12 per 1,000 live births and as high as 10.2–11% in extremely low birth weights, occurring most commonly within the first few days of life. The most common risk factor associated with PH is prematurity. PH is typically seen in babies weighing less than 1,500g, who often have a patent ductus arteriosus (PDA), have been treated with surfactant, and are ventilated. Other risk factors predisposing to PH include intrauterine growth restriction, chorioamnionitis, coagulopathy, and respiratory disorders. Clinically significant hemorrhage appears as the presence of hemorrhagic endotracheal fluid, associated with hypoxia or pulmonary edema in most cases. If the hemorrhage continues, the infant can develop apnea, generalized pallor, become cyanotic, with concomitant bradycardia and hypotension from hypovolemic shock.

This report presents a rare case of a newborn who presented a pulmonary hemorrhage 6 hours after cesarean delivery; the pregnancy was described as physiologic: the expectant mother, a 37-year-old healthy woman in the 38th week of gestation, referred to a local hospital after undergoing a Nonstress Test, which was non-reactive. A few hours later, the woman showed a prelabor rupture of the membranes with slightly meconium-stained amniotic fluid, so physicians decided to perform a C-section, extracting a male baby weighted 2,820g. The Apgar of the newborn scored 8 at 1 minute and 9 at 5 minutes after the delivery, indicating substantial wellbeing. Six hours after birth, the newborn presented with cyanosis and breathing difficulties and was immediately transferred to the NICU (Neonatal Intensive Care Unit) where his clinical conditions worsened quickly, requiring endotracheal intubation. Soon, he showed an abundant leak of hemorrhagic endotracheal fluid and despite the prolonged resuscitation maneuvers, the newborn died a few hours later. Parents presented a claim for medical malpractice against the physicians involved, and a full autopsy was performed. No congenital malformations were identified nor macroscopic pathological findings except for a bilateral massive pulmonary hemorrhage. Histological examination on lung samples confirmed the great amount of hemorrhage (associated to septal and bronchial ruptures).

During the trial, no medical liabilities were identified, confirming physicians' adherence to clinical guidelines. Our case shows how spontaneous pulmonary hemorrhage can occur even in the absence of known risk factors. Forensic analysis, especially histological ones, can provide useful information to understand actual pathological mechanisms in order to detect potential diagnostic tools and therapeutic protocols. Furthermore, new frontiers on such diseases can have an impact on medical liability, helping reduce claims in the pediatric setting.

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**Pulmonary Hemorrhage; Newborn; Infant Mortality**



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**I125 An Unexpected Pulmonary Embolism in an Adolescent: A Case Report and Literature Review**

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**Learning Objective:** After attending this presentation, attendees will increase their awareness of factors predisposing a child or adolescent to a rare natural cause of death that may present for forensic autopsy.

**Impact Statement:** This presentation will impact the forensic science community by reviewing current literature on detection and prevention of death from pulmonary embolism in adolescents with relevant family history.

A 15-year-old African American boy, who had complained of leg pain for a few days, was found down by maintenance workers in his family's apartment. The front door was unlocked to allow the maintenance workers entry, but there was no evidence of struggle, robbery, or signs that anyone else had been in the apartment. He was lying supine atop a broken coffee table; he had white foam at the nose and mouth.

Past medical history included attention deficit disorder treated with dextroamphetamine preparations and clonidine, asthma treated with budesonide-formoterol and albuterol, and anxiety. There was no drug or alcohol history. His recent leg pain had not been brought to medical attention. The family of the decedent reported a history of "inherited blood disorders." On external examination, he was obese (BMI 39.3kg/m<sup>2</sup>), without injuries, with white foamy fluid originating from his mouth and nose. Internal examination was significant for a ~20cm saddle pulmonary embolism; there was right popliteal deep vein thrombosis without Baker cyst. Histology confirmed pulmonary embolism, quiescent asthma without acute exacerbation, and deep venous thrombosis.

Pulmonary embolism (PE) secondary to deep venous thrombosis (DVT) can be difficult to diagnose in pediatric populations, especially since symptoms are often vague and the only diagnostic clue may be unclear familial risk factors. The diagnosis of PE in children is difficult due to its relative rarity, with an annual incidence of 0.5/10,000 children, with a bimodal peak in infancy and adolescence.<sup>2</sup> Children with PE may complain of non-specific symptoms such as tachycardia, tachypnea, acute shortness of breath, or leg pain. Due to the morbidity and relatively high mortality (5.5–18%) of PE in children, raising the index of clinical suspicion and improving diagnostic tools is important in prevention.

Despite the rarity of PE in children, it can be diagnosed with relative accuracy using the same clinical decision-making tools used for adults. The clinical studies used to predict PE are D-dimer (sensitivity 79–88%, specificity 13–69%) or Well's score (sensitivity 72–86%, specificity 60%).<sup>3</sup>

Our patient had three risk factors that could increase his risk of thromboembolic disease: race, obesity, and family history. Stein et al. found that PE, DVT, and venous thromboembolic disease (VTE) were more common in Black children than White.<sup>3</sup> Obesity has also been associated with increased risk of VTE; Stein et al. determined that 47–52% of children greater than age 12 diagnosed with VTE were obese.<sup>3</sup> He carried no thrombophilia diagnosis, but his mother had known occurrences of "thrombi;" Stein et al. found that up to 19% of children over 12 diagnosed with pulmonary embolism had an inherited thrombophilia.<sup>3</sup> More specific family history was not available for this decedent; although coagulation studies such as D-dimers cannot be utilized on postmortem blood, genetic testing may have confirmed a shared abnormality. If the significance of his leg pain with this family history had been recognized, either by his family or by his treating physician, his death might have been prevented.

Cases such as these contribute to recognition of the rare diagnosis of PE in children and may increase the index of clinical suspicion in children and adolescents with familial risk factors.

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**Forensic Sciences; Embolism and Thrombosis; Adolescent Medicine**



**J1      The Challenges Facing the Applicability of Psychological Autopsy in the Middle East Region for Female Deaths: A Brief Review**

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**WITHDRAWN**

## J2 The Role of Graphology in the Study of Suicides and Homicides and in the Analysis of Self and Hetero-Aggressive Behaviors

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**Learning Objective:** After attending this presentation, attendees will understand how graphology can provide a contribution to investigations in suicide cases through to the graphological analysis of personality and in those cases where an analysis of the manuscripts of the alleged suicide is necessary to compare and verify their source (forensic graphology).

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the usefulness of graphology as a suitable tool for reconstructing the writer's personality and self-harm or hetero-aggressive ideations.

Girolamo Moretti, founder of the modern graphology in Italy, in his 1962 work, "Mental imbalance, anomalies of the psyche and graphology," wrote that "the fundamental sign of suicide is ink spotting or intozzata secondo modo (in high degree)," adding, however, that the presence of other signs is necessary. Therefore, graphological analysis studies the graphic layout in its complexity and, in this scenario, aims to identify the combinations of signs that represent, contextualized, the deterioration of the survival instinct.

Often, one who commits suicide will leave a letter, and the study of such manuscripts, in doubtful cases, supports the need for further investigation. On the other hand, few researchers have studied letters related to murders. The purpose of the graphological investigation is to validate a suicidal reality that can be inferred and found from the graphic context or to exclude it in cases of murder and imitations of graphics.

We report four cases of suicide and one of homicide-suicide for which the manuscripts left on the scene were subjected to graphological analysis to detect the "serious anomalies" of the writing as indicators of the suicide. We used the Morettian graphological method.

We analyzed the manuscripts in the cases of suicide (a woman by hanging, a woman by suffocation and inhalation of gas, a man by explosion of a homemade explosive device, and a man by hanging), observing the presence of different signs (Staccata, Rovesciata, Discendente, Titubante, Ripassata, Intozzata Secondo modo, narrowness of the graphics and large signs between words), as well as other alterations (line direction, pressure, caliber, inclination, widths, margins). Such variants are indicative of the psychic, affective, emotional, and relational state of the subject.

We examined the graphic space, which, according to Max Pulver, represents the environment where the writer symbolically moves, as well as the signature when present, which represents the social identity. In the fifth case, we finally analyzed the manuscripts of a young man who committed suicide after matricide. 300/5,000. The graphological analysis of the note written before the murder showed an uncontrolled emotionality with an amplification of the feeling of the ego (huge caliber of writing). also emerged of the letters T in the shape of "sharp swords" (symbol of mythomania) with delirium of omnipotence.

In conclusion, the graphological study does not allow us to make a diagnosis or predict human behavior (therefore, it cannot predict suicide but only confirm it *a posteriori*). Nonetheless, graphological analysis can offer an indication of the writer's personality and hemotivity (as an example aggression and a sense of guilt in the same graphism). Furthermore, a careful graphological analysis can identify warning signs about suicidal intentions, present at the time of death, especially in serious situations such as in the cases analyzed above.

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**Forensic Sciences; Graphology; Suicide**

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## **J3      The Aurora, Colorado, Movie Theater Shooting: The Significance for Forensic Mental Health Professionals**

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**Learning Objective:** After attending this presentation, attendees will gain a better understanding of the factors that contributed to James Holmes' decision to open fire on a movie theater full of patrons there to see the Batman film *The Dark Knight Rises* in 2012. Attendees will understand how some of Holmes' offense preparations, social media presence, and beliefs may have impacted the outcome of his criminal trial.

Reconstructing a timeline of Holmes' mental health treatment and prior comments he made to treatment providers combined with comments he made online and preparatory behaviors will help forensic mental health professionals conceptualize how to best intervene with their own potentially violent patients. Tarasoff warnings and their diversity among different states will be explored, giving practitioners insight into how to best support their patient while keeping the community at large safe.

**Impact Statement:** This presentation will impact the forensic science community by delving into considerations important in potential Tarasoff warnings, psychiatric and psychological treatment of potentially violent individuals, and forensic assessment of criminal defendants raising a Not Guilty by Reason of Insanity defense.

This presentation will explore divergent expert opinions regarding Holmes' criminal responsibility/legal sanity and contrast that with lay opinions regarding what constitutes severe mental illness. The forensic science community will gain a better understanding of important factors that influence decisions regarding Not Guilty by Reason of Insanity. This presentation will also explore how different forensic mental health practitioners can reach different conclusions about a defendant's legal sanity.

While the Aurora, CO, movie theater shooting was a terrible tragedy, the intricacies of James Holmes' mental health treatment, preparatory behavior, online presence, and insanity defense provide a wealth of educational material for forensic mental health clinicians, whether they are providing clinical mental health treatment or expert opinions.

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**Not Guilty by Reason of Insanity; Tarasoff; Mass Murder**

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## J4 An Area of Conflict—Forensic Psychiatry in Germany

*Klaus Neudecker, MD\*, Landshut, Bayern; GERMANY*

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**Learning Objective:** The goal of this presentation is to provide attendees with the knowledge to differentiate between their own forensic system and those established in Germany. The information given will help them to reflect on their own roles as part of a process that has the challenge to play a key role either in the juridical system as (in Germany) the psychiatric care.

**Impact Statement:** This presentation will impact the forensic science community by presenting information about the forensic psychiatric system in Germany, its advantages, and boundaries.

After attending this presentation, attendees will be able to: (1) identify key aspects of the German forensic psychiatric system; (2) compare them with their home system of dealing with mentally ill offenders; (3) rate the advantages; and (4) identify the disadvantages. Furthermore, the presentation will highlight the role of the forensic expert in the courtroom and in his/her role as forensic counselor.

Forensic psychiatry is a sophisticated part of the psychiatric system in Germany. It has the task of identifying criminally ill offenders and providing expert opinions to the court to help judges decide whether an offender is fully responsible for his actions, not responsible because of reason of insanity, or has a diminished responsibility. This expert opinion often marks a turning point in the criminal proceedings because it determines the future of where and how long a convict will be penalized or treated.

The forensic psychiatrists, among other occupational groups, treat the offenders in special branches of the psychiatric hospitals and in prison. Forensic hospitals in Germany are medical facilities connected to general forensic clinics. They provide treatment options for addicts who have committed a crime as well as for mentally ill offenders who were found not guilty by reason of insanity or had a diminished responsibility at the time of the crime. During and at the end of a mostly long course of forensic treatment, experts play a key role in providing a (hopefully) valid prognosis to the parole board. Because of historical aspects, especially their fatal role as a crucial part of the euthanasia project carried out at the time of Nazism in the past century, forensic experts in Germany are since then not allowed to decide a lot of questions on their own but instead help the courts/judges in making these decisions. Besides their role in the criminal trial and forensic treatment, forensic psychiatrists have duties and responsibilities in civil cases (such as assessing the ability of a person to make a will), assessment of the capacity of taking legal actions, and many others regarding court proceedings.

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### Forensic Psychiatry; Requirements and Procedures; Germany

## J5 The Pitfalls in the Assessment of Insanity Due to Dissociative Disorders

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**Learning Objective:** Attendees of this presentation will: (1) recognize the scientific evidence refuting societal stereotypes about dissociative disorders; (2) understand the relevance and admissibility of expert testimony in insanity defense claims involving dissociation; and (3) appreciate the unique challenges of expert evidence about dissociative disorders in insanity defense cases.

**Impact Statement:** This presentation will impact the forensic science community by improving the ability of experts and legalists to consider the strengths, limitations, and misconceptions associated with insanity defenses based on dissociative disorders. In doing so, it will improve the use of expert opinion evidence in attendees' practices.

Dissociative disorders are “characterized by a disruption of the normal integration of consciousness, memory, identity, emotion, perception, body representation, motor control, and behavior,” and are frequently found in the aftermath of psychological trauma.<sup>1</sup> In this presentation, the term will be used to refer to conditions including but not limited to Dissociative Identity Disorder (DID), Post-traumatic stress disorder (PTSD) with dissociation, dissociative amnesia, and depersonalization/derealization disorder.

Dissociative symptoms have historically been viewed with skepticism by the public. Scientific evidence supporting dissociation as a symptom of trauma-related and other mental illnesses has grown enormously over the past three decades, yet media portrayals continue to focus on sensationalized stereotypes of violent and manipulative individuals with DID (formerly Multiple Personality Disorder).<sup>2</sup> Even mental health clinicians are hypothesized to harbor biases against the “authenticity” of these illnesses and have been found likely to misdiagnose or avoid diagnosing certain dissociative disorders.<sup>3</sup>

With this context, it is perhaps unsurprising that a review of case law suggests that insanity defenses based on dissociation often fail. In fact, the courts have questioned if dissociative disorders such as DID are even applicable to the insanity defense and whether expert witness testimony about dissociative disorders meets the standard for evidentiary admissibility.<sup>4</sup>

Dissociation-based insanity defenses may be found lacking in a variety of ways. Often, these defenses are deemed unconvincing when experts fail to establish the link between dissociative symptoms and the legal standard for insanity. Additionally, symptoms of dissociation can be challenging to convey to laypersons. For instance, an individual experiencing overwhelming sensations that the surrounding world is not “real” may have little or no observable change in their behavior.

This presentation analyzes the relevant scientific literature and law to assist experts and legalists in identifying legitimate claims of dissociative disorders, evaluating their potential impact on criminal responsibility, and when applicable, presenting dissociative symptoms as the foundation for a well-reasoned insanity defense.

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### Insanity; Dissociation; Expert Testimony

## J6 Police Suicides in Turkey

*Nevzat Alkan, MD\*, Istanbul Tıp Fakültesi, Caba, Istanbul, TURKEY*

**Learning Objective:** Attendees will gain important information about the problems experienced by the police officers working in an important field of forensic sciences in the Republic of Turkey and their suicidal behavior resulting in death.

**Impact Statement:** Suicidal behavior resulting in death is an important field of study of forensic medicine and behavioral sciences. Thanks to this presentation, literature information about the problems experienced by the police officers working in the Republic of Turkey and the reasons that lead them to suicidal behavior resulting in death will be created.

Turkey is a Eurasian country with a population of approximately 90 million. There is significant economic distress and youth unemployment in the country. In the past 20 years, especially with the increase in the number of universities, unemployment of young university graduates is at a high level. In the hope of getting rid of this helpless situation, these young people who are exposed to unemployment may turn to occupational groups that are not suitable for their own personality structures and resistance mechanisms.

In countries such as the Republic of Turkey, where security policies are a priority, the number of soldiers, police, guards, and private security personnel is high. Due to the high unemployment of university graduate youth in the country, university graduates tend toward the security sector, especially as police officers, where employment is always higher.

Being a police officer is a difficult and tiring profession. In addition to this, considering their youth and the fact that they carry guns, we come across an occupational group that needs good social and psychological support. In Turkey, especially in the past five years, suicides of police officers with guns have increased at a significant and remarkable rate. This situation is currently an important area of discussion at the level of decision makers in the Republic of Turkey. Developing countries such as the Republic of Turkey are among the communities where people have difficulty in coping with their living conditions.

This presentation will address the increasing number of suicides among police officers, the causes, ways and methods of suicide, the distressing structure of the profession, and the mobbing approaches that are claimed to be present at a significant levels. Discussion will also include what can be done to prevent such suicides and the tragic reflections of the issue in society through some specific cases.

### Police Officer Suicides; Mobbing; Forensic Medicine

## J7 An Examination of a Police Officer's Quality of Life 23 Years After Surviving a Penetrating Gunshot Wound to the Head: A Case Report

Gregory Mack, PhD\*, Clinical, Police, & Forensic Psy Svcs PLLC, Shrub Oak, NY

**Learning Objective:** After attending this presentation, attendees will be able to describe the survival rate of patients with gunshot wounds to the head. Attendees will also be able to describe specific factors that affected an officer's recovery from a devastating injury, including the role of individual characteristics and the availability of peers, family, and social support.

**Impact Statement:** The forensic science community will be impacted by the resiliency of this officer and his ability to survive this devastating injury.

**Introduction:** Within the past few years, there has been an alarming increase in violence perpetrated against members of our law enforcement community.<sup>1,2</sup> The National Fraternal Order of Police reports that as of April 1, 2022, 17 police officers have been shot and killed in the line of duty (LOD). Additionally, 84 officers have been wounded by a firearm in the LOD.<sup>3</sup> Not surprisingly, a gunshot wound to the head is the deadliest of all gun injuries.<sup>4</sup> According to the research literature, the survival rate of patients with gunshot wounds to the head is only 9%.<sup>5</sup> Despite advances in modern medicine, these injuries are still associated with a high mortality. Factors associated with surviving this type of injury include the level of consciousness and the presence or absence of brain spill.

**Presentation of case:** This presentation utilizes an interpretive phenomenological case study methodology.<sup>6</sup> LCJ, a 54-year-old Black male police officer from a large metropolitan police department suffered a cranial trauma 28 years ago when a suspect shot him and his partner with a .380 caliber handgun.<sup>7</sup> His partner was shot in the leg. LCJ was struck by a single round that entered the left frontal side of his head without exiting.<sup>8</sup> The officer sustained severe neurological damage. During his initial recovery and rehabilitation, LCJ had to relearn how to speak and ambulate despite partial paralysis of the right side of his body.

**Discussion:** This presentation will provide an overview of the of circumstances that led up to the October 30, 1993, attempted murder of two police officers. After attending this presentation, attendees will gain an appreciation of the miraculous recovery LCJ made and the obstacles that he had to face. We will discuss the nature and extent of the rehabilitation process as well as the physical and psychological recovery processes.<sup>9</sup> Last, a review of available medical records, outcomes from recent psychological testing, and a quality-of-life measure will also be summarized and evaluated.

**Conclusion:** Law enforcement officers are the street soldiers patrolling our communities and serve as the "guardians" of our democratic ideals. An assault on a police officer is an assault on our democracy. As a result of the recent uptick in felonious assaults against members of the law enforcement community, many of our protectors have sustained career-ending injuries.<sup>10-12</sup> The ongoing care and support of these individuals' rests not only on the shoulders of their brothers and sisters in blue but also on the broad shoulders of society.

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### Traumatic Brain Injury; Police Officer Resiliency; Penetrating Gunshot Wound

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## J8 Family Massacres Perpetrated by the Man of the House in Turkey

*Nevzat Alkan, MD\*, Istanbul Tıp Fakültesi, Caba, Istanbul, TURKEY*

**Learning Objective:** This presentation will inform the forensic science community about family massacres that have started to emerge in Turkey in recent years.

**Impact Statement:** Attendees will be informed about a tragedy that has started to occur in Turkey in recent years regarding the murder-suicide association, which is a special type of violence, in numbers, case types, and what can be done to prevent it.

The Republic of Turkey is a Eurasian country with a population of approximately 90 million. In the past five years, there has been significant economic distress and a significant refugee problem in the country. These refugees generally come to Turkey from Syria, Iraq, Afghanistan, Pakistan, and the African continent as well as countries in Central Asia. This situation significantly increases unemployment and economic problems in the country.

Suicidal behavior resulting in death is a problem that we encounter from time to time all over the world. Here, the person usually cannot cope with life's problems adequately and gets rid of them by ending his own life. Again, domestic violence, violence against women, violence against the elderly, violence against children, or violence against vulnerable groups are among the fields that forensic science professionals deal with.

However, we have started to encounter a different situation in the Republic of Turkey, especially in the past five years: The man of the house first kills his wife, then kills his children, and commits suicide. Detective investigations conducted after the massacre in question generally point to inextricable economic problems, especially if there are elements such as a suicide note. Studies conducted in such cases reveal that in a country with a patriarchal society, the man could not cope with the economic difficulties of his family, found his salvation in a suicide attempt that resulted in death, but before such an attempt, he did not want his wife and children to stay in these difficult living conditions and killed them as well.

The approach of the government in Turkey to such tragic events is an attempt to prevent such events from being heard in public. For this purpose, a ban on reporting to the press is imposed on most of these painful events. However, of course, the solution to the tragic situation in question lies not in hiding the problem.

In this presentation, the prevalence of these unfortunate events in the Republic of Turkey in recent years, special tragic case groups and what can be done to prevent this tragedy will be conveyed.

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**Family Massacres; Forensic Medicine; Turkey**

## J9 A Persistent Deficit of Attention: The Inadequate Treatment of Attention Deficit Hyperactivity Disorder (ADHD) in Correctional Settings

Sundeep Madireddi, MD\*, University of Southern California, Los Angeles, CA

**Learning Objective:** After attending this presentation, attendees will understand: (1) which subset of individuals with ADHD in correctional custody may benefit from treatment with medication; (2) whether restricting the use of stimulant medications in the treatment of detainees with ADHD constitutes a violation of existing law; and (3) best practices for stimulant prescribing for ADHD in correctional settings.

**Impact Statement:** Attendees of this presentation will be able to: (1) identify situations in which the medical treatment of detainees with ADHD will help their rehabilitation; and (2) safely and effectively prescribe medication targeting ADHD symptoms in this vulnerable population.

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder, characterized by persistent inattention, hyperactivity, and impulsivity.<sup>1</sup> The prevalence of ADHD in the general population is estimated to be five to ten percent of children and one to six percent of adults; most estimates suggest the prevalence of ADHD is even higher in correctional populations.<sup>2</sup> In the community, stimulant medications are the first-line treatment for ADHD. However, this is not the case in most correctional settings, where prescribing these medications carries several risks, especially with respect to diversion into the prison barter economy and misuse.

In the subset of detainees with ADHD whose uncontrolled symptoms are impairing their present functioning, the benefits of prescribing stimulants may outweigh the risks. For example, when untreated ADHD impairs the ability of detainees to take advantage of educational and vocational programming, this diminishes their rehabilitative potential. Additional functional consequences of inadequately treated ADHD include impulsivity, which may result in a loss of privileges, disciplinary action, and even additional criminal charges. For these reasons, correctional health services for one state prison system implemented a treatment protocol to ensure safe access to stimulant medications for eligible detainees.<sup>3</sup> Nonetheless, treating detainees with stimulants for ADHD remains controversial and not broadly practiced.

Correctional facility policies that prohibit stimulant prescribing without exceptions arguably violate legislation protecting individuals with disabilities from discrimination (such as the Americans with Disabilities Act) as well as constitutional guarantees.<sup>4</sup> Therefore, the implementation of stimulant-prescribing protocols in correctional health systems can serve to mitigate the risks involved while bringing treatment of ADHD in line with the standard of care. This presentation will outline best practices for prescribing medications to detainees with ADHD. Improving management of ADHD in correctional settings has the potential to reduce rates of recidivism of crimes and infractions related to impulsivity, including those that occur while in custody.

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### ADHD; Treatment; Corrections

## J10 The Eight Best Practices to Improve Forensic Psychological Assessment

Tess Neal, PhD\*, Arizona State University, Glendale, AZ; Kristy Martire, PhD\*, University of New South Wales, Sydney Australia, SydneyNSW, AUSTRALIA; Randy Otto, PhD, University of South Florida, Tampa, FL

**Learning Objective:** The content of this presentation outlines several recommendations intended to improve the current state of psychological practice in legal contexts. It is our hope that, by applying these best practices, psychologists and legal professionals may better critique, improve, and uphold high standards in the field of forensic psychological assessment: (1) describe eight best practices for the validity of forensic psychological assessments, (2) analyze the foundational and applied validity of psychological assessment tools, (3) identify how to minimize bias in assessment practices, and (4) critique one's own practices to align with and exceed standards of practice and promote improvement in the field.

**Impact Statement:** This presentation will impact the forensic science community by outlining recommendations intended to improve the current state of psychological practice in legal contexts.

We review the state of forensic mental health assessment. The field is in much better shape than in the past; however, significant problems of quality remain, with much room for improvement. We provide an overview of forensic psychology's history and discuss its possible future, with multiple audiences in mind. We distill decades of scholarship from and about fundamental basic science and forensic science, clinical and forensic psychology, and the law of expert evidence into eight best practices for the validity of a forensic psychological assessment. We argue these best practices should apply when a psychological assessment relies on the norms, values, and esteem of science to inform legal processes. The eight key considerations are: (1) foundational validity of the assessment; (2) validity of the assessment as applied; (3) management and mitigation of bias; (4) attention to quality assurance; (5) appropriate communication of data, results, and opinions; (6) explicit consideration of limitations and assumptions; (7) weighing of alternative views or disagreements; and (8) adherence with ethical obligations, professional guidelines, codes of conduct, and rules of evidence.

From the perspective of the law, psychological expertise can be valuable in numerous ways. In broad terms, forensic psychological assessments seek to measure, predict, or describe an individual's emotional, behavioral, or cognitive functioning as it affects some legal issue in dispute. Psychologists' expert opinions about relevant psycho-legal issues, and the data underpinning those opinions, are communicated to the court through reports and/or testimony to assist the trier of fact in reaching a better-informed decision. Although the practice of forensic psychology has advanced over the years, and the evidence that psychologists can present in court has improved in quality, not all psychologists rely on high-quality methods. And unfortunately, the courts have struggled to hold psychologists and other mental health professionals accountable. We draw upon scholarship from multiple domains and sources of information to identify contemporary best practices for forensic psychologists. The current state of forensic psychological assessment is, on the whole, better than in the past as the field and its scientific underpinnings have evolved. Still, significant variation in quality could be improved by the actions of both psychologists and the courts. As such, we briefly overview the history and possible trajectory of the field and offer suggestions for improvement. Specifically, we distill eight best practice principles for forensic psychological assessments to aid psychologists in bringing better evidence to court, but also to aid courts in holding psychologists accountable for the quality of their evidence.

The ideas in this paper are currently in press for publication in the *Annual Review of Law and Social Science*<sup>1</sup>.

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### Forensic Evaluation; Psychological Assessment; Best Practices

## J11 Cocaine Craving, Desperate Search for Intoxicants, Settling of Scores, and Murder

Bernat-Noël Tiffon Nonis, PsyD\*, Consultoria En Psicologia Legal Y Forense, Barcelona, Catalonia, SPAIN

**Learning Objective:** The goal of this presentation is to inform novice professionals and professionals with experience to learn the scope of aggressive, hostile, and criminological behavior that leads to the hopelessness of the search for toxic substances.

**Impact Statement:** This presentation will impact the forensic science community by describing how a clinical-symptomatic phenomenology of a state of withdrawal can lead to the scope of criminal behavior.

**Introduction:** This profile of subjects affected by dual pathology with chronic consumption of toxic substances, presents, from the juridical point of view, an incessant search for the drug (“craving”) and a pathological impulsivity (“acting out”) that supposes a partial and/or significant rupture, rather than total, of the inhibitory mechanisms of behavior. This negatively influences their cognitive abilities and, at the same time, their volitional-motivational abilities in the form of an emotional, toxic-induced outburst. It would be difficult for a legal practitioner to determine a complete defense as a modifying circumstance of criminal responsibility, solely based on the aforementioned psychopathological diagnostic categories.<sup>3-7</sup>

Impulsivity is a well-known and widely addressed phenomenon in the addiction clinic so that impulsivity prior to drug abuse increases the susceptibility to relapse after abstinence.<sup>1,2,9</sup> Likewise, compulsive behavior would be characterized by the exacerbation of the traits of lack of premeditation and lack of perseverance; as well as the tendency to commit impulsive acts under states of discomfort or stress, and it has been associated with indicators of severe consumption.<sup>8</sup> It is possible that this facet of impulsive behavior is more closely linked to neuropsychological dimensions dependent on affective feedback, for example, decision-making.<sup>10</sup> This could explain the possible pernicious influence to develop disorganized behaviors from the harmful influence of a pattern of use, abuse, and acute consumption of toxic substances.

The present case illustrates how cocaine craving and, therefore, the incessant search for the intoxicant by the perpetrators can reach the behavioral magnitude of criminal and delinquent behavior from the origin of potentially compulsive-aggressive and criminological behaviors (and in a vindictive way “settling the score”) by developing disorganized and altered behaviors.

**The case:** According to the wording of the sentence, and being by unanimity of the jury, it was considered as proved that the pair of defendants (male and female, the latter being the one who maintained a sentimental relationship with the victim) went to the victim’s home. Once inside the house, and for unknown reasons, an argument began in the course of which both perpetrators in an agreement to end the victim’s life, tied his hands and feet in order to ensure the success of their action, grabbed him by the neck with the intention of strangling him, and hit him with great force on the head, causing a craniocerebral traumatism, and left the house leaving him moribund. Because of the very serious injuries to the skull and, subsequently, to the brain parenchyma caused by the blows, the victim died in the hospital. At the trial and before the beginning of the presentation of evidence, the defendants acknowledged their participation in the criminal acts and that they shared interests in multiple drug use (toxico-philia).

In essence, the case is contextualized in the context of cocaine consumption and the incessant search (“craving”) for this substance by the perpetrators, among other drugs. Whatever the motive for the discussion between the actors in the criminal scenario, the perpetrators felt frustrated at not having obtained the toxic substance they craved so much and decided to subdue the victim so that later, out of revenge, they could take his life (“settling the score”).

Next, images of the crime scene and the victim’s autopsy are presented to illustrate the potentially hostile and aggressive behavioral consequences of subjects with cocaine craving. In this case, the perpetrators chose to engage in vindictive “score-settling” behavior of criminal magnitude.

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### Murder Drug-Dependent Victimizer; Cocaine Craving; Disorganized Criminal Behavior

## J12 A Cocaine-Induced Psychosis, Frustrated Suicide With a Firearm, and Subsequent Conviction for Attempted Homicide

Bernat-Noël Tiffon Nonis, PsyD\*, Consultoria En Psicologia Legal Y Forense, Barcelona, Catalonia, SPAIN

**Learning Objective:** This presentation is for novice professionals and experienced professionals who will learn the scope of aggressive, hostile, and criminological behavior that leads to the despair of a subject with suicidal behavior.

**Impact Statement:** This presentation will impact the forensic science community by describing how the clinical-symptomatic phenomenology of a subject with suicidal behavior can lead, paradoxically, to a behavioral state of heteroaggressiveness.

**Introduction:** Major depressive disorders of severe intensity are the psycho-pathological clinical entities that have a greater probability of leading to suicide. In the development of this type of suicide-related thoughts, there is the pernicious influence of a lack of hope. Thus, hopelessness or negative expectations about the future are possibly the most important predictors of suicide risk in depressive disorders that present suicidal contemplations.<sup>1-6</sup>

The subject, a 42-year-old man, had recently been released from his last prison term. He felt lost and disoriented in life and decided to commit suicide "so as not to be another hindrance in the life of my family." He said that he had planned everything to commit suicide: he had acquired a pistol and bought about 100 grams of cocaine. During the three days prior to his suicide (frustrated), he consumed about 70 grams of cocaine (20-25g/day). He wanted to travel to a mountain near the town where he lived to commit suicide, but for this he had to travel by motorcycle.

Throughout the interview, the subject said that "it was very clear to me I had to shoot myself in the heart, since in prison they always told me that if I put the gun to my head, the bullet might just graze me, and I might not die. After three days ingesting 70-75 grams of cocaine, I was high and totally paranoid. So, I got on my motorcycle and headed up the mountain. A car was repeatedly approaching me at each red light, and it seemed to me that it was chasing me. I noticed how the occupants of the car continually laughed at me and spoke in another language. I remember losing patience. I took the pistol and fired directly and indiscriminately at the wheels of the car. I put the gun inside my jacket and started the bike again to go straight to the mountain. With my nerves, I lost my balance and fell off the bike a few meters from them. I crashed my motorcycle and due to the fall, I broke my femur in an open fracture. The bone was sticking out of my flesh, and I was in a lot of pain. The gun was thrown 3 or 4 meters from me and I crawled up to it. I got up as best I could but fell in pain as the open fracture prevented me from standing up. On seeing that I could not continue to my destination, I decided to do it right there, on the street. I crawled to where the gun was, touched my chest to make sure where my heart was, put the barrel against it and pulled the trigger, firing at myself. After four or five days, and to my regret, I woke up in the hospital, surrounded by police officers. I did not want to do any harm to anyone, and everything had gone wrong. Now, the Public Prosecutor accuses me of four frustrated attempts of homicide of the four occupants of the car I shot."

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### Toxic Psychosis Cocaine; Frustrated Suicide; Homicide Attempt

## J13 Forensic Psychiatric Implications of the Psychedelic Renaissance

Brian Holoyda, MD, MPH\*, Martinez Detention Facility, Portland, OR

**Learning Objective:** In this presentation, attendees will learn about the history of psychiatric research on psychedelics, current experimental applications of psychedelic treatments in psychiatric conditions, studies assessing the risk of violence associated with psychedelic use, and practical considerations in the forensic assessment of psychedelic users.

**Impact Statement:** This presentation will impact the forensic science community by summarizing available research on an emerging, experimental treatment in the field of psychiatry and delineating useful recommendations for forensic psychiatrists who evaluate psychedelic users.

Psychedelics are a unique category of psychoactive substances. Their effects are varied and can include alteration of sensory perception, depersonalization, and transcendent experiences resulting from completely altered consciousness. Various investigators studied the therapeutic potential of LSD and other psychedelic compounds on conditions including addiction and depression until the 1960s. The federal government's categorization of LSD as a Schedule I substance under the Controlled Substances Act of 1970 caused clinical research on psychedelics to come to a standstill. Today, however, psychedelics are again seeing their day in clinical research trials for conditions including addiction and terminal illness-related anxiety with resultant widespread public attention. In May of 2019, Denver, CO, decriminalized the possession of psilocybin-containing mushrooms. Since that time an increasing number of jurisdictions in the United States have followed suit.

There has been a comparative silence regarding the forensic implications of the psychedelic renaissance. Questions regarding psychedelic use and interpersonal violence and other forms of criminal behavior remain largely unanswered, though new research is beginning to better elucidate the relationship. With increasing public interest in and decriminalization of psychedelics, psychiatrists may be increasingly likely to encounter subjects with a history of use and will require a framework to assess the use of this unique category of compounds.

A search of the PubMed® and PsychINFO® databases to identify peer-reviewed literature assessing the relationship between psychedelic use, violence, and other criminal behavior yielded 11 case reports and numerous studies of varying quality. Early research spearheaded by Timothy Leary in the 1960s sought to determine if psychedelic use reduced criminal recidivism of prisoners from Massachusetts; later re-analysis of his work demonstrated no effect. Around the same time, case reports in the medical literature suggested that psychedelic use may result in homicide and other violent behavior. More recently, large-scale epidemiologic studies indicate the opposite, however, that a history of psychedelic use may reduce one's risk of violent and other criminal behavior.<sup>2</sup>

In the absence of a clear relationship between psychedelic use and negative legal and behavioral outcomes, the forensic evaluator should understand the unique characteristics of psychedelics and how to assess evaluatees' use based on "set" and "setting."<sup>1</sup> In addition, there are practical concerns related to assessing subjects' psychedelic use in the context of competency and criminal responsibility evaluations that forensic evaluators should know. While research on these substances and their effects continues to grow, thoroughness and caution should guide the evaluator.

This presentation will review the history of psychedelic research in the United States, including early efforts to understand the role that psychedelics may play in criminal behavior and recidivism. It will summarize the current body of literature assessing psychedelics' potential benefit in the treatment of various psychiatric maladies. It will evaluate the relationship between psychedelic use, violence, and other criminal behavior. Lastly, it will provide forensic psychiatrists a framework for evaluating psychedelic users in forensic contexts.

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### Psychedelics; Violence Risk Assessment; Forensic Psychiatric Evaluation



## J14 Internet Gaming Disorder: A Sine Materia Intoxication That Can Make You Not Guilty

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**Learning Objective:** This presentation aims to debate if a new form of addiction recognized by the scientific community in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*, the Internet Gaming Disorder (IGD), impairs the subject's capacity of self-control and the forensic consequences.

**Impact Statement:** The presentation will impact the forensic science community by presenting the approach in framing the imputability in patients suffering from gaming addiction, comparing it from a neuro-biological point of view to other forms of addiction.

Internet Gaming Disorder (IGD) is a pattern of potential non-substance addictive behaviors, mentioned for the first time by the psychiatrist Yvan Goldberg as "internet addiction." According to the *International Classification of Disease, 11<sup>th</sup> edition, Clinical Modification (ICD-11)* and *DSM-5*, IGD is related to a pattern of behaviors such as the need to play, over-use, abstinence, addiction, and negative effects on individuals' health and social interactions.<sup>1</sup>

Clinical research shows that IGD is associated with cognitive impairments and neurofunctional alterations.<sup>2-6</sup> More specifically, it is related to impairments in executive function, attention, memory, decision-making, or learning disabilities. Functional magnetic resonance imaging (fMRI) investigates mechanisms of these altered cognitive systems, showing that IGD stimuli induce an enhanced activation of frontolimbic and mesocorticolimbic reward systems. fMRI demonstrates decreased gray matter in people with IGD relative to controls in all the brain areas involved in cognitive control, error processing, decision-making, and reward, such as the inferior frontal lobe, insula, or amygdala. These are the same brain areas involved in craving in other addictions. Therefore, clinical research demonstrates that gamers don't pay attention to negative long-term consequences and that the gamers' brain impulse management is also comparable with the one displayed by individuals with alcohol use disorder. Considering this scientific premise, the question remains as to how this status does and should affect the liability for criminal behavior related to addiction.

Within the forensic area, we must understand if subjects affected by IGD can exert choice about antisocial behaviors related to addiction. If IGD is a psychopathology based on irrational and impulsive behaviors, alterations in executive functioning, and deficits in self-regulation ability such as in other kinds of addictions (e.g., alcohol and substances), subjects affected by IGD make constrained choices to satisfy an allegedly overpowering desire. If so, there is the possibility that their capability to be juridically liable for their own actions is reduced and a careful evaluation on a case-by-case basis is consequently needed to assess a lack of capacity for self-control ("will power").

### References:

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### Gaming; Intoxication; Imputability



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## J15 Forensic Pathology for Forensic Psychiatrists

*Anita Rajkumar, MD\*, Livingston, NJ*

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**Learning Objective:** This presentation will educate forensic psychiatrists on the basics of forensic pathology reports.

**Impact Statement:** This presentation will impact the forensic science community by improving forensic psychiatrists' skills and abilities to understand and analyze pathology evidence.

Forensic pathology is the subspecialty that focuses on determining the cause of death by examining a corpse. A postmortem examination is performed by a medical examiner or forensic pathologist, usually during the investigation of criminal cases, but also in civil cases in some jurisdictions. The autopsy also provides an opportunity for other issues raised by the death to be addressed, such as the collection of trace evidence or determining the identity of the deceased. Autopsies are performed when an unexpected death occurs, when someone dies while not under the care of a physician, to solve criminal cases, when a mass disaster occurs and requires the identification of the victims, and upon request by the family or loved ones of the deceased. Typically, autopsies can cost anywhere from \$3,000 to \$5,000; however, the price can vary from country to country. The forensic pathologist examines and documents wounds and injuries, along with the possible cause of those injuries, at autopsy, at the scene of a crime, and occasionally in a clinical setting, such as rape investigation or deaths in custody. Forensic pathologists collect and examine tissue specimens under the microscope (histology) to identify the presence or absence of natural disease and other microscopic findings such as asbestos bodies in the lungs or gunpowder particles around a gunshot wound. They collect and interpret toxicological specimens of body tissues and fluids to determine the chemical cause of accidental overdoses or deliberate poisonings. Forensic pathologists work closely with the medicolegal authority for the area concerned with the investigation of sudden and unexpected deaths: the coroner (England and Wales), procurator fiscal (Scotland), or coroner or medical examiner (United States).

The outline of the presentation is as follows: autopsy report structure and terminology; external examination; a general description of the decedent, including scars, tattoos, and unique natural anomalies (remote absence of a foot, etc.); injuries (blunt force, gunshot wounds, sharp force, strangulation, etc.); internal examination; and ancillary studies.

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### Forensic Pathology; Autopsy; Cause and Manner of Death

## **J16 A Case of Münchausen Syndrome By Proxy: The Role of Legal Medicine in Child Abuse Management in Emilia-Romagna**

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**Learning Objective:** After attending this presentation, attendees will understand the key role of Legal Medicine and the importance of multidisciplinary teamwork in a rare case of Münchausen Syndrome by Proxy, approached according to the 2014 Emilia-Romagna recommendations about suspected child abuse/mistreatment.

**Impact Statement:** This presentation will impact the forensic science community by proposing a model for child abuse/mistreatment management. Moreover, this report could be meant as a starting point for more specific protocols in complex cases of Münchausen Syndrome by Proxy.

The purpose of this presentation is to show the key role of Legal Medicine in hospital multidisciplinary teamwork in a rare case of Münchausen Syndrome by Proxy (MSBP) and to highlight the importance of specific guidelines regarding the investigation of suspected child abuse/mistreatment.

According to research literature, MSBP, or Factitious Disorder Imposed on Another (FDIA), is considered a form of child abuse in which a caretaker, usually the mother, feigns or induces symptoms in a child that result in unnecessary medical testing and procedures. This maltreatment occurs in approximately 0.5 to 2.0 per 100,000 children younger than 16 years old, with a mortality rate ranging from 6% to 35%. It is estimated that MSBP is significantly underdiagnosed all over the world due to the variety of symptoms (real or fabricated) the children could show and because the illness is the outworld manifestation of an adult psychiatric disorder.

In November 2018, a 27-year-old woman was admitted to the Emergency Department of the University Hospital of the city of Ferrara, in northern Italy, with her 2-month-old daughter, describing a history of blood streaks in the child's diapers. The woman, a teacher, gravida-2 para-2, obese (BMI=40), gave birth to the baby in the same hospital; during the pregnancy, gestational diabetes was diagnosed at month 6. The pregnancy had a physiological course with uncomplicated delivery.

During observation, the 2-month-old child looked healthy, and no evident urogenital lesions or other causes of bleeding were found. However, some of the diapers' spots showed "weird" shapes and colors and the mother referred seizures and appetite loss, never observed by the hospital personnel. Since child abuse could not be excluded, pediatricians alerted the Hospital Legal Medicine Service, in order to reassess the case and then notified the Judicial Authority.

Forensic genetic analyses of the suspected bloodspots allowed the identification of a hematic origin for a group of stains. DNA analysis (Short Tandem Repeat; STR) revealed the mother's genetic profile. Questioning the mother, she confirmed their suspicions, and, consequently, it was possible to reach the diagnosis of Münchausen Syndrome by Proxy. Therefore, the Judicial Authority activated the social service department: the baby was entrusted to another family and the mother started psychological therapy.

According to 2014 regional Emilia-Romagna recommendations about child abuse/mistreatment management, a fast resolution of this case was only possible thanks to the interdisciplinary approach between clinicians and legal authorities. These recommendations define the role of Legal Medicine Service in hospitals as a bridge between the health care system and law. The medical examiner consultancy is crucial not only for proving/excluding child abuse/mistreatment but also for ensuring medical records to the trial court. As reported in the recommendations, when child abuse/mistreatment cannot be excluded, the pivotal point is represented by a proper and early clinical and forensic assessment, divided into four steps: (1) triage anamnesis; (2) clinical and instrumental examination; (3) documentation of the relevant details in a medical report; and (4) providing clinical and psychological support (victim/offender). Additionally, the application of these recommendations has strong implications for forensic and clinical practice: to prevent invasive clinical tests and encourage information sharing between health personnel and parents. More specific protocols could be useful in complex cases of MSBP.

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### **Münchausen Syndrome by Proxy; Legal Medicine Recommendations; Hospital Legal Medicine Service**

## J17 Homicide-Suicide in Older Couples

*Laura Renard, MD\*, CHU Angers, Angers, Pays de la Loire, FRANCE; Nathalie Jousset, MD, Hospital of Angers, Angers, Centre, FRANCE*

**Learning Objective:** The goal of this presentation is to highlight the different characteristics of homicide-suicide, notably among elderly couples, and to emphasize the particularity of the suicidal mechanism used in these cases and the underlying psycho-social context.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the importance of screening for depression in the elderly and not neglecting suicidal actions.

Homicide-suicide (HS) has traditionally been defined as homicide followed by the suicide of the perpetrator. Different types exist. In the family context, the most common forms are the so-called spousal HS with “altruistic” motives, also known as uxoricide (designating a wife’s murder by her spouse). Such cases often occur in the context of depression, chronic alcoholism, domestic violence and/or jealousy, with suspicions of adultery, and/or a desire of the woman to separate from her husband. It represents 20% of all homicides (i.e., approximately 170 deaths per year in France in 2019). Spousal HS with “mercy” motives often concerns an elderly couple (the perpetrator is 55 years old or older), with at least one of the spouses suffering from a serious medical or physical condition. The symptoms reported are often associated with chronic pain, rheumatology, or cancer, which become difficult to bear (and for which patients do not always receive sufficient care). The conditions may also be psychological, requiring even more specific medical care (hospitalization). The other member of the couple (“in good health”) is likely to become the primary caregiver. It is worth mentioning that, unlike “altruistic” spousal HS, the couples in the “mercy” case are not in conflict. The act of homicide is therefore conceived as some form of deliverance, lessening the other’s sufferings, a form of “homicide by compassion,” quickly followed by suicide, so that the couple leave “together” with the possibility of meeting again or obtaining rest in the afterlife. This act often takes the form of a “suicide pact”. Evidence suggests that the perpetrator is often a man experiencing depression. According to the epidemiological data reviewed, the incidence of all HS in France is estimated at 0.2 to 1.5 per 100,000 inhabitants per year; in England, at 0.05 per 100,000, or 23 cases per year; and in the United States, at 0.27 to 0.38 per 100,000, approximately 1,000 to 1,500 deaths per year.

Spousal homicide-suicide refers to the discovery of two bodies in the same location and, in most cases, where a wife is killed by her husband. It involves a particularly violent crime scene. Among the objectives of the forensic analysis, the estimation of the chronology of the events leading to the suicide and the determination of the causes of deaths, which appear as more or less self-evident at first glance, sometimes require an autopsy, if this is the direction that the investigators choose to follow. In this presentation, we describe the homicide-suicide of an elderly couple by mechanical asphyxia. A postmortem examination was carried out on site and the intervention of a third party was formally ruled out.

### Homicide-Suicide; Older Couple; Asphyxia

## J18 A Multidisciplinary Approach in a Sexual Homicide Scene

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**Learning Objective:** After attending this presentation, attendees will better understand the importance of a multidisciplinary approach in crime scene investigations to successfully disguise the true nature of the events in sexual crime scene.<sup>1</sup>

**Impact Statement:** This presentation will impact the forensic science community by showing that the interpretation of findings from external examination and autopsy should always be considered with considerable caution, especially in psychopathological and criminological field.

A 76-year-old man, obese, with a diagnosis of “psychotic disorder of the paranoid type, with depressive episodes, social maladjustment and sexual identity disorder,” was found dead in the bedroom of his apartment. The body was completely naked, except for a scarf around his neck, and lying in a supine position on his bed.

On external examination, three scalp lacerations on the right parietal side (4cm in length), on the occipital side (3cm in length), and skin abrasions on the chin were found. The cause of death was related to the combined effects of positional asphyxia (smothering with a pillow) and blunt head injury. At the death scene, women’s clothes were found next to the body. In the bedroom, a coffin lid was leaning against the wall, with a nude male porn star poster attached on it. There was no sign of forced entry or burglary in the house; the deceased’s wallet was in the house. A pillow with bloodstains was found beneath the victim’s head. Forensic investigators collected several DNA profiles from the coffin, which the victim used for his sexual intercourse fantasies of killing his partners. Surveillance cameras showed a young man entering the victim’s apartment before his death, eventually identified as a 28-year-old drug addict and prostitute with a criminal record. Four days later, police arrested the man while he was trying to escape with the victim’s laptop and phone. He was charged with murder. The prosecutor assumed that the killer lured the victim on dating apps, and killed him during an attempted robbery. The killer confessed the homicide, but he claimed self-defense. He had gone for a massage, but the old man sexually assaulted him. He hit him on the head with a bottle and the victim fell onto the bed with his face pushed on the pillow, dying shortly after by smothering. Obesity and pre-existing pulmonary diseases had contributed to the death from asphyxia.

The findings from the crime scene investigation and the criminological evaluation permitted his murder charge to be reduced to manslaughter during the criminal trial. The in-depth behavioral analysis of the relationship between the victim (a psychotic patient with a perverse and destructive sexuality) and the murderer (suffering from a personality disorder with antisociality and sexual promiscuity) played key roles in influencing sentencing law changes.

### Reference:

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### Crime Scene Investigations; Homicide; Paraphilia

## J19 Citation Analysis of Misinformation Regarding Parental Alienation Theory

*William Bernet, MD\*, Vanderbilt University School of Medicine, Brentwood, TN; Shenmeng Xu, PhD, Vanderbilt University, Nashville, TN*

**Learning Objective:** After attending this presentation, attendees will be able to explain how specific misinformation regarding parental alienation theory occurred repeatedly in professional journals and presentations over many years. Also, attendees will be able to describe the use of citation analysis—a type of bibliometric research—to display the flow of (mis)information through many publications and presentations over many years.

**Impact Statement:** This presentation will impact the forensic science community because it explains how errors in family law—affecting many children and families—may occur because of widespread published misinformation.

Misinformation regarding parental alienation (PA) frequently occurs in journals, books, and presentations. The purpose of this study was to identify recurrent publication of the same misinformation (i.e., variations of the statement: “Parental alienation theory assumes that the favored parent has caused parental alienation in the child simply because the child refuses to have a relationship with the rejected parent, without identifying or proving alienating behaviors by the favored parent.”) This is a false statement regarding PA theory. The recurrent misinformation reported here is not trivial; these statements are significant misrepresentations of PA theory. Instead, PA theory states that PA is only one of several possible causes of contact refusal (i.e., not all children who refuse contact with Parent B were influenced by Parent A to reject a relationship with Parent B).

The authors searched for examples of misinformation in published papers, conference presentations, book chapters, government documents, and legal briefs. The search involved reviewing citations among relevant documents. These documents were identified through Google® Scholar and Web of Sciences®. Eighty-seven examples of the same misinformation were published or presented between 1994 and 2022. The publications and presentations were subjected to citation analysis using Gephi software, which exhibits the links between citing material and cited material. Thus, the genealogy of this recurrent misinformation is clearly portrayed visually. Citation analysis is a useful way to illustrate the relationships among a large number of published papers and presentations.

This recurrent publication of the same misinformation suggests a systemic flaw has occurred among the authors, peer reviewers, editors, and publishers of mental health and legal literature. There are plausible explanations for this trail of misinformation: pertaining to the psychological mindset of the authors (i.e., confirmation bias); and pertaining to their writing skills (i.e., persistent use of secondary sources for their information). There are important implications regarding family law in the United States and other countries. Since many examples of this false information occurred in legal journals and briefs, it seems inevitable that these erroneous beliefs will percolate into trial and appellate cases, thus creating flawed judicial decisions that may harm many children and families.

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### Parental Alienation Theory; Misinformation; Retraction

## **J20 Parental Alienation Relational Problems and the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR)**

*William Bernet, MD\*, Vanderbilt University School of Medicine, Brentwood, TN*

**Learning Objective:** After attending this presentation, attendees will be able to explain the standards for adding a relational problem to the chapter of *DSM-5-TR* called “Other Conditions That May Be a Focus of Clinical Attention.” Also, attendees will understand the criteria for the diagnosis of parental alienation relational problem and will be aware of the extensive qualitative and quantitative published research regarding parental alienation.

**Impact Statement:** This presentation will impact the forensic science community by providing key information regarding the possibility of including a new diagnosis—parental alienation relational problem—in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision*.

The purpose of this research project was to determine whether there is sufficient research to include parental alienation relational problem (PARP) in the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR)*. The following steps were followed: (1) collect criteria for adding a new relational problem to *DSM*; (2) collect qualitative and quantitative research published regarding PARP; and (3) compare (1) and (2) to see if PARP meets criteria for a new relational problem in *DSM*.

PARP is a serious mental condition that sometimes occurs when a child’s parents are engaged in a high-conflict separation or divorce. The proposed diagnosis for PARP has the following definition: his category may be used when a child—whose parents are engaged in a high-conflict separation or divorce—allies strongly with one parent and rejects a relationship with the other parent without a good reason. The diagnosis of parental alienation relational problem usually requires five criteria: (1) the child actively avoids, resists, or refuses a relationship with a parent; (2) the presence of a prior positive relationship between the child and the now-rejected parent; (3) the absence of abuse or neglect or seriously deficient parenting on the part of the now-rejected parent; (4) the use of multiple alienating behaviors by the favored parent; and (5) the manifestation of behavioral signs of alienation by the child.

An extensive review of pertinent literature found that journal articles regarding PARP have appeared in the professional literature in more than 50 countries. Most of these articles report qualitative research, such as descriptions of PARP in various societies and cultures. However, many articles also reported quantitative research, which pertained to standardized definitions and diagnostic criteria for this mental condition, and to the reliability and validity of the components of this condition. The validity of Factors 1, 2, and 3 are untestable; they comprise the definition of PARP. The validity of Factors 4 and 5 was established in numerous studies. It has been estimated that about 35% of parents in the United States reported being targets of parental alienating behaviors; about 0.5% of children in the United States experience PARP.

It was noted that several *DSM-5-TR* diagnoses may be confused with PARP in some circumstances. These conditions can be distinguished in a careful evaluation: child affected by parental relationship distress; parent–child relational problem; child psychological abuse; and delusional symptoms in the context of relationship with an individual with prominent delusions. It was concluded that the qualitative and quantitative research regarding PARP fulfills the criteria for this mental condition to be added to *DSM-5-TR*.

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### **Parental Alienation Relational Problem; *DSM-5-TR*; Diagnosis**

## J21 Temporary Psychopathy in Teen Accomplices

*Katherine Ramsland, PhD, MA\*, DeSales University, Hellertown, PA*

**Learning Objective:** After attending this presentation, participants will better understand how predators can induce defensive strategies in adolescent accomplices that temporarily suppress prosocial behavior.

**Impact Statement:** This presentation illustrates to the legal and clinical communities the benefit of understanding the vulnerability of malleable adolescent personalities to predators who recruit them as partners.

Some youthful offenders who were lured onto murder teams have wondered, post-arrest, how they could have committed certain acts. Afterward, they barely recognized the heartless person they became. To comply with the ego-dystonic criminal behavior, they had adopted strategies that suppressed prosocial emotions. Freed from the predator's influence, some have recovered their former moral frame.

Few researchers have focused on the nuances of a reluctant partner's psychological devolution. It involves a vulnerable personality, cognitive reframing, and incentive manipulation. For the reluctant teen offender, the shift into psychopathic acts can be temporary. Thus, rehabilitation efforts can use the provisional frame to support their hope to recover and positively evolve.

Brent Koster was 15 when he helped serial killer Danny Ranes rape and murder three young women. In prison, he improved himself via rehab programs and earned a law degree. He told a parole board, "If I had not met Danny Ranes, I know in my heart that I would have never become involved in crimes like this."

"Beltway Sniper" Lee Boyd Malvo was recruited and trained by a dominant criminal role model, John Muhammad, who exploited Malvo's need for a stable parent. Under Muhammad's influence, Malvo participated in nearly a dozen fatal shootings when he was 17. He recalls his blind trust in Muhammad. "Whatever he was or was not, he was consistent ... I absorbed his personality ... I was nothing without him." In prison, Malvo has made progress in understanding how Muhammad restructured his values. With support, Malvo has reclaimed his former sense of self.

Elmer Wayne Henley, Jr., was a Bible-reading 15-year-old who hoped to make some money. Dean Corll, 31, baited him with lies and leveraged him into committing multiple murders of other boys. Henley complied for a year and a half until he ended Corll's control by killing him. He has described the experience as having two competing selves during his killing period, with the good one finally winning. He hopes to understand how Corll's grooming had breached his sense of self.

This is the early part of an exploratory qualitative study that applies neuroscience to the process of a groomed teen's compliance. Adolescence is a time of exploring identity, aided by a reward-responsive, malleable brain. Predators who seek partners gradually replace the teen's belief system with one that supports callous acts, luring them into crimes they would not initiate on their own. With the right incentive, they adapt to deviance. Yet this "learned psychopathy" in a reluctant accomplice lasts only as long as the predator's influence. That is, the person who acts in a psychopathic manner under provisional conditions might not be a psychopath, because under circumstances without the predator, their prosocial inclinations may resurface.

This defensive maneuver does not absolve criminal responsibility, but the concept of provisional psychopathy can help recovering accomplices and their counselors with future behavioral improvement.

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### Psychopathy; Adolescent Brain; Serial Killer



## J22 The Pathological Impulsivity of a Homicidal Juvenile With Severe Attention Deficit Hyperactivity Disorder (ADHD) and Borderline Intellectual Function

Bernat-Noël Tiffon Nonis, PsyD\*, Consultoria En Psicologia Legal Y Forense, Barcelona, Catalonia, SPAIN

**Learning Objective:** The goal of this presentation is to show attendees how to diagnose an aggressive and hostile type of Attention Deficit Hyperactivity Disorder.

**Impact Statement:** This presentation will impact the forensic science community by describing how Attention Deficit Hyperactivity Disorder can manifest a serious symptomatology of burdensome scope to reach the magnitude of criminal behavior.

**Introduction:** Attention Deficit Hyperactivity Disorder (ADHD) is one of the most common neurodevelopmental disorders in children and adolescents. The core symptoms are inattention and hyperactivity/impulsivity, and it is frequently accompanied by learning difficulties, low self-esteem, anxiety, and behavioral problems.

In general terms, in blood crimes perpetrated by subjects with pathological impulsivity, there are no conditions for an attenuation of the sentence, unless it is accompanied by some other type of serious mental disorder.<sup>9</sup> From the juridical point of view, pathological impulsivity implies a partial or significant, but not total, breakdown of the inhibitory mechanisms of behavior, negatively influencing cognitive capacities and, at the same time, volitional-motivational capacities.<sup>10</sup>

About ADHD, made some classifications considering the existence of an aggressive subtype ADHD and an anxious subtype ADHD.<sup>1</sup> Thus, and following the studies, the data suggest that people with an ADD that is co-morbid with a dissocial disorder tend to have a lower IQ, present greater deficits in reading/learning, evidence of neuropsychological alterations, and high levels of familial susceptibility.<sup>2</sup> Additionally, depending on the social situations, “some ADHD subjects act with a peculiar aloofness, giving little evidence of interest or emotional connection to those around them; others relate very awkwardly, feeling strange as they become intimate. Often, these children and adolescents impress both peers and adults as being ‘strangers’ because, surprisingly, they seem to be distant from even the most basic social expectations.”<sup>1</sup>

Emotional expression in these children, adolescents, and adults with atypical characteristics “may be very different from that of most other people, including others with ADD. They may react to seemingly minor frustrations with sudden and sustained ‘catastrophic’ emotions (e.g., inconsolable or violent crying, threatening rage). In others in this group, emotional expression is in principle absent; even in situations where intense pleasure, sadness, or anger might be expected, the individual may simply withdraw or appear totally unaffected.”<sup>1</sup>

The uniqueness of the casuistry lies not only in the criminological impact of the murder itself, but it also pivots on the mental state of the perpetrator and for which (condemnatory) sentence—based on the Mental Health professionals of the public sector, as well as the Forensic Doctors and other circumstances surrounding the casuistry—do not appreciate the modifying circumstance of criminal responsibility (MCCR) based on the existence of psychic alteration.<sup>3-7</sup>

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**Attention Deficit Hyperactivity Disorder (ADHD); Pathological Impulsivity; Homicidal Intentionality**

## J23 Insight: The Missing Link

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**Learning Objective:** Insight is a critical part of the mental status examination. When we do evaluations for the courts, be they civil or criminal, we are required to assess insight as part of the process. Many of the statutes and much of the case law relevant to psychiatric or psychological expert opinions allude to the insight of the evaluatee. Yet in our formal training, this key concept is often brushed over. The link between an individual's insight and his or her capacity in any form is a crucial component in drawing expert opinions. In this presentation, we will explore the way various courts have indirectly referred to this issue, the recent literature on the concept of insight in mental health, and our ethical responsibility to fully consider insight (often lumped together with judgment) when we provide our expert opinions to the courts.

**Impact Statement:** This presentation will impact the forensic science community by elucidating what is probably the most important part of the mental status examination and the psychiatric/psychological expert opinion, yet is mostly ignored both by evaluators and the courts. We can only perform our work responsibly and ethically by bringing our specialized knowledge and abilities to the table. Only when we understand what truly differentiates the mind of the mentally ill from the intact mind can we fully and appropriately assess an individual's capacity, criminal responsibility, testamentary capacity, or any of the myriad of psychiatric-legal questions that confront us in doing this work. By clarifying, understanding, and applying our ability of assessing insight to legal matters, we will greatly increase our usefulness to the legal arena and to the individuals we assess.

Insight is probably the most important mental ability that experts assess as part of any forensic psychiatric or psychological evaluation. Probably the best definition I have found is from Markova, who states, in part: Insight can be defined not only in terms of people's understanding of their illness, but also in terms of understanding how the illness affects individuals' interactions with the world.<sup>1</sup> The term "insight" encompasses a complex concept that should not be considered as an isolated symptom that is present or absent. Instead, it may be more appropriate to think of insight as a continuum of thinking and feeling, affected by numerous internal and external variables. Different psychiatric disorders involve different mechanisms in the process of impairment of insight; this may influence the ways in which insight should be assessed in clinical practice.

In my own book (2016) I write: Assessment of insight is crucial for considering competence and capacity for all legal issues, even when competence and capacity are not actually being considered.<sup>2</sup>

The concept of insight remains fuzzy and is frequently ignored in mental health education.<sup>3</sup> There is no single accepted definition of insight and little research has been done on the subject. However, virtually every legal application of psychiatry requires an assessment of insight. Because the concept is hazy and not well understood or explained in the literature or in psychiatric and even in forensic psychiatric training, many experts are hesitant to even address this critical concept in their evaluations. Yet, without our specialized knowledge and our ability to discern an individual's rational and factual understanding of the legal situation, we cannot possibly provide an adequate assessment.

In this presentation, I will explore the traditional way the concept of insight is taught and understood. I will then review the literature to examine how various authors have interpreted this idea. Next, I will look at some of the common psychiatric-legal questions that we face daily in our work as psychiatric experts and determine where and how the concept of insight is addressed in the statutes and case law. I will then continue on to identify the often very subtle way in which the requirement to assess insight is hidden in many of these statutes and case law. I will review some recent cases and see how various experts addressed or ignored this critical issue, and how the courts demonstrated their understanding of capacity with or without specifically considering insight. Group participation will be encouraged, and attendees will be invited to share cases and questions from their own work.

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### Insight; Capacity; Expert Witness

## J24 $\Delta$ 8-Tetrahydrocannabinol ( $\Delta$ 8-THC) Is Equipotent to $\Delta$ 9-Tetrahydrocannabinol ( $\Delta$ 9-THC) in a Mouse Model of Cannabis-Like Intoxication

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**Learning Objective:** Attendees will learn about the standardized behavioral assay used to evaluate cannabinoid compounds in rodent models. Additionally, this presentation will highlight the utility of the model to assess novel cannabinoids and determine their potency compared to well-established compounds such as Delta-9-THC. Last, the attendees will learn of our assessment of Delta-8-THC vs. Delta-9-THC and how it contradicts anecdotal reports of the potency of Delta-8-THC.

**Impact Statement:** The presentation will provide translational relevance and information on the evaluation of novel drugs of abuse that are emergent in the forensic and commercial fields, and their behavioral effects in whole organisms.

Emergent use of delta-8-tetrahydrocannabinol ( $\Delta$ 8-THC) has been of concern following the widespread availability of this compound “over the counter,” which is structurally and functionally similar to delta-9-tetrahydrocannabinol ( $\Delta$ 9-THC), the primary intoxicating constituent of cannabis. While both compounds produce effects consistent with cannabis intoxication, anecdotal reports claim that  $\Delta$ 8-THC is less potent, or “mild,” compared to the naturally occurring  $\Delta$ 9-THC.

In the present study, we assessed whether  $\Delta$ 8-THC and  $\Delta$ 9-THC differ in potency in a mouse model highly sensitive to the *in vivo* pharmacological (cannabimimetic) effects of cannabis. Cumulative dose-response curves were evaluated for  $\Delta$ 8-THC and  $\Delta$ 9-THC in eliciting the following three dependent measures: (1) catalepsy (bar test)—front paws were placed on a bar with hind paws on ground and time immobile is recorded; (2) antinociception—tail was submerged in 52°C water bath and time to withdraw the tail from the water is recorded; and (3) hypothermia—body temperature was recorded from rectal thermometer. Sixty minutes following the final dose of the drug, the mice were euthanized, and blood was collected. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) was used to quantitate the parent compounds and their respective 11-OH- and -COOH metabolites. The respective ED<sub>50</sub> (95% confidence limits) for  $\Delta$ 8-THC and  $\Delta$ 9-THC for each measure were: catalepsy (37mg/kg (20-69) vs. 39mg/kg (18-80)); antinociception (88mg/kg (32-239) vs. 48mg/kg (26-90)); and hypothermia (19mg/kg (14-26) vs. (18mg/kg (14-23)). The potency ratio between the two compounds did not differ for each measure. The blood concentrations for  $\Delta$ 8-THC (10,699ng/mL  $\pm$  5,953) and  $\Delta$ 9-THC (16,756ng/mL  $\pm$  10,271) did not significantly differ ( $p = 0.62$ ). Additionally, similar concentrations were found for the 11-OH (161ng/mL  $\pm$  23 vs. 108ng/mL  $\pm$  160) and -COOH (129ng/mL  $\pm$  32 vs. 178ng/mL  $\pm$  81) metabolites of  $\Delta$ 8-THC and  $\Delta$ 9-THC. These results indicate that contrary to the anecdotal claims,  $\Delta$ 8-THC possesses similar potency as  $\Delta$ 9-THC in eliciting cannabimimetic effects. Likewise, in 1993, it was also reported that  $\Delta$ 8-THC and  $\Delta$ 9-THC have approximately the same potency in measures of spontaneous activity, catalepsy, tail-withdrawal, and body temperature. More generally, this study underscores the utility of evaluating novel cannabinoid compounds in pre-clinical models of cannabis-like intoxication.<sup>1</sup>

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### THC; Cannabinoid; Behavior

## J25 Adolescent Nitrogen-Assisted Suicide Emulation: Coincidence or “Werther Effect”?

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**Learning Objective:** After attending this presentation, attendees will know more about the Werther effect.

**Impact Statement:** This presentation will impact the forensic science community by providing an example of a suicide emulation.

In social sciences and in medicine, the term “Werther-effect” refers to suicidal behavior associated with the effects of media-induced self-immolation.<sup>1</sup>

This study illustrates the unusual case series of the deaths of two young men (aged respectively 48 and 16) that occurred in the same town (15,000 inhabitants) using the same self-created device, within a time interval of 16 months. Both events had identical dynamics: the head and neck of the victims were surrounded by the device, consisting of a plastic bag, tied to the neck, connected by a pipe to a large cylinder of industrial nitrogen gas. In both cases, the cause of death was assumed to be the anoxia induced by the combined action of nitrogen gas inhalation and mechanical asphyxia for the plastic wrap covering the face.

Nitrogen gas, also known as laughing gas, is a euphoriant and is an inhalant drug, often used for recreational purposes for its easy and legal availability. Death from suffocating gases is caused not by their toxicity, but because of displacement of oxygen by the breathed gas mixture.

By a physiopathological analysis, the effect of the inhalation of nitrogen is able to fill up, after a few breaths, the alveolar spaces, causing fatal asphyxia.

The internet provides a quantity of information about a number of suicidal purposes and ways of achieving the target.<sup>2</sup> For example, the “Hemlock Society,” an American right-to-die and assisted suicide advocacy organization, provides information about several suicidal paradigms: among the proposed suicidal modalities, a very suggestive one is nitrogen-induced asphyxia. The internet has also been the information vehicle for “Sarco” (short for sarcophagus), the 3D-printed machine that causes suicide by inhaling nitrogen. This device was invented by an Australian euthanasia activist and became famous in 2018, by the public information activity made by the founder of “Exit International Association.”<sup>3</sup>

A recent systematic review showed that the second most common cause of death in adolescents is suicide and correlates the rise of incidence with depression related to the increased use of social media.<sup>4</sup> In the case presented, several local newspapers had reported the suicidal episode, describing the details of the suicidal mode. The second man had recently been involved in sentimental affairs with reactive depression symptoms, as reported by his parents.

The mechanisms that cause suicide are still uncertain, particularly when the diffusion of the information about the suicide modality is spread over by the main mass media. The aim of future research should be to clarify what factors contribute to strengthening the suicide inclination of depressed individuals.

The chronological and topographic circumstances of these deaths and the use of the same self-made device open the field to a larger discussion.

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### Suicide Emulation; Nitrogen Inhalation; Werther Effect

## J26 Advanced Magnetic Resonance of the Brain in Antisocial Behavior

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**Learning Objective:** After attending this presentation, attendees will understand the contribution of Magnetic Resonance Imaging (MRI) to the brain examination of a person with antisocial behavior.

**Impact Statement:** Antisocial personality disorder (ASPD) is a mental condition in which a person shows little regard or respect for other people. They often violate the norms of right and wrong, and they may engage in criminal or harmful behavior. An estimated 1–4% of people in the United States have ASPD. The objective individualization with a non-invasive method will contribute to a better understanding of motive and modus operandi of patients with antisocial behavior.

Antisocial behavior (AB), a complex behavioral phenotype that includes violence, aggression, and rule-breaking, is central to the psychiatric diagnosis of Antisocial Personality Disorder (APD) in adults and Conduct Disorder (CD) in youth. AB encapsulates a striking failure to respect the rights of others and conform to societal laws, and predicts a host of negative sequelae, including crime, substance use, and poor physical and mental health. Moreover, AB is harmful and financially costly to victims, families, and communities.

Magnetic resonance is a non-invasive examination using the properties of body protons placed in a magnetic field and stimulated with radiofrequency pulses. The behavior of proton-related energy depends on the tissue properties allowing magnetic resonance sequences to provide information on morphological and functional aspects of the brain.

The advanced MRI consists of 13D T1W anatomical images, depicting the brain not only in 2D but in 3D. Changes in the cortical surface, the area where the gray matter covers the cerebral hemispheres and where the higher nervous system centers are located, can be detected using a reconstruction software that provides color images reflecting those changes: cortical integrity images based on cortical thickness. The neurons in the gray matter are the producers of brain activity and transmitting the impulses and connecting with each other through the white matter tracts. Measurement of white matter integrity can be obtained with diffusion tensor imaging and measurement of fractional anisotropy and parallel and perpendicular diffusivity. Fractional anisotropy measures how compact the white matter fibers are. Parallel diffusivity measures integrity of the axons, the nerve fibers that are covered by a layer of an insulator, the myelin. Damage to the myelin is measured by perpendicular diffusivity. This method shows abnormalities especially in the uncinate fasciculus, connecting orbitofrontal structures to the temporal lobe. The cortical centers, clusters of neurons, and the nerve cells producing or receiving information have reciprocal connections through white matter tracts. Cortical and white matter lesions have as an end result the abnormal functioning of the brain networks. When checking brain connectivity, a decrease in the Default Mode Network can be seen in patients with AB. We will provide examples of these methods in homicides, rapists, and drug dealers.<sup>1-7</sup>

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### Antisocial Personality Disorder; Advanced Magnetic Resonance Imaging of the Brain; Brain Lesions



## J27 Crime as an Outcome of Conflicts Between Subcultural and Symbolic Dimensions

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**Learning Objective:** After attending this presentation, attendees will better understand the complexity of the issues underlying affective and cultural codes, relational patterns, processes of change and evolution in family contexts, in relation to their traditions and historical, tradition-based, and rural characteristics.

**Impact Statement:** This presentation will impact the forensic science community by showing how processes of change in these contexts can trigger aggressiveness that culminates in conflicts with outcomes of criminological, medicolegal, psychological, and forensic psychiatry concern.<sup>1,2</sup>

The case involves two brothers from a rural family living in northern Italy, the owners of a farm. The older brother was true to the family traditions and lived in Italy with his two children. The younger brother, in disagreement with his older brother about questions of the succession and not wishing to work on the family farm, emigrated to France, where he became a successful entrepreneur. Their subsequent relationship and contacts were sporadic.

The elder brother's daughter, growing up in the rural context, refused to become an agricultural worker. Her father was angry with her and associated her with her "traitor" uncle. At the age of 19, the girl went to France to meet her uncle, aged 48 years, and was struck by the great cultural differences. Her uncle introduced her to "his world," allowing her to have experiences she had never had in Italy. After about one month, they became lovers. The affair ended after about six months by mutual agreement, when the girl had started a relationship with a north-African Muslim waiter working for her uncle. The uncle accepted the relationship.

The girl went back to Italy to present her new companion to the family. The father was very angry about the relationship, due to racist issues. In revenge, the daughter revealed her previous relationship with her uncle.

The man invited his brother to come to Italy, to talk about questions related to their inheritance. During lunch, the older brother strongly criticized his brother for having a relationship with his niece. In response, the younger brother criticized him for his narrow-mindedness and backward culture. At the height of the quarrel, the elder brother struck the younger in the neck with a knife, severing the right recurrent laryngeal nerve.

The prosecutor initially accused the man of attempted homicide dictated by questions related to the inheritance. During the trial, however, all the underlying family motives emerged, demonstrating that the true motive for the attempted homicide was the ancient rivalry and competition between the two brothers, together with the daughter's attempted emancipation. She had colluded with her uncle and become an accomplice, while her mother was relegated to a dependent, peripheral role. We will discuss the difficulties and active psychological disorders, attributable to a rigid, archaic, and traditional system that cannot accept change and transformation of any family members.

A complete understanding of the criminal act during the brothers' quarrel thus derives not only from the medicolegal assessments and forensic psychology-psychiatry evaluations, but also from an overall examination of the family history and cultural and anthropologic relations of the enlarged family nucleus, and of all the actors in the background of the crime scene.

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### Homicide; Psychiatric Assessment; Incest

## J28 From Game to Death Is a Moment: The “Copycat Effect” in a Case of Atypical Murder

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**Learning Objective:** After attending this presentation, attendees will understand the copycat effect through the description of the forensic investigations carried out in an atypical murder case reported by our experience.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the risk related to the simulation of dangerous scenes by patients with psychiatric diseases as well as the importance of a careful examination of the crime scene and clues for the recognition of cases with suspected copycat effect.

The copycat effect is a phenomenon first identified in 1974, initially called the “Werther effect,” referring to the famous novel by J. W. Goethe, *The Sorrows of Young Werther*. In the novel, the young Werther decides to kill himself due to his impossible love with Carlotta. Goethe tells us that the protagonist was sitting at his desk when exactly at midnight, he shoots himself. After its publication, many young people, seeing themselves in Goethe’s words, decided to kill themselves following exactly the same procedure, committing suicide right in front of a copy of the book. Since then, numerous cases of killings following the modus operandi of characters belonging to the world of television, comics, or video games have been identified. An example can be the case of Lionel Tate, a 12-year-old who killed his 6-year-old playmate to emulate the stunts of televised wrestling.<sup>1</sup>

**Case report:** We report the case of a woman found dead in her home. The body showed numerous slash injuries on her head and stab wounds on her wrists. The crime scene was atypical as it showed, in the son’s bedroom, various clues, including a comic, a playing card (ace of spades), and numerous handwritten letters. In the same day, the woman’s son was also found dead due to suicide from falling from a bridge. In this case, there were numerous doubts about the motive and dynamics of this atypical murder-suicide.

**Materials and methods:** A thorough examination of the primary and secondary crime scene was carried out with photographic surveys. The investigators examined the boy’s room with evaluation and seizure of the clues left behind, namely the comic, the playing card, and the various letters. Subsequently, an autopsy was performed on the mother and son. The data collected at the scene and at the autopsy were compared.

**Results and discussion:** The autopsy showed that the murder had been perpetrated in a first phase with the violent use of an axe that caused a traumatic head injury. After death, the man raged on the victim’s corpse with a cutter, causing multiple cuts on the wrists and severing the vessels. The boy’s autopsy confirmed polytrauma due to falling from a great height. In this case, the crime scene analysis and especially the examination of the man’s bedroom was essential to understand the context and the psychological background of the event. The bedroom showed numerous horror movies, books, and videogames with violent content. The letters found had threatening content aimed at the victim. The playing card was a highly symbolic element as the “signature of the killer” since in cartomancy, its meaning refers to death. The crucial element for resolving the case was also the comic left open in the room containing an episode of DareDevil. The characters in this tale are the antagonist Bullseye fighting Elektra, DareDevil’s lover. In the episode, Bullseye, taking out a playing card, an ace of spades, wounds Elektra in her throat and subsequently kills her with a stabbing weapon. In the case described, it is clear how the murder was perpetrated with a copycat effect and how the killer had intentionally left various clues with the signature of the crime. Literature has shown that the copycat effect is frequent in subjects with psychiatric diseases, mental illnesses, or histories of violence. We emphasize the prevention of these dangerous simulations through careful control over the psychological, social, and environmental context of these patients. In the forensic field, we suggest an accurate collection and comparison of the clues for a better understanding of the psychological background and the motive of the event.

### Reference:

1. Helfgott, Jacqueline B. "Criminal behavior and the copycat effect: Literature review and theoretical framework for empirical investigation. " *Aggression and violent behavior* 22 (2015): 46-64.

### Forensic Sciences; Copycat; Murder



## J29 The Presence of Drugs in Firearm Suicides in Oakland County, Michigan

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**Learning Objective:** Attendees will learn from suicide data in Oakland County, MI, wherein certain drugs have been identified in the toxicology reports of suicide victims. There will be a review on the drug distribution of both firearm and non-firearm suicides to see which drugs are more prevalent with each method of suicide.

**Impact Statement:** The topic of suicide has been discussed heavily in the past couple of years, and the issue of drug use has been discussed for even longer. The purpose of this presentation is to display the findings of two years' worth of suicide data from Oakland County, MI, and discuss the presence of drugs in the body at the time of death. Sharing this information could bring new awareness to the problem of drug-related suicides with both firearms and non-firearms, and how drugs may influence decision-making in these crucial times.

The links between substance abuse and suicide have long been established in previous research. However, there has been little examination on the correlation between specific drugs and certain methods of suicide, as well as the decision-making that alcohol and drugs can impair. Suicides involving firearms are by far the most popular method. According to the Educational Fund to Stop Gun Violence, firearms are used in half of suicide deaths, and suicides make up 60 percent of firearm deaths. These national statistics are consistent with the findings of this report, in which of the 294 suicide cases in Oakland County, 151 of them, which comes out to 51 percent, were committed with a firearm. Michigan is also ranked 36<sup>th</sup> in state firearm suicide rates, with 6.98 individuals committing suicide via firearm per 100,000 people. Furthermore, in a 2013 report by the Office of National Drug Control Policy, in the years 2009–2010, Michigan was one of the top ten states for illicit drug use among persons aged 26 or older. Approximately 10.37 percent of Michigan residents reported past-month use of illicit drugs, while the national average was 8.82 percent.

In this report, using data from the Oakland County Medical Examiner Database, suicide cases are analyzed to determine what drugs are most closely associated with suicides involving a firearm. Using suicide case data from 2020–2021 in Oakland County, MI, the analysis shows that firearm suicides are most closely associated with alcohol and cocaine, whereas other methods of suicide are associated with THC, antidepressants, and benzodiazepines. This report also analyzes drug distribution between firearm and non-firearm suicide categories, which gives further insight into the drugs of choice for victims of suicide. In addition, this report analyzes the demographic data given by the Oakland County Medical Examiner Database to build a profile of the suicide victim in Oakland County, MI.

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**Suicide; Toxicology; Drug Abuse**

## J30 Psychosis in Cocaine Overdose: An Interesting Case

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**Learning Objective:** Attendees will learn what a cocaine overdose is, what cocaine-related psychosis in chronic drug abuse is, and the correlation between unusual and violent behavior and cocaine metabolite levels in the blood.

**Impact Statement:** Psychotic cocaine-related episodes are more likely with heavier, more frequent, and long-term misuse of cocaine, as is addiction. It follows that cocaine use disorder commonly co-occurs with cocaine-induced psychosis. This presentation serves the purpose of finding a solid correlation in drug-abuser and psychotic behavior to prevent the risk of death and protect society.

Cocaine overdose is a condition that causes an increase in blood pressure, heart rate, and depth of breath. After an initial excitation phase, the overdose state can lead to death via acute and massive pulmonary edema.

Cocaine consumption also causes a wide and well-known neuropsychiatric symptomatology that is characterized by incomprehensible behavior, confused and disordered thoughts, and paranoia. Cocaine psychosis in overdose may be related to a massive drug intake such as alternate routes of entry other than nasal inhalation.

Cocaine-induced psychosis is a type of mental illness in which psychotic symptoms are triggered or worsened by such intoxication. Risk factors include heavy, long-term cocaine use or early first use of cocaine. Cocaine-induced psychosis is an episode of psychotic symptoms, which may include delusions, hallucinations, disordered thinking, and unusual behaviors and emotional responses. This kind of psychosis may be a short episode, but it can also become chronic. Psychotic episodes are more likely with heavier, more frequent, and long-term misuse of cocaine, as is addiction. Cocaine has numerous effects on many important neurotransmitters in the brain; however, the most dramatic effect is on the increase as well as the release of dopamine. Dopamine is thought to be the primary neurotransmitter involved in the pleasure centers of the brain. Its release is associated with pleasure, a sense of well-being and is often a “reward” for certain behaviors. Excessive dopamine levels have also been hypothesized to be associated with anger, aggressiveness, hallucinations, delusions, and other psychotic symptoms.

This work shows a specific case in which cocaine poisoning and overdose caused the death of the subject, preceded by psychotic and extremely violent behavior.

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### Cocaine Overdose; Psychosis; Bizarre Behavior

## J31 The Appropriateness of Insertion of Psychiatric Offending Patients in the Forensic Communities

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**Learning Objective:** After attending this presentation, attendees will have a better understanding regarding the appropriateness of placing psychiatric offending patients in forensic facilities.

**Impact Statement:** This presentation will impact the forensic scientific community by presenting evidence about the treatment provided to psychiatric offending patients based on the level of therapeutic safety they need.

In Italy in recent years, the treatment of psychiatric patients who committed crimes has undergone significant changes. DPCM (Prime Minister's decree) April 2008 transferred health responsibilities from the Ministry of Justice to the Ministry of Health. Over a period of less than ten years, responsibility for forensic inpatient treatment was transferred to 20 regional public health authorities. This change entailed the establishment of the Residences for the Execution of the Safety Measurement (REMS) and the transfer to the regional psychiatric structures of all those patients with a profile of social dangerousness.

For the Puglia Region, a special commission has planned the discharge of inpatients from "legacy" High Security Hospitals (OPGs) originating in the region to the two new REMSs already activated and ten low security community forensic facilities. More than ten years after the Prime Ministerial Decree, we examined the state-of-the-art of this transformation and whether the forensic inpatients received a correct level of security treatment.

The Structured Professional Judgment Tools for Admission, Urgency, Treatment Completion and Recovery Evaluation Triage (DUNDRUM) Toolkit is a set of four different structured professional judgement and assessment tools consisting of four different scales used with forensic patients for evaluation and treatment purposes.<sup>1</sup> In particular, DUNDRUM 1 is an evaluation tool for allocating forensic patients to higher or lower levels of therapeutic security according to need. The DUNDRUM 2 includes items that are designed to provide a structured professional judgment tool to give priority to those admitted from the waiting list. This has been validated as matching best practice in Ireland, the United Kingdom, Belgium, Australia, New Zealand, and Canada.

The authors use DUNDRUM 1 and 2 to evaluate whether its own structures for inpatients placed in the different types of settings in Italy because of a legal finding of social dangerousness corresponded to the assessed need using this instrument. We assessed whether legal placements matched or mismatched the international criteria provided by the DUNDRUM toolkit. The first data of this study will be presented.

### Reference:

- <sup>1</sup>. Kennedy, H. G., O'Neill, C., Flynn, G., Gill, P., & Davoren, M. (2016). *The DUNDRUM Toolkit V1*. 0. 30. 2010-2016. Central Mental Hospital. Dublin.

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### Treatment; Forensic Settings; Correct Level of Security Treatment

## K1 An Examination of Questioned Documents Using Digital Grid Overlays

*Mark Goff, BA\*, Michigan State Police Lansing Laboratory, Lansing, MI; McKenzie Weyh, BS, Michigan State Police Lansing Laboratory, Lansing, MI*

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**Learning Objective:** After attending this presentation, attendees will have an understanding of the options available for the digital grid overlay examination of suspected altered documents and the applicable current Forensic Document Examination standard.

**Impact Statement:** This presentation will demonstrate and evaluate the use of digital grid overlays in the examination of questioned documents for insertions, interlineations, and copy/paste alterations. Additional instruction will be given on how these examinations conform with conventional grid examination methods and ANSI/ASB Standard 035, Standard for the Examination of Documents for Alterations.

Many methods of document alteration involve the insertion of additional lines of text to informational aspects of the document or the creation of a document montage by combining elements such as signature blocks and notary blocks from legitimate documents with fabricated elements to attempt to legitimize false documents. With these types of altered documents, it is extremely difficult to obtain precise horizontal and vertical alignment of the various additions or insertions. Conventional document examination methods include examining documents for alignment using transparent typewriter grids wherein the examiner selects a grid of similar spacing to the document in question and notes any deviations from the established grids. Limitations to this examination include the grid spacing is limited to common typewriter spacing and documenting the grid alignment. Another conventional method of examination is the creation of custom grids based upon the physical measurements of the document spacing and printing this grid to a transparency to be overlaid on the questioned document. This method can be more accurate in the examination of interlineations due to the custom nature of the comparison grid; however, it is limited by the accuracy of measurement of the physical document and, as above, preservation of the overlay results. Additionally, conventional methods rely on the examination of physical documents even though many documents only exist in a digital format in the current document environment.

The current ANSI/ASB Standard 035, Standard for the Examination of Documents for Alterations allows for the use of grid and software examinations, so a method for combining these options for digital grid overlays was explored. In this method, a physical document is scanned in high resolution and imported into a raster graphics editing program (Adobe® Photoshop®), or a digital document is directly imported the raster graphic editing program and examined by applying a grid overlay image. This method allows for precise grid creation and alignment and preservation of the digital image overlay in the examination record.

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### Alteration; Grid; Forensic Document Examination

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## K2 The Security Design of the Next Generation U.S. Passport, U.S. Emergency Passport, and U.S. Bridge Visa

*Traci Moran, MFS, US Department of State, Washington, DC; Joel Klotnick, MS\*, US Department of State, Washington, DC*

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**Learning Objective:** Attendees will learn about security printing and security features of three identity and travel documents designed and issued by the Department of State. Additionally, design and manufacturing priorities that preceded each release will be discussed.

**Impact Statement:** This presentation will provide forensic-level knowledge to questioned document examiners who may come across these types of documents in their case work.

High-value security documents such as passports, visas, and banknotes remain valid and in circulation for years. For example, the United States e-Passport was first issued in 2006 and will remain in circulation until approximately 2033. The United States Lincoln visa will have a similar lifespan. Because of their long lifespans and the need to adapt to evolving technological environments, it is critically important that these documents are well-designed to resist both current and future security risks related to counterfeiting and alteration.

Using a strategic approach to implement security technologies in a purposeful way, such as layering various security elements, is a sound method of building a secure document. Design is important. A document overloaded with security features is not necessarily secure because security features without considering feature design and context does not necessarily make counterfeiting or alteration harder. Overlapping and integrating different document technologies achieves two purposes. First, features can be tied to some specific document, which creates a problematic hurdle for counterfeiters that harvest genuine elements from authentic documents to repurpose them into counterfeits. Second, tight integration of materials, design, and manufacturing processes creates a security document that would require a counterfeiter to access all three of these elements and not just specific security materials. Counterfeiters will always attempt to simulate or alter United States documents, but designing them to resist and/or reveal these attempts in the most obvious way should be the goal of the designer.

The United States Department of State is responsible for designing and issuing many high-value security documents to include the United States Passport, Visa, Passport Card, Emergency Passport, Border Crossing Card, and Consular Report of Birth Abroad. The Department has released new versions of the United States Passport (referred to as the Next Generation Passport) in 2021, the Emergency Passport in 2021, and the Bridge Visa in 2022. The redesign of each of these types of documents (whether book, sticker, card, or paper) is a complex manufacturing challenge that requires several years of preparation. This presentation will reveal security features and security printing utilized in the construction of the Next Generation Passport, the Emergency Book, and the Bridge Visa and will discuss the design and manufacturing priorities that preceded each release.

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### Security Document Design; Security Features; Security Printing

## K3 The Characterization of Polymers of Counterfeit Identity Documents of European Union (UN) Member States

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**Learning Objective:** The goal of this presentation is to show attendees what types of polymers are used for the production of counterfeit identity documents in card format that enables individualization by laser engraving.

**Impact Statement:** This presentation will impact the forensic community by showing the correlation between the different types of polymers used for the production of counterfeit documents and the ways of their individualization.

Modern identification and travel documents, such as identity cards, driver's licenses, residence permits, and passports are made entirely or partially of polycarbonate. European Union regulations stipulate that security documents issued by Member States in card format (ID-1) should be made of polycarbonate (PC) or an equivalent synthetic polymer (which can last at least ten years). Security documents made of polycarbonate are well secured because, due to their very good characteristics, it is possible to incorporate various modern fraud-resistant security features. Due to the wide range of temperature resistance, polycarbonate documents can be individualized by laser engraving, which is considered one of the most reliable ways of integrating the owner's data and image into the body of the cards.

Until recently, various printing techniques, such as thermal, laser, or inkjet printing, have been used for the individualization of counterfeit travel and identity documents made of polymers. However, in recent times, a new form of forgery of documents made of polymers has appeared on which the bearer's photo and data are laser-engraved, as is the case with legally issued documents made of polycarbonate.

In the present study, more than 50 samples of different types of counterfeit European Union MS documents made of polymer were analyzed (identity cards, driver's licenses, residence permits), using non-destructive methods. IR-spectroscopy was used for the analysis of the qualitative chemical composition of the polymer of counterfeit documents. The obtained IR-spectra were compared with the IR-spectra available in the European Network Forensic Science Institute database (ENFSI/EPG) and in the internal databases, while the search was performed using "KnowItAll" software. Video-spectral and stereomicroscopic analysis were used for the determination of techniques used for the individualization process on counterfeit documents.

This study will show that the technique used to individualize polymers counterfeit documents in card format depends on the type of polymers. As most of laser engraved polymer counterfeit documents have imitated security features of very good quality, it is necessary to be careful during the examination of such documents, especially at the first line of border control, and bear in mind that the personalization of a polymer document by laser engraving does not mean that it is legally issued.

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### Counterfeit Documents; Polymers; IR Spectroscopy

### **K4      Scientific Instruments to Forensically Examine Booklet Perforations**

*George Virgin, MS\*, HSI Forensic Laboratory\*, Mclean, VA*

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**WITHDRAWN**



## K5 Fraud and the United States Postal Service (USPS): A Focus on Stamps

*Lisa Tolli, MS, US Postal Inspection Service, Forensic Laboratory Services, Dulles, VA; Patricia Manzollilo, MSFS\*, US Postal Inspection Service, Dulles, VA; Stephanie Kingsbury, MSFS, US Postal Inspection Service, Dulles, VA; Haley Elliott, MS, US Postal Inspection Service Forensic Laboratory Services, Dulles, VA*

**Learning Objective:** After attending this presentation, attendees will have a better understanding of how USPS stamps are produced, best practices for the examination of suspected counterfeit USPS stamps, and security features currently integrated into USPS stamps.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees and FDEs how to learn best practices on how to examine these types of complex cases.

The United States Postal Service (USPS) has issued United States postage stamps since 1847, and for many years stamps were the primary source of revenue for the USPS. Most individuals think of the “Forever” postage stamps used to mail a First-Class letter, currently valued at \$0.60, but stamps are available for a variety of amounts ranging from \$0.01 to a \$26.00 Priority Mail Express stamp. Due to their monetary value, stamps have increasingly become a prime target for counterfeiting and fraud. Over the past few decades, there has been a shift in the type of revenue fraud cases involving stamps that have been investigated by the United States Postal Inspection Service (USPIS). Previously, many cases involved stolen or washed genuine stamp stock. Due to advances in technology, whether utilized by USPS in an attempt to combat fraud or by those attempting to defraud the public, there has been an increase in counterfeit stamps available for purchase. With the increase in online shopping, many consumers are moving toward purchasing stamps from large online vendors, especially when sales are advertised. Unfortunately, many of these counterfeit stamps are high quality and are produced using printing processes similar to genuine United States postage, making counterfeit determination that much more difficult. For the Forensic Document Examiners (FDEs) of the United States Postal Inspection Service, a substantial reference collection, intimate knowledge of USPS security features, and ongoing training are essential to our successful resolution of these fraud cases.

The aim of this presentation is to provide a brief history of the different types of stamp fraud and to show the quality of the counterfeit stamps that are available on the market today. Best practices in conducting both visual and instrumental USPS stamp examinations in order to mitigate challenges associated with production and printing methods will also be discussed. A variety of examples of genuine and counterfeit stamps will be reviewed, including many different designs and years of production.

To understand USPS stamps more fully, stamp security features will be considered, including the printing processes of genuine stamps, the placement of microtext into the design of stamps, and the addition of an ultraviolet-reactive taggant. By requiring genuine stamps to be designed and printed by high-end commercial means, the counterfeiter has a more difficult time in procuring this equipment to replicate the stamp successfully. The addition of microtext to the design of a stamp makes the stamp more difficult to accurately counterfeit by increasing the complexity for a counterfeiter in re-creating a stamp’s design fully. Finally, the addition of an ultraviolet taggant serves as a tool for USPS to sort mail as well as providing security to the stamp itself.

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### Fraud; Counterfeit Stamps; Forensic Document Examination

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## K6 An Update on Standards Development Activities Related to Forensic Document Examination

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

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**Learning Objective:** After attending this presentation, attendees will better understand the status of the current and upcoming Forensic Document Examinations standards being developed by the OSAC Forensic Document Examination Subcommittee and the ASB Forensic Document Examination Consensus Body.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of the current standards development activities related to Forensic Document Examination. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

During this presentation, an update will be provided on the standards development activities related to Forensic Document Examination. This will begin with an introduction to OSAC and ASB, and an overview of the standard development process as it relates to both organizations.

Second, this presentation will discuss Draft OSAC Proposed Standards at various stages of development at OSAC. The OSAC FDE Subcommittee is currently working on Draft Proposed Standards related to the Collection of Known Writing, Conclusions, Destructive Ink Examinations (including writing inks, inkjet inks, and miscellaneous inks), Non-Destructive Ink Examinations (including writing inks, inkjet inks, and miscellaneous inks), Document Triage, and Torn Paper Examinations.

Third, this presentation will discuss Draft ASB Documents (including both Standards and Technical Reports) at various stages of development at ASB. The ASB FDE Consensus Body is currently working on the Standard for Minimum Training Requirements for Forensic Document Examiners, the Standard for Examination of Mechanical Checkwriters and Their Impressions, and a Technical Report on Forensic Document Examination Terms and Definitions.

Fourth, this presentation will discuss completed ASB Standards. As of the writing of this abstract, the ASB has published the Standard for Examination of Documents for Indentations, the Standard for the Examination of Documents for Alterations, and the Standard for Examination Stamping Devices and Stamp Impressions. The ASB Forensic Document Examination Consensus Body has also completed work and await publication of 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.

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.*

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**Standards; Forensic Document Examination; OSAC and ASB**

## K7 The Forensic Document Examination (FDE) Steering Committee

*Timothy Campbell, BSc\*, Canada Border Services Agency, Ottawa, ON, CANADA*

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**Learning Objective:** Attendees will be introduced to the new Forensic Document Examination Steering Committee and be briefed on its initial tasking or assessment of core versus referential topics of knowledge.

**Impact Statement:** The Forensic Document Examination Steering Committee is assessing the state of the profession and how it will develop in the future. It is an example of the evolution of a forensic discipline that has existed for a long time yet remains very relevant to current and upcoming trends.

The discipline of Forensic Document Examination (FDE) has existed for over a century, spanning a time of immeasurable technological progress. Unlike some recently conceived forensic fields, a practitioner is required to be trained in a spectrum of possibilities that may be encountered in the line of case work since any given case could involve either contemporary or archival items. While historical materials have given way to modern ones, and traditional communication techniques to advanced messaging, what has not changed is the need for humans to communicate with one another and exchange business transactions.

In February 2022, the American Academy of Forensic Sciences (AAFS) Questioned Document Section and the American Society of Questioned Document Examiners (ASQDE) agreed to strike a Steering Committee to analyze, discuss, and make recommendations on the current state of FDE and chart a course for future development of new examiners and, by extension, the profession as a whole. This is the first time these two organizations have conducted such a joint venture. Three members of each organization were selected with the addition of a digital forensic investigator to represent that field's knowledgebase.

The first task undertaken by the FDE Steering Committee has been to review the topics covered in the Syllabus of the draft ANSI/ASB Standard 155 Standard for Minimum Training Requirements for Forensic Document Examiners, Section 4.7.<sup>1</sup> Each topic was given a designation of either core working knowledge or referential knowledge, whereby an examiner is expected to either know the topic by rote memory, in the former, or understands enough to research the topic as needed, in the latter. Beyond existing topics, current technologies such as digital documents and digital signatures have also been considered for their place in this ever-evolving discipline. A position paper is to be written and issued to provide guidance for existing and future practitioners.

**Reference:**

<sup>1</sup>. DRAFT ANSI/ASB Standard 155 - Standard for Minimum Training Requirements for Forensic Document Examiners, Section 4.7.

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### Questioned Documents; Steering Committee; Training

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## K8 The Forensic Document Examination (FDE) Forum

*Carl McClary, MS\*, ATF, Atlanta, GA; Atlanta, GA; Karen Nobles, MFS, Forensic Document Services, LLC, Pensacola, FL; Samiah Ibrahim, BA, BSc, ForensInk, Ottawa, ON, CANADA*

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**Learning Objective:** After attending this presentation, attendees will know various opinions and insights into current issues facing the modern forensic document examiner through a panel discussion led by three experienced examiners—one employed by the Federal government, one retired (former state government), and one retired (former Canadian government).

**Impact Statement:** The panel presentation/group discussion will impact the forensic science community by providing varying opinions to both old and new issues facing a forensic document examiner. Usually, the audience is composed of examiners of varying experience and backgrounds, including government employees, private consultants, and often trainees.

The concept of an FDE Forum is not new to the Questioned Document community. In this author's experience, this format was first utilized at the Southeastern Association of Forensic Document Examiners (SAFDE) meetings in the early 1990s as a method of opening and discussing certain topics of interest to members. At these meetings, there were discussion starters on subjects upon which there may have been disparate opinions. Through forums of this type in other organizations, there have been many lively discussions on topics such as the strength of opinions on examinations involving photocopies or rubber stamp impressions. Other topics that could be considered are opinion terminology, board certification, standardized training, court testimony, or specific requirements found in our standard methods. A popular theme in the past has been what type of evidence is required to achieve a particular conclusion in handwriting examination, for instance, the elimination of a subject as having written a particular body of writing. Not all discussions must necessarily be centered on issues facing examiners. Some discussions could focus on attendees' thoughts on and interpretations of quotes from texts in the field by authors such as Harrison, Osborn, and Hilton. Other discussions could begin with the opinions of some of these authors, and others in criminal and civil cases of notoriety, past and present. This type of forum is an excellent way to poll attendees on their preferences on methods of testimony, court presentations, and laboratory requirements regarding testimony.

It is requested that any potential attendees submit topics/questions to Carl McClary at [carl.mcclary@atf.gov](mailto:carl.mcclary@atf.gov) or Karen Nobles at [karenjnobles@gmail.com](mailto:karenjnobles@gmail.com). This year's meeting theme is "Science Works," and it is requested that any topics submitted beforehand could relate to the theme or to international issues. Questions pertinent to our theme would be especially appreciated as questions around the reliability of science and medicine, particularly in our COVID-19 environment, are front and center today.

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### Forensic Document Examination; Opinions; Panel Discussion

## K9 Black-Box Metamodeling Between Feature Combinations for Paired Comparisons of Documents of Forensic Handwriting Data

Cami Fuglsby, MS\*, South Dakota State University, Brookings, SD; Christopher Saunders, PhD, South Dakota State University, Department of Mathematics and Statistics, Brookings, SD; Danica Ommen, PhD, Iowa State University, Ames, IA; JoAnn Buscaglia, PhD, FBI/Laboratory, Research and Support Unit, Quantico, VA

**Learning Objective:** Attendees will learn strategies to characterize relationships between feature sets of handwritten characters obtained from a black-box automated handwriting identification system. They will learn about the dependency structure induced by the pairwise comparisons and how data visualization techniques for large-scale inference can be used to communicate the relationships of feature sets.

**Impact Statement:** This presentation will impact the forensic science community by providing an understanding of how to investigate feature relationships from an automated handwriting identification system. This may have an impact on future studies on automated handwriting data pertaining to the explainability of the output of black-box systems.

Many automated handwriting identification systems use complex, black-box sets of algorithms to extract various features from a given handwriting sample, which differ depending on the system. In the original CEDAR-FOX system, features were grouped into macro- and micro-feature sets.<sup>1,2</sup> FLASH ID<sup>®</sup> and handwriter extract sets of features based on graphical structure and orientation.<sup>3</sup> For the language-independent FLASH ID<sup>®</sup> system, the node structure of a handwritten graph is the “isokey,” the orientation is a “shapecode,” and the combination of an isokey and a shapecode is a “caddy.” In recent work, a set of pairwise scoring rules (known as the vector of counts (VOC) method) was developed that can be decomposed across the observed caddies in a given pair of documents.<sup>4</sup> The overall goal is to understand how the FLASH ID<sup>®</sup> system works for pairwise comparisons by finding which caddy-specific VOCs are related to each other.

A subset of the West Virginia University (WVU) dataset was used. Subjects provided two different types of documents in their natural handwriting: the modified London Letter and a “freeform” document, which allowed subjects to write anything they conceived in their minds, but provided prompts in order to focus their attention toward a coherent sample. Thirty documents, each with a different writer, from the freeform set were selected for this research.

Pairs of caddy-specific VOCs from the WVU subset are being studied using a class of new statistical regression techniques developed and extended under two National Institute of Justice grants to provide a statistically rigorous characterization of the dependency between the different VOC-caddy pairwise similarity scores.<sup>5,6</sup> The dependency is based on the number of documents that contain a given caddy pair. These new statistical techniques are based on using least squares methods on the set of all pairwise comparisons between writers with the same given caddy pair (for all caddy pairs), and accounts for the new dependency structure between the comparisons. This process results in about 15,000 pairs of caddies in the WVU subset, and about 30,000 regression models. To allow for the comparison of many fitted regression models that use different sets of information, large-scale inference techniques on the estimated models are implemented, and graphical displays of these results will be presented.

### References:

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### Handwriting; Automated Identification Systems; Explainability

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## **K10 Fontastique! Framework for the Comparison and Evaluation of Currently Available Typeface Identification Applications for the Modern Forensic Document Examiner**

*Zain Bhaloo, MSc, BScH\*, Canada Border Services Agency, Ottawa, ON, CANADA*

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**Learning Objective:** Attendees will learn the proposed approach to be employed to identify the basic list of applications, the evaluation of the features that are most desired, and the considerations that will be taken while evaluating these features. Attendees will also learn of the possible and hopeful outcomes of this research once completed.

**Impact Statement:** This presentation aims to evaluate the currently available font identification applications specifically with a lens that will determine what, if any, features and characteristics are the most useful to the Forensic Document Examiner, and which application, if any, has all or most of these useful features and can be used to increase the effectiveness of the FDE in their work.

Typefaces have come a long way since the Blackletter typefaces that were used at the onset of printing to mimic the handwriting style popular at the time. Much has changed since then, especially with the jump from print typography to digital typography. The ability and ease with which typefaces can currently be created has increased significantly with the ability to employ applications and software packages to create new fonts with a couple of clicks of a mouse, and therefore so has the concomitant need for the modern FDE to be able to identify these typefaces accurately and quickly. Commercial digital tools are currently available for the purpose of identifying typefaces based on photographs and/or scans of paragraphs of text and even using only individual characters. However, an FDE's requirements are, and will be, much more focused than simply narrowing down the list of possibilities. For example, considerations such as accuracy and precision, in combination with reproducibility, are of concern to the FDE, as well as protection of privacy and sufficiency of sample in terms of quantity and quality.

The purpose of this presentation, and ultimately this study, is to present the framework to identify the typeface identification applications available and to compare and contrast their various capabilities for use by the modern day FDE.

The exact features and capabilities that are available and that will be assessed will depend on the available features within each application but could include, but not be limited to, assessment of the capability to consistently correctly and accurately identify a typeface, assessment and comparison of the reproducibility of the results, evaluation of the possibility and ease of their use both online and offline, evaluation and comparison of the minimum requirements allowable for input for correct identification and assessment of the range of typefaces that they have available to them.

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**Font Identification; Typeface Identification; Questioned Documents**

## K11 Handwriting Variability in Adult Writers With Attention Deficit Hyperactivity Disorder (ADHD) Pre- and Post-Medication: A Pilot Study

Christopher Burke, MS\*, East Tennessee State University, Auburn, WA; Heidi Harralson, MA, East Tennessee State University, Tucson, AZ; Larry Miller, PhD, Department of Criminal Justice/East Tennessee State University, Johnson City, TN

**Learning Objective:** In this presentation, attendees will learn about the symptoms and treatments of attention deficit hyperactivity disorder (ADHD) in adults with emphasis on how graphomotor skills of ADHD influences handwriting.

**Impact Statement:** Establishing intra-writer variance is an important task in forensic handwriting examination. This study helps to characterize the graphomotor variability of ADHD adult writers and gives insight into the type of intra-writer variance that can be expected in adult writers with ADHD, especially pre- and post-medication.

Research has shown that those with ADHD have increased intra-individual variability in graphomotor tasks such as handwriting.<sup>1,2</sup>

Due to the variability in their graphomotor skills, adults with ADHD have increased variance and dysfluencies in their handwriting. In this study, it was hypothesized that adult writers with ADHD may have common handwriting characteristics (e.g., dysfluency, handwriting errors, illegibility, irregularity). Further, it is predicted that adult writers with ADHD will show change in their handwriting pre- and post-medication with an improvement in handwriting variance post-medication. Medications such as mixed amphetamine salts, commonly prescribed to those with ADHD, reduces inattentiveness and hyperactivity-impulsivity.<sup>3</sup>

In this preliminary study, 16 adult participants voluntarily stated in a survey they had been diagnosed with ADHD. All participants were between the ages 29–50 (average age was 35) and included 9 women and 7 men. Of the participants, 15 were right-handed and 1 was left-handed. Ten participants stated they did not take medication for their ADHD. Six participants stated they took medication for their ADHD symptoms. Medications reported by participants included mixed amphetamine salts, bupropion, and venlafaxine, all of which are common medications for treating ADHD.

In the testing conditions, all participants were instructed to write a standard handwriting paragraph on plain white, unruled paper. Participants were instructed to write the paragraph in printscript. For the non-medicated participants, they were instructed to write the paragraph in printscript. The medicated participants were instructed to write two paragraphs: (1) prior to taking medication, write the paragraph in printscript; (2) after medication had taken full effect, write the same paragraph again in printscript. Some participants wrote the same paragraph in cursive in addition to printscript.

All samples were evaluated for dysfluency, errors, proportion, baseline alignment, and both letter and word spacing. Most of the samples, both pre- and post-medication, displayed characteristics of dysfluency, spatial irregularities, alignment irregularity, and/or errors. Irregularities in word spacing and letter size were present in several of the samples. Changes in handwriting were observed in pre- and post-medicated samples. In comparison to the post-medicated samples, the pre-medicated samples displayed an increase in dysfluency, errors, erratic spacing between letters and words, erratic letter proportions, and/or poor letter formation, including illegibility.

An increase in intra-writer variability was observed between pre- and post-medicated samples. This finding is important in forensic handwriting examination as dysfluent and variable handwriting may increase difficulty in handwriting comparison and identification tasks.

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### Forensic Handwriting; ADHD; Forensic Document Examination



## K12 Quantifying Bayes Factors for Forensic Handwriting Evidence

*Anyesha Ray, BS\*, CSAFE, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA*

**Learning Objective:** Attendees will gain a greater understanding of how statistical analysis, specifically the Bayes factor obtained using objective features extracted from handwriting evidence, can support a forensic document examiner's subjective writership determination.

**Impact Statement:** Bayes factors are highly recommended as a statistically sound method of interpreting forensic evidence, and this presentation will provide forensic document examiners with specific instructions on how to compute this statistic for determining the source of handwriting evidence.

Questioned Document Examiners (QDEs) are tasked with analyzing handwriting evidence to make source (or writership) determinations. The Center for Statistics and Applications of Forensic Evidence (CSAFE) has previously developed computational methods to automatically extract quantifiable handwriting features and statistical methods to analyze handwriting evidence to aid QDEs.<sup>1-3</sup> The method developed by Crawford et. al uses a K-means clustering algorithm and Bayesian hierarchical model to perform closed-set writer identification.<sup>2</sup> This means a questioned document is assigned to its most likely writer from a set of known writers but does not allow for the possibility of the questioned document to be written by someone not included in the set. Another method developed by Johnson and Ommen utilized machine learning techniques and score-based likelihood ratios (SLRs).<sup>3</sup> SLRs have been criticized for a variety of shortcomings, including a lack of coherence and ability to incorporate the rarity of the features.

Our goal is to develop a method that supports feature-based open-set writer identification while avoiding these issues. We implement an approach to quantify the value of forensic handwriting evidence using Bayes factors and Markov chain Monte Carlo (MCMC) computational techniques like those described in Collins and Ommen.<sup>4</sup> There are two paths to consider depending on the forensic question: the common source and the specific source identification problems. We demonstrate the approach for each identification problem using documents from the CSAFE Handwriting database, which consists of documents of various lengths from over 240 writers: the London Letter is the longest, followed by an excerpt chosen from the book *The Wonderful Wizard of Oz*, and the phrase "The early bird may get the worm, but the second mouse gets the cheese" is the shortest.<sup>5</sup> Handwriting features are extracted using the "handwriter" system, clustered using K-means, and subsequently used to quantify the Bayes factor. The performance of the methods is assessed using cross-validation and rates of misleading evidence (among other measures).

### References:

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**Handwriting; Statistics; Bayes Factors**

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## K13 Quantifying Writer Variance Through Rainbow Triangle Graph Decomposition of the Common Word “the”

*Alexandra Arabio, BS\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA; Alicia Carriquiry, PhD, CSAFE - Iowa State University, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA*

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**Learning Objective:** After attending this presentation, attendees will have a further understanding of the concept behind rainbow triangle graph decomposition and how it is used to quantify discriminating features for the analysis of handwriting.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating a new method for analyzing handwriting that could be used in conjunction with current practices to better quantify results.

Handwriting comparative analysis is based on the principle that no two individuals can produce the same writing and that an individual cannot exactly reproduce his/her handwriting. This project aims to assess and quantify the natural variations produced by a distinct writer. In an attempt to support traditional examination with objective measures, this project provides results from a study where features of handwriting are examined through graph decomposition and rainbow triangulation. Using this method to examine handwriting samples, more specific information can be obtained from each exemplar and can be standardized to be compared both within a writer and between different writers. Each type of characteristic or landmark of each handwriting sample are marked as a different color node in a graph, including the location that a pen stroke begins (blue), the location that a pen stroke ends (orange), any location where a pen line overlaps itself (pink), the highest location that a pen stroke reaches (green), and the lowest location that a pen stroke reaches (purple). Triangles can provide information on angles, edge slopes, edge lengths, and areas that all prove useful for quantitative and comparative analysis. By forming rainbow triangles over these samples, it is possible to gauge the variation within a single writer and to compare these quantitative values to other samples of unknown sources. Rainbow triangles are formed so that each vertex or node within a triangle set has a unique color, and each edge is unique to its triangle so that it is not to be used to form a different triangle in another set.

Using this information, the study aims to form a quantitative analysis of handwriting samples and to calculate how similar or dissimilar two samples are from one another. One of the study's main goals is to form these triangles from multiple samples from several different writers and to group, identify, and accurately determine what samples came from which writer. Finally, multiple summary statistics are explored to determine whether any can be used to discriminate between inclusions and exclusions using data where ground truth is known, such as a true match. This project hopes to impact the forensic community by demonstrating a new method for analyzing handwriting that could be used in conjunction with current practices to better quantify and support results regarding the source of a questioned document.

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### Rainbow Triangles; Decomposition; Natural Variation

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## K14 Training, Education, and Certification of Forensic Document Examiners in the United States

*Lauren Turnacioglu, BA\*, Duquesne University, West Windsor, NJ; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA; Gary Licht, MS, Iowa Division of Criminal Investigation Criminalistics Laboratory, Ankeny, IA; Khody Detwiler, BS, Lesnevich & Detwiler, Roaring Spring, PA*

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**Learning Objective:** After attending this presentation, attendees will understand the status of education, training, and certification, the trends that exist, and the uniformity of training within the field of forensic document examination.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the need for further research into the status of training, certification, laboratory accreditation, and specialization within the field, and indicating the amount of uniformity that exists.

Whether examiners are found reliable varies case by case and is based on the examiner's knowledge, training, and experience, which is often influenced by whether they complete training in accordance with published standards. The field of forensic document examination has outline-style or general overview standards that address the content of the training, one of which dates back to the Army Crime Lab Forensic Document Examination two-year training program. A separate federally funded FBI study that addresses the reliability of forensic document examiners is currently being done.

The purpose of this study was to investigate the training, certification, and education of forensic document examiners in the United States. A survey was conducted using Qualtrics™ software. The survey included questions focused on demographics, certification, training, education, and proficiency testing. Recruitment emails were sent to forensic document examiners across the United States by distributing it to forensics organizations such as the American Academy of Forensic Science (AAFS), the American Board of Forensic Document Examiners (ABFDE), the Mid-Atlantic Association of Forensic Scientists (MAAFS), the Association of Forensic Document Examiners (AFDE), and the American Society of Questioned Document Examiners (ASQDE). Qualitative and quantitative comparisons were used to determine if there was significant variation among examiners employed at different laboratories. It was concluded that the field lacked uniformity in how training standards were applied, as respondents' answers differed. Also, vast variations in timing existed for the time since last certification and proficiency test. Additionally, the type of education and training required by different organizations varied greatly. Another notable trend found during analysis was related to the status of accreditation based on the type of laboratory where the examiner was employed. All respondents who were employed at a government laboratory indicated the laboratory was accredited, while most examiners employed privately indicated the laboratory was not accredited.

This research provides an overview of the differences in training, education, and certification for questioned document examiners that can be used to inform the development of future standards.

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### Forensic Document Examination; Training; Standards

## K15 Twin Convolutional Neural Networks to Classify Writers Using Handwriting Data

*Pilhyun Lim, MS\*, Iowa State University, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA*

**Learning Objective:** Attendees will learn how forensic examiners compare questioned documents and the limitations of this process. This presentation will discuss the architecture of one of many black-box models called twin convolutional neural networks and how this method provides an alternative for the traditional methods of comparing questioned documents used by forensic examiners. Attendees will be able to understand the best ways to process and structure the questioned documents into scanned images in order to optimize the classification accuracy of this model by seeing how variations in image processing and model architecture influence classification accuracy. The constraints of this model primarily brought on by limitations in technology and the nature of the data will be examined.

**Impact Statement:** This presentation will shed light on ways in which statisticians are developing and using black-box algorithms to create an automated and more accurate process to classify similar and different writers between two questioned documents. Given that a twin convolutional neural network has a high classification accuracy and there is increasing trust for the inner workings of this model to be applied to forensic applications, classification of documents will be performed at a much faster rate than examiners, and more consistent examinations to compare questioned documents can be made by removing bias that comes from the subjectivity of each individual examiner. Therefore, this presentation will impact the forensic community by sharing the use of an algorithm that has reasonably high accuracy that can improve traditional techniques in questioned document examination in speed and accuracy to reduce the cases in which incorrect conclusions are made for the comparison of questioned documents.

Identifying the source of handwriting is an important application in the field of forensic science that addresses questioned document evidence found in criminal cases and civil litigation. It is difficult, given the idiosyncrasies of a person's handwriting, to recognize the exact writer of a piece of handwriting based only on its physical properties. Even more so is trying to classify a writer without any prior database containing handwriting characteristics of such writer.

Data sets containing handwriting samples from different sources are used to investigate how well a convolutional neural network can classify writers from unseen sources. Comparisons between scenarios modeled after real-world situations with varying degrees of complexity, which are adjusted by whether and from which source the samples from the suspects have been collected to train the model, are made to examine the extent to which twin convolutional neural networks can successfully classify similar and different writers.

This presentation primarily aims to compare data processing and modeling methods to improve classification on whether two pieces of handwriting are from the same or different writers, in the context where every potential writer has never been seen before. The structure of a twin convolutional neural network allows such comparisons between two images by passing them through identical convolutional neural networks and defining a metric that merges their outputted feature vectors to obtain a similarity score. As model limitations in this presentation are driven by memory and available data, various pre-processing and sampling methods are compared to maximize classification accuracy. On this optimized data set, a custom model that is developed for this analysis is shown to outperform various top-performing architectures in image classification problems with a classification accuracy of 85.5 percent on a test set with similar structure to the training set and 82.8 percent on a data set collected from a different database. Results show that as long as a large-enough number of samples are available to train the model, comparisons between the writers of questioned documents can be classified with over 80 percent accuracy.

### References:

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### Handwriting Classification; Deep Learning; Algorithms

## K16 A Comparison of FLASH ID and Handwriter: Two Handwriting Analysis Programs

*Stephanie Reinders, PhD\*, Center for Statistics and Applications in Forensic Evidence, Ames, IA; Danica Ommen, PhD, Iowa State University, Ames, IA; Alicia Carriquiry, PhD, CSAFE - Iowa State University, Ames, IA*

**Learning Objective:** After attending this presentation, attendees will have a better understanding of FLASH ID and handwriter—two forensic handwriting analysis programs. Attendees will learn the basics of how these programs work and see their accuracy rates on different lengths of questioned handwriting documents.

**Impact Statement:** This presentation will impact the forensic science community by introducing them to two forensic handwriting analysis programs: FLASH ID and handwriter. They will see how these programs perform on different lengths of questioned handwriting documents.

FLASH ID is commercial forensic handwriting analysis software sold by Sciometrics.<sup>1</sup> It is used in forensic labs in the United States and elsewhere. The Center for Statistics and Applications in Forensic Evidence (CSAFE) is currently developing a free open-source R package for handwriting analysis called handwriter.<sup>2</sup> Both programs compare a questioned handwritten document Q to handwriting samples from a set of writers. FLASH ID ranks the writers from most likely to least likely to have written the questioned document. As an example, FLASH ID allows the user to make a statement like, “Writer C is the most likely writer of the questioned document.” The R package handwriter uses a statistical model called a Bayesian hierarchical model to calculate the posterior probability that each known writer wrote the questioned document. For example, handwriter enables the user to make a statement like, “The posterior probability that Writer C wrote the questioned document is 98%.” Both programs take scans of handwritten documents and split the writing into component shapes. The programs compare shapes by looking at the number of edges, vertices, and loops, as well as the angle and the distances between parts of the shapes. Under the hood, both programs compare handwriting samples by first splitting the writing into component shapes. Then the programs compare the component shapes by looking at the features like the number of edges, vertices, and loops, as well as the angle and the distances between parts of the shapes.

This presentation will compare the performance of FLASH ID and handwriter on the same questioned documents. In a preliminary experiment, FLASH ID and handwriter were both given three London Letter handwriting samples from 90 writers as known handwriting samples and one *Wizard of Oz* prompt from each of the 90 writers as questioned documents. FLASH ID correctly ranked the true writer as the most likely writer for all 90 questioned documents. The handwriter package correctly assigned the highest posterior probability to the true writer for 88 of the 90 questioned documents. This presentation will explore the programs’ performance on more questioned documents, including documents of different lengths. The questioned documents will come from three publicly available handwriting datasets: the CSAFE Handwriting Database; CLV-Database; and the IAM Handwriting Database.<sup>3-5</sup>

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### Forensic Statistics; Handwriting Analysis; Hierarchical Modeling

## K17 Determining Authorship Among the Handwriting of Fifth Graders: A Case Study

*Sandra Raudabaugh, BS\*, SLM Forensic Consultants, Harrisburg, PA*

**Learning Objective:** Attendees will learn that the comparison of writings during childhood developmental stages can produce meaningful results in actual casework.

**Impact Statement:** This presentation will impact the forensic science community by reaffirming through actual casework that individualizing characteristics are present in the handwriting of elementary school-aged children.

The development of handwriting and the appearance of individualizing characteristics among elementary school-aged children is a progressive process robustly studied and the results published in 2016.<sup>1</sup> This study, among others, will be reviewed and practically applied to an actual case that involves this developmental stage of writing. This case study incorporates a fundamental principle of handwriting that no two people write exactly alike and aims to determine if sufficient individuality exists at this developmental stage to arrive at meaningful results from comparison examinations. “Meaningful results” is defined as that which is useful and helpful to the end user (client).

This actual case study involves questioned, hand-printed text discovered inside an elementary school locker to be compared to the submitted known writings of ten 5<sup>th</sup> graders to possibly determine the author of the questioned, hand-printed text.

Due to the time-sensitive nature of this case, a screening process was conducted to narrow the field of suspect writers. Three writers were selected for further examination and additional known writing of the three writers was requested and received. Comparison examinations were subsequently conducted.

The outcomes of this study concluded the known writings of 5th graders were sufficiently individualizing to not only differentiate between the writers, but also to support that the known writing was sufficiently individualizing to opine that one of the writers probably did write the text in question. The results were deemed by the client to be meaningful.

Some limitations were introduced to these examinations in the form of a poor image of the questioned text, the writing position within a cramped space, and the use of a felt-tipped writing instrument. There were also some deficiencies noted in the comparability of the submitted known writings where some of the irreconcilable features between the writings were attributed to: the writing position and spatial constraints of the questioned text; incomparable known writing; and/or accidental or developmental occurrences at the moment of execution of the questioned text.

This actual case study supports and is supported by the research in the progression of individuality of handwriting among elementary school-aged children who are still developing their handwriting proficiency.

### Reference:

<sup>1</sup>. S. N. Srihari, L. Meng, L. Hanson, Development of Individuality in Children’s Handwriting. *J. Forensic Sci.* 61, 1292-1300 (2016).

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### Handwriting; Childhood Development; Progression of Individualizing Characteristics

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## K18 Handwriting Comparison Examinations With Limited Known Writing Samples

*Donald Moryan, BS\*, USACIL, Forest Park, GA*

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**Learning Objective:** This presentation will inform attendees that given handwriting cases with limited amounts of handwriting samples can still be examined with positive results.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that even though at first glance what looks like not enough evidence to conduct a positive examination, full scrutiny is needed in all handwriting comparison examinations.

Given limited writing samples to compare to questioned notes usually prevents the forensic document examiner from drawing a positive conclusion (either toward or away from the writer of the known samples) in a handwriting comparison examination. To say that two writings, the questioned and known writing, were made by the same individual, it is necessary to show that the two writings have the same similarities and contain no significant differences. For this to occur, it is obvious that the best writing samples must include dictated known writing samples and pre-existing or course-of-business writing samples. The writing samples must also be in the same style as the questioned writing—cursive vs. cursive, hand printing vs. hand printing, upper case letters vs. upper case letters, etc. It is also essential that the known writing samples be contemporaneous. That is known writing produced around the same time frame as the questioned writing. The amount of writing necessary for comparison differs in different cases but, if possible, enough should always be obtained to clearly show the writing habits of the one whose writing is under scrutiny.

In this case study, three different handwriting comparison cases are examined. All three cases involved the examination of questioned handwritten notes with a limited number of known standards submitted for examination. Also, in all three cases, only known writing samples of the course-of-business variety were submitted. No dictated writing samples were available for submission. One case involved limited known writing from company forms that was used to compare to a full-paged note, including a signature. Another case involved a full-paged question note and only three small known writing samples were submitted, some of which were not comparable to the questioned writing. The last case involved another full-paged note and the known writing consisted of one page of dubious writing and one distorted signature.

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### Handwriting Comparison; Known Writing Samples; Questioned Writing



## K19 Signature Verification Algorithms: What They Are, How They Are Used, and How to Evaluate Them

*Kevin Kulbacki, MSFS\*, KDX Forensic Consulting, LLC, Chicago, IL; Nikolaos Kalantzis, MSc, Chartoularios Institute, Palaio Faliro, Attiki, GREECE; Batya Fuchs, MSc, DIFS Israel Police, Jerusalem, ISRAEL*

**Learning Objective:** After attending this presentation, attendees will better understand what Signature Verification Algorithms (SVAs) are, how they are used, and what factors must be considered when conducting validation studies of SVAs. This presentation will further discuss additional research necessary for understanding the implications of SVA implementation in both forensic and non-forensic deployments.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of Signature Verification Algorithms (SVAs).<sup>1</sup>

Over the past decade, there has been a significant shift toward conducting business in a paperless and digital manner. As part of this transition, the collection of Digitally Captured Signatures (DCS; also known as biometric signatures, online signatures, or dynamic signatures) has increasingly replaced traditional static signatures written on paper. Traditionally, digitization of signatures has been accomplished with flat (2-dimensional) scanning of a signature executed on paper that leads to the creation of a bitmap file (i.e., static signatures). While static signatures represent visual information in the X and Y coordinates only (and hence lack information regarding the dynamic aspects of signing), dynamic signatures capture the X and Y coordinates as well as Time and Force.

In conjunction with the faster pace of the digital world, businesses have had to adapt their fraud prevention efforts to handle not only more transactions, but also more sophisticated attempts at fraud. While static signatures allow for the comparison of geometric comparison metrics, the inherent numerical nature of the DCS data allows the contemplation of statistical methods to characterize a group of “known” DCS and then compare a “questioned” DCS versus that group, effectively performing a statistical evaluation of authenticity. Various companies have invested in the creation of such Signature Verification Algorithms (SVAs) to potentially determine whether or not a given signature was actually prepared by the purported author.

During this presentation, attendees will be introduced to what SVAs are, how they are used, and what factors must be considered when conducting validation studies of SVAs. This presentation will also discuss further additional research necessary for understanding the implications of SVA implementation in both forensic and non-forensic deployments.

This presentation is intended to only provide an introduction and overview of SVAs and will not discuss the results of any particular SVA validation study.

### Reference:

- <sup>1</sup>. Kulbacki, K. P., Kalantzis, N., & Fuchs, B. M. (2022). Technical Note: Signature Verification Algorithms: What They Are, How They are Used, and How to Evaluate Them. *Journal of the American Society of Questioned Document Examiners, Inc.*, 25(1), 3–8.

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### Handwriting; Signatures; Algorithms

## K20 Signature Verification Algorithms: Potential Forensic Uses and Bias Considerations

Kevin Kulbacki, MSFS\*, KDX Forensic Consulting, LLC, Chicago, IL; Nikolaos Kalantzis, MSc, Chartoularios Institute, Palaio Faliro, Attiki, GREECE; Batya Fuchs, MSc, DIFS Israel Police, Jerusalem, ISRAEL

**Learning Objective:** After attending this presentation, attendees will be aware of potential forensic applications of Signature Verification Algorithms (SVAs) as well as the potential cognitive bias implications of SVA usage in any application.

**Impact Statement:** This presentation will impact the forensic science community by introducing potential forensic applications of Signature Verification Algorithms (SVAs) as well as by highlighting the potential cognitive bias implications of SVA usage in any application.<sup>1</sup>

Over the past decade, there has been a significant shift toward conducting business in a paperless and digital manner. As part of this transition, the collection of Digitally Captured Signatures (DCS, also known as biometric signatures, online signatures, or dynamic signatures) has increasingly replaced traditional static signatures written on paper. While pen and paper static signatures can be digitized via imaging/scanning, the resulting image represents visual information in the X and Y coordinates only (and hence lack information regarding the dynamic aspects of signing). Conversely, dynamic signatures capture the X and Y coordinates as well as Time and Force. In conjunction with the faster pace of the digital world, businesses have had to adapt their fraud prevention efforts to handle not only more transactions, but also more sophisticated attempts at fraud. As part of these efforts, various companies have invested in the creation of Signature Verification Algorithms (SVAs). SVAs compare presented (questioned) and known signatures to potentially determine whether or not a given signature was prepared by the purported author. While SVAs can be developed for both static and dynamic (DCS) signatures, the numerical nature of the DCS captured data allows for more in-depth analysis by an SVA.

SVAs are currently deployed in the banking sector as a form of authentication, providing live feedback regarding the authenticity of a signature executed at the teller or alternatively as a means of subsequently reviewing transactions. SVAs are currently deployed as a means of access control in military facilities. These are not the only possible applications of SVAs. This presentation will explore the possible uses, and subsequent requirements, for utilizing an SVA within a forensic context. Such forensic implementations could potentially include either assisting the Forensic Document Examiner in their analysis task or alternatively serving as an extra peer reviewing safeguard. Furthermore, this presentation will discuss the potential cognitive bias implications of SVA usage in any application.

### Reference:

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### Signature Verification Algorithms; Digitally Captured Signatures; Cognitive Bias

## K21 The Robert Durst Case: Revisiting Myths of Hand Printing Examinations

*Linton Mohammed, PhD\*, Forensic Science Consultants, Inc, Poway, CA; Lloyd Cunningham, Alamo, CA*

**Learning Objective:** Attendees will learn about a high-profile case that involved hand printing. Myths involving hand printing examination will be presented, together with information about how to deal with them.

**Impact Statement:** The forensic science community will receive information about misunderstood handwriting examination techniques. This information will hopefully strengthen the admissibility of hand printing examinations.

Robert Durst was a multi-millionaire who prospered from funds received from his family's successful New York real estate business. He met a young lady by the name of Kathy McCormack, and they eventually got married. She attended medical school and during that time, Robert became very abusive, which led her to tell him that she wanted a divorce. Just prior to graduating from medical school, Kathy disappeared, never to be seen again. During the investigation, police found the "Dig List" in Durst's home trash can. This "Dig List" was comprised of the words, "Town – dump," "bridge," "dig," "boat," "other," shovel or, check car – truck or trunk/rent."

Robert had a very close friend, a confidante named Susan Berman who lived in Beverly Hills, CA. Apparently, Susan had knowledge of what really happened to his wife, Kathy. As the investigation heated up, investigators contacted Susan and asked if they could interview her about the disappearance of Kathy. She agreed to the interview, then she contacted Robert and told him about the pending interview; she also told him that she needed money. Apparently, Robert was very concerned that Susan would provide the investigators with information that he was complicit in the disappearance of Kathy, so he arranged to visit Susan at her Beverly Hills home.

Susan was found dead from a 9mm gunshot to the back of her skull. The next day, the Beverly Hills Police Department received an anonymous letter that was hand printed with uppercase letters that read, "1527 Benedict Canyon—Cadaver." This hand printing became vital to the case, especially as Durst admitted that whoever wrote the "Cadaver" note was the killer.

Robert Durst adamantly denied that he wrote the "Cadaver Letter," and he also adamantly denied that he was even in Los Angeles at the time of the murder of his best friend, Susan Berman. There was also an inference from Durst's team of lawyers that hand printing cannot be identified.

At the request of the LA District Attorney's Office, Lloyd Cunningham and Linton Mohammed were retained to conduct examinations of the "Cadaver" envelope and note, and the "Dig Note." Both FDES identified Robert Durst as the writer of the questioned entries.

Professor Mark Denbeaux was hired by the defense to try to get the handwriting testimony excluded. Denbeaux wrote a lengthy declaration that was filled with erroneous statements about handwriting examination.

The judge presiding over the Durst murder trial ordered Lloyd Cunningham and Dr. Linton Mohammed to prepare a joint rebuttal declaration in opposition to Professor Denbeaux's declaration. The order from the court included nine specific questions from the defense about information included in Lloyd Cunningham's formal report. These nine questions were to be answered with the utmost detail. Dr. Mohammed and Lloyd Cunningham prepared a 26-page rebuttal declaration, and it was submitted to the Superior Court Judge so he could make the decision to allow or reject the hand printing evidence at trial.

The judge ruled that the forensic hand printing evidence would be admissible. The defense then admitted that their client Robert Durst was in Los Angeles at the time of the murder and that he did write the Cadaver letter. They also admitted that Durst wrote the Dig List that was related to the disappearance of his wife, Kathy.

Durst was found guilty of first-degree murder September 17, 2021, and sentenced to life in prison without the possibility of parole. Durst died shortly afterward, and his conviction was vacated posthumously due to his death during a pending appeal that resulted in a cancellation of conviction.

This presentation will describe the handwriting examination and discuss the rebuttal report.

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**Hand Printing Examination; Forensic Document Examination; Handwriting Examination**

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## K22 A Failure Analysis of Ink Samples Using Multiple Modes of Pyrolysis-Gas Chromatography/Mass Spectrometry (PY-GC/MS)

*Athena Nguyen, BS\*, Frontier Lab America, Katy, TX*

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**Learning Objective:** After attending this presentation, attendees will learn the technique of detecting and identifying differences in like polymer materials.

**Impact Statement:** As detecting and identifying differences in like polymer materials are key factors in failure and deformation analysis and reverse engineering, the forensic science community will benefit by learning a technique (Pyrolysis-GC/MS) that expands the application in GC/MS. It is an easy solid sample introduction that provides precise and reproducible results without the need for solvent extraction.

Detecting and identifying differences in like polymer materials are key factors in failure and deformation analysis and reverse engineering. A simple technique that can be used is Pyrolysis-Gas Chromatography/Mass Spectrometry (Py-GC/MS). Py-GC/MS is an easy solid sample introduction technique that expands the application areas of GC/MS. Frontier Lab developed a micro-furnace pyrolyzer based on the vertical micro-furnace design. The multi-mode Micro-Furnace pyrolyzer with different modes of operations, including Evolve Gas Analysis (EGA), Flash pyrolysis (single-shot), Thermal Desorption (TD), Double-shot (thermal desorption followed by flash pyrolysis), and Heart-Cutting (HC) can be operated in the same system. The method requires very little sample preparation. It is very straightforward and efficient. Depending on the sample type, appropriate sampling tools can be chosen and used. The sample is placed into the sample cup and can be analyzed as-is; there is no need for any solvents or solvent extraction. The first step is to perform an EGA. This technique provides information about the thermal temperature behaviors of the sample. The EGA thermogram is then used to determine the next steps in the separation analysis process, which include obtaining the optimum temperature for flash pyrolysis as well as thermal zones.

In this presentation, deformation and failure analyses were performed on two ink samples. Each ingredient in good and bad ink was compared to determine the cause of failure. The ink contains solvent, resin, and dye. Resin powders are weighed, placed into the cup, and analyzed. Ink and dye liquid samples were taken by a needle and placed into the cup and analyzed. Evolve gas analysis (EGA-MS), Flash pyrolysis (single-shot), and Heart-cutting (HC) modes of operations were utilized. Data analysis was performed using the F-search libraries, which provides the compound name, its structure, and polymer source. The obtained pyrograms and thermograms provide detailed information about the differences.

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### Py-GC/MS; GC/MS; Sample Preparation

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## K23 A Spectroscopic Evaluation of Two Black Pen Inks Using Video Spectral Comparator

*Rafal Turek, MS\*, Internal Revenue Service, Chicago, IL*

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**Learning Objective:** The goal of this presentation is to present the dual properties of two black ink colors.

**Impact Statement:** This presentation will impact the forensic science community by making examiners aware that the spectroscopic evaluation of similarly colored inks should be considered as a supplementary tool for sample characterization.

Dual color properties of two black inks were observed and characterized using the spectrometry tool of the Video Spectral Comparator. It is fundamental for a forensic document examiner to be able to use a non-destructive tool that delivers useful information. The present study demonstrates the usefulness and prospective application of a spectrophotometer tool for analysis of inks in handwritten documents. The method presented in this study is a non-destructive technique and allows examiners to compare and analyze various colorants.

Spectroscopic examination was conducted on three pages of a contract agreement. Comparisons were made between ink entries on each page, then between ink entries on all three pages, and spectra were made.

The multiple spectrograms reveal intensity of reflectance differences when a sample is taken from the same ink on different points. This observation could be directly proportional to the opacity of the colorant in those points. The spectrograms also show a very similar graph profile in the visible region.

While there were some conspicuous similarities between the spectra in the visible range, there were differences in the range above 700nm. The dual color properties area is observable above 700nm. Since light outside of this range is not perceived by the human eye, deeper study of this area under spectrophotometer helps in ink differentiation for the samples of similar color that are not separated in the visible range. Whereas there were minute differences in intensity of reflectance of the graphs in the visible area, there were perceptible and often different profile graphs in the area above 700nm. This phenomenon helps to distinguish inks having similar color visible by the human eye.

Those inks may have a similar spectral profile in the visible range, but they could differ vastly in the area above 700nm.

This presentation will make examiners aware that spectroscopic evaluation of similarly colored inks should be considered as a supplementary means for sample characterization.

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**Color; Spectra; Ink**

## **L1      A Hospital Serial Murder by Benzodiazepine Poisoning**

*Pietro Zuccarello\*, University of Catania, Catania, Italy; Nunziata Barbera, University of Catania, Catania, Italy; Giulia Carnazza, University of Catania, Catania, Italy; Massimiliano Esposito, University of Catania, Catania, Italy; Giuseppe Cocimano, University of Catania, Catania, Italy; Cristoforo Pomara, University of Catania, Catania, Italy*

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**WITHDRAWN**

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**L2     The Quantitation of Five Benzodiazepines and Zolpidem in Whole Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

*Ivana Antunovic, MSc\*, FSC Ivan Vucetic, Zagreb, Grad Zagreb, CROATIA; Sandra Mudrić, MSc, Forensic Science Centre Ivan Vucetic, Zagreb, Grad Zagreb, CROATIA; Tomislav Houra, MSc, Forensic Science Centre Ivan Vučetić, Ministry of the Interior of the Republic of Croatia, Zagreb, Grad Zagreb, CROATIA; Elizabeta Stojaković, MSc, Ministry of the Interior, Forensic Science Centre Ivan Vučetić, Zagreb, Grad Zagreb, CROATIA; Maja Jelena Čop, PhD, Forensic Science Centre, Zagreb, Grad Zagreb, CROATIA*

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**Learning Objective:** Concerned with the wide use of benzodiazepines and zolpidem in the local population, an effective LC/MS/MS method for quantification of five common benzodiazepines and zolpidem in whole blood was developed.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees of a fast quantification of five common benzodiazepines and zolpidem in whole blood samples from cases regarding driving under the influence, suspicious deaths, and other crimes.

**Methods:** For method development, 0.5mL OF drug-free blood was spiked with standards (Lipomed) of the six analytes (diazepam, nordazepam, oxazepam, alprazolam, midazolam, and zolpidem) to achieve the nine calibration samples (concentration range 5-1,200ng/mL); respective deuterated internal standards in 250ng/mL concentration were also used. Samples were extracted using Solid-Phase Extraction (SPE) Evolute Express CX 60 mg/3mL (Biotage®) and GX-271 Automated Solid Phase Extraction Cartridge (ASPEC) System (Gilson®). The extract was evaporated and reconstituted, then injected into the LC/MS/MS system (1290 Infinity® II binary pump, a thermostatted multisampler, a temperature controlled column compartment, and a 6470 triple quadrupole mass spectrometer by Agilent® Technologies). Chromatographic separation was performed at 55°C using an Agilent® Poroshell 120 EC-C18, 2.1 x 50mm, 1.9µm. Duration of the analysis was 1.9min. The mobile phase consisted of water (0.01% formic acid and 5mM ammonium formate) and methanol (0.01% formic acid) at a flow rate of 0.90mL/min. Mass spectrometric analysis was performed using electrospray ionization in positive-ion Multiple Reaction Monitoring (MRM) mode. Limit of quantitation for all analytes is 5ng/mL. Calibration linearity (r<sup>2</sup>) is above 0.99. The same protocol was applied to the analysis of case specimens. The protocol described above was evaluated and found to achieve the required experimental criteria. Commercially available control samples were in permitted range.

**Conclusion:** Benzodiazepines are one of the most frequently prescribed medications in the country and, therefore, are often present in biological samples that require toxicological analysis. In the past five years, cases that require quantification of benzodiazepines are on the rise. The method described above was successfully implemented in the routine work. This method is more efficient, sensitive, and with a shorter run time than the method previously used (gas chromatography/mass spectrometry in single ion mode) for these kind of analyses. There is no need for derivatization of the samples.

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**LC/MS/MS; Benzodiazepines; Blood**



### **L3     The Quantitation of Fentanyl, P-Fluorofentanyl, and Xylazine in Postmortem Blood by Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)**

Michael Truver, PhD\*, University of Florida, Gainesville, FL; Sara Brogan, BS, University of Florida, Gainesville, FL; Amy Kinsey, MS, University of Florida, Gainesville, FL; Jennifer Hoyer, MS, University of Florida, Gainesville, FL; Chris Chronister, PhD, University of Florida, Gainesville, FL; Bruce Goldberger, PhD, University of Florida College of Medicine, Gainesville, FL

**Learning Objective:** After attending this presentation, attendees will have a better understanding of a method validated for 4-ANPP, acetyl fentanyl, fentanyl, p-fluorofentanyl, norfentanyl, and xylazine in blood by LC/MS/MS. This method was then utilized to determine concentrations in postmortem blood.

**Impact Statement:** This presentation will impact the forensic science community by filling a knowledge gap regarding fentanyl, p-fluorofentanyl, and xylazine in postmortem blood.

The opioid epidemic still is prominent in the United States. Fentanyl concentrations recently have been detected in combination with compounds such as p-fluorofentanyl and xylazine. The purpose of this study was to develop and validate an analytical method for the quantification of 4-ANPP, acetyl fentanyl, fentanyl, p-fluorofentanyl, norfentanyl, and xylazine in blood samples utilizing LC/MS/MS. Blood samples previously analyzed for acetyl fentanyl, fentanyl, and p-fluorofentanyl by Gas Chromatography/Mass Spectrometry (GC/MS) were retested by the newly developed LC/MS/MS method. A Waters Acquity™ Ultra Performance Liquid Chromatography (UPLC) system equipped with an Electrospray Ionization (ESI) source and TQ detector was utilized.

Chromatographic separation was achieved using an Agilent® RX-SIL column (1.7µm, 2.1 x100 mm). The analytes of interest were 4-ANPP, acetyl fentanyl, fentanyl, p-fluorofentanyl, norfentanyl, and xylazine. M-fluorofentanyl and o-fluorofentanyl were qualitatively monitored. Deuterated internal standards were used for all six analytes of interest. The method was validated in accordance with the Academy Standards Board (ASB) Standard Practices for Method Development in Forensic Toxicology.<sup>1</sup> Blood samples ( $n=58$ ) previously quantitated by GC/MS were analyzed by the newly developed LC/MS/MS method.<sup>2</sup> Blood samples for this study were selected based on test ordered and/or case history.

The lower Limit Of Quantitation (LOQ) for all analytes was 0.1ng/mL, with the exception of xylazine (0.2ng/mL). The upper LOQ was 100ng/mL. Calibration curves for 4-ANPP, acetyl fentanyl, fentanyl, and norfentanyl were fit using a linear regression model with 1/x weighting; calibration curves for p-fluorofentanyl and xylazine were fit using a quadratic regression model with 1/x weighting. Analysis of ten blank blood samples revealed no interferences. Dilution integrity of control samples in blood at 2x, 10x, and 100x dilution indicated % target concentrations ranging from 82-105%. Stability was assessed at three different concentrations for 72h in the autosampler unit at 10C and the % difference was -5% to 3%. Bias and imprecision did not exceed  $\pm 13.1\%$ . 4-ANPP carried-over following the highest calibrator, but carry-over was mitigated with an additional solvent wash. Matrix effects in blood were  $\leq \pm 25\%$  for all analytes, with the exception of xylazine (61%). The enhancement of signal was deemed acceptable as the deuterated internal standard also demonstrated comparable enhancement and did not compromise the method parameters including LOQ, precision, and bias.

Reanalysis of 58 blood samples by LC/MS/MS produced comparable results in 53 samples with a concentration range of fentanyl of 0.27-66ng/mL. Six samples were negative by GC/MS, and five samples were negative by LC/MS/MS. The concentration of the outlier was 0.27ng/mL, below the GC/MS lower LOQ. Concentration ranges of the other analytes were: 4-ANPP, 0.11-12ng/mL; acetyl fentanyl, 0.18ng/mL ( $n=1$ ); p-fluorofentanyl, 0.14-33ng/mL; norfentanyl, 0.14-98ng/mL; and xylazine, 0.24-108ng/mL. Compared to GC/MS, the LC/MS/MS improved the detection of p-fluorofentanyl (7 samples v. 20 samples). Finally, xylazine was identified in 22% of the samples, and 61% of the xylazine-positive samples were also positive for p-fluorofentanyl.

In conclusion, an LC/MS/MS method was developed for the quantitation of 4-ANPP, acetyl fentanyl, fentanyl, p-fluorofentanyl, norfentanyl, and xylazine in blood. Validation studies were conducted and the method met all ASB validation criteria. This method can be used to establish the prevalence of synthetic opioids such as p-fluorofentanyl and xylazine in postmortem casework.

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2. Truver MT, Chronister CW, Kinsey AK, Hoyer JL, Goldberger BA. (2022) Toxicological analysis of fluorofentanyl isomers in postmortem blood. *Journal of Analytical Toxicology*, <https://doi.org/10.1093/jat/bkac014>.

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#### **Fentanyl; Xylazine; Para-Fluorofentanyl**

## **L4 A New Approach for Identifying the Postmortem Formation of Ethanol: The Development and Validation of a Salt-Assisted Headspace/Gas Chromatograph/Flame Ionization Detector (HS/GC/FID) Method for the Determination of Ethanol in Vitreous Humor**

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**Learning Objective:** After attending this presentation, attendees will have received detailed information on the importance of using Vitreous Humor (VH) as an alternative biofluid for detecting ethanol in cadaveric specimens. The present study is aimed at the assessment of the postmortem formation of ethanol as a result of putrefactive phenomena.

**Impact Statement:** This presentation will impact the forensic science community by providing a new approach for accurately evaluating the content of ethanol also in a cadaveric alternative biofluid, such as VH. The proposed method has the potential to offer an alternative approach for the assessment of postmortem ethanol formation using a rapid salt-assisted HS/GC/FID method.

In Western countries, ethanol is the most used and abused substance, and it is by far the most frequently detected psychoactive compound in cadaveric specimens involving homicides, suicides, work-related accidents, motor vehicle crashes, drownings, and other violent deaths.<sup>1</sup> In such cases, the estimation of Blood Alcohol Concentrations (BAC) is needed not only as evidence of acute intoxication but also to comply with the laws on the fitness to drive and work. Considering the adoption by the law of threshold limits of concentration, the used analytical methods require the highest accuracy in view of the legal consequences related to the BAC interpretation. In addition to the analytical validation, the pre-analytical phases (specimen collection, storage, etc.) also have in this context a particular relevance. Of crucial importance in the pre-analytical chain is the need for avoiding specimen/analyte degradation. As per ethanol, special care has to be given to hinder the activation of fermentation and/or putrefaction phenomena, which for deaths of forensic interest often affect the body and the biological specimens collected during postmortem. In fact, several authors reported that, in the presence of carbohydrates, various bacteria, yeast, and fungi are capable of synthesizing ethanol in vitro as well as in tissues, potentially causing a spurious increment of blood ethanol concentration that, in some cases, may lead to “positive” results in an otherwise “negative” specimen.<sup>2-4</sup> Although the integrity of blood specimens can be adequately guaranteed after the sampling, the quantification of ethanol in cadaveric blood can be affected by postmortem fermentative phenomena occurring between the time since death and the sampling of biofluids. Consequently, the interpretation of BAC values in postmortem specimens requires particular attention to avoid misleading conclusions. In comparison to blood, vitreous humor is less affected by putrefactive phenomena, allowing compound determination and its use as an alternative biological matrix. The present work was aimed at developing and validating a method using the salting-out effect and based on HS/GC/FID for detecting ethanol in vitreous humor.

The reported analytical method is based on a simple VH pre-treatment consisting of a dilution (1:9) with a solution of 2.5 mol/L K<sub>2</sub>CO<sub>3</sub> and 0.0012 mol/L tert-butanol (internal standard). After 1 minute of incubation, part of the specimen evaporated in the headspace (2,000 µL) is injected into the chromatographic system and analyzed in isothermal mode (40°C), with a chromatographic time of 1.6 minutes. Total run time was 2.6 minutes.

The method was validated in terms of selectivity, lowest limit of detection, intraday and total imprecision, and trueness (bias). The determination of ethanol in vitreous humor and blood was carried out in 75 cases. The correlation between the two matrices was confirmed in 61 cases. However, 14 vitreous humor specimens showed lower ethanol concentrations, and in the related blood specimens, it was possible to identify the signal of n-propanol, a typical product of postmortem microbial fermentation, that could justify the excess of ethanol in the blood specimens.

In conclusion, this presentation will show the results of the validation and application to real cases of the developed method for determining ethanol in vitreous humor as an alternative biofluid in forensic cases, which, because of putrefactive phenomena, could have produced postmortem ethanol affecting the blood alcohol concentration.

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### **Vitreous Humor; Ethanol Postmortem Formation; Postmortem Forensic Toxicology**

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**L5 Comparing Density Functional Theory (DFT) Calculations and Surface-Enhanced Raman Spectroscopy (SERS) Results to Differentiate Structurally Similar Fentanyl Analogs**

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**Learning Objective:** New synthetic opioids, especially fentanyl and its analogs, are causing the most recent acceleration in opioid abuse. The presence of fentanyl analogs as mixtures in illicit drugs makes it hard to estimate their potencies. There are so many structurally similar fentanyl analogs with different potencies. Thus, it is important to differentiate these analogs from similar molecules in order to track and identify trends in illicit distribution. In this presentation, we develop methods for the differentiation of structurally similar fentanyl analogs using theoretical and experimental methods.

**Impact Statement:** The ultimate goal of this presentation will be to assist law enforcement in identifying and differentiating unknown fentanyl analogs individually and in drug mixtures. The experimental results obtained in this project can be readily implemented in field applications and in smaller laboratories, where inexpensive portable Raman spectrometers are often present and are used in drug analysis.

The detection and differentiation of fentanyl analogs is critically significant. Most of the screening methods in current use have difficulty in detecting the full range of opioid analogs due to a wide variety of structural variations. However, Raman spectroscopy, specifically SERS is quite capable of detecting and identifying previously known and/or unknown fentanyl analogs. The SERS technique uses Raman spectroscopy combined with colloidal metal nanoparticles to yield highly sensitive SERS spectra. It can also differentiate structurally similar fentanyl analogs due to its ability to yield spectroscopic fingerprints for the detected molecules. Certain fentanyl analogs, such as carfentanil, furanyl fentanyl, acetyl fentanyl, 4- fluoroisobutyryl fentanyl, and cyclopropyl fentanyl, have gained popularity and constitute 76.4 percent of the fentanyl analogs identified in drug seizures. Several of these have been already described using Raman spectroscopy. However, there are many other fentanyl analogs that are structurally similar to 4- fluoroisobutyryl fentanyl or cyclopropyl fentanyl. Thus, it is important to differentiate these analogs from similar molecules in order to track and identify trends in illicit distribution.

In this presentation, we develop methods for the differentiation of structurally similar fentanyl analogs using theoretical and experimental methods. To do this, a set of fentanyl analogs were examined using Density Functional Theory (DFT) calculations. These results were then compared with Normal Raman and SERS techniques and analyzed using statistical methods. Structurally similar fentanyl analogs have been able to be differentiated from each other.

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**Fentanyl Analogs; SERS; DFT**

## L6 The Simultaneous Determination of 24 Designer Benzodiazepines in Hair Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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**Learning Objective:** After attending this presentation, attendees will be aware of a method that can be helpful to investigate the illegal abuse of 24 designer benzodiazepines and sedative-drug use in suspected rape victims or drugged drivers using hair analysis.

**Impact Statement:** This presentation will impact the forensic science community by helping attendees understand LC/MS/MS analysis of 24 new designer benzodiazepines in human hair.<sup>1,2</sup>

In the field of forensic science, hair is one of the critical samples for detecting abused or misused drugs in Drug-Facilitated Crime (DFC), which includes Drug-Facilitated Sexual Abuse (DFSA) or Driving Under the Influence of Drugs (DUID), including long-term exposure.<sup>3</sup> Designer benzodiazepines are one group of New Psychoactive Substances (NPS) with several side effects such as drowsiness, dizziness, strong sedation, short-term memory loss, or slight euphoria. Due to these sedating effects, especially hypnosis and amnesia, they can be used for DFC. In this study, we developed an LC/MS/MS method for the simultaneous determination of 24 new different designer benzodiazepines in hair; adinazolam, bromazolam, diclazepam, flualprazolam, flubromazepam, flubromazolam, meclonazepam, metizolam, brotizolam, camazepam, cloxazolam, delorazepam, fluclozepam, fludiazepam, flutoprazepam, halazepam, ketazolam, loprazepam, mexazolam, oxazolam, phenazepam, pinazepam, quazepam, and tetrazepam.<sup>1</sup>

Hair samples were put into a reservoir and accurately weighed to about 10mg, followed by mounting the reservoir in the manifold. Then the samples were separately washed with 3mL of methanol, 3mL of distilled-deionized water, and 3mL of methanol. After drying, the samples were finely cut, and diazepam-d<sub>5</sub> of the internal standard was added. The samples were incubated in 2mL of methanol with a magnetic stirrer 38°C for 16h. The extracts were evaporated, then the residue was reconstituted, filtered, and injected into the LC/MS/MS. Selectivity, linearity, the Limit Of Detection (LOD), the Limit Of Quantification (LOQ), precision, accuracy, matrix effects, recovery, and process efficiency were evaluated, and all results were acceptable. The LOD and LOQ ranged from 0.1 to 1pg/mg in hair and 0.5 to 3pg/mg in hair, respectively. Linearity was achieved within the range of 0.5-500pg/mg in hair, except oxazolam, and correlation coefficients (R<sup>2</sup>) ranged from 0.97 to 0.99. From both the intra- and inter-day analysis, the precision (CV %) values ranged from 0.36 to 12.1 %, and the accuracy (bias %) ranged from -5.3 to 15.0 % at low, medium, and high QC samples. The matrix effect values ranged from 63.2 to 184.7 % (mean 95.0%) in three QC samples examined. The values of recoveries and process efficiencies were mean 86.9 % and 81.6 %, respectively. This method can be helpful to investigate these new benzodiazepines in the hair of common drug abusers as well as suspected rape victims and drugged drivers using hair analysis.<sup>3,4</sup>

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### Designer Benzodiazepines; Hair; LC/MS/MS

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**L7     Evaluating Cannabinoid Stability in Different Conditions and Validating THC-O and THC-P for Oral Fluid Analysis**

*Cody Paseur, MSFS\*, Huntsville, AL; Curt Harper, PhD, Alabama Department of Forensic Sciences, Hoover, AL; Elizabeth Gardner, BS, PhD, University of Alabama at Birmingham, Department of Criminal Justice, Birmingham, AL*

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**Learning Objective:** This presentation will show the impact of the 2018 Farm Bill on the rise of novel cannabinoids in Alabama that need to be validated for analysis in matrices such as blood and urine. It will highlight the stability and other characteristics of Tetrahydrocannabinol (THC) and novel cannabinoids such as THC-O and THC-P in oral fluid. Finally, this presentation will illustrate several factors that could affect the accuracy of cannabinoid analysis in oral fluid.

**Impact Statement:** This presentation will impact the forensic science community by illustrating the validation of THC-O and THC-P to be added to an existing method of analyzing cannabinoids in oral fluid. This is the first study to evaluate  $\Delta 9$ -THC and  $\Delta 8$ -THC stability in reanalyzed cases at two years of storage at 4°C and provide stability data for THC-P and THC-O in different conditions up to 120 days. This study will also add to the existing literature of the possibility for Cannabidiol (CBD) conversion to other cannabinoids prior to analysis.

Due to the 2018 Farm Bill, cannabinoids derived from *Cannabis sativa* plants with a  $\Delta 9$ -THC concentration  $\leq 0.3\%$  are technically legal. This has led to the manufacture of novel cannabinoids such as THC-O and THC-P for users who desire a legal high. THC-O and THC-P have been reported to be about 3 and 30 times more potent than  $\Delta 9$ -THC, respectively. These novel cannabinoids are being sold in products such as vaping liquids. Oral ingestion, smoking, and vaping allows drugs to concentrate into the oral fluid. At the Alabama Department of Forensic Sciences (ADFS), oral fluid specimens are collected with the Quantisal® collection device and sent to laboratory for confirmatory testing. The objectives of this study include: (1) to add novel cannabinoids  $\Delta 9$ -THC-P,  $\Delta 8$ -THC-P,  $\Delta 9$ -THC-O, and  $\Delta 8$ -THC-O to the previously validated ADFS cannabinoid oral fluid extraction method; (2) determine corrected  $\Delta 9$ -THC concentrations in previously analyzed ADFS cases using the Van Der Linden formula; (3) analyze the stability of THC-O, THC-P, and  $\Delta 9$ -THC in oral fluid at room temperature, 4°C, and -20°C for up to 120 days of storage; (4) evaluate stability of previously analyzed  $\Delta 9$ -THC- and  $\Delta 8$ -THC-positive oral fluid samples that have been stored for two years; and (5) evaluate CBD conversion to other targets in oral fluid.

Samples will be extracted by liquid-liquid extraction and analyzed using a validated method for evaluating cannabinoids in oral fluid on the Agilent® 6460 and/or 6470 Triple Quadrupole Mass Spectrometer. Limits Of Detection (LOD) for the novel cannabinoids  $\Delta 9/\Delta 8$ -THC-P, and  $\Delta 9/\Delta 8$  THC-O in oral fluid will be the lowest concentration analyzed in duplicate that meets Academy Standards Board (ASB) Standard 036, *Standard Practices for Method Validation in Forensic Toxicology*, criteria in 75% of samples in at least three batches. Matrix interference was evaluated by analyzing blank matrix samples from ten different sources. Analyte interference was evaluated by spiking oral fluid with all validated targets and other novel cannabinoids. For concentration correction, concentrations of previously analyzed  $\Delta 9$ -THC oral fluid cases was evaluated using the Van Der Linden formula. Stability will be evaluated by spiking oral fluid samples in triplicate with novel cannabinoids at simulated casework concentrations and Quantisal® collection will be simulated. Samples will be stored at room temperature, 4°C, and -20°C and analyzed at time points between 0-120 days of storage. Previously analyzed cases positive for  $\Delta 9$ -THC and/or  $\Delta 8$ -THC were and will continue to be reanalyzed after two years of storage at 4°C. CBD conversion will be evaluated by spiking oral fluid with mixes that do and do not contain CBD at equal concentrations.

LODs were 2ng/mL, 1ng/mL, 2ng/mL, and estimated to be 2ng/mL for  $\Delta 8$ -THC-P,  $\Delta 8$ -THC-O  $\Delta 9$ -THC-P, and  $\Delta 9$ -THC-O, respectively. Results show that the novel cannabinoids do not interfere with any validated targets, and there is no matrix interference. The average percent change between uncorrected and corrected  $\Delta 9$ -THC concentrations was 8.8% and the median was -0.5%.  $\Delta 9$ -THC concentration percent change for previously analyzed cases stored at 4°C was -44% at two years.

This study has validated a method to detect THC-P and THC-O using an existing cannabinoid oral fluid extraction method. The mean percent change after concentration correction lies within 18% uncertainty of measurement for the cannabinoids in oral fluid method. Stability data shows that  $\Delta 9$ -THC is not stable in oral fluid after being stored for one year at 4°C (Maxwell, 2022). This is the first study to evaluate  $\Delta 9$ -THC and  $\Delta 8$ -THC stability in reanalyzed cases at two years of storage at 4°C and provide stability data for THC-P and THC-O in different conditions up to 120 days.

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**Cannabinoids; Stability; Oral Fluid**

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**L8 Substance-Related Pediatric Deaths Examined at the West Tennessee Regional Forensic Center: An Analysis of the Past 16 Years**

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**Learning Objective:** This presentation will give attendees an overview of drug/substance toxicity related to pediatric autopsy examinations performed at the West Tennessee Regional Forensic Center with a discussion of demographics and substances involved.

**Impact Statement:** This will impact the forensic science community by providing greater insight into substance-related deaths, which is also affecting the most vulnerable portion of our population—children.

Substance-related deaths are an important public health burden globally. Drug-related deaths have increased in the United States and worldwide over the past decade. Per the National Center for Health Statistics, the ages of 35 to 44 have the highest number of drug-related deaths; however, pediatric cases also occur.<sup>1</sup> Since 2013, there is an unprecedented increase in fentanyl-related deaths in the United States, which also includes the pediatric population.

This study analyzes data from pediatric (0 days to 18 years) autopsies performed at West Tennessee Regional Forensic Center in Memphis, TN. The Death Investigation and Decedent Information (DIDI) database at the West Tennessee Regional Forensic Center was utilized and 1,807 pediatric autopsies were performed between 7/3/2006 to 6/30/2022. The cause of death for 38 cases (2.1 %) was drug/substance related to include prescription medication, illicit drugs, and alcohol. The most common manner of death was accident (60%), followed by undetermined (29%), suicide (8%), and homicide (3%), respectively. Sixty-one percent were male and thirty-nine percent were female. The age distribution was as follows: 13-18 years, 61%; 0-1 years, 16%; 2-4 years, 13%, and 5-12 years, 10%. Race distribution was 60% White, 29% Black, and 11% designated as other race. In this 16-year cohort, 50% of the deaths took place in the past five years. The most common substance responsible for pediatric deaths was fentanyl and fentanyl analogs detected in 39.5% of the cases, followed by heroin, morphine, and oxycodone at 10.5% each. In 21% of the cases, fentanyl (and metabolites) was the only drug detected. It was combined with fentanyl analogs (acetyl fentanyl or para-fluorofentanyl) at 10.6% and other drugs in 7.9% of cases. Among 15 fentanyl-related deaths, 13 of them occurred in the last five years. The age distribution of fentanyl-related deaths was as follows: 13-18 years, 53.3%; 2-4 years, 26.7%; 5-12 years, 13.3%, and 0-1 years, 6.7%. The average peripheral blood concentration of fentanyl in deaths was 22.88ng/dL, with the highest being 68ng/dL and the lowest being 6.2ng/dL. Substance-related deaths have substantially increased in our region recently, as seen in the United States overall. Pediatric substance-related deaths are also rising, most of them in our region associated with fentanyl.

**Reference:**

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**Pediatric; Toxicology; Fentanyl**



## L9 Is a Subdural a Time Capsule?

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of how subdural clots are susceptible to diffusion and the elimination of ethanol from the body.

**Impact Statement:** This presentation will impact the forensic science community by providing case studies that demonstrate subdural clots are not as useful as traditional specimens for postmortem ethanol analysis due to pharmacokinetics.

Traditionally, postmortem ethanol toxicology is performed on ocular fluid and peripheral blood. On occasion, subdural clots are submitted for toxicology analysis. Historically, it was thought that subdural clots were less vulnerable to diffusion and therefore could provide toxicological results at time of injury or clot formation. However, subdural clots are susceptible to diffusion and pharmacokinetics. Therefore, a subdural clot may not serve as a time capsule or provide an exact snapshot of the alcohol in a person's body at the time of the trauma. Twenty-two postmortem cases were documented over 16 months to examine the toxicology results of subdural clots compared to antemortem blood or postmortem blood and ocular fluid. Four cases are shown to demonstrate that subdural clots undergo diffusion and the results were similar to those of postmortem peripheral or heart blood. Additionally, two cases had hospital results taken upon admission.

**Case 1:** The decedent was found unresponsive and taken to a hospital. Hospital testing indicated the decedent's ethanol concentration was 0.29g/dL upon admission. The decedent died inpatient approximately three days later. In the postmortem toxicology testing, no ethanol was found present in the subdural clot.

**Case 2:** The decedent was involved in a motor vehicle collision and taken to a nearby hospital. Hospital testing indicated the decedent's ethanol was 0.18g/dL upon admission. The decedent died later the same day. In this case, antemortem blood specimens and postmortem specimens were analyzed. Toxicology results revealed the following ethanol concentrations: 0.03g/dL in subdural clot, 0.14g/dL in antemortem blood, 0.05g/dL in ocular fluid, and 0.02g/dL in heart blood.

**Case 3:** The decedent was pronounced dead on scene after a motor vehicle accident. In this case, the subdural clot, ocular fluid, and peripheral blood were tested. Toxicology results revealed the following ethanol concentrations: 0.14g/dL in subdural clot, 0.18g/dL in ocular fluid, and 0.15g/dL in peripheral blood.

**Case 4:** The decedent was found in the woods and later pronounced dead by emergency services. In this case, the subdural clot, ocular fluid, and peripheral blood were tested. Toxicology results revealed the following ethanol concentrations: 0.20g/dL in subdural clot, 0.21g/dL in ocular fluid, and 0.19g/dL in peripheral blood.

It was concluded after examining these cases that subdural clots may not act as a time capsule at the time of injury. The toxicological results demonstrated that subdural clots were similar to the results of the peripheral/heart blood. Therefore, subdural clot specimens were not found to be as beneficial as testing antemortem or traditional postmortem specimens to determine the ethanol concentrations in an individual.

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### Subdural; Postmortem Toxicology; Ethanol



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**L10 The Detection of Ethanol Consumption Markers and Drugs in Urine Samples From Inmates of Two German Prisons**

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**Learning Objective:** After attending this presentation, attendees will understand that this study, which is based on the detection of substances in urine samples of inmates, provides a representative estimation of drug use and alcohol consumption in jail, showing a high percentage of non-prescribed drug use, while alcohol consumption seems to play a minor role in German jails.

**Impact Statement:** The estimation of the prevalence of substance misuse in prison population could be useful to improve the surveillance measures and suggest public health interventions. According to our numbers, jails are a high-risk environment for non-prescribed drug use. Therefore, this presentation will impact the forensic science community by initiating and establishing techniques for regular drug checks in jail utilizing validated toxicological analyzing methods; these could be an important part of the prevention strategy against non-prescribed drug use and associated problems among prisoners.

**Introduction:** Prison population differs greatly from the general population in their experience of substance misuse, showing a higher prevalence. Despite the prison setting requiring rigorous abstinence from alcohol and drugs, the use of these substances among inmates is a well-known problem. While drugs are brought into prisons from the outside by smuggling, alcohol can be easily produced by fermenting processes within the prison. Different measures have been adopted to prevent and reduce the substance misuse in jails and associated problems (e.g., internal controls, drug detection dogs, etc.). A realistic estimate of the prevalence of substance misuse in prison could be useful to improve the surveillance measures and suggest public health interventions. However, literature provides data mainly based on surveys, which might not give reliable information. The present study aims at estimating the prevalence of substance consumption in prisoners through the detection of ethanol consumption markers, drugs, and their metabolites in urine samples.

**Materials and methods:** After obtaining the approval of an ethical review committee, voluntarily submitted urine samples from male inmates of two German prisons were collected and tested for previous ethanol consumption (EtG/EtS) as well as drugs (opiates, opioids, amphetamine, cocaine, cannabinoids) and their metabolites. Analyses were performed by validated Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) and Gas Chromatography/Mass Spectrometry (GC/MS).

**Results:** The participation rates in the two included German jails were 70–75 % and 30.6 %, respectively; 675 urine samples were analyzed for alcohol markers and drugs, of which 238 (35.3 %) tested positive. One or more drugs were found in 227 samples (95.4 %), EtG/EtS were detected in 4 samples (1.7 %), and 7 samples contained both substances (2.9 %). The most commonly detected substances were Tetrahydrocannabinol (THC) (44.5 %), buprenorphine (31.1 %), and methadone (31.1 %). Non-prescribed medication was found in 188 urine samples (79.0 %).

**Conclusion:** This cross-sectional study, based on the detection of substances in urine samples by validated analytical techniques, allowed us to obtain objective data about substance consumption in prisoners, showing a high percentage of illegal and non-prescribed drugs, while alcohol consumption seems to play a minor role in German jails.

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**Prison; Drug Use; Alcohol Consumption**

## L11 Quantitative and Qualitative Analysis of Fentanyl and Its Analogs in Urine Using Disposable Pipette Extraction

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**Learning Objective:** While attending this presentation, attendees will learn about the benefits of using Disposable Pipette eXtraction (DPX) tips on an automation platform to extract fentanyl and its analogs from urine. Additionally, attendees will be made aware of the abundance and relative concentrations of fentanyls in urine specimens from court-ordered drug monitoring and drug rehabilitation programs.

**Impact Statement:** This presentation will impact the forensic community by providing laboratories with an automated extraction method for the detection and quantitation of fentanyl and its analogs.

Fentanyl is a potent, fast-acting synthetic opioid that has become one of the more potent analgesics on the market. Overdose deaths related to synthetic opioids such as fentanyl has increased 10-fold since 2015, which is due to the drug's tolerance and physical-dependence liability.<sup>1,2</sup> Fentanyls have a high affinity for mu opioid receptors and relatively low affinity for kappa and delta opioid receptors.<sup>3</sup> Its potency is due primarily to its lipophilic nature, which allows fentanyl and its analogs to readily cross the blood-brain barrier where they have definitive effects on the central nervous system, including heightened euphoria and severe respiratory depression.<sup>1</sup> Rapid metabolism occurs in the liver, primarily through N-dealkylation, leading to the inactive metabolite norfentanyl.<sup>1</sup> Automated liquid-handling systems allow for high-throughput sample preparation with improved accuracy and precision compared to manual sample preparation. Dispersive extraction tips are automation tips that contain a loosely packed sorbent material and a tightly packed frit at the end of the tip. Mixing within the tip is achieved using a disperser that allows for efficient interaction between the analytes and the sorbent. Dispersive extraction Strong Cation Exchange (SCX) tips contain a sulfonated stationary phase with the styrene divinyl benzene ideal for binding with basic compounds such as fentanyl and its analogs (pKa~ 8.4).<sup>4,5</sup> A method was developed and validated that extracted fentanyl, norfentanyl, acetyl fentanyl, acryl fentanyl, furanyl fentanyl, isobutyl fentanyl, butyl fentanyl, valeryl fentanyl, para-fluorofentanyl, tetrahydrofuran fentanyl, and methoxyacetyl fentanyl. Fentanyl, norfentanyl, and acetyl fentanyl are quantitatively analyzed in this study, while all other analogs are qualitative.

Method validation studies included accuracy, precision, Limit of Detection/Lower Limit of Quantification (LOD/LLOQ), Upper Limits Of Linearity (ULOL), carryover, matrix effects, stability, recovery, dilution integrity, and interference from over 100 related and non-related drugs. Specimen preparation involved Solid Phase Extraction (SPE) (Strong Cation Exchange DPX 1mL) clean-up of 0.5mL of the sample. Urine specimens were poured into 8mL polypropylene tubes and loaded onto the Hamilton® Nimbus HD, where 0.5mL aliquots were transferred into source plates (96 deep-well plates). Source plates are transferred from the Hamilton® Nimbus HD to the Hamilton® Nimbus 96, where sample clean-up occurs. The analysis is performed on a Prominence Liquid Chromatograph (Shimadzu) coupled to a Sciex™ API 3200 Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) by injecting 3µL onto Kinetex® 2.6µm Phenyl Hexyl 100Å column. Mobile phases are made of 0.1% formic acid with 2mM ammonium formate in deionized water and 0.1% formic acid with 2mM ammonium formate in acetonitrile. Data are acquired between 0.2 to 4.4 minutes with a total acquisition time of 6 minutes. The LLOD and LOQ of all analytes were 0.35 ng/mL and 0.5 ng/mL, respectively.

The ULOL for quantitative analytes (fentanyl, norfentanyl, and acetyl fentanyl) was 500ng/mL, resulting in  $R \geq 0.99$ . Inter- and intra-day precision were 89% for all analytes. Ionization suppression was within  $\pm 25\%$  for all analytes, and analysis of clinical and proficiency specimens showed acceptable agreement with a validated, manual liquid-liquid extraction procedure.

This report demonstrates an automated SPE method for fentanyl and its analogs using DPX tips, developed and validated for this laboratory. Detection and quantitation are valid over a linear range of 0.5–500ng/mL for fentanyl, norfentanyl, and acetyl fentanyl, with all other analogs validated qualitatively with a 0.5ng/mL cutoff.

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### Fentanyl; Automation; SPE

## L12 Machine Learning Approaches to Make Data Driven Decisions in Forensic Science Application by Using Fourier Transform Infrared (FTIR) Spectroscopy

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**Learning Objective:** The attendees will have an insight about a unique combination of analytical and chemometric method and its real-life application in the field of forensic science investigation

**Impact Statement:** We will be introduced the forensic science community with a novel, noninvasive, rapid and non-destructive analytical system with advance machine learning approach that has promising potential to provide statistically high confidence result. The significance of this study is it could increase the reliability of the results and reduce the “human factor” such as biasness from investigator, analyst, or expert witness, which would be beneficial for consideration of evidence in criminal justice system.

Attenuated total reflection Fourier transform infrared (ATR FT-IR) spectroscopy is a technique that has been widely used to study the chemical composition of biological materials. This technique is fast, simple, non-destructive and it requires only small quantities of measured material with minimal to no sample preparation.<sup>1</sup> A significant advantage of ATR FT-IR is it provides a specific spectral signature for different sample types based on their biochemical composition.<sup>2</sup> We are applying this technique for forensic purposes to analyze trace evidences. Different biological substances, chemicals, and drugs are accumulated and stored in nails, where they can be detected and measured by using different techniques.<sup>3</sup> Testing nail specimens is highly beneficial and have been used in toxicological studies for decades. The examination of nail specimens has been typically used for drug and alcohol testing.<sup>4</sup> We developed a new approach by using machine-learning tools (ML) to leverage the potential and enhance the selectivity of the instrument. Machine learning (ML) algorithm was applied to create classification models, which provide invaluable information that is saved in human nails with high statistical confidence. The primary goal of this research is to determine whether an individual’s age, sex, race, smoking and alcohol status can be determined based on ATR FT-IR spectra collected from human nail clippings using ML computational framework.

The most powerful forensic analysis is DNA analysis, which could provide critical information about an unknown individual and has been used in forensic science for decades.<sup>5</sup> However, this technique is time consuming, sometime destructive to the sample, and expensive.<sup>5</sup> Moreover, the results are only of any use when a match is found in DNA database, a combined DNA index system (CODIS).<sup>5,6</sup> To the best of our knowledge, our study would be the very first study of human nails specimen in forensic science field, which combine the analytical method with new advance chemometric methods. It would be extremely beneficial to apply our developed chemometric methods to obtain high statistically confidence results. We have developed support vector machine discriminant analysis (SVM-DA) with smoothly clipped absolute deviation (SCAD) method to create classification models and reduce the risk of overfitting. This proof-of-concept study demonstrated the promising potential of predicting organism’s characteristics by using information collected from the nails clipping ATR-FT-IR spectra. In this study, the observable traits are age, gender, race, smoking and alcohol consumption status. We believe it would be a break through study in phenotype profiling in the field of forensic science.

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### Machine Learning; Multivariate Analysis; ATR/FTIR

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**L13 The Analysis of Depressants and Psychedelic Drugs From Vitreous Humor Using Liquid-Liquid Extraction (LLE) and High-Performance Thin-Layer Chromatography (HPTLC) Plates**

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**Learning Objective:** After attending this presentation, attendees will better understand drugs of abuse and the significance and usefulness of vitreous humor biofluid in toxicology investigations and a simple and reliable method for extraction of various drugs from vitreous humor and its analysis using HPTLC plates.

**Impact Statement:** This presentation will impact the forensic science community by providing a simple and reliable method for extraction and analysis of various drugs from vitreous humor using HPTLC plates. The determination of drugs is one of the most requested analyses in forensic toxicology laboratories and thus will play a vital role in determining the cause of death and a fair trial.

Drug abuse is a common issue and a growing concern all over the world. Impairment by drugs is one of the major contributing factors in suicides, road crashes, and violent crimes, and determination and interpretation of drugs is an important requisite in toxicology laboratories. Blood and urine samples are generally used for the analysis of drugs. This study highlights the importance of the use of vitreous humor in cases where blood and urine samples cannot be obtained due to severe trauma, putrefaction, contamination, etc.

Vitreous humor is an important biofluid due to its easy accessibility, lower protein content, low contamination, and high stability of drugs.<sup>1-3</sup> A vitreous humor sample was spiked with various acidic and basic drugs. Then drugs were extracted from vitreous humor using liquid-liquid extraction. Acidic drugs were extracted using glacial acetic acid and diethyl ether, whereas basic drugs were extracted using ammonium hydroxide, diethyl ether, and chloroform. The aqueous layer was collected, concentrated, and analyzed using HPTLC plates. The solvent system used for extracted acidic drugs was ethyl acetate:methanol:ammonia (8.5:1:0.5) and for extracted basic drugs was chloroform:methanol (9:1), respectively. Developed HPTLC plates were viewed under Ultraviolet (UV) light at 254nm and 365nm.

The proposed method was used for the extraction of drugs in the case of a 62-year-old male who was found dead at his home. The deceased had a history of drug abuse. During the postmortem examination, a vitreous humor sample was collected for analysis of drugs. The proposed method was used for the extraction of drugs and analyzed using HPTLC plates. Marijuana was successfully detected in the vitreous humor sample of the deceased using the proposed method. Vitreous humor is a useful biofluid in postmortem forensic toxicology. The proposed method for analysis of drugs from vitreous humor is a simple, rapid, and inexpensive procedure that can be used in any toxicology laboratory.

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**Drug Abuse; Vitreous Humor; HPTLC**

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**L14 Comparing the Performance of Liquid Chromatography/Triple Quadrupole/Mass Spectrometry (LC/QqQ/MS) vs. Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/QTOF/MS) for Targeted Analysis of NPS With a Test Mixture of 40 New Psychoactive Substances (NPS) and Metabolites in Whole Blood, Urine, and Oral Fluid**

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**Learning Objective:** After attending this presentation, attendees will have a scientific comparison of two effective methods of targeted analysis for NPS using a complex mixture of substances in relevant biological matrices.

**Impact Statement:** This presentation will impact the forensic community by presenting scientific evidence showing which instrument and method is most effective in targeted analysis of NPS in a biological sample.

**Background:** To combat the growing use of NPS due to the abundance of information available to the public, laboratories need to become more sophisticated in their screening and identification of compounds. Currently, immunoassays are the most common technique for screening drugs of interest, but chromatographic separation coupled with MS is becoming a more widely used technique for screening of multiple NPS, due to its selectivity, specificity, and sensitivity. Both low- and high-resolution instrumentation, such as LC/QqQ/MS and LC/QTOF/MS, can monitor specific precursor to product ion fragmentations and determine accurate mass, respectively, for specific NPS compounds. However, there is currently a lack of data that compares the potential of these methods for use in NPS screening.

**Objectives:** This work provides evidence to show the effectiveness of using LC/QqQ/MS and LC/QTOF/MS with Data-Dependent Acquisition (DDA) for the analysis of multiple NPS. In this analysis, a 40-compound test mixture of NPS containing various structures and pharmacological classes was used to spike authentic matrices of whole blood, urine, and Oral Fluid (OF). The mixture included closely eluting NPS analytes, isomers, and metabolites that have a wide range in polarity to allow for assessment of selectivity and other identification algorithms from the data obtained by each instrument. The performance of both acquisition methods was evaluated using figures of merit (Limit of Detection [LOD], dynamic range, linearity, selectivity, precision), carryover, and matrix effects.

**Methods:** Individual standard solutions of NPS were spiked in MeOH to create a mixture with a final concentration of 200ng/mL for each compound. The mixture was further diluted with blank matrix to yield concentrations of 1, 2, 5, 10, 20, 50, and 100ng/mL for determination of LOD, dynamic range, linearity, etc. for the targeted MS studies. Two extraction methods were used for specimens, dilute-and-shoot for urine and crash-and-shoot for whole blood and OF. The LC/QqQ/MS used a dynamic Multiple Reaction Monitoring (dMRM) mode as a measure of selectivity, where specific MRM data was acquired to identify each compound based on their retention time, and transitions which included specific collision energies, fragmentor voltages, and product ion selections for each compound. The LC/QTOF/MS used a targeted MS/MS mode that specifically targeted the precursor, retention time, and collected a range of collision energies to confirm a compound. The fragmented product ions from the range of energies were analyzed in the TOF and both full scan and MS/MS data are acquired. Both instruments used positive and negative Electrospray Ionization (ESI) ionizations for all of the compounds.

**Results:** The performance of these targeted methods was evaluated by their sensitivity, linear range, precision, matrix effects, and specificity in whole blood, urine, and OF. For spiked whole blood, a second approach was studied using a series of “dummy analytes” to mimic the presence of interferents in the matrix. Results were measured using statistical significance testing and a scoring system designed to summarize each analyte for each analytical method in positive and negative ESI modes. This was to present these methods as more effective in screening an evolving list of new substances that are difficult for older methods used in forensic laboratories.

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**Targeted Methods; New Psychoactive Substances; Forensic Toxicological Analysis**



## L15 Evaluating Cannabidiol (CBD) for Potential Interference in Six Commercial Homogeneous Cannabinoid Urine Screening Kits

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**Learning Objective:** After attending this presentation, attendees will understand how Cannabidiol (CBD) and its biological urine metabolites potentially interfere in six homogenous urine cannabinoid screening kits at 20/25ng/mL and 50 ng/mL cutoff concentrations.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees about potential interferences from CBD and its metabolites in cannabinoid urine drug screening.

Following federal legalization of hemp with the passage of the Agricultural Improvement Act of 2018, there has been an increased presence and use of cannabinoids. Currently, the United States Food and Drug Administration (FDA) has an approved prescription formulation of CBD (Epidiolex®) to treat some forms of epilepsy. CBD and its major metabolites have a similar chemical structure to Δ9-Tetrahydrocannabinol (Δ9-THC) and its major metabolites, which presents the potential for interferences with current immunoassay screening methods regularly employed in forensic, clinical, or pain management testing laboratories that are used for the detection of Δ9-THC-COOH in urine. CBD is metabolized to the carboxylic acid (CBD-COOH), 6-OH-CBD, and 7-OH-CBD. Additionally, CBD has a regioisomer known as Abnormal CBD (Abn-CBD). The federal screening cutoff for Δ9-THC-COOH is 50ng/mL; however, in certain cases, a lower cutoff (20/25ng/mL) is used to further extend the detection window.

Six urine homogeneous immunoassay kits (Abbott™ Cannabinoids–Abbott™ Diagnostics; LZI Cannabinoids (cTHC) Enzyme Immunoassay–Lin-Zhi International; DRI® Cannabinoid Assay and CEDIA™ THC–Thermo Fisher Scientific™; ONLINE DAT Cannabinoid II–Roche Diagnostics; and Syva EMIT®IIPlus–Siemens Healthineers) were evaluated for their ability to detect cannabinoids. The analysis was performed on an Abbott™ Architect Plus c4000 Each kit was programmed at two different cutoff concentrations: 20/25ng/mL and 50ng/mL. CBD and its metabolites were evaluated individually. The limit of detection for each compound was evaluated by preparing samples at 20, 50, 100, and 1,000ng/mL in urine. Prepared samples were analyzed at both cutoff concentrations in order to determine if the compounds could be detected at one/both immunoassay cutoff(s). If the compound was not detected at 1,000ng/mL for a given cutoff, the compound was considered not detectable. If the compound was detected, the appropriate concentration was used as the decision point to determine the precision at the immunoassay's cutoff using Academy Standards Board (ASB) Standard 036 guidelines. To assess precision, three QC pools of the individual compound were prepared (-50%[QCN]), decision point, and +100%[QCP]). Each pool was analyzed in five different runs ( $n=3$ ) along with the respective immunoassay's control materials. The total mean ( $n=15$ ), total Standard Deviation (SD), and total percent Coefficient of Variation (%CV) were calculated for each QC concentration. A decision point was considered valid if the %CV for the QC didn't exceed  $\pm 20\%$  for each concentration, and the total mean of the QCN and QCP $\pm 2SD$  didn't overlap the mean of the decision point.

CBD, CBD-COOH, and Abn-CBD were not detected by any of the six immunoassays at either the 20/25ng/mL or 50ng/mL cutoffs. 6-OH and 7-OH-CBD were not detected by any of the six immunoassays at the 50ng/mL cutoff, and only at 1,000ng/mL. 6-OH-CBD was detected at 1,000ng/mL by Abbott®(20), DRI®(20), LZI(25), and ROCH(20). 7-OH-CBD was detected at 1,000ng/mL by Abbott®(20), DRI®(20), LZI(25).

Funded in part by National Institute of Justice (NIJ) Research and Development in Forensic Science for Criminal Justice Purposes Grant (15PNIJ-21-GG-04188-RESS).

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### Cannabidiol (CBD); Immunoassay; Interference Studies

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**L16 The Revival of Opioids Among Young People: An Overview on Toxicological, Epidemiological, and Social Aspects**

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**Learning Objective:** The goal of this presentation is to explain that numerous cases of deaths resulting from overdoses from new substances, if deepened, could bring out new social dynamics related to drug use and new emerging social problems.

**Impact Statement:** This presentation will impact the forensic science community by starting from an unusual and very interesting case in toxicological matters that should stimulate some reflections on the close connection between the restrictive measures of the pandemic, the social and psychological unease of young people also conveyed through music, and the dynamics regarding the supply of substance abuse.

**Introduction:** It has now been three years since the COVID-19 pandemic has upset everyone's lives. The impact of the virus, we know, has had a devastating impact in terms of human lives and also in terms of sequelae on physical health. While the strictly "organic" side of the COVID disease had to be dealt with, the restrictive measures adopted to contain the contagion—the lockdown—have determined a real psychological disruption in many individuals. Numerous studies have shown a surge in the incidence of psychiatric diseases during the lockdown, especially in people of developmental age. Moreover, the use of new substances of abuse has also emerged, most of these easily available in pharmacies, since removal from home during the lockdown was difficult, if not for work or health reasons. Our presentation starts from an interesting toxicological case, then extends the reflection on this broader field of psychiatric pathologies and the "new" drugs of abuse. Surprisingly, they opened our eyes to the world of Italian trap music, which carries dangerous messages and explains what drugs to use for this new Saturday night high.

**Case report:** In September 2021, a 19-year-old boy was found dead in his bedroom. During the inspection of the place, the judicial staff found cartons of drugs with empty blisters of alprazolam, oxycodone + paracetamol, quetiapine, sertraline, and two bottles of codeine syrup. The judicial authority ordered the execution of the autopsy to detect the cause of death. The clinical history examination showed a story of borderline personality disorder and a suicide attempt by stab wounds one year earlier. The boy had embarked on a path of psychotherapy, then interrupted and occasionally resumed electronically during the third wave of COVID-19. The presence of codeine bottles, which the boy's mother reported was not part of his standard therapy, and carbonated drinks raised the suspicion of lethal intoxication with "purple drank," a recreational new drug composed of codeine, promethazine, and soda. During the autopsy examination, a urine sample and a blood sample from the inferior vena cava were taken for the execution of appropriate toxicological tests. The search for substances was performed by qualitative analysis with Gas Chromatography/Mass Spectrometry (GC/MS) and Liquid Chromatography/Mass Spectrometry (LC/MS). Quantitative analysis was performed by LC/MS. Investigations did not find codeine but rather a lethal cocktail of multiple drugs: alprazolam (344ng/ml), quetiapine (47ng/ml), sertraline (290ng/ml), and oxycodone (154ng/ml). The results of the toxicological investigations showed that the blood concentrations of alprazolam, sertraline, and oxycodone exceed the therapeutic range. The simultaneous intake of alprazolam, sertraline, and oxycodone is suitable to determine an inhibitory effect on the central nervous system, with associated severe respiratory depression and consequent death. The doses found in this case were compared with those found in other studies in the literature. As the "purple drank," suspected at first, oxycodone, and other opioids have been the protagonist of a growing interest among young people in recent years, becoming the new "Saturday night high." The present case, which started from an interesting toxicological analysis of the combined use of psychotropic drugs and synthetic opioids, intersects with new social and public health problems: the pandemic, isolation, the increase in psychiatric pathologies, and the increase in the abuse of prescription substances. Numerous cases of deaths resulting from overdoses from new substances, if deepened, could bring out new social dynamics related to drug use and new emerging problems.

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**Oxycodone; Opioids; Psychotropic Drugs**



## L17 A Combined Death by Food Aspiration and Acute Citalopram Intoxication

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**Learning Objective:** After attending this presentation, attendees will appreciate how useful the toxicologist’s assessment is in medicolegal investigations. Combined death is not common. Furthermore, this presence can be of fundamental importance for health care professionals working in nursing homes to improve the management of frail patients.

**Impact Statement:** This case report will impact the forensic community by demonstrating that the coroner should not stop at identifying the most obvious first cause of death without considering the possibility of combined death mechanisms.

Pulmonary aspiration is one of the major health risks for older adults in Nursing Homes (NH), which could lead to respiratory infections, aspiration pneumonia, and sudden bolus death.<sup>1</sup> Dysphagia, neurological disorders, and drug-sedation comprise the commonest risk factors for aspiration-related deaths.<sup>2</sup> Over the years, there has been an increase in the use of antidepressants in NH, especially among residents with dementia or cognitive impairment.<sup>3,4</sup> In NH, misconduct in medication therapy may represent a potential risk of adverse drug reactions and poisonings.<sup>5</sup>

Reported here is the toxicological evaluation of a case of death by food aspiration. In this case, concentrations of citalopram close to lethality were found in the organism.

An 89-year-old man, suffering from dysphagia and Alzheimer’s and a resident in a nursing home, was being fed with liquefied food directly injected into mouth by a syringe. The man was being treated with escitalopram film-coated tablets. No information about the manner of administration was reported.

One evening, after receiving the meal in the usual way, the man complained of sudden illness, with vomiting and breathing difficulties, a SatO<sub>2</sub> of 72%, hypotension, and tachycardia. Carried to the emergency room, the man died about three hours later. First, the autopsy and histopathological examinations were ordered. Sections of the main organs were stained with hematoxylin and eosin for microscopic examination.

The toxicological investigation was subsequently ordered. Escitalopram analyses were carried out on femoral blood, brain, lung, and gastric content samples. The samples were extracted by Liquid-Liquid Extraction (LLE) and analyzed by Gas Chromatography coupled with Mass Spectrometry (GC/MS) using an Agilent® GC/MS. Precision and accuracy were less than 20% for each matrix. The autopsy ascertained that the trachea and bronchia were filled with exogenous material, probably food. The histological findings also revealed that the presence of this exogenous material was up to the finest bronchial branches. The toxicological examination revealed the presence of high escitalopram concentrations: in femoral blood 1,972ng/mL, in the brain 4,657ng/g, in the gastric content 2,317ng/mL, and, especially, in the lungs 21,771ng/g.

It was hypothesized that death occurred by a combined mechanism between food aspiration and escitalopram toxic action, which likely led to arrhythmias, respiratory depression, and hypotension. Escitalopram blood concentrations about 360ng/mL could be considered dangerous.<sup>6</sup> Moreover, after escitalopram intake, no conversion to its R-enantiomer occurs.<sup>6,7</sup> Given the high escitalopram concentrations mainly in the lungs and blood, it was suspected that the tablet was crushed and dissolved in liquefied food administered by syringe and accidentally aspirated. The bioavailability of the drug absorbed by the respiratory tract is far greater, also due to the lack of first pass hepatic metabolism.<sup>8</sup>

This intuition and the toxicological data led the magistrate to gather further information by questioning the nursing staff of the NH. The practice of nurses about dissolving the escitalopram tablet in food and administering it by syringe directly into the mouth was revealed. Therefore, the high escitalopram concentrations did not relate to an overdose but to the reaching of the drug into the deep respiratory tract. This caused an acute intoxication, which, combined with aspiration, led to the man’s death.

The present report clearly highlights the importance of the toxicologist in forensic investigations, although this does not always occur as soon as in the case presented here. The close collaboration between the different forensic skills is fundamental to the resolution of even apparently simple cases. In this investigation, without the involvement of the toxicologist, doubts would have remained about the pathogenetic mechanism of death and the possible misconduct of the nurses.

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**Combined Death; Poisoning; Pulmonary Aspiration**

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**L18 Donepezil Concentrations in Postmortem Toxicology**

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**Learning Objective:** After attending this presentation, attendees will better understand the toxicology of donepezil and the meaning of the blood concentrations in postmortem casework.

**Impact Statement:** This presentation will impact the forensic science community by evaluating postmortem distribution of donepezil to aid in interpretation of postmortem blood concentrations.

**Introduction:** Donepezil is approved for the treatment of Alzheimer's disease alone and in combination with memantine. It has a large volume of distribution, 10-15L/kg, and high lipophilicity, indicating a potential for postmortem redistribution. With an estimated six million Americans suffering from Alzheimer's disease, donepezil may be encountered in postmortem toxicology.<sup>1</sup> Few studies have investigated postmortem donepezil concentrations, and none have compared paired antemortem and postmortem concentrations. Nagasawa et. al. evaluated donepezil postmortem drug levels in seven cases and found elevated concentrations in both central and peripheral blood.<sup>2</sup> This study aims to evaluate donepezil blood concentrations in paired antemortem and postmortem specimens as well as comparing central and peripheral blood specimens.

**Method:** Postmortem blood specimens submitted for donepezil confirmation by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) between January 2015 and May 2022 (2,045 cases) were reviewed. The laboratory confirms donepezil via a liquid/liquid extraction, analyzed on an LC/MS/MS method with a reporting limit of 5.0ng/mL. Testing may be ordered as a directed test or reflexed from a Liquid Chromatogram/Time Of Flight/Mass Spectrometer (LC/TOF/MS) screen with a reporting limit of 10ng/mL. Cases where testing was performed on multiple blood specimens will be presented as case studies.

**Results:** There were 1,797 positive donepezil postmortem blood confirmations between January 2015 and May 2022. Donepezil values ranged from 5.7–6,700ng/mL (mean 177ng/mL, median 120ng/mL) for all postmortem blood types. These ranges were 5.7–4,800ng/mL (mean 168ng/mL, median 110ng/mL) for peripheral blood ( $n=1,330$ ) and 6.4–6,700 (mean 183ng/mL, median 120ng/mL) for central blood ( $n=409$ ). Nine cases performed donepezil testing on multiple blood sources, five of these included antemortem and postmortem specimens. One case study revealed a femoral blood donepezil concentration of 500ng/mL and a subclavian blood concentration of 550ng/mL for a central to peripheral blood ratio of 1.1. Although the donepezil concentration was elevated, the medical examiner determined that it did not contribute to the cause of death of atherosclerotic cardiovascular disease.

**Conclusion:** This study found that 77% of postmortem blood donepezil concentrations were higher than the proposed clinical plasma therapeutic range of 30–75ng/mL (21–53ng/mL whole blood). Elevated blood donepezil concentrations have been reported in postmortem cases where cause of death was unrelated. Postmortem donepezil concentrations should be interpreted with caution in the context of a comprehensive case history.

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**Donepezil; Postmortem Distribution; LC/MS/MS**

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**L19 The Development of an Extraction Method for the Analysis of Synthetic Opioids in Bone Samples Using the Bead Ruptor**

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**Learning Objective:** Attendees will understand the importance of Novel Synthetic Opioid (NSO) detection in uncommon toxicological matrices like bone, discover a new method for bone extractions, and learn about a more suitable animal model when correlating blood:bone drug concentrations.

**Impact Statement:** This presentation will impact the forensic science community by discussing the development of a novel validated bone extraction for synthetic opioids that saves both money and time and can become an important established method for forensic labs. Establishing a correlation between blood and bone for drug concentration will also help when determining cause of death when due to overdose.

NSOs have been attributed to an increasing number of overdose deaths in recent years. Detecting NSOs in toxicological matrices can prove difficult due to their high potencies leading to low concentrations in the body. In instances where bone is the only available toxicological matrix, the correlation between blood:bone drug concentration is important when determining cause of death in skeletal remains due to overdose. To achieve this, correlation studies of animal models are necessary, and a careful choice of a suitable animal model is required. Rabbits, as opposed to rodents, are the most appropriate choice, due to their exhibition of spontaneous cortical bone remodeling, a natural bone process that creates and destroys bone mass and helps to incorporate substances inside the bone tissue, that is not present in rodents. Previous research has not yet been able to establish such a correlation using rodents, leading to studies with a new choice of animal model for appropriate correlation data. Therefore, the hypothesis is that the difficulty in achieving a successful correlation is due to the physiological characteristics of the animal model chosen for the experiments. The aim of this work is to develop a fast and suitable method for the extraction of synthetic opioids from bone samples to help establish a blood:bone drug correlation using rabbits as the chosen animal model.

Development of the extraction protocol utilized commercial drug-free rabbit bone samples that were fortified with the following synthetic opioid standards: fentanyl, norfentanyl, bucinnazine, and AP-238 in a concentration range from 1 to 1,000ng/g of bone. Fentanyl-d5 and norfentanyl-d5 were included as internal standards. For the fortification process, bones were spiked with 25µL of the analytes solution and left drying naturally; 500mg of fortified bone samples were homogenized in 4mL methanol using the Omni International Bead Ruptor Elite. The homogenized samples were centrifuged, and the supernatant was collected. The supernatants were filtered through a 0.45µm syringe filter, then diluted to 30mL with pH 10 deionized water to prepare for Solid-Phase Extraction (SPE), where a 3cc flangeless Oasis® MCX cartridge was utilized. After loading, the samples were first washed with 2mL 0.1 M HCl, followed by a 2% formic acid in methanol solution. The analytes were eluted with a 5% ammonium hydroxide in methanol solution, which was evaporated and reconstituted with 50µL ethyl acetate for Gas Chromatography/Mass Spectrometry (GC/MS) analysis. A GC/MS method was developed to identify and quantitate the selected NSOs, and calibration curves were prepared using the standard addition method.

Yields for all analytes varied from 20% to 65%, with norfentanyl having the lowest yield and AP-238 having the highest. The use of the Bead Ruptor for the extraction during the homogenization process showed to be efficient with less time and cost spent, when compared to the traditional methods for bone analysis that consist of drying the samples, gridding them, and letting them soak in solvent for 24 hours.

The developed extraction and GC/MS methods were capable of extracting, detecting, and quantitating the target opioids. Once animal experiments begin, these methods can be applied to the bone samples obtained from the rabbits for the determination of the blood:bone correlation of synthetic opioids.

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**NSO; Animal Model; Extraction**

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**L20 The Chemical Characterization of Cannabidiol-Based Products Using Attenuated Total Reflectance/Fourier Transform Infrared (ATR/FTIR), Gas Chromatography/Mass Spectrometry (GC/MS), and Gas Chromatography/Tandem Mass Spectrometry (GC/MS/MS)**

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**Learning Objective:** After attending this presentation, attendees will better understand the application of ATR/FTIR, GC/MS, and GC/MS/MS for the analysis of Cannabidiol (CBD) oil samples.

**Impact Statement:** CBD-based products have become very popular in the United States, but the majority of them are not approved by the Food and Drug Administration (FDA). Therefore, the investigation into the contaminants, mislabeling, and by-products that can appear within these products will provide insight and aid in the creation of safe restrictions in the production process. This presentation describes the application of an analytical workflow combining ATR/FTIR, GC/MS, and GC/MS/MS and the potentialities and limitations to the analysis of CBD-based products.

**Background:** CBD is one of the hundreds of compounds found within *Cannabis sativa*. The compound does not hold any psychoactive properties and its medical properties are well recognized. Over the past few years, products containing CBD have become more common in the United States. However, CBD-based products are not approved by the FDA and cannot be marketed and sold for medical purposes. Moreover, there is a growing concern about the levels of CBD as well as the presence of other substances in these products, which can be of interest to forensic practitioners.<sup>1</sup>

**Objective:** The objective of this study was to explore an analytical workflow based on ATR/FTIR, GC/MS, and GC/MS/MS to document the chemical profile of commercially available CBD tincture oil samples.

**Methods:** Three samples acquired from Arizona, Colorado, and North Carolina were tested. According to the label, the concentration of CBD in each sample ranged from 30mg/mL–60mg/mL. Initially, samples were analyzed using the Thermo Scientific™ Nicolet® 6700 ATR/FTIR, using 32 scans and a resolution of 4cm<sup>-1</sup>. No sample preparation was required. Library search and IR Spectral Interpretation tools were used for analysis and a solid CBD standard was analyzed as a reference. Samples were also analyzed using a Thermo Trace GC Ultra coupled with a Thermo TSQ Quantum XLS MS. The temperature gradient was as follows: an initial temperature of 100°C with a hold time of 1 minute, followed by a 20°C/min increase to the final temperature of 280°C with a hold time of 3 minutes. Injector, transfer line, and ion source temperatures were 280°C, 280°C, and 250°C, respectively. Helium was used as carrier gas. Helium and argon were used as carrier gas and collision gas, respectively. After dilution with hexane/ethyl acetate (70:30), samples were screened using Full Scan GC/MS. For confirmation, an Selected Reaction Monitoring (SRM) GC/MS/MS method was developed, and samples were prepared using dilution followed by acid-base extraction.<sup>2</sup>

**Results and discussion:** ATR/FTIR analysis revealed peaks at 2923.64cm<sup>-1</sup>, corresponding to aliphatic hydrocarbons. Strong peaks at 1741.39cm<sup>-1</sup> corresponding to aliphatic propionate esters were present only in the CBD samples, associated with the oil components. A strong characteristic peak at 3467.18cm<sup>-1</sup>, potentially corresponding to the OH groups of CBD, was present in the standard but not in the sample's spectra. The library search did not match CBD in any of the samples. In regard to GC/MS screening, CBD was detected in all samples (retention time: 10.23min). In two samples, glycerol triacrylate was also detected (retention time: 11.3min). GC/MS/MS was successfully used to confirm the presence of CBD in the samples, monitoring the transitions m/z 231–174 and m/z 231–145.

**Conclusion:** ATR/FTIR analysis did not lead to direct identification of CBD within the samples but did show similarities within the reference spectra. Screening by GC/MS allowed the preliminary identification of CBD, which was further confirmed by GC/MS/MS. The methods used in this study combining GC/MS and GC/MS/MS were effective in detecting the presence of CBD in the samples, whereas ATR/FTIR provided general information about large concentration components of the oil.

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**Cannabidiol; Cannabidiol-Based Products; GC/MS**

## **L21 A Report of Isolated Para-Fluorofentanyl Deaths in Allegheny County, Pennsylvania**

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**WITHDRAWN**

## L22 Color Me Stable: Choosing an Adequate Blood Collection Tube for Venous Blood Toxicological Analyses

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**Learning Objective:** After attending this presentation, attendees will be able to discern the best blood collection tubes for the purpose of toxicological analyses in venous blood and summarize elements contributing to this decision.

**Impact Statement:** This presentation will impact the forensic science community by providing tools to practitioners to select blood samples leading to the most reliable results (reflecting xenobiotic concentration at time of sampling). Forensic experts will better be able to consider and communicate the impacts different blood tubes may have on their testing and results interpretation.

Blood collection devices, including their handling and storage, play a crucial role in the reliability of forensic toxicology results. Degradation, neoformation, or adsorption events may modify the xenobiotic concentration present at the time of sampling. Concerns about this eventuality are especially acute for Driving Under the Influence (DUI) cases where a legal threshold is specified in the law or Criminal Code.

At the Laboratoire de Sciences Judiciaires et de Médecine Légale (LSJML), Quebec, Canada's, provincial forensic science laboratory, approximately 90% of blood samples collected in DUI cases use a so-called "grey top" tube (BD Vacutainer® #367001 with 100mg sodium fluoride and 20mg potassium oxalate), part of an approved collection kit for this purpose. However, in the remaining cases, blood samples collected for medical purposes are seized by police officers for toxicological analysis. In such cases, it is common to receive multiple samples in different blood collection devices. The forensic toxicologist then faces the question: which tube will yield measurements best reflecting xenobiotic(s) concentration(s) at the time of sampling? To answer this question, long-term stability of 76 analytes in 6 different BD Vacutainer® tubes commonly encountered was evaluated.

Whole blood specimens were collected from ten voluntary donors in BD Vacutainer® #367001 (grey top, sodium fluoride/potassium oxalate), #36863 (lavender top, EDTA), #366430 (red top, silica particles–clot activator), #367886 (green top, heparin), #367986 (gold top, silica particles and plasma separation gel), and #369714 (light blue top, sodium citrate), for a total of 60 collecting tubes. Ethics approval was obtained from Université du Québec à Trois-Rivières (CER-19-263-07.12). Samples were handled as directed in a hospital setting (e.g., waiting time, centrifugation). Within 15 hours of collection, plasma (gold top tubes) or whole blood (all other devices) samples were spiked at low and high concentration levels with 76 xenobiotics and stored at 4°C. Duplicate analysis was carried out at T=0 week, 1 week, 2 weeks, 4 weeks, and 6 months. Samples were extracted by protein precipitation and analyzed by targeted screening on a 5500 QTRAP® LC/MS/MS (SCIEX™), a fully validated method accredited under ISO/IEC 17025:2017 and the Standards Council of Canada norms.

An analyte was considered stable if the measured concentration at T0 overlapped with the measured concentration at a subsequent time point, within the limits of their Measurement Uncertainty (MU) (calculated with a 95% confidence level). While every analyte had its own (in)stability pattern, as a general rule it was observed that xenobiotics were stable four weeks in grey, light blue, green, and lavender top tubes. Gold and red top tubes suffered severe losses in almost all analytes, starting as soon as one week in most cases. This may be associated with the absence of any preservative to stave off bacterial growth and/or adsorption to plasma separating gel. Furthermore, some analytes displayed marked instability in several blood collection tubes: diazepam, cocaine, zopiclone, methylphenidate, and THC-COOH. No significant neoformation event was observed for any analyte, including GHB, in any of the studied collection devices. Detailed results per analyte will be presented in the poster.

Faced with a choice between several blood samples in different collection devices, grey, light blue, green, and lavender top tubes should generally be preferred to red and gold top tubes. A more granular approach (per analyte) could also be used, relying on the results of the present study to select the sample of choice.

### Blood Collection Tubes; Drug Stability; Driving Under the Influence of Drugs



## L23 Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds: “Nitazenes”

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**Learning Objective:** After attending this presentation, attendees will have gained a general knowledge of synthetic 2-benzylbenzimidazole “nitazene” compounds and will have learned about an optimized solid-extraction procedure of these analytes from blood and urine. Attendees will also better understand the analytical challenges associated with the extraction of nitazenes.

**Impact Statement:** As forensic scientists continue to battle the continuously changing environment of Novel Psychoactive Substances (NPS), this study presents one of the first solid phase extraction methods for a panel of nine nitazene compounds. This procedure can be used by medical examiners and toxicologists who suspect an overdose from these potent synthetic opioids.

First synthesized in the 1950s by a Swiss pharmaceutical company, etonitazene and its structurally related analogs were investigated as potential analgesics.<sup>1</sup> However, these 2-benzylbenzimidazole “nitazene” compounds were never approved for clinical use. Today, they are re-appearing as new potent non-fentanyl-like synthetic opioids. Isotonitazene, also known as “iso” or “toni,” first appeared in Canada and Europe in March 2019. In July of 2019, these compounds were first detected in biological samples in the United States.<sup>2</sup> In August 2020, the United States Drug Enforcement Administration (DEA) temporarily placed isotonitazene in Schedule I.<sup>3</sup> In December 2021, the DEA issued temporary scheduling of seven additional nitazene compounds.<sup>4</sup> Nicknamed “Frankenstein” opioids due to their highly potent nature, these compounds have been identified in numerous states and the number of cases continues to rise.<sup>5</sup> This presentation outlines methods for extracting nine nitazene compounds from urine and blood utilizing a polymeric cation exchange Solid Phase Extraction (SPE) sorbent.

Nine nitazene compounds (butonitazene, clonitazene, etonitazene, etonitazepyne, flunitazene, isotonitazene, metodesnitazene, metonitazene, and pronitazene) were extracted from human urine and blood utilizing UCT, Inc.’s Styre Screen® BCX (SSBCX) SPE cartridges: 1mL biological samples were prepared at a concentration of 25ng/mL using a stock solution of the nine nitazene compounds; 1.7mL of phosphate buffer (pH 7.0, 0.1M) followed by 300uL of acetonitrile were added for sample dilution and pH adjustment. The SSBCX SPE cartridges were conditioned with 3mL of methanol and equilibrated with 3mL of pH 7.0 phosphate buffer. Matrix interferences were removed by washing the columns with two washes of 3mL of deionized water and two washes of 3mL of 50% methanol. The target analytes were eluted with 3mL of 2% ammonium hydroxide in methanol. After fully drying under a gentle stream of nitrogen at 30°C, the extracts were reconstituted in 1mL of 50:50 methanol:water. Samples were analyzed using a Shimadzu® Nexera® LC-30AD with MS-8050 operated in positive ion mode. Analytes were separated using a SelectraCore® C18 Column (100mm x 2.1mm, 2.7um) with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B.

Analytes were successfully extracted from urine and blood with high recoveries and low matrix effects. Recoveries were determined by comparing pre-spiked samples to post-spiked samples. The matrix effect was determined by comparing post-spiked samples to spiked solvent samples. Extraction recoveries of analytes from urine ranged from 95-99% and the matrix effect for all analytes was within ±25%. Extraction recoveries of analytes from blood ranged between 87-100% and the matrix effect for all analytes was within ±25%.

Due to their novelty, much information was gained about this class of drugs and how they behave under specific conditions during method development. For example, a sizeable amount of some of the non-polar analytes, such as butonitazene and isotonitazene, remain in the test tube after loading the sample onto the SPE cartridge. Also, a significant loss of some analytes was observed during evaporation of the elute under certain conditions. Potential solutions were tested to combat these issues. An extraction method has been successfully optimized to extract nine nitazene compounds from urine and blood using Styre Screen® BCX SPE cartridges.

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### Nitazenes; Novel Psychoactive Substances; Solid Phase Extraction

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**L24 The Quantitative Analysis of Cadmium in Blood Using Flame Atomic Absorption Spectrometry (FAAS)**

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**Learning Objective:** After attending this presentation, attendees will better understand the FAAS technique, its applications in toxicology, and the method for the quantitative determination of cadmium in blood using FAAS.

**Impact Statement:** This presentation will impact the forensic science community by serving as a key aspect in the cases of cadmium poisoning encountered in clinical and forensic toxicology. The proposed method will help in the quantitative determination of cadmium using FAAS.

Cadmium (Cd) is a non-essential heavy metal, found in nature, which is toxic for humans as well as for fauna. Cadmium, a pollutant, is commonly derived from anthropogenic sources. The carcinogenic nature of cadmium makes it a worldwide health concern. Excessive exposure to cadmium results in osteodystrophy, as it accelerates the synthesis of metallothionein in the liver, causing a disease called as Itai-Itai (Ouch-Ouch) disease. The main routes for cadmium poisoning in humans are inhalation of fumes, ingestion, and cigarette smoke. Atomic Absorption Spectrophotometry (AAS) and Inductively Coupled Plasma/Atomic Emission Spectrometry/Mass Spectrometry (ICP/AES/MS) are the analytical techniques that can be used to detect cadmium concentration in biological samples. A method was developed for the determination of cadmium in blood with an absorption technique using the FAAS.

A total of 10 samples of blood were collected, after written consent, from the laboratory staff of the general population, who were not occupationally exposed to cadmium. Each 1ml of blood sample was spiked with 1ml of cadmium with a concentration ranging from 0.01 to 1ppm, vortexed, and left overnight. The sample was digested by the wet digestion method (1ml sample, 7ml nitric acid, and 1ml H<sub>2</sub>O<sub>2</sub>) and left overnight. After digestion, the sample was made up to 50ml with distilled water, calculated as dilution factor, in order to obtain the amount of sample required to run on FAAS. Cadmium was detected by using FAAS. Cadmium standards were prepared at concentration 0.02ppm, 0.04ppm, 0.08ppm, and 0.1ppm. The flame was ignited, and a blank value was recorded; values of the standard were also recorded at the wavelength of 228.8nm with lamp current 4 and slit size 0.4. The method showed excellent linearity with a correlation curve ( $r^2$ ) of 0.99382. After the completion of standard values, the sample was run on flame through a nebulizer, and the value was recorded. The same sample was run for the second time to measure the accurate value of the sample. The average concentration of cadmium was calculated in the sample accordingly.

The toxicity from cadmium starts with a concentration of more than 0.005ppm, whereas it becomes lethal at 2.5ppm. Hence, it becomes desirable to develop a method to detect levels of cadmium within this range, which is convenient and effective. In the present study, the cadmium concentration was successfully detected in various spiked blood samples with concentrations ranging from 0.02 to 1ppm, with the lowest detection limit of 0.02ppm. FAAS can be used for the quantitative determination of cadmium in the blood owing to its specificity, reliability, and reproducibility. The proposed method is simple, reliable, and can be used for clinical and forensic toxicology purposes.

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**Cadmium Toxicity; FAAS; Toxicology**

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**L25 The Evaluation of Spontaneous Overtime Methemoglobin Production in Postmortem Blood Samples From Real Cases in Critical Storage Conditions for an Application in Cases of Suspected Nitrate Poisoning**

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**Learning Objective:** After attending this presentation, attendees will understand the importance of methemoglobin determination in postmortem blood samples.

**Impact Statement:** This presentation will impact the forensic science community by discussing a simple and fast method to assess spontaneous methemoglobin formation and the time limits in which this analysis should be done to avoid artifacts due to storage conditions.

Several cases of potassium nitrate self-poisoning have been reported in Italy in the past two years. Nitrate can be converted into nitrite in the human intestine and oral cavity.<sup>1</sup> Nitrites interact with hemoglobin, causing its oxidization to methemoglobin, which is incapable of transporting oxygen, therefore causing progressive death by hypoxia.<sup>2</sup>

Chemical-toxicological determination of nitrate poisoning is complex since a direct method for its determination is not commonly present in a forensic laboratory as routine analysis, and it may be requested even months after autopsy. A valuable option is, therefore, methemoglobin quantitation. Even though some studies have been carried out to assess the best storage parameters for blood samples dedicated to methemoglobin determination, few have been done to evaluate the maximum threshold of spontaneous overtime methemoglobin formation in postmortem specimens in typical storage conditions.

This study aims to evaluate the spontaneous overtime degradation of postmortem blood samples in the storage conditions typical of a toxicology laboratory (i.e., without any preservatives, in a freezer). The method used in this study is an easy and fast one, carried out with a Ultraviolet/Visible (UV/VIS) spectrophotometer.<sup>3</sup> Specifically, two solutions were prepared from blood samples: solution A—adding 100µl of blood to 9,900µl of pure deionized water; solution B—adding 100µl of blood and 50µl of potassium ferricyanide (K<sub>3</sub>Fe[CN]<sub>6</sub>) to 9,850µl of pure deionized water, thus obtaining a 100%-methemoglobin-sample. Eight equally spaced values of absorbance were registered for both solutions. Those points were then interpolated by a fourth-grade regression function and its first derivative was calculated at  $\lambda=645\text{nm}$  for both specimens. The total percentage of methemoglobin was then obtained by a ratio of the two first derivative values. Intra- and inter-assay precision and trueness were assessed and found to be satisfactory (RSD < 20%, bias < 20%). One hundred blood samples from subjects deceased in 2018, 2019, 2020, and 2021 for causes not related to high methemoglobinemia were analyzed with the described method. Blood samples had been stored in the freezer since the date of the autopsy. Mean MetHb% values for blood samples from 2018 (78%) were found to be significantly higher than the ones from 2019 (61%), 2020 (59%), and 2021 (43%), while mean values for 2019 and 2020 were significantly higher than the ones from 2021. The samples did not show a maximum MetHb threshold. Fresh blood samples were analyzed as well, both once a week and every day. Both showed a rapid increase in MetHb%, even though it was higher for samples unfrozen every day, suggesting that the freezing-unfreezing cycle could influence MeHb spontaneous formation.

Those findings suggest that MetHb analysis with this or an analogous fast method should be introduced as a screening procedure immediately after blood sample collection for all those cases in which nitrate poisoning is suspected. Indeed, a later determination of MetHb may be invalidated by spontaneous formation, leading to false positive findings.

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**Methaemoglobin; Nitrate Suicide; Toxicology**

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**L26 A New Assay for Phosphatidylethanol (PEth) in Blood Via Gas Chromatography/Electron Impact/Mass Spectrometry (GC/EI/MS)**

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**Learning Objective:** After attending this presentation, attendees will understand that ethanol in the form of PEth can be incorporated into Red Blood Cells (RBCs), extracted, derivatized, and assayed by GC/EI/MS.

**Impact Statement:** This presentation will impact the forensic science community by presenting a new method of analysis for phosphatidyl ethanol (PEth), a key long-term drug biomarker.

We report a new method to assay PEth in blood via derivatization and GC/EI/MS analysis. The goal was to develop a “point of care” means of detection and quantitation of PEth to identify alcohol misuse of patients in a clinical setting that was faster and less expensive than liquid chromatography/tandem mass spectrometry.<sup>1,2</sup> Derivatization creates a chemical species consisting of an ethyl phosphate headgroup with two TMS moieties. Thus far, quantitation has been performed in whole and Dried Blood (DBS). Samples were extracted with various combinations of methanol, isopropanol and chloroform. DBS samples were hole punched as single units and sonicated in extracting solvent. The supernatant was filtered, evaporated to dryness, and derivatized. Derivatization of phosphatidylethanol 16:0/18:1 (PEth) and phosphatidylethanol-d5 16:0/18:1 (PEth-d5) with BSTFA or MSTFA yielded the corresponding phosphate ester-di-trimethylsilyl product.<sup>3-5</sup> Samples were analyzed by GC/EI/MS in full scan/Selected Ion Monitoring (SIM) dual mode on an Agilent® 7890A/5975C GC/MSD fitted with an Rtx-5ms GC column and helium carrier gas. Chromatographic separation of PEth-di-TMS and several other PEth phosphate ester di-TMS analogs used as internal standards was surprisingly good. Most work was focused on PEth-d5 as the internal standard. Analysis of whole and dried blood samples were linear in the range of 100-1,500ng/mL using PEth-d5 as internal standard. Method precision and accuracy have not yet been established. This new method is effective at measuring the total amount of ethanol incorporated into the blood phospholipids. Unlike existing LC/MS/MS methods that directly measure individual PEth species, our new method captures all ethanol incorporated into the RBC phospholipids by forming a single, derivatized ethylphosphate analyte. This new approach has several advantages over prior methods: the cost of instrument purchase and operation and the flexibility to detect ethanol in whole, dried, and potentially decomposed blood (ex. postmortem). This work is based on United States patent 11,085,939 B2 titled “Quantifying Phosphatidylethanol from Blood Samples” published in 2021.

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**Phosphatidyl Ethanol; Derivatization; Drug Biomarker**

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**L27 Death Following the Accidental Ingestion of E-Liquid**

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**Learning Objective:** After attending this presentation, attendees will be more familiar with the clinical manifestations, associated autopsy and histopathology findings, and toxicology results following accidental ingestion of concentrated liquid nicotine or “e-liquid.”

**Impact Statement:** This presentation will impact the forensic science community by presenting an uncommon case of accidental ingestion of e-liquid with a fatal outcome. This presentation can provide valuable and helpful information in managing similar cases.

Electronic cigarette (e-cigarette) use is growing and becoming increasingly popular in society, resulting in both intentional and unintentional exposures to e-liquid. E-cigarettes vaporize e-liquid—a solution composed of nicotine, propylene glycol, and vegetable glycerin. The nicotine-containing solution can be refilled, and such e-liquid refill cartridges are easily accessible. The acceptable nicotine concentration in e-liquids can vary from 20mg/ml to 60mg/ml. Fatalities following ingestion of e-liquid are extremely rare but can affect people of different ages or even children.

A 30-year-old man accidentally ingested an unknown amount of e-liquid. He kept the liquid in his refrigerator in a plastic bottle. He confused the liquid with alcohol that he kept in a similar bottle in the same place. Immediately after ingestion, he tried to spill out the substance and additionally tried to vomit, but without any visible result. He took activated carbon with water. The onset of symptoms was sudden, so he and his girlfriend called an ambulance. The physician recommended hospital admission, but the man refused and decided to stay home. According to his girlfriend, his condition worsened in the following hours, and during the night he was shaking, having convulsions, and eventually died.

A second ambulance was called, but the subject was pronounced dead on the scene. The postmortem examination revealed only evidence of severe brain and lung edema. An excessive amount of transparent liquid with small grayish particles was present in the stomach and intestines, consistent with activated carbon ingestion. The toxicology report showed a high nicotine concentration in the deceased's blood—3.3µg/mL. The nicotine concentration in the ingested liquid was 125mg/ml. The cause of death was acute nicotine toxicity, and the manner of death was an accident.

The easily accessible refill cartridges containing e-liquid give people a chance to make their own “homemade” and more concentrated e-liquids. The inappropriate conservation of such substances is a potential risk factor for accidental ingestion and sudden death, as in the case presented.

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**Nicotine Intoxication; E-Liquid Ingestion; Sudden Death**

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**L28    The Evaluation of the Cross-Reactivity of Cannabidiol (CBD), Delta-Tetrahydrocannabinols (Delta-THCs), Olivetol, and Metabolites in Whole Blood Using a THC Enzyme-Linked Immuno-Sorbent Assay (ELISA) Kit**

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**Learning Objective:** After attending this presentation, attendees will better understand the cross-reactivity of some forensically relevant CBDs in whole blood to a THC direct immunoassay.

**Impact Statement:** This presentation will impact the forensic science community by providing information that can be useful to those who use ELISA assays as screening methods for  $\Delta^9$ -THC in whole blood.

With the legalization/decriminalization of cannabis starting with Colorado in 2012 and the passage of the 2018 Farm Bill (Hemp Bill), there has been an increase in the availability of THC analogs, both natural and synthetic, CBD, and other cannabis-related compounds such as olivetol. The major psychoactive compounds of cannabis include  $\Delta^9$ -THC and CBD. Non-traditional cannabinoids such as  $\Delta^8$ -THC and  $\Delta^{10a,6a}$ -THC are psychoactive cannabinoids and often synthesized from THC and/or CBD. Olivetol is a precursor in the biosyntheses of THC and has been antidotally reported as a Cannabinoid Receptor One (CB1) antagonist. ELISA are often used as a non-specific preliminary test for the analysis of whole blood specimen in human performance (i.e., suspected Driving Under the Influence of Drugs [DUID]) and postmortem cases.

The cross-reactivity in whole blood of  $\Delta^9$ -THC,  $\Delta^8$ -THC, and their 11-hydroxy (11-OH-THC) and carboxylic acid (THC-COOH) metabolites, the enantiomers 9(R) and 9(S)  $\Delta^{10a,6a}$ -THC, CBD and its 6- and 7-hydroxy (7- or 6-CBD-OH and carboxylic acid (CBD-COOH) metabolites along with olivetol and olivetolic acid (Cerilliant®) were evaluated. The analysis was performed using an Immualysis™, ELISA for forensic matrices, Cannabinoids (THC) kits on a Bio Tek Synergy H1 Hybrid Multi-Mode Monochromator Fluorescence Microplate Reader. The immunoassays parameters were set according to manufacturer specifications, including the volumes and incubation times. The cutoff was established using 10ng/mL  $\Delta^9$ -THC-COOH in blood.  $\Delta^9$ -THC-COOH was analyzed at 10 concentrations ranging from 5–500ng/mL ( $n=3$ ). The other cannabinoids were analyzed at 10, 20, 50, 100, 500, and 1,000ng/mL in triplicate depending on their determined cross-relatively.

Using a 10ng/mL  $\Delta^9$ -THC-COOH as the cutoff, it was determined that at 1,000ng/mL CBD and metabolites, olivetol and olivetolic acid and 100ng/mL of 9(R) and 9(S)  $\Delta^{10a,6a}$ -THC did not cross-react with the assay. Both  $\Delta^9$ -THC and  $\Delta^8$ -THC produced positive results at 500ng/mL but not at 100ng/mL. 11-OH- $\Delta^9$ -THC, 11-OH- $\Delta^8$ -THC, and  $\Delta^8$ -THC-COOH produced positive results at 20, 50, and 10ng/mL, respectively. These results were consistent with those listed on the packet insert, except the 11-OH-THC metabolites observed at a 10x greater cross-reactivity.

The Immualysis™ ELISA for forensic matrices THC kit can be used as an effective preliminary test in whole blood for the analysis of THC metabolites but not THC parent compounds with a low potential of false positives in the presences of CBD and/or its metabolites.

*Funded in part by the National Institute of Justice (NIJ) Research and Development in Forensic Science for Criminal Justice Purposes (15PNIJ-21-GG-04188-RESS) and National Institute on Drug Abuse (P30 DA033934).*

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**ELISA; THC; Cross-Reactivity**



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**L29 A Review of Illicit Substances Detected in Homicidal Deaths in Memphis, Tennessee, Over a Three-Year Period**

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**Learning Objective:** This presentation will give attendees an overview of illicit substances detected in homicidal deaths in Shelby County, TN, based on autopsy examinations performed at the West Tennessee Forensic Science Center over the course of three years. This study benefits the forensic community by providing insight into illicit substances detected on toxicology in homicidal deaths.

**Impact Statement:** The presentation will impact the forensic science community by providing insight into drug and substance incidence in homicidal deaths in a high crime-volume major city.

Memphis, TN, has been ranked as one of the most dangerous cities in the United States with homicide rates continuing to increase. Memphis Crime Organization reported a 7.2% increase in homicides in 2021 compared to 2020 alone.<sup>1</sup> Drug-related deaths and crimes have also increased. This study analyzes toxicology reports for homicides in Memphis, TN, to identify the incidence of illicit substances detected in homicidal deaths. Analysis was performed to categorize cause of death and to analyze illicit substances including stimulants, depressants, or a combination of both. We subsequently aim to evaluate circumstances of the deaths in a subset of cases.

The Death Investigation and Decedent Information (DIDI) database was utilized to identify homicidal deaths. A total of 848 homicides were identified from 2019–2021 with the cause of death breakdown as follows: gunshot wound-related, 769 cases (91%); blunt force trauma, 30 cases (4%); sharp force injury, 30 cases (4%); strangulation, 3 cases (< 1%); more than one injury type, 4 cases (< 1%). Toxicology was performed in 804 cases. Toxicology testing was negative in 143 cases (18%), with 661 cases having either illicit substances and/or ethanol detected. Cases with ethanol and metabolites of marijuana were excluded from the study. A total of 243 cases with illicit substances were found with a breakdown as follows: stimulants only, 131 (54%); depressants only, 86 (35%); combined stimulant and depressant, 26 (11%). Fentanyl was the most common illicit depressant and was detected in 50 cases. The average fentanyl concentration among all cases was 28ng/mL (Range: .22ng/mL–370ng/mL). Cocaine was the most common stimulant, comprising 100 cases with other stimulants to include methamphetamine and MDMA. Stimulants were the most common illicit substances in gunshot-related injuries and blunt force injuries. Sharp force injuries most commonly had a combination of at least one depressant and one stimulant.

The findings of this study demonstrate the significant presence of illicit substances detected in homicidal deaths and highlights the increase of the recreational use of fentanyl. This is an evolving study and we aim to review circumstances in all homicides with fentanyl detected.

**References:**

<sup>1</sup>. Memphis Crime Rate: Crime Statistics Memphis TN: MSCC. Crime Commission, <https://memphiscrime.org/the-stats/>.

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**Homicide; Toxicology; Fentanyl**



**L30 The Extraction, Isolation, and Identification of Sildenafil From Urine Using Thin Layer Chromatography (TLC)**

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**WITHDRAWN**

## **L31 The Development of New Solvent Systems for the Analysis of Clonazepam in Blood Using High-Performance Thin-Layer Chromatography (HPTLC) Plates**

*Naagar Sunil, MD\*, AIIMS New Delhi, New Delhi, INDIA; Rohit Kanojia, PhDc, Department of Chemistry, University of Delhi, New Delhi, INDIA*

**Learning Objective:** After attending this presentation, attendees will better understand a new method for the analysis of clonazepam in blood with the help of a new solvent system by using a TLC technique.

**Impact Statement:** Clonazepam is the most common drug prescribed to depressed people worldwide, and many cases of clonazepam toxicity are reported annually. This presentation will impact the forensic science community by showing a new method for the analysis of clonazepam in blood with the help of a new solvent system by using a TLC technique.

Clonazepam is a benzodiazepine medication used to prevent and treat seizures, panic attacks, anxiety, and akathisia and is typically taken orally. This medicine has a few common side effects, including poor coordination, sleepiness, and agitation. Chronic use normally results in the developments of high tolerance and dependence, and withdrawal symptoms may occur if it is stopped abruptly. It belongs to class of psychoactive drugs. Overdose of this drug causes mental confusion, nausea, impaired reflexes, dizziness, respiratory depression, low blood pressure, and eventually coma. These are frequently encountered in clinical and forensic caseworks involving drug overdoses. These Benzodiazepine (BZD) drugs have hypnotic, tranquilizing, and anticonvulsant properties in general. Patients usually taking the drug for four weeks and above develop a dependence on it, and results in overdosing of the same.

In this study, an attempt has been made to develop a new solvent system for the analysis of clonazepam in blood using HPTLC plates by a TLC technique. A Dispersive Liquid-Liquid Micro Extraction (DLLME) method was used for the extraction of drug from blood. TLC plates were viewed under Ultraviolet (UV) light at 254nm and 365nm; iodine fuming was performed to confirm the results. A solvent system with different ratios taken was analyzed for their retention factors, and the best solvent system was chosen. Among all the solvent systems taken, the best solvent systems were used for the analysis of clonazepam in blood, which are ethyl acetate; methanol:chloroform; ethyl acetate:methanol. These solvent systems showed clear spots and good Retention factor (Rf) value. Spots were clear and Rf values were similar in the case of analysis of the drug in blood as was in the standard. The detection of clonazepam in blood by TLC technique, using HPTLC plates, proved to be a convenient method, being cheaper and cost effective. The method can be used in any laboratory using the above solvent system.

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**Clonazepam; Thin-Layer Chromatography; Benzodiazepines**

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## **L32 The Analysis of Vitreous Humor: Enhanced Toxicological Testing Potential in Postmortem Examination**

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**WITHDRAWN**

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**L33 The Generation of Methamphetamine From Amphetamine by N-Methyltransferase in Human Cytosol: Implications for Therapeutic Amphetamine Patients**

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**Learning Objective:** The goal of this presentation is to demonstrate the potential for enzymatic methylation of Amphetamine (AMPH) and explore the potential significance for therapeutic AMPH patients.

**Impact Statement:** This research focuses on the potential for small concentrations of Methamphetamine (MAMP) to be generated by enzymatic methylation in circumstances of elevated AMPH levels. This presentation will impact the forensic science community by exploring the potential for such findings to be interpreted as MAMP abuse.

The relatively rapid N-demethylation of MAMP, producing AMPH, is well recognized as the basis for the presence of AMPH in biological fluid and hair samples of individuals using MAMP as a recreational drug. In contrast, there is no expectation that individuals either abusing, or receiving AMPH for therapeutic purposes (e.g., as treatment for ADHD), will generate any significant levels of MAMP, and its presence in drug abuse monitoring samples is routinely presumed to reflect MAMP use. Such MAMP-positive results can lead to significant medical or legal consequences.

We have recently investigated a number of cases where the interpretation of very low levels (e.g., < 50ng/mL) of MAMP in urine drug monitoring samples (confirmed by Gas Chromatography/Mass Spectrometry [GC/MS]) as legitimately indicating MAMP abuse in individuals receiving therapeutic AMPH was challenged. (We recognize that such challenges may not be justified). In response, we have investigated the ability of an S-adenosylmethionine dependant N-methyl transferase in human hepatic S-9 cellular fraction to generate MAMP in circumstances of therapeutic levels of AMPH.

We have modified an enzymatic method for phenylethanolamine-N-methyl transferase originally published in 1962, to allow for small volume incubation, using an S-9 cellular fraction, with subsequent extractions of neurotransmitters and analogs (e.g., AMPH and MAMP) and analysis via GC/MS.<sup>1,2</sup> We have then investigated the ability of the enzyme to generate MAMP and the impact of product inhibition on the equilibrium between the methylation and demethylation pathways.

Our results indicated that human cytosolic N-methyl transferase, normally functional in neurotransmitter synthetic pathways, is capable of generating MAMP from AMPH. Accumulation of significant levels of MAMP in cases of therapeutic AMPH administration is limited by the competing action of an NADPH-dependent N-demethylase. The equilibrium between the N-methyl transferase and the N-demethylase is markedly in favor of the demethylation process and also reflects product inhibition of the N-methyl transferase. This relation between the enzyme pathways is consistent with the practical observations that significant levels of AMPH are regularly observed in cases of MAMP abuse but, in contrast, significant levels of MAMP are not similarly observed in individuals receiving therapeutic AMPH.

Our results further suggest that in some cases, in which very low levels of MAMP are detected in the face of much higher AMPH levels (corresponding to a therapeutic regimen), investigating personnel might wish to consider the possibility that the ratio reflects an equilibrium between AMPH and MAMP, rather than necessarily being indicative of MAMP abuse.

**References:**

- <sup>1.</sup> Axelrod J. The Enzymatic N-Methylation of Serotonin and Other Amines. *J. Pharmacol Exper. Therap.* 1962; 138 (1): 28-33.
- <sup>2.</sup> Axelrod J. Purification and Properties of Phenylethanolamine-N-methyl Transferase. *J. Biol. Chem.* 1962; 237(5):1657-1660.

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**Methamphetamine; Amphetamine; N-methyltransferase**

## L34 Molecular Dynamics Simulations of Novel Synthetic Opioids Binding to the Opioid Receptors (ORs)

Uzoma Okafor, PhD\*, Albany State University, Albany, GA

**Learning Objective:** The presentation will highlight various recent data on the medicolegal cases of opioid abuse, the role of opioid receptors in synergistic response to opioid dependence and withdrawal syndrome, and the binding affinities of various novel synthetic opioids.

**Impact Statement:** The presentation seeks to provide the basis for synthetic opioid identification via molecular dynamics simulations

The US Centers for Disease Control and Prevention's recent data on opioid abuse showed that opioid abuse had become one of the country's most critical public health crises.<sup>1</sup> Various research has identified 3 major opioid receptors, and recently another receptor; Opioid Receptor Like 1- ORL 1 (also called nociception, or orphaninFQ receptors). It has been reported that ORL 1 receptors are the youngest members of the ORs.

Post-translational processing and other environment episodes (in brain reward pathways) are significant contributors to the synergism in cooperativity binding in opioid receptors to various opioids resulting in difficulties in the treatment and relapse in opioid addictions.<sup>2</sup> Opioid withdrawal syndrome results from chronic opioid use which usually leads to the advancement of possibly devastating opioid dependence. Recent data showed that the misuse, or abuse of opioids, is a major contributing factor in reported medicolegal cases of opioid-related cases involving fatalities.<sup>3</sup>

Well-known opioid receptors (ORs- Mu, Kappa, and Delta) and the subtypes or isoforms are mostly G-protein coupled receptors and activate G-protein inhibitory activities.<sup>4</sup> To demonstrate the specificity of some selected novel opioids to the 3 classical ORs, the molecular dynamics simulations of various novel opioids to the ORs ligands upon binding were investigated in this study. To further explain biases in specificity in binding of the ligands, the binding pockets of the ORs were examined for the nature of amino acids present and the whole amino acids sequence of the receptors was aligned using sequence alignment in Molecular Evolutionary Genetics Analysis (MEGA) software.<sup>5</sup> Also, the binding simulations of the selected novel opioids to Opioid Receptor-Like 1 (ORL 1) were studied. Thus, elucidating any possible molecular affinity of novel opioids binding to ORL 1 opioid receptor targets. ORL1 receptors could likely be considered a candidate for the future of opioid pharmacology if there is a strong binding affinity. The sequence of the ORs (Mu, Kappa, and delta) were aligned using Molecular Evolutionary Genetic Analysis (MEGA 11) software, to generate a phylogenetic tree to establish an evolutionary relationship amongst the receptors. The molecular docking was performed using a virtual screening software for computational drug discovery -PyRx software.

Lofentanil a Fentanyl derivatives binding affinity to the ORs studied was used as baseline binding scores for the binding of the nascent synthetic opioids. Lofentanil generated low binding scores, as compared to the synthetic opioids used in the research having noticeable increased binding scores. The results of the binding affinities reveal an important relationship between the binding scores of opioids. The binding affinities show that the molecular binding dynamics methodology can be used to precisely predict the binding strength of nascent synthetic opioids or unidentified fentanyl derivatives or analogs at various ORs or OR1 which can be used to support in vitro analysis data. There is a need to urgently identify new opioids on the street and properly scheduled them as controlled substances; this may help reduce the menace of opioid abuse and availability on the street thus reducing the risk of exposure and death as a result of fentanyl or fentanyl derivatives exposure. This molecular approach can also assist in the development of a potent and functional antagonist that can compete with fentanyl for the binding site on the ORs, thus providing a quick and effective method to reverse the activity of the fentanyl or its derivatives.

The molecular dynamic simulations studies of novel opioids to various ORs and ORL1 opioid receptors will provide an understanding of the binding affinity of the selected novel opioids to the ORs or ORL 1, and efficiency concerning the potency, receptor affinity, ORs subtype selectivity, and other structural and molecular characteristics.

### References:

1. Zhennan Zhao, Tingting Huang, Jiazhong Li (2020) Molecular Dynamics Simulations to Investigate How PZM21 Affects the Conformational State of the  $\mu$ -Opioid Receptor Upon Activation. *Int. J. Mol. Sci.*, 21, 4699.
2. Piotr FJ Lipiński, Małgorzata Jarończyk, Jan Cz. Dobrowolski and Joanna Sadlej (2019). Molecular dynamics of fentanyl bound to  $\mu$ -opioid receptor. *Journal of Molecular Modeling* 25: 144.
3. Ream Al-Hasani, Michael R Bruchas. (2011). Molecular Mechanisms of Opioid Receptor-Dependent Signaling and Behavior. *Anesthesiology*. 115(6): 1363–1381.

### Opioid Receptors; Synthetic Opiates; Molecular Dynamics Simulations

## L35 The Detection of $\Delta^9$ -Tetrahydrocannabinol (THC) in Oral Fluid Using the Fast Blue BB (FBBB) Test

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**Learning Objective:** After attending this presentation, attendees will understand the concept of a colorimetric and fluorometric test for the differentiation of marijuana-type and hemp-type cannabis and its translation to the oral fluid platform for application in the field, including the figures of merit as method proof of principle.

**Impact Statement:** This presentation will impact the forensic science community by developing a new possibility for the screening of individuals under possible cannabis influence using oral fluid as a biological matrix.

Cannabis is currently one of the most abused substances in the world: around 200 million users were reported in 2019.<sup>1</sup> In addition, there is an increasing number of jurisdictions that have legalized or decriminalized the recreational use of marijuana in the United States. The FBBB screening test was recently validated for the analysis of plant extracts for the differentiation of marijuana-type cannabis ( $\geq 0.3\%$  w/w THC) from hemp-type cannabis ( $< 0.3\%$  w/w THC). The test is based on both the formation of a chromophore and the formation of a fluorophore, presenting a red color and fluorescence when reacted with THC. It presents an orange color and absence of fluorescence when reacted with Cannabidiol (CBD).<sup>2-4</sup> The fluorescence of THC and FBBB enhances the selectivity of the test. Oral Fluid (OF) has become a suitable biological matrix for drugs of abuse detection, and its easy sample collection facilitates for a test with field applicability, such as roadsides or workplace scenarios.

The aim of this project is to translate the FBBB test for the analysis of OF samples and THC detection. The test optimization included the evaluation of different substrate materials for a miniaturized reaction, such as Planar Solid Phase Microextraction (PSPME), silicon filter paper, Whatman® number 3 filter paper, and glass fiber filter paper, which was the chosen substrate. A calibration curve was prepared of different concentrations of THC diluted in OF, and the Limit Of Detection (LOD) for THC—in which fluorescence could be observed—was established in approximately 30ng. The potential to report quantitative concentrations of THC in OF using the FBBB test is explored. Interference studies for other cannabinoids and metabolites, and for other substances that could potentially be found in the oral cavity, such as caffeine, nicotine, teas, juices, and sodas, were conducted. Among these substances,  $\Delta^8$ -THC and the metabolites THC-COOH and THC-OH also presented red color and fluorescence in the presence of FBBB. The stability of the testing platform is also explored. Initial studies show that the red color and fluorescence on the spots are stable for at least 12 days, when protected from light.

In conclusion, the FBBB test is an inexpensive, sensitive, and rapid field test that can detect the presence of THC in OF. It could improve the identification of drivers, workers, or athletes, for example, that have recently smoked marijuana.

### References:

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2. HS Franca, A Acosta, A Jamal, W Romaoa, J Mulloor, JR Almirall. Experimental and ab initio investigation of the products of reaction from  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and the fast blue BB spot reagent in presumptive drug tests for cannabinoids. *For. Chem.*, 2020, 17, 100212. <https://doi.org/10.1016/j.forc.2019.100212>.
3. A Acosta, JR Almirall. Differentiation between Hemp-type and Marijuana-type Cannabis using the Fast Blue BB Colorimetric Test. *For. Chem.*, 26, 2021, 1000376. <https://doi.org/10.1016/j.forc.2021.100376>.
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### THC; FBBB; Oral Fluid

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**L36 The Elution and Recovery of Cannabinoids From an Impaction Filter Device**

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**Learning Objective:** After attending this presentation, attendees will better understand the use of impaction filter devices for the collection of exhaled breath aerosols from cannabis users in the field and challenges specific to the quantitative analysis of cannabinoids recovered from these devices.

**Impact Statement:** This presentation will impact the forensic scientific community by describing methods to improve quantitative recovery of cannabinoids from impaction filter devices, with a focus on  $\Delta^9$ -Tetrahydrocannabinol (THC), Cannabidiol (CBD), and Cannabinol (CBN). The presentation will primarily describe the analysis of laboratory samples where THC, CBD, and CBN concentrations are known a priori, but will also include analysis of authentic samples.

Cannabis has been decriminalized or legalized across the United States, with a coinciding increase in the use of recreational and medicinal cannabis. Cannabis induces decreases in executive function and motor control that make drivers more likely to be involved in crashes than unimpaired drivers.<sup>1</sup> Breath is a non-invasive matrix that is suitable for roadside sampling and/or detection and  $\Delta^9$ -THC has been detected in breath samples collected with a variety of devices. However, due to its low volatility, THC is expected to be carried by aerosol particles generated by the mechanical disruption of the airway lining fluid during inhalation.<sup>2</sup>

From 2020 to 2022, our team analyzed breath samples from legal-market cannabis users collected with an impaction filter device. The device consists of a wide mouthpiece followed by three parallel collectors. The cylindrical collectors are 22mm long, have a 10mm internal diameter, and contain 8 baffles perpendicular to the main direction of the flow that dictate the velocity profile. Breath samples were collected from participants 1h after they smoked THC-dominant cannabis. Following sample processing, we detected THC, CBD, and CBN in one or more eluates.<sup>3</sup> While our results validated the hypothesis that cannabinoids are carried by aerosols, the effect of sample processing on cannabinoid recovery remained unknown. In this work, cannabinoids were recovered by eluting each collector with methanol containing ethylene glycol as a keeper, combining the eluates from all three collectors, and vacuum concentrating the eluate for analysis by liquid chromatography with tandem mass spectrometry. If each collector could be analyzed separately, one could potentially be analyzed in the field with rugged instruments, another in the laboratory with high sensitivity instruments, and the third could be archived.

To identify sample processing factors that significantly impact THC, CBD, and CBN recovery from an impaction filter device, we conducted a sensitivity analysis with six factors. These factors included target compound concentration, extraction solvent, keeper, agitation, container material, and the temperature during vacuum concentration. These factors address the potential for cannabinoids to degrade with time or temperature, the efficiency of the extraction method, and the interaction of cannabinoids with surfaces. Single collectors were removed from the device and prepared for analysis by adding cannabinoids within a small volume (20 $\mu$ L) of ethanol; this solvent wets the top and bottom of each baffle within the collector without pooling in the bottom of the vial. Each collector was then dried at room temperature in the dark. Our sensitivity analysis utilized a fractional factorial design to identify significant factors with an efficient number of experiments. Sensitivity analysis has been used in the optimization of other developing methods to investigate robustness and the precision of sample processing or instrumental methods.<sup>4</sup> This presentation will conclude by describing quantitative analysis of authentic samples from cannabis users who smoked THC-dominant, CBD-dominant, or a mixed cannabis strain. This work is an essential step in validating breath aerosol collection as a sensitive and precise method for the quantitation of cannabinoids in breath.

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2. Lovestead TM, Bruno TJ. Determination of cannabinoid vapor pressures to aid in vapor phase detection of intoxication. *Forensic Chemistry* 2017, 5, 79-85.
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**Breath Aerosols; Cannabis; Impaction Filters**



**L37 The Characterization of Spice Products: Barely Legal, Voodoo Child, Brainstorm and Their Effects on Human A549 Lung Cells**

*Oluseyi Vanderpuye, PhD\*, Fayetteville State University\*, Fayetteville, NC; Miron Gilbert, PhD, Tifton Circuit Public Defender Office, Tifton, GA*

**Learning Objective:** After attending this presentation, attendees will have learned about the analysis of the Spice products Barely Legal, Voodoo Child, and Brainstorm by Thin-Layer Chromatography (TLC) and Fourier Transform Infrared (FTIR) spectroscopy and their effects on human lung A549 cells as assessed by fluorescence microscopy with lectins and antibodies to cannabinoid receptors and complement system regulators and Western blotting with lectins to assess cell surface glycoproteins.

**Impact Statement:** This presentation will impact the forensic science community by increasing awareness of the use of human lung A549 cells for studying the effects of Spice synthetic cannabinoid products on human lung cells. Attendees will also see how different Spice products vary in analysis by TLC and FTIR spectroscopy.

**Hypothesis/Proposition:** “Spice” refers to brands of designer drugs consisting of various plant materials on which different synthetic analogs of  $\Delta$  Tetrahydrocannabinol (THC) have been detected in some analyzed samples.<sup>1</sup>  $\Delta$  THC is the main psychoactive compound in marijuana. There are serious forensic and health problems with Spice. The first is that the actual composition and type of synthetic cannabinoid of many brands and even different samples of the same brand may vary thus constituting problems in analysis and identification. Second, the toxicology and range of biological effects of the Spice brands, and particularly the emerging ones, is incompletely understood and even unknown in the case of some newer synthetic cannabinoids.<sup>1</sup> Third, the use of Spice is a global problem that is not covered under international conventions on drug trafficking and has been of serious concern to the United States armed forces. Spice products have been reported to contain a variety of synthetic cannabinoids that cause hallucinations, euphoria, paranoia, and numerous other side effects similar to those of delta-9-THC.<sup>1,2</sup> However, for three different brands of Spice—Barely Legal, Brainstorm, and Voodoo Child—there is no information in the peer-reviewed literature concerning their composition and biological activities and toxicology. In particular, there is no knowledge of the effects of these brands on a target tissue such as lung cells or important endocrine cells such as those of the adrenal cortex. For these reasons, it is important to investigate and develop methods for analysis of Spice products and their toxicological effects on cells. It is hypothesized that the Spice brands Barely Legal, Brainstorm, and Voodoo Child contain different compounds, overlap in their biological activities with  $\Delta$  THC, and affect the function, protein expression profiles, and morphology of cells.

**Synopsis of the content/statement of methods:** Methanol and chloroform extracts of the Spice brands Barely Legal, Brainstorm, and Voodoo Child were examined by using UV-visible, FTIR, and Nuclear Magnetic Resonance (NMR) spectroscopy and thin-layer chromatography. Human A549 lung cells and SW-13 adrenal gland cells were cultured in the absence and presence of the three Spice brands. Lectin-binding fluorescence, immunofluorescence microscopy, and gel electrophoresis were used to characterize A549 and SW-13 cell proteins in the presence and absence of the three Spice brands and  $\Delta$  THC.

**Results:** The results showed that UV-visible and FTIR spectra of the three Spice samples showed many similar absorption peaks but also some differences. TLC showed fluorescent spots common to all three samples with similar mobilities but also differences among the three Spice brands possible biomarkers for the effects of Spice; fluorescence microscopy showed that A549 and SW-13 cells contain cannabinoid receptor type 2 proteins but not type 1 receptors. The plasma membrane complement system regulatory proteins CD46 and CD59 but not CD55 were on A549 cells. Glycoproteins of these cells were identified by carbohydrate-binding proteins (lectins) for further detection of the biological effects of the Spice brands on cells. Comparison of the glycoprotein composition of control cells and ones exposed to the Spice products and  $\Delta$  THC revealed only subtle or no changes by lectin staining of proteins separated by electrophoresis. Exposure to the cannabinoids  $\Delta$  THC and Barely Legal Spice caused stronger Con A lectin binding by fluorescence microscopy to A549 lung cells than exposure to Brainstorm Spice or control cell culture media.

**Conclusion:** The different Spice products can be distinguished by TLC and FTIR spectroscopy. Concanavalin A lectin bound more strongly to A549 cells after treatment with Barely Legal and  $\Delta$  THC. Little difference in glycoprotein composition occurs in A549 cell lines after exposure to the Spice products under the conditions used in this study.

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**Synthetic Cannabinoids; A549 Cells; Lectins**

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**L38 A Sip of Blue Wine: Fatal Intoxication by Dimethoate**

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**Learning Objective:** After attending this presentation, attendees will have learned that classical biological matrices cannot always be used to determine acute intoxication by substances that occurred months earlier.

**Impact Statement:** This presentation will impact the forensic science community by stimulating debate about the use of traditional methods in unconventional cases and encouraging researchers to publish their results to build a reference database.

**Case report:** A 72-year-old man was in the backyard of his house when he saw his neighbor walking by with brightly colored plastic bottles filled with a dark liquid. Later, he saw the same bottles lying on the ground outside his brother's door. Thinking it was wine, he drank an unspecified amount of it, only to realize he was mistaken and spit out the blue liquid. Shortly thereafter, he developed abdominal pain and lost consciousness. He was rushed to the nearest emergency room, where gastric lavage was performed. Police seized the bottle and identified the contents as an insecticide called Rogor L40 ST2020, which is an organophosphorus compound (dimethoate). The patient underwent various diagnostic tests, including a negative test for psychotropic substances, and blood tests that showed a non-specific increase in inflammatory indices and a progressive decrease in pseudokinesterases within a few hours. Atropine and pralidoxime were immediately administered, and the patient was artificially ventilated due to respiratory distress. However, despite therapy, the man suffered cardiac arrest. The patient was resuscitated, intubated, and admitted to the intensive care unit for one month, where he was also tracheostomized. The man then remained in the hospital for another two months, during which he developed various infections and died immediately after emergency surgery for an abdominal abscess. The Judicial Authority ordered an autopsy and extensive toxicological testing to determine whether the patient's progressive clinical deterioration and death were a direct result of the unwitnessed ingestion of an unknown amount of dimethoate.

**Results:** The autopsy findings were unremarkable. In addition to a purplish skin color, examination of the body revealed signs of prolonged hospitalization such as marked sarcopenia, numerous fluid-filled blisters on the lower limbs, and an advanced decubitus ulcer on the sacrum with exposed bone. Internally, there was a serous effusion of blood in the pleural cavities, pericardium, and hepatic cavity. All organs appeared to be moderately congested. The only macroscopically visible changes were cardiac hypertrophy, hepatic nodules, esophageal hyperemia, and a tracheoesophageal fistula. The abdomen showed signs of open surgery and a polyp on the top of the bladder dome. Tissue specimens (blood, hair, and organ samples) were collected for histologic and toxicologic studies. Histological examinations were affected by marked autolytic phenomena and only confirmed already-known pathological conditions such as cardiac hypertrophy, cirrhosis, and chronic obstructive pulmonary disease. Toxicological tests had to be performed on the collected biological samples to detect dimethoate poisoning. However, it was decided to analyze the keratin matrix of hair since the classical biological matrices such as blood, urine, liver, kidney, and brain could not be used due to the long hospital stay. This experimental approach, never described in literature for this organophosphorus compound, was performed by liquid chromatography combined with high-resolution mass spectrometry (HPLC-HRMS) and showed the presence of dimethoate at a concentration of 0.48ng/mg. Unfortunately, the concentration found in the hair could only confirm the ingestion of dimethoate by the subject, but given the lack of data in the literature, it was not possible to determine the exact amount of substance ingested. However, these positive findings on the keratin matrix scientifically prove dimethoate intoxication, which is consistent with the signs of organophosphorus poisoning observed in the emergency room, such as the progressive decrease in pseudokinesterases.

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**Dimethoate; Organophosphate; Poisoning**

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**L39 Standards Development Activities in Forensic Toxicology**

*Marc LeBeau, PhD\*, FBI Laboratory, Quantico, VA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the status of discipline-specific standards pertinent to the field of forensic toxicology.

**Impact Statement:** This presentation will impact the forensic science community by creating greater awareness of standards development activities pertinent to forensic toxicology. It will also increase awareness regarding training, tools, and resources that support implementation, compliance monitoring, and broader understanding.

The Organization of Scientific Area Committees (OSAC) for Forensic Science was created to strengthen the nation's use of forensic science by promoting the use of discipline-specific forensic science standards. To this end, the OSAC drafts standards that are forwarded to Standards Developing Organizations (SDOs) that further develop and publish them. The OSAC also reviews published 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 forensic toxicology will be presented.

These include:

(1) standards that have been added to the OSAC Registry:

- ANSI/ASB 017: Standard Practices for Measurement Traceability in Forensic Toxicology;
- ANSI/ASB 036: Standard Practices for Method Validation in Forensic Toxicology;
- ANSI/ASB 037: Guidelines for Opinions and Testimony in Forensic Toxicology;
- ANSI/ASB 053: Standard for Report Content in Forensic Toxicology;
- ANSI/ASB 054: Standard for Quality Control Programs in Forensic Toxicology Laboratories;
- ANSI/ASB 119: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Medicolegal Death Investigations;
- ANSI/ASB 120: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Blood in Impaired Driving Investigations;
- ANSI/ASB 121: Standard for the Analytical Scope and Sensitivity of Forensic Toxicological Testing of Urine in Drug-Facilitated Crime Investigation; and
- ANSI/ASB 152: Standard for the Minimum Content Requirements of Forensic Toxicology Procedures

(2) published standards from the Academy Standards Board (ASB) that have yet to go through the OSAC Registry approval process:

- ANSI/ASB 098: Standard for Mass Spectral Data Acceptance in Forensic Toxicology;
- ANSI/ASB 113: Standard for Identification Criteria in Forensic Toxicology;
- ANSI/ASB 153: Standard Practices for Proficiency Testing for Forensic Toxicology Laboratories; and
- ANSI/ASB 156: Guidelines for Specimen Collection and Preservation in Forensic Toxicology

(3) documents currently in development by the ASB:

- ASB 055: Standard for Breath Alcohol Measuring Instrument Calibration;
- ASB 056: Standard Practices for Evaluating Measurement Uncertainty of Quantitative Measurements in Forensic Toxicology;
- ASB 118: Standard for Breath Alcohol Instrument Specifications;
- ASB 122 Best Practice Recommendation for Performing Alcohol Calculations in Forensic Toxicology; and
- ASB 173 Standard for Education and Training of Forensic Toxicology Personnel

(4) documents currently being drafted at the OSAC:

- Quality Management Systems in Forensic Toxicology Laboratories;
- Standard Method for Blood Ethanol Identifications and Quantitations; and
- Human Factors Considerations for Forensic Toxicology Laboratories

(5) priorities for new documents or work products, and other highlights. Opportunities for supplemental training related to discipline-specific standards will be presented, as well as additional resources and tools designed to facilitate gap analysis, compliance monitoring, and outreach efforts.

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**OSAC; ASB; Standards**

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**L40 2021 National Forensic Laboratory Information System (NFLIS) Toxicology Laboratory Survey Findings**

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**Learning Objective:** After attending this presentation, attendees will understand the findings from the Drug Enforcement Administration's (DEA's) National Forensic Laboratory Information System (NFLIS) 2021 Toxicology Laboratory Survey.

**Impact Statement:** This presentation will impact the forensic science community by providing updated testing practices of Toxicology Laboratories (TLs) across the United States that participated in the survey.

The NFLIS is a program of the DEA, Diversion Control Division. In 2018, DEA expanded the NFLIS program to include drug testing results from TLs (NFLIS-Tox). To assist in the recruitment of TLs, in 2017 DEA conducted its first Toxicology Laboratory Survey (NFLIS-Tox Survey). In 2021, the second NFLIS-Tox Survey was conducted to collect updated information on toxicology caseloads, policies, and practices for calendar year 2019. Data for calendar year 2019 were collected because many laboratories were affected by the COVID-19 pandemic, making data for 2020 atypical. Selected results were published on the NFLIS website. The purpose of this presentation is to provide the community with information on toxicology testing practices that were not reported in the survey publication. This information can benefit laboratory management decisions as well as aid interpretation of national statistics.

TL respondents were asked to report their qualitative and quantitative testing frequency as routinely, sometimes, or rarely for specific drugs and drug classes. Data from TLs are reported by laboratory ownership (public or private) and by caseload size of the responding laboratory. This presentation will also report on respondents' use of toxicology reference laboratories. Where possible, comparisons will be made between the two survey years.

TLs reported "routinely" conducting qualitative testing for the following drugs or drug classes more than 75% of the time: alcohol, amphetamines, benzodiazepines, cocaine, fentanyl, marijuana, opiates and opioids (other than heroin or fentanyl), and Phencyclidine (PCP). More than half of TLs reported "rarely" testing phenethylamines, piperazines, synthetic cannabinoids, and synthetic cathinones. Higher percentages of large and private TLs (both 18%) than medium, large, and public TLs (each less than 10%) reported rarely testing fentanyl.

TLs reported "routinely" quantifying the following drugs or drug classes more than 75% of the time: alcohol, amphetamines, benzodiazepines, cocaine, fentanyl, heroin, marijuana, and opiates and opioids (other than heroin or fentanyl). More than half of TLs reported "rarely" quantifying inhalants/volatiles, over-the-counter medications, phenethylamines, piperazines, synthetic cannabinoids, and synthetic cathinones. Higher percentages of small (22%) than medium and large TLs (18% and 5%) and public than private TLs (17% vs. 12%) reported rarely testing fentanyl.

The DEA regularly publishes data from the NFLIS program that can benefit crime laboratories, toxicology laboratories, medical examiner and coroner offices, and public health agencies through various reports throughout the year. Data sharing and integration among complementary datasets help to provide a larger picture of drug use and diversion. NFLIS provides a resource for the community to identify and respond to drugs trends.

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**NFLIS; NFLIS-Tox; Laboratory Survey**

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**L41 The Continually Expanding World of Designer Benzodiazepines**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the increasing scope and prevalence of Designer Benzodiazepines (DBZD) encountered in forensic toxicology casework since 2016.

**Impact Statement:** This presentation will impact the forensic science community by providing a comprehensive timeline and evaluation of DBZD reported in toxicological casework in a nearly six-year time period.

DBZD are a subclass of Novel Psychoactive Substances (NPS). Structurally related to traditional benzodiazepines such as alprazolam or lorazepam, DBZD retain the anxiolytic, sedative-hypnotic, muscle relaxant, and anticonvulsant properties while potentially being more harmful with unknown pharmacological and toxicological profiles. One of the characteristic trends of NPS is that within the subcategories, such as synthetic cannabinoids or synthetic stimulants, new substances enter the market and old, newly controlled, and/or those out-of-favor exit the recreational market. The DBZD subclass has expanded over time to include more and more substances that require surveillance, including testing for metabolites to increase the ability to detect exposure to DBZD.

Many DBZD exhibit good cross-reactivity with commercial benzodiazepine immunoassays; thus it is important for confirmatory assays to include the most current emerging DBZD. At NMS Labs, targeted screening for DBZD involves primarily Liquid Chromatography/Time Of Flight/Mass Spectrometry (LC/TOF/MS). Confirmation is performed via a quantitative Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) panel that initially included etizolam, flubromazolam, clonazolam, diclazepam, delorazepam, meclonazepam, bromazepam, flubromazepam, deschloroetizolam, pyrazolam, and phenazepam, with a subsequent test update to add flualprazolam and alpha-hydroxyetizolam in 2020. Testing capabilities were added for 8-aminoclonazolam and bromazolam in 2020 and 2021, respectively. Data from toxicological testing, including death investigation and Driving Under the Influence (DUI) casework, was compiled to investigate positivity of DBZD in blood specimens between 2016 and 2022, with focus on quantitative results.

Between October 2016 and June 2022, a total of 9,102 total findings of one or more of the above-listed DBZD were reported in 6,804 blood samples submitted during the course of forensic investigations. Detection of DBZD has increased over time: 231 blood samples submitted in CY2017 reported one or more DBZD compared to 2,356 submitted blood samples from CY2021. Etizolam and its metabolite alpha-hydroxyetizolam account for 59% of all DBZD findings in 56.7% of the studied population. Etizolam has remained prevalent for several years, demonstrating continued longevity. Flualprazolam, despite emerging around 2019, accounts for an additional 19% of findings in 26% of the studied population. Other positivity included flubromazolam (7.7%), clonazolam/8-aminoclonazolam (3.5%), bromazolam (3.4%), diclazepam/delorazepam (3.0%), flubromazepam (2.0%), and bromazepam (1.2%). Flubromazolam was present in 10% of the studied population, clonazolam/metabolite and bromazolam around 5%, and diclazepam/delorazepam, flubromazepam, and bromazepam at 2.7%, 2.7%, and 1.6%, respectively. Phenazepam, pyrazolam, deschloroetizolam, and meclonazepam were reported less than 17 times each. In 2016, a total of 8 different DBZD were reported. By CY2021, a total of 15 different DBZD (with 3 metabolite pairs) had been reported.

DBZD are a subclass of NPS with increasing prevalence. Their appeal lies in possessing the same effects as traditional benzodiazepines, but they typically circumvent routine drug testing and scheduling. DBZD pose a threat to public health and safety, particularly due to their potential for impairment to cognitive and physical faculties or exacerbating central nervous system depression, especially when used in combination with opioids, resulting in increased risk for adverse outcomes. DBZD continue to pose challenges to forensic laboratories as they are increasing in both variety and positivity, requiring laboratories to continually update their testing protocols.

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**Designer Benzodiazepines; NPS; Toxicology**

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**L42 Mixed Intoxications Involving the Novel Synthetic Opioids Methoxyacetylfentanyl and U-47700: A Study of Three Cases**

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**Learning Objective:** After attending this presentation, attendees will have gained additional knowledge on the autptic, histopathological, and toxicological features of deaths due to mixed acute intoxications involving Novel Synthetic Opioids (NSOs).

**Impact Statement:** This presentation will impact the forensic science community by presenting preliminary data on half-life and tissue distribution of NSOs in three cases of death by demonstrating the usefulness of the toxicological significance score as a means for a multidisciplinary evaluation of data and by presenting a refined standard addition method approach, as applied on three cases of death.

NSOs represent an emerging group of Novel Psychoactive Substances (NPS) acting as potent agonists at the opioid receptors, which have been linked to several acute intoxications and deaths.<sup>1</sup> NSOs include fentanyl-related compounds, (e.g., Methoxyacetylfentanyl [MeACF]), as well as non-fentanyl analogs (e.g., the so-called “U-compounds,” particularly U-47700).<sup>2,3</sup> The spread of NSOs in Europe is considered much more limited than in the United States since only some outbreaks of death have been reported, and classical opioids (heroin and its substitutes) likely still play a major role in fatalities. Here we present three cases of death involving MeACF and U-47700, with particular reference to preliminary half-life and tissue distribution data. In all cases, a complete postmortem examination, including external examination, internal section, and histological evaluation, were performed. General unknown screenings and analysis of drugs of abuse were done on postmortem samples by means of immunoassays, gas chromatography/mass spectrometry, and liquid chromatography/mass spectrometry by applying validated methods.

To quantify the analytes of interest in postmortem central and peripheral blood and tissues (brain, liver, kidney, stomach content), the standard addition method was used. A toxicological significance score, which is a scale that allows weighing the contributory role of the NSOs in death cases, was assigned.<sup>4</sup>

Autptic, histological, and toxicological data on the three cases of acute mixed intoxication will be presented, together with the assigned toxicological significance score.

Case 1 died at the hospital after consumption of U-47700, methadone, tilidine, and benzodiazepines. In Case 2, U-47700, together with methadone, flubromazepam, and diazepam, were detected. In Case 3, methoxyacetylfentanyl, furanylfentanyl, 4-ANPP, alprazolam, and alpha-hydroxyalprazolam were quantified in femoral blood. In all cases, despite the presence of additional intoxicants, mostly pertaining to the categories of benzodiazepines and opioids, the NSOs were assigned a toxicological significance score of 3 (i.e., the NPS likely contributed to the death [TSS=3]).

NSOs appear to often be consumed in settings of poly-drug use, especially in combination with other opioids and benzodiazepines, leading to synergistic effects. The standard addition method appears to be most reliable when analyzing postmortem samples, and forensic toxicological results should always be evaluated in a synopsis with circumstantial and autopsy data.

**References:**

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4. Elliott S, Sedefov R, Evans-Brown M (2018) Assessing the toxicological significance of new psychoactive substances in fatalities. *Drug Test Anal* 10:120–126. <https://doi.org/10.1002/dta.2225>.

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**Novel Synthetic Opioids; Tissue Analysis; Standard Addition**



**L43 Phase I Metabolism of New Psychoactive Substances Via Alternative *In Vitro* Methods**

*Leonardo Maya, MS\*, Florida International University, Homestead, FL; Anthony DeCaprio, PhD, Florida International University, Miami, FL*

**Learning Objective:** After attending this presentation, attendees will better understand the biotransformation reactions and metabolites of two fentanyl New Psychoactive Substances (NPS) via different *in vitro* methods.

**Impact Statement:** This presentation will impact the forensic science community by presenting research that explores alternative *in vitro* metabolomic approaches to assist in the identification of appropriate metabolites for two fentanyl NPS to aid monitoring in forensic toxicological screening and confirmation.

NPS are modified, or synthesized, drugs designed to mimic pharmacological effects produced by classical illicit substances. Fentanyl analogs, a group of opioid-type NPS, are created from modifications performed on the fentanyl structure. These analogs can be several-fold more potent, producing lethal sedative effects at low dosages. Ideally, published literature and testing standards are relied upon for detection and quantitation of illicit substances.<sup>1,2</sup> In addition to this, the metabolite panels of some NPS are not entirely mapped out, which can result in metabolites that are unaccounted for and undetected in analytical screenings. Even inactive metabolites are encountered in paraphernalia, each with the potential to metabolize, exhibiting further need to conduct extensive biotransformation studies. This study takes a multidimensional approach on the Phase I metabolism of two NPS fentanyl analogs, para-Chloroisobutyl Fentanyl (pCIBF) and Despropionyl para-Fluorofentanyl (DpFF), via Human Liver Microsomes (HLM) and Electrochemical Oxidation (ECO) assays and an *in silico* metabolism prediction tool, MetaSite. HLMs contain high concentrations of metabolizing enzymes, known as cytochrome P450 enzymes. ECO metabolism studies investigate redox reactions that may occur on the structure of an analyte through heterogenous electron transfers. These experiments involve a three-electrode setup. The MetaSite metabolism prediction tool utilizes a unique algorithm that is independent of the training dataset to predict sites of metabolism and biotransformation based on chemical reactivity with CYP450 (CYP) isoforms.

Metabolism of pCIBF and DpFF was successfully predicted utilizing MetaSite's liver enzyme model and 40 common CYP reaction mechanisms in addition to five uncommon reaction mechanisms. An initial prediction produced primary-generation metabolites. Primary generation metabolites were subjected to a second round of prediction. Metabolites, of the second round, that surpassed a 20% probability of formation were considered as second-generation metabolites. All *in silico* generated metabolites were tabulated and arranged by their respective biotransformation reaction and monoisotopic mass. Each metabolite was manually scanned for in subsequent Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS) analysis of HLM and ECO experiments. HLM experiments incorporated 2mM of drug with 0.5mg/mL HLM with cofactors such as 1mM NADPH, 1.5mM glucose-6-phosphate, and 1.5mM magnesium chloride. These conditions were reconstituted with 10mM ammonium bicarbonate buffer solution, at physiological pH of 7.4, incubated for 4 hours at 37 °C, and analyzed via LC/qTOF/MS. ECO experiments involved the use of a gold, platinum, and glassy-carbon working electrode, a saturated calomel reference electrode, to a platinum/titanium counter electrode. Utilizing full-scan in LC/qTOF/MS, a preliminary screening was completed, and the potential encountered metabolites were documented. Confirmation of presumptive metabolites was conducted via structural elucidation of LC/qTOF/MS/MS fragmentation for each metabolite. The *in vitro* assays conducted in this study successfully produced metabolites for pCIBF and DpFF.

MetaSite produced 25 and 24 total primary metabolites for pCIBF and DpFF, respectively. Hydroxylated, dealkylated, and dehydrogenated were the three most popular types of metabolites formed for pCIBF. Hydroxylated, dealkylated, and carbonylated products were the three most popular types of metabolites formed for DpFF. Each NPS was subjected to a second round of biotransformation to form 20 and 25 secondary-generation metabolites for pCIBF and DpFF, respectively. *In vitro* assays produced a total of 8 metabolites for pCIBF and 11 metabolites for DpFF, each coinciding with *in silico* data. Several metabolites not previously reported were discovered in this study. These results suggest that *in silico* analyses of NPS are advantageous in metabolite discovery. This study proves it is useful to complement with different *in vitro* assays to develop comprehensive metabolomic profiles to aid in detection and identification of NPS in forensic toxicology.

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1. Stanley TH. The Fentanyl Story. *Journal of Pain* 2014, 15 (12), 1215-1226.
2. Jolanta BZ. An Expanding World of Novel Psychoactive Substances: Opioids. *Frontiers in Psychiatry* 2017, 8.

**New Psychoactive Substances; Metabolism; Toxicology**



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**L44 N-Pyrrolidino Etonitazene: A Newly Emerging Nitazene Compound in the Synthetic Opioid Landscape**

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**Learning Objective:** After attending this presentation attendees will better understand the importance of the high-potency synthetic opioids, including N-Pyrrolidino Etonitazene (PYRO), and how the drug(s) potentially influence medicolegal death investigation, specifically cause and manner.

**Impact Statement:** This presentation will impact the forensic science community by providing case histories and toxicology results in case reports of deceased individuals in Denver, CO, with PYRO being detected in the blood. The first confirmed case in Denver, CO, is that of a middle-aged man who died of the combined toxic effects of methamphetamine, amitriptyline, oxymorphone, and PYRO.

In 2021, the Denver Office of the Medical Examiner completed 996 autopsies. Of those cases, 437 were drug-related deaths, primarily driven by methamphetamine and fentanyl. In comparison to 2020, this represented a 28.2% increase in drug-related deaths. As our nation stands in the midst of a booming fentanyl epidemic, cases with PYRO highlight an up-and-coming synthetic opioid that poses a serious threat to individuals who use illicit substances, as the toxic effects of designer opioids pose relatively unknown potency and adverse effects. PYRO pills are manufactured to mimic the appearance of a fentanyl “M30” tablet and may be encountered unbeknown to the user. The case presented below is the result of a single individual ingesting ½ of a PYRO pill, which ultimately contributed to his death.

The decedent was a 44-year-old male with a history of opioid use, hypertension, diabetes mellitus, and cystic fibrosis who was found unresponsive in a motel room. Evidence of illicit drug use was identified at the scene, including a glass smoking apparatus and syringes.

At autopsy, there was no evidence of significant trauma. There was moderate pulmonary edema and minimal cerebral edema. Femoral blood samples were collected and submitted for an expanded forensic analysis. Results revealed the decedent’s femoral blood was positive for methamphetamine at 180ng/mL, amitriptyline at 180ng/mL, and oxymorphone at 220ng/mL. The forensic pathologist was notified by the toxicology lab personnel that there was an out-of-scope finding and inquired if we wanted to pursue additional testing. Confirmatory testing was approved by the forensic pathologist. PYRO was detected by **Liquid Chromatography/Time-Of-Flight (LC/TOF)** and confirmed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) in femoral blood at 3.5ng/mL.

Although the cause of death was stated as a combined toxic effect of multiple substances, this was the first confirmed fatality involving PYRO in the state of Colorado. As of the date of this submission, Denver has three confirmed deaths involving PYRO. Many current cases are pending toxicology testing, so that number is expected to increase. The designer drug landscape challenges any medicolegal death investigation system with increased workload, demands on the budget, and public health concerns. The Denver Office of the Medical Examiner is committed to understanding this new substance, and additional emerging designer compounds, with the goal of significantly impacting public health, improving toxicological approaches to forensic cases, and uniting the forensic community against this raging epidemic.

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**Synthetic Opioid; Overdose; Nitazene**

## L45 Temperature and pH-Dependent Stability: Identifying Fentanyl Degradation Pathways

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**Learning Objective:** After attending this presentation, attendees will better understand fentanyl analog (fentanyl) degradation pathways in a controlled environment and their application to more complex biological matrices.

**Impact Statement:** This presentation will impact the forensic science community by providing additional information concerning pH-dependent degradation of fentanalogs and the identification of potential biomarkers in toxicological specimens.

Fentanalogs are synthetic opioids that can be challenging to detect in biological matrices due to their high potency. Analytical methods may require subnanogram limits of detection. The collection, storage, and transport of samples prior to analysis is of utmost importance. A variety of factors affect stability, including but not limited to temperature, pH, matrix composition, analyte characteristics, and the presence of preservative. Short-term refrigerated (4°C) or long-term frozen (-20°C) storage is recommended for the majority of biological matrices. Unlike antemortem blood (pH 7.4), urine has a much wider range of associated pH that can be attributed to diet, medical conditions, and storage/environmental effects (pH 4-9). Temperature and pH-dependent stability of analytes is an important consideration because breakdown products may serve as potential biomarkers in the absence of parent drug or metabolite.<sup>1-3</sup>

An accelerated stability study was performed using several fentanalogs ((+)-cis-3-methylfentanyl, 4-ANPP, acetylfentanyl, alfentanil, butyrylfentanyl, carfentanil, p-fluorobutyrylfentanyl, p-fluoroisobutyrylfentanyl, o-fluorofentanyl, furanylfentanyl, β-hydroxythiofentanyl, norfentanyl, norcarfentanil, remifentanyl, sufentanyl, and valerylfentanyl) over a wide range of pH (2–10) and temperature (20–60°C) over 24 hours. Dilute aqueous buffer systems were used to investigate temperature and pH-dependent kinetics using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). An Agilent® Technologies 1290 Infinity II autosampler and 6460 triple Quadrupole (QqQ) system was employed using positive Electrospray Ionization (ESI) mode. Separation was achieved using an Agilent® InfinityLab Poroshell 120 EC-C18 column (3.0mm x 100mm, 2.7μm) with a gradient elution consisting of deionized water/acetonitrile containing 0.1% formic acid. The method was previously optimized and validated in accordance with published standards (American National Standards Institute/Academy Standards Board [ANSI/ASB] Standard 036). The abundance of each fentanyl was normalized to T0 with a ±20% bias for stability. Compounds were considered unstable or degraded after dropping below the 80% threshold. The T0 sample was monitored throughout the experiment to ensure no further degradation occurred in the refrigerated autosampler.

N-dealkylated metabolites (norfentanyl and norcarfentanil) were notably more stable than their parent compounds. All fentanalogs except alfentanil, acetylfentanyl, and β-hydroxythiofentanyl underwent some degradation at extreme pH and temperature during the accelerated stability study. All fentanalogs were stable at pH 2, 4, and 6 with the exception of remifentanyl. Notable differences were observed in acidic and basic environments. Degradation was generally more rapid in alkaline environments (pH 8 and 10) with remifentanyl degrading in the first half hour and falling below 20% of T0 within two hours under some conditions. Due to the use of a dynamic Multiple Reaction Monitoring (MRM), these data only indicate the decrease in the parent drug. Degradation products were further investigated using Liquid Chromatography/quadrupole/Time-Of-Flight/Mass Spectrometry (LC/qTOF/MS). Using this approach for 17 fentanalogs with a wide variety of chemical substituents, degradation pathways and potential biomarkers for new and existing fentanalogs might be identified.

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### Fentanalogs; Stability/pH; LC/MS/MS

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**L46 An Unusual Case of Misrepresented Cocaine Powder That Resulted in Fatal and Non-Fatal Intoxications Involving the Novel Synthetic Opioid Etodesnitazene**

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**Learning Objective:** After attending this presentation, attendees will be able to assess the negative impacts of Novel Synthetic Opioids (NPS) (e.g., nitazene analogs) in toxicology samples after people consume recreational drugs.

**Impact Statement:** This presentation will impact the forensic science community by expanding knowledge of novel synthetic opioids and cases of misrepresented drug materials.

The recreational drug supply in the United States remains volatile and increasingly toxic. The 21<sup>st</sup> century has observed increasing drug overdose deaths, paired with the emergence and proliferation of NPS. Currently, fentanyl is the primary driver of drug-related deaths, while increasing trends for cocaine and methamphetamine are being observed. To a lesser yet significant extent, other synthetic opioids (e.g., fentanyl analogues, nitazene analogs) have caused substantial mortality across various parts of the country. As drug trends evolve, there remains a constant—misrepresentation, adulteration, cutting, and/or dilution of drug materials occurring to increase profits and produce better effects or highs along with reducing potential unwanted side effects. These scenarios challenge forensic practices and can place strains on forensic pathologists, forensic toxicologists, forensic chemists, and public health officials.

In October 2021, three individuals were found unresponsive in a park after snorting what they believed to be cocaine powder. All three individuals were transported to the hospital for medical care. Two individuals were successfully revived following naloxone administration and survived. The third individual required advanced life support for persistent comatose state suspected to be precipitated by an opioid overdose. A hospital urine drug screen was positive for amphetamine, cocaine, and benzodiazepines and negative for opiates; testing for fentanyl was not performed. The patient died three days later. The body was transported to the Santa Cruz County Sheriff-Coroner's Office. The pathologist performed an external examination. Hospital admission blood and urine samples were sequestered for toxicological analysis. Toxicology testing performed at NMS Labs for traditional drugs uncovered positive results for fentanyl, methamphetamine, and cocaine in urine; the blood was negative for fentanyl. A review of medical records revealed that fentanyl was administered by medical staff prior to urine collection. Since the toxicology results were insufficient to explain the cause of death, further investigation was pursued revealing that three white powders were recovered at the scene.

The three white powders were submitted to the Center for Forensic Science Research and Education (CFSRE) to determine the drug culprit. The powders were prepared via a simple methanol dilution and analyzed by Gas Chromatography/Mass Spectrometry (GC/MS). Two white powders contained cocaine, and one white powder contained etodesnitazene, a novel synthetic opioid. Blood and urine samples were submitted to the CFSRE for quantitation of etodesnitazene. Samples were prepared via Liquid-Liquid Extraction and analyzed by Liquid Chromatography Tandem Quadrupole Mass Spectrometry (LC/QQQMS). Quantitation was determined by standard addition (internal standard: fentanyl-D5). The blood sample contained 72ng/mL of etodesnitazene, and the urine sample contained 68ng/mL.

Etodesnitazene is a new synthetic opioid that is not medically approved for use. Limited information and data exist regarding its presence and toxicity in humans. At the CFSRE, etodesnitazene has been quantified in 11 death investigation cases. The average concentration in blood was 33ng/mL (median 11ng/mL, range 0.53–120ng/mL). Given the information regarding deaths involving etodesnitazene paired with case circumstances, autopsy findings, and other toxicology results, the pathologist ruled the manner of death to be an accident and the cause of death related to acute etodesnitazene intoxication.

This unusual case of mistaken cocaine powder consumption stresses the importance of thorough medicolegal death investigation paired with expanded toxicology testing, especially for new synthetic opioids in cases where preliminary toxicology findings do not match circumstances. Forensic pathologists and forensic toxicologists must continue to work together and remain vigilant of emerging drug trends, turning to analysis of drug materials when available. Misrepresentation and adulteration of drug products will likely continue, especially with visually indistinguishable white powders; therefore, processes and protocols to combat these scenarios are vitally important.

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**Cocaine; Etodesnitazene; Toxicology**

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**L47 I Believe I Can Fly: Hallucinogenic/Psychedelic Drugs Found in Fall-From-Height Cases in Miami-Dade County, Florida**

*Marissa Finkelstein, MS\*, Miami-Dade Medical Examiner Department, Miami, FL; George Hime, MS, Miami-Dade Medical Examiner, Miami, FL; Diane Boland, PhD, Miami-Dade Medical Examiner Department, Miami, FL*

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**Learning Objective:** After attending this presentation, attendees will better understand the impact that hallucinogenic/psychedelic drugs have on cases in which a decedent has jumped or fallen from a significant height as well as possible implications this could have on the determination of manner of death.

**Impact Statement:** This presentation will impact the forensic science community by explaining the effects of hallucinogenic/psychedelic drugs and the impact they have on postmortem investigative toxicology.

From 2011 to 2021, the Miami-Dade County Medical Examiner Department (MDME) had a total of 266 Fall-From-Height (FFH) cases, and the manner of death in these cases was ruled a suicide or accident, 92.1% and 7.9%, respectively. The toxicology results of these cases showed that drugs with hallucinogenic and/or psychedelic properties were detected in 25 of the 266 (9.4%) FFH cases. The term hallucinogen refers to a large class of psychoactive drugs that alter a person's state of awareness, as well as their thoughts, moods, and feelings. They are commonly divided into two categories: classic hallucinogens (Lysergic Acid Diethylamide [LSD], N,N-Dimethyltryptamine [DMT], 3,4-Methylenedioxymethamphetamine [MDMA], mescaline, psilocin, and psilocybin) and dissociative drugs (Phencyclidine [PCP], ketamine, and dextromethorphan). The physiological effects of these drugs have been found to include hallucinations; increased heart rate, body temperature, and blood pressure; intensified feelings and sensory experiences; disorientation; seizures; anxiety; panic; paranoia; and psychosis. Due to the nature of these psychoactive drugs, a study was performed to determine the impact they may have on FFH cases and the determination of manner of death. The analytes included in this study were ketamine, dextromethorphan, MDMA, LSD, and psilocin. Also included was zolpidem due to its documented somnambulism at therapeutic concentrations as well as other adverse effects that include auditory, visual, or tactile hallucinations at higher concentrations. A retrospective study of all FFH cases at the MDME from 2011–2021 was completed. The initial query was followed by an in-depth case analysis for each FFH case in which an analyte of interest was detected. The case analysis included age, sex, race, history of suicidal ideations, cause of death, manner of death, and if the fall or decedent's actions prior to the fall were witnessed.

The hallucinogenic/psychedelic drugs were detected in 25 cases at the MDME by solid phase extraction and either gas chromatography/mass spectrometry, liquid chromatography/ion trap mass spectrometry, or both. The cause of death in all 25 cases was determined to be blunt force trauma/injuries, and the manner of death in these cases was suicide ( $n=17$ ), accident ( $n=7$ ), and undetermined ( $n=1$ ). The age range in FFH cases was 14–93 years old, and the decedents were primarily White males (72%). Most of the decedents in FFH cases that were classified as suicides had a previous history of suicide attempts, had expressed suicidal ideations, or suffered from depression, whereas the decedents classified as accident had exhibited erratic behavior such as throwing things, hearing voices/screaming, isolation, and paranoia.

When determining the manner of death in FFH cases in which hallucinogenic/psychedelic drugs are detected, the ability to distinguish between an accidental death and suicide is not always obvious. In some cases, the intent of the decedent may be to end one's life; however, there are situations in which hallucinogenic/psychedelic drugs may truly lead a person to believe they can fly. Based on this study, all cases in which concrete evidence of the intent to harm oneself were classified as a suicide. Cases without that intent were classified as accidental based on bizarre or erratic behavior alone. Ultimately, every case is unique, and all medicolegal investigation should be carefully considered to successfully determine manner of death in FFH cases.

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**Hallucinogenic/Psychedelic Drugs; Postmortem Forensic Toxicology; Manner of Death**

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**L48 Synthetic Cannabinoids Adulterated With Brodifacoum—Part II: The Florida Experience**

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**Learning Objective:** After attending this presentation, attendees will have gained an appreciation for the challenges associated with dangerous adulterants in synthetic cannabinoids. Attendees will have learned how to coordinate public health and public safety to identify the root cause of serious patient presentation to hospitals and subsequent treatment after use of synthetic cannabinoids adulterated with brodifacoum, a superwarfarin rodenticide.

**Impact Statement:** This presentation will impact the forensic science community by alerting forensic toxicologists, pathologists, and drug chemists to the adulteration of synthetic cannabinoids with the superwarfarin brodifacoum.

On December 3, 2021, 2 patients arrived in emergency departments in the Tampa, FL, area, suffering from bleeding disorders. On December 4, 2021, 11 additional cases appeared in area hospitals. Over the next week, an additional 43 confirmed cases, 9 probable cases, and 1 suspect case appeared. Some combination of increased International Normalized Ratio (INR), hematuria, abdominal/back pain, vomiting blood, bloody gums, blood in stool, vaginal bleeding, and bruising were commonly observed. These cases mirrored a similar outbreak of >150 cases in 2018 originating in the Midwest United States that spread to other geographical areas of the United States. Coordinated efforts between Medical Toxicologists at the Florida Poison Information Center Tampa (FPIC), the Centers for Disease Control and Prevention (CDC), poison centers affiliated with the Midwest outbreak, and the Florida Department of Health (FD DOH) led to an orderly root cause analysis and patient care efforts.

Qualitative toxicological analysis of blood specimens from patients in the Tampa cases led to the identification of the causative agent, brodifacoum, a superwarfarin rodenticide, the same causative agent in the 2018 cases. Brodifacoum is tasteless and odorless, has an elimination half-life of several months, and is estimated to be approximately 100 times more potent than warfarin. While complex in form, its mechanism of action can be summarized as inhibition of vitamin K-dependent steps in the synthesis of multiple clotting factors, thus leading to hemorrhaging. Part of effective treatment is administration of continuous vitamin K, and this can last for months. In prior cases, it was determined that treatment should continue until the blood brodifacoum concentration is < 10ng/mL. Laboratory testing results were directly reported to hospitals, poison control, and local health departments for treatment and surveillance purposes. In the Florida cases, after identification of the agent, toxicological testing focused on quantitative analysis with the goal of monitoring patients until blood brodifacoum concentrations were < 10ng/mL. Some patients were interviewed, and all had in common the use of synthetic cannabinoids. Product was available in some cases and testing demonstrated the presence of brodifacoum as well as the synthetic cannabinoids 4-F-MDMB-BUTICA and ADB-BUTINACA. Unfortunately, there were five deaths associated with the Florida outbreak. However, the coordinated efforts of FPIC, FL DOH, and testing laboratories led to a coordinated response that quickly identified the critical causative agent and facilitated successful patient care in most cases.

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**Brodifacoum; Synthetic Cannabinoids; Bleeding**

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**L49 Adverse Effects of Veterinary Pharmaceuticals as Toxic Adulterating Agents in Illicit Drug Deaths**

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**Learning Objective:** After attending this presentation, attendees will be able to describe examples of dangerous adulterants being found in combination with Schedule I and II drugs and their toxicological effects.

**Impact Statement:** This presentation will impact the forensic sciences by providing awareness of an additional pharmacological contribution to be considered in deaths related to illicit drugs.

Many street drugs, especially more potent drugs such as opioids, are frequently cut with other materials, including pharmacologically inert substances such as microcrystalline cellulose, starch, and sugars, to add bulk and dilute the drugs, but are also cut with toxic adulterating substances with active pharmacological effects. These have traditionally included the anthelmintic agent levamisole/tetramisole, analgesic agents phenacetin, acetaminophen, quinine, lidocaine, tramadol, metamizole/dipyrone, but more recently xylazine and acepromazine, veterinary large animal tranquilizers, and phenylbutazone, a veterinary analgesic, have appeared. As veterinary products, these drugs may not be included in routine targeted drug screens in forensic toxicology. This presentation considers these drugs, their pharmacological effects, and reports on recent trends in positivity in human postmortem forensic toxicology.

A list of toxic adulterating substances of concern was generated from a review of the literature, and data was submitted by an international laboratory director's consortium through the Colombo Plan.<sup>1,2</sup> These substances were then monitored in forensic toxicology casework samples by a Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/QTOF/MS) screening method, which includes over 1,000 therapeutic and abused drugs and adulterants. For this presentation, selected prominent adulterants of interest—levamisole, phenacetin, and xylazine—were tracked as a percentage of positivity per number of cases analyzed, between 2018 and 2022, as were the major drugs of abuse, fentanyl, cocaine, heroin, and methamphetamine. Levamisole positivity fluctuated between 5% and 14% between 2018 and 2021 but showed a recent spike in positivity in 2022 at 17% of cases evaluated. Levamisole was most frequently found in combination with cocaine, which was present in between 8% and 28% of cases in this series during the same time period. Levamisole, a de-worming agent, has adverse side effects of agranulocytosis, leukopenia, purpura, and visible necrotized skin tissue. Data from prior studies at the Center for Forensic Science Research & Education (CFSRE) demonstrated a typical average daily dose of levamisole at 178mg in chronic cocaine users, in excess of the estimated 150mg threshold for toxicity. It is estimated that in excess of 35% of regular cocaine users would have exposure to levamisole above this toxicity threshold.

The second major adulterant was phenacetin, which was removed from the United States pharmacopeia in 1983 due to its adverse effect profile of urologic and renal diseases, including cancers, and death due to cardiovascular effects. Phenacetin had been largely absent from the illicit drug supply in the United States until 2020, but currently is present in approximately 8% of cases, most frequently associated with fentanyl and other opioids, in the Northeastern United States.

The third most commonly emerging toxic adulterant is the veterinary tranquilizer xylazine, which was never approved for use in humans due to its adverse effect profile of hypotension and bradycardia; however, disorientation, drowsiness, respiratory depression, and the development of ulcers and lesions at the site of injection are increasingly reported. Xylazine was historically present in 1-4% of cases, but since early 2020 has been increasing in positivity and in the second quarter of 2022 was present in approximately 18% of postmortem cases, frequently in combination with fentanyl.

Other emerging veterinary adulterants that will be discussed include acepromazine and phenylbutazone. Many veterinary drugs are not approved for therapeutic use in humans because of their adverse effects, but their use as adulterants in the illicit drug supply exposes drug users to additional risks and harms and should be taken into account in the interpretation of apparent drug-related deaths or adverse events.

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2. Singh VM, Browne T, Montgomery J. The Emerging Role of Toxic Adulterants in Street Drugs in the US Illicit Opioid Crisis. *Public Health Rep*. 2020 Jan;135(1):6-10. doi: 10.1177/0033354919887741. Epub 2019 Nov 18. PMID: 31738861; PMCID: PMC7119254.

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**Toxicology; Adulterants; Adverse Effects**



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**L50 A Compilation of Postmortem Vitreous Fluid Data for Pediatric Cases**

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**Learning Objective:** The goal of this presentation is to provide ranges for vitreous fluid electrolytes in the pediatric population.

**Impact Statement:** In current pediatric casework, there is no standard data range for vitreous fluid electrolyte concentrations. This presentation will impact the forensic science community by providing data for six measured vitreous electrolytes in several pediatric age groups and comparing them against known adult reference ranges.

Vitreous humor testing is a useful postmortem chemistry tool available during autopsy to potentially identify Cause Of Death (COD) or substance use. Vitreous fluid is in a well-protected organ (the eyeball) with limited blood supply, leaving a lower chance of degradation and putrefaction compared to other postmortem matrices. Currently, there are no standard ranges for electrolyte vitreous fluid concentrations in the pediatric population (ages 0–19 years). Overall, this study will provide insight into vitreous electrolyte values for defined pediatric age groups: neonates (0–8 days), infants (1mo–11mo), toddlers (1yr–5yr), school-aged children (6yr–12yr), and adolescents (13yr–19yr). These data are compared to literature values established from the adult population to analyze trends and provide ranges for electrolyte data for the pediatric postmortem population. More defined and established ranges may provide medical examiners and coroners with more accurate tools for determining Postmortem Intervals (PMI) and cause of death.

Postmortem pediatric vitreous electrolyte data from a large reference laboratory was compiled over a 2.5-month time period, from mid-April 2022 through June 2022.<sup>1</sup> A total of 375 cases were recognized, de-identified, and categorized into the aforementioned age groups. Cases were evaluated for the following six electrolytes: glucose, urea nitrogen, potassium, creatinine, sodium, and chloride.

Compared to the adult standards, urea nitrogen and potassium have above-average values, specifically in the early childhood cases. However, creatinine and chloride have values similar to the adult literature values. With sodium, the averages in the younger age groups are lower than the adult averages, but stabilizes by school-age and adolescence. Glucose trends are difficult to establish. There is a high variability in reported values due to various factors, such as time of last meal, disease state (e.g., diabetes), and poor analytical stability. Based on the preliminary data, trends indicate that the younger pediatric populations, especially neonates, infants, and toddlers, have noticeable differences in some electrolyte ranges. These differences suggest that special consideration needs to be given when assessing this type of postmortem casework, particularly for pediatric deaths. Moreover, establishing general ranges for these age groups may help to more easily identify when outliers occur and indicate when additional investigation may be needed. Overall, this study provides a better understanding of the differences in vitreous analyte concentrations of the pediatric postmortem population compared to the more well-known and utilized adult values.

**Reference:**

- <sup>1</sup>. Rose KL, Collins KA. (n.d.). Vitreous postmortem chemical analysis. *News Path Pathology News for the Medical Community*. Retrieved July 31, 2022, from [https://webapps.cap.org/apps/docs/newspath/0812/vitreous\\_postmortem\\_chemical\\_analysis.pdf](https://webapps.cap.org/apps/docs/newspath/0812/vitreous_postmortem_chemical_analysis.pdf).

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**Vitreous Fluid; Pediatrics; Postmortem**



## L51 The Postmortem Formation of Ethanol: Is N-Propanol a Reliable Marker? A Proof-of-Concept Study Using a Real-Environment Experimental Set-Up

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**Learning Objective:** After attending this presentation, attendees will have information on: (1) postmortem ethanol formation and the factors affecting the process; (2) possible strategies to identify postmortem ethanol production; and (3) the meaning of n-propanol determined in cadaveric blood.

**Impact Statement:** This presentation will impact the forensic science community by outlining the reliability of n-propanol as a marker of postmortem production of ethanol, as demonstrated by a proof-of-concept study carried out in a real-environment experimental setting.

**Background:** Ethanol is the psychoactive substance identified most frequently in postmortem specimens. Unfortunately, the interpretation of postmortem ethanol concentrations can be difficult because of postmortem alcohol redistribution and the possibility of postmortem alcohol neogenesis. Indeed, in the time interval between death and sample collection, the decedent may be exposed to non-controlled environments for an extended period, which may promote microbial colonization. As a result of microbic action, several postmortem biochemical processes affect the concentration of many compounds, such as ethanol and other xenobiotics. Many authors report that in the presence of carbohydrates, various species of bacteria, yeast, and fungi are capable of synthesizing ethanol *in vitro* as well as in the tissues.<sup>1-3</sup> In this setting, a concentration of n-propanol higher than 0.001g/L has been recently reported as evidence of postmortem ethanol production.<sup>4</sup>

**Methods:** The “putrefactive cadaveric blood” was obtained starting from blood samples collected in 6mL Vacutainer Tube EDTAK3 from healthy living people after the measurement of blood glucose and alcohol by a Headspace/Gas Chromatography/Flame Ionization Detector (HS/GC/FID) method. Three-hundred milligrams of cadaveric tissues, namely fragments of intestinal mucosa and liver, at different Postmortem Intervals (PMI) (PMI 48h and 21days) were then added to blood sample tubes. Finally, a layer of paraffin oil was introduced in the headspace of the tubes, which were kept at environmental temperature and at 4°C for 9 days. The “putrefactive cadaveric blood” was analyzed for alcohols daily using an HS/GC/FID validated method and a Shimadzu GC2030 head pace GC analyzer with an FID and equipped with an SH-Rxi-5ms-wide bare column (30m x 0.23mm x 0.25µm. Fifty µL of “putrefactive cadaveric blood” sample was added to 200µL of a solution of tert-butyl alcohol at 0.0975g/L. A headspace volume of 1,250µL was injected in the system. The temperature of the syringe was kept at 80°C, and an isotherm separation was carried out at 40°C. An internal quality standard with an ethanol concentration of 0.5g/L was also measured every five samples. The method allowed for identification and quantification of ethanol (Limit Of Quantitation [LOQ]: 0.05g/L) and n-propanol (LOQ: 0.001g/L). The formation of ethanol and n-propanol and their relationship were evaluated taking into consideration different parameters: (1) putrefactive stage of the added fragments; (2) blood glucose concentration; (3) storing temperature; and (4) storing time. The statistical analysis was performed by applying the unpaired T-test and simple linear regression.

**Results:** Concerning the putrefactive stage, the higher postmortem ethanol production using fragments from cadavers at the emphysematous stage—PMI > 48h and < 7days—was probably due to the higher concentration of fermenting anaerobic bacteria. As expected, samples with higher blood glucose concentrations showed greater ethanol production. The incubation of samples at environmental temperature (20°C) showed greater ethanol production than samples incubated at 4°C. The evaluation of ethanol production according to storing time showed a rapid increase of ethanol concentration up to day 3 of incubation, then a slight decrease over the next four days, and finally a further rise. All the samples that showed ethanol production had concomitant n-propanol formation with a close correlation between the two alcohols (R<sup>2</sup>=0.918, P< 0.0001).

**Conclusion:** The present study evaluates, at an experimental base, the impact of body putrefactive stage, antemortem glycemia, storing temperature, and time on the postmortem ethanol production process. In addition, it confirms the feasibility of using n-propanol as marker of postmortem ethanol production.

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1. Blackmore DJ. The Bacterial Production of Ethyl Alcohol. *J. Forensic Sci. Soc.* 8 (1968) 73–78.
2. Boumba VA, Ziavrou KS, Vougiouklakis T. Biochemical pathways generating post-mortem volatile compounds co-detected during forensic ethanol analyses. *Forensic Sci. Int.* 174 (2008) 133–151.
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4. Boumba VA, Kourkoumelis N, Ziavrou K, Vougiouklakis T. Estimating a reliable cutoff point of 1-propanol in postmortem blood as marker of microbial ethanol production. *J. Forensic Sci. Med.* 5 (2019) 141-146.

### Postmortem Ethanol Production; N-propanol; Forensic Toxicology

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**L52 A Quantitative Analysis of Fentanyl and Major Metabolites From Empty Puparia and Adult Blow Flies Via Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Analysis**

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**Learning Objective:** After attending this presentation, attendees will better understand a QuEChERS extraction protocol utilized for the quantitative analysis of fentanyl and metabolites from a late-stage insect tissue specimen coupled to a targeted detection system using LC/MS/MS.

**Impact Statement:** This presentation will impact the forensic science community by providing a comprehensive analytical workflow for the isolation of fentanyl and metabolites extracted from empty puparia and adult fly tissue using a safe method that reduces matrix interferences while maintaining high sensitivity.

After death, the natural decomposition process degrades or eliminates access to traditional toxicology biological matrices like blood, urine, and liver tissue, increasing the opportunity for insects to be used as an alternative matrix. The use of insects as a surrogate toxicological matrix is generally accepted; however, the interpretation of major findings in medicolegal death investigations is still debatable. With a lack of comprehensive studies regarding metabolism, feeding behavior, and correlation of drugs found in the insect tissue to those in the human tissue, further research is necessary to understand insect tissue in relation to human tissue concentrations. The hypothesis behind the accumulation of xenobiotics in insect tissues follows a natural mechanism of entrapment. When drugs are deposited into the chitin exoskeleton, this allows for the materials to remain in the insect body for months and years after all other tissues used for toxicology analysis are no longer available. When an adult fly emerges, the puparia cases left behind can be extracted to provide a qualitative observation of drugs present in the body while the insect was feeding after soft tissues and traditional biological fluids are not present.

To evaluate the persistence and prevalence of fentanyl and metabolites in the late-stage insect tissue and the ability to extract the drugs from the insect tissue, fentanyl was spiked into 200g aliquots of human liver homogenate to evaluate four concentrations: negative control (0µg/kg), low (10µg/kg), medium (100µg/kg), and high (350 µg/kg) concentrations. The 200g treatment portions were aliquoted as seven 25g aliquots onto aluminum foil sheets inside 8oz plastic containers with sand as a substrate for pupation. To each aliquot of the liver, approximately 70–90 eggs (by mass) were placed and allowed to feed undisturbed until the time of collection. The containers were housed in the Percival I36LLVLC8 incubator controlling for temperature, relative humidity, and photoperiod. Entire insect cohorts (each treatment: control, low, medium, and high) were collected after emergence (day 21). This was repeated for three replicates. In addition, during the third replicate, an authentic postmortem liver specimen with a fentanyl concentration of 111µg/kg, norfentanyl concentration of 8.1µg/kg, and a 4-ANPP concentration of 5.8µg/kg was homogenized to be treated as a fifth treatment or validation group.

Fentanyl was detected in both empty puparia and adult flies for all treatments across all three replicates but was below the **Lower Limit of Quantification (LLOQ)** for the low treatment in the empty puparia. For the adult flies, as the concentration of the fortified liver treatment increased, the concentration of fentanyl observed in the adult fly extractions also increased. Fentanyl and metabolites were extracted from the empty puparia and adult flies feeding on liver tissue fortified with fentanyl.  $\beta$ -hydroxyfentanyl was detected in the empty puparia but not in the adult flies. The detection of fentanyl and norfentanyl from the empty puparium and the adult flies also indicates that a portion of the drug is left behind in the fly exuviate but also persists to the adult fly.

These findings strengthen the utility of empty puparia for the detection of fentanyl and metabolites collected from tissues fortified with fentanyl. This is promising for the use of late-stage insect tissue for the detection of postmortem tissue drug presence.

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**Fentanyl; QuEChERS; Empty Puparia**

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**L53 Toxicology Laboratory Findings From the 2018 Census of Medical Examiner and Coroner Offices**

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**Learning Objective:** After attending this presentation, attendees will understand the toxicology laboratory findings from Bureau of Justice Statistics' (BJS') 2018 Census of Medical Examiner and Coroner Offices (CMEC).

**Impact Statement:** This presentation will impact the forensic community by providing updated information of the state of postmortem toxicology services in the United States based on an 80.9% response rate of the 2018 CMEC.

In 2021, the United States BJS published results for the MEC, 2018, that provided an update on the medicolegal death investigation system in the United States (www.bjs.gov, November 2021, NCJ 302051). The first iteration of the CMEC collected data from 2004 (www.bjs.gov, June 2007, NCJ 216756). In 2018, there were more than 1.3 million cases referred to MEC offices. Of referred cases, 45.9% of cases were accepted for further MEC investigation, which equated to an average of 80 cases accepted per full-time equivalent personnel. Like the first CMEC iteration, much of the data collected on toxicology laboratory operations, casework, and practices were not included in the published BJS report. Publicly accessible BJS data were analyzed by RTI International (RTI) to report on 2004 toxicology findings.<sup>1</sup> The present analysis of toxicology findings provides the community with an update to this work using the 2018 CMEC data of 1,648 MEC offices that responded.

The 2018 CMEC was conducted by RTI on behalf of BJS (2017-MU-CX-K052) from June 2019 through March 2020. The questionnaire was developed based on review and update of the 2004 CMEC, expert panel review, and pilot testing. Mixed mode collection of data was conducted by mail, online, and email. Data were obtained from the BJS publicly accessible website and evaluated in 2022 for any remaining information that was not reported in the 2021 BJS Report. Questions and data around the operation of toxicology laboratories within a MEC office or specific to toxicology testing were analyzed and will be discussed. For example, the 2018 CMEC captured salaries by profession. Respondents reported an average minimum of \$41,825 and an average maximum \$65,388 for forensic toxicologists. Moreover, 65% of responding ME offices reported that some of their forensic toxicologists were certified by the American Board of Forensic Toxicology, while coroner offices had more than 10% less certified forensic toxicologists. Similar data were analyzed for certification of forensic analysts with comparative results. When respondents were asked which staff performed the duty of determining which cases received forensic toxicology testing, two-thirds (66.2%) indicated that coroners or non-physicians made this determination, as opposed to autopsy pathologists, death investigators, or other internal staff. As much as possible, results will be compared to the 2004 CMEC. Additionally, the data from several new toxicology-related questions will be presented.

Results from this study include information on operations, workload of toxicology laboratories within these MEC offices, toxicology retention time schedule, analyst toxicology certification, screening and confirmation toxicology testing practices, and evidence-retention practices. These data are important to understand the postmortem toxicology policies and practices and how these practices have evolved since the 2004 CMEC, as available, and are of national importance considering the ongoing overdose crisis contributing to more than 100,000 deaths in 2021. These data can help inform BJS as it has begun planning for the 2022 CMEC. Continued data collection of forensic toxicology through a national census can provide comparability over time, document contextual insight for needs, and provide necessary information to better support the state and local MEC offices operating nationwide.

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- <sup>2.</sup> CDC, National Center for Health Statistics, Office of Communication. *U.S. Overdose Deaths In 2021 Increased Half as Much as in 2020—But Are Still Up 15%*. May 11, 2022. (301) 458-4800. [https://www.cdc.gov/nchs/pressroom/nchs\\_press\\_releases/2022/202205.htm](https://www.cdc.gov/nchs/pressroom/nchs_press_releases/2022/202205.htm).

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**Overdose; Postmortem Toxicology Laboratory Practices; Medical Examiner and Coroner Offices**

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**L54 Acute Psychotic Episodes in Impaired Driving Cases Involving Tetrahydrocannabinols ( $\Delta 8$  and  $\Delta 9$ )**

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**Learning Objective:** After attending this presentation, attendees will increase their competence in the interpretation of the uncommon effects of cannabinoids in impaired driving investigations.

**Impact Statement:** This presentation will impact the forensic science community by outlining the increasing incidence of acute psychotic episodes in cases involving cannabinoids.

**Introduction:** Substantial research has been presented on the impairing effects of Tetrahydrocannabinols (THCs) and their impacts on driving. Impairment to reaction time, perception, short-term memory, attention, motor skills, tracking, and divided attention tasks are typical. Conversely, relatively few reports have described acute psychotic episodes due to THCs in impaired driving investigations. Herein two cases from the Southeastern United States are presented.

**Methods:** A volatile analysis was performed by Headspace/Gas Chromatograph/Flame Ionization Detector/ Mass Spectrometry (HS/GC/FID/MS). Screening for other drugs was conducted using a basic extraction with scan GC/MS and an 11-panel Enzyme-Linked Immuno-Sorbent Assay (ELISA). All positive results were confirmed with GC/MS and/or Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The LOQ for delta 9 THC, delta 8 THC, delta 10 THC and hydroxy THC was 1.0 ng/mL. The Limit Of Quantitation (LOQ) for carboxy THC was 5.0ng/mL. Delta 8 and delta 10 THC metabolites were not included.

**Results: Case 1:** A 26-year-old male was involved in a single vehicle crash where he drove into the median of a divided roadway. There was no airbag deployment. When officers arrived, the driver was awake and alert with his eyes closed. His statements and demeanor demonstrated a detachment from reality and paranoid delusions. At times he was making guttural noises/chanting in what seemed to be a different language, although his English was clear and free of any real accent. After the incident the driver contacted the officer and stated that he purchased a delta 8 THC vape (not submitted) from a smoke shop after the clerk recommended it to him to alleviate his anxiety. The driver advised he smoked the vape throughout the week leading up to the incident and that it made him feel like he was not in control of himself. Acetone and delta 8 THC at 1.8ng/mL were identified in the blood specimen collected ~2 hours after the crash. **Case 2:** A 23-year-old female was involved in multiple hit-and-run crashes, including striking two pedestrians, one on the sidewalk and one in a crosswalk, other vehicles, and a telephone pole before the car was disabled. Upon initial contact the driver appeared to be unconscious, then convulsed and made seizure-like movements. She exited the vehicle and, while standing, was unresponsive and stared off into space. When taken to the ambulance for evaluation, her eyes appeared to roll back in her head, she began to scream and fight with medics stating she was "God" and "Harry Potter," and rolled herself off of the stretcher she was on. She made unintelligible utterances that medics described as speaking in tongues, started shaking, and would not let the medics help her back on the stretcher. Shortly after, she stood up and sat down on the stretcher like nothing happened. Subsequently, 400mg ketamine was administered to sedate her. She had gel blister packs labeled THC Cannabis. Analysis of one gel by the seized drug unit identified delta 9 THC. Toxicology analysis of a blood specimen collected ~4.5 hours after the crash detected amphetamine at 68ng/mL, delta 9 THC at 4.5ng/mL, hydroxy THC at 2.6ng/mL, and carboxy THC at 63ng/mL. Ketamine and norketamine were tentatively identified, but not confirmed due to noted administration after the incident.

**Discussion/conclusion:** THCs, including delta 8 and delta 9 THC, can produce acute psychotic episodes in some individuals. Highly concentrated vape oils and edibles of various THCs have proliferated the market and may contain little-to-no cannabidiol. This may increase the risk and incidence of these episodes. Not only does this pose a risk for users of these products, but can also result in significant driving impairment, which poses an increased risk for traffic safety.

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**THC; DUID; Psychosis**

## L55 Drug Testing and Traffic Safety: What You Need to Know

*Amy Berning, MA, PhD\*, National Highway Traffic Safety Administration, Fairfax, VA; Ryan Smith, PhD, National Transportation Safety Board, Washington, DC; Kathryn Wochinger, United States Department of Transportation/National Highway Traffic Safety Administration, Washington, DC; Morgan Drexler, MPH, American Medical Group Association, Arlington, VA*

**Learning Objective:** Information will be presented on the importance of drug toxicology results in the nation's traffic safety database for crashes involving a fatality (which many likely do not know exists). These data are used by cities, states, federal agencies, researchers, advocacy groups, and legislators and can lead to important policy decisions. This presentation discusses inconsistencies with states obtaining and reporting drug test results and the limitations in use of that dataset.

**Impact Statement:** A focus of this presentation is the importance of drug test results, and consistency in obtaining and reporting results, for the traffic safety community. This is crucial for improving data that is widely used and often misinterpreted. This presentation will advise attendees regarding the improvements the National Highway Traffic Safety Administration (NHTSA) is making, and their efforts to reach out to those in the toxicology community. We are hoping to improve coordination between those involved in testing, state highway safety offices, and other partners.

Drugs-and-driving is a traffic safety issue of great concern. As attention on this very complex issue has risen, so have discussions about the limitations and use of data on drug prevalence among road users. This presentation continues that discussion by examining the process of obtaining and reporting the drug use data from people involved in motor vehicle crashes that are included in the NHTSA's Fatality Analysis Reporting System (FARS), a national census of fatal motor vehicle crashes in the United States. The presentation will describe challenges in drug testing and reporting and how that impacts the drug data in FARS. The limitations identified here are not necessarily unique to drug testing, or to FARS, and are presented to inform discussions on drugs and driving and lay the groundwork for improving data collection and reporting internationally.

The research team obtained information from multiple sources to examine how drug tests, toxicology results, and drug data are collected and, ultimately, received by the FARS analysts. The team visited toxicology laboratories and medical examiner's offices and reviewed the original documentation of drug test results for nearly 1,200 FARS cases to learn about the quantity and quality of drug toxicology information submitted for inclusion in FARS. The lack of standardization in toxicology testing and in the process of transferring data to FARS analysts make the drugged driving data entered into FARS largely incomplete and incomparable across states. The drug result documentation provided to FARS often does not include the full drug panel, whether screening or confirmatory testing was performed, or the matrix tested. This research identified inconsistencies in toxicology testing, how drug information is provided to FARS analysts, and how these data are ultimately entered in FARS. The limitations constrain the use and interpretation of drug test results in FARS, but also provide targeted areas for improvement and discussion. There are significant barriers and inconsistencies to the collection and entry of drug information on fatal crashes in the United States. This leads to confusion about the usage and interpretation of these data. Lessons learned from this research can not only be used to improve drug data within the United States, but also internationally. The NHTSA is continuing in its efforts to improve the data in FARS, including working with toxicologists across states to increase awareness of reporting issues.<sup>1</sup>

### References:

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### Drug Testing; Traffic Safety; Toxicology



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**L56 1,1-Difluoroethane in Driving Under the Influence (DUI) Cases—Intoximeter® DMT Dual Sensor and Draeger Alcotest 7110 Aiding Officers in Inhalant Cases**

*Jasmine Maxwell, MSFS\*, Alabama Department of Forensic Sciences, Pelham, AL; Greg Turner, PhD, Alabama Department of Forensic Sciences, Pelham, AL; Curt Harper, PhD, Alabama Department of Forensic Sciences, Hoover, AL*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of 1,1-difluoroethane in Driving Under the Influence of Drugs (DUID) cases using the Intoximeter® DMT and Draeger Alcotest 7110.

**Impact Statement:** This presentation will impact the forensic science community by presenting interference data for 1,1-difluoroethane using evidential breath testing instruments, Intoximeter® DMT and Draeger Alcotest 7110. Additionally, information about inhalant dangers while driving will be presented.

1,1-Difluoroethane (C<sub>2</sub>H<sub>4</sub>F<sub>2</sub>), also known as DFE, is an odorless, colorless gas that is easily ignited. DFE is commonly used as an aerosol propellant, such as liquefied gas or an air duster. DFE falls under the inhalant category of the seven drug categories on the Drug Recognition Expert (DRE) matrix. Inhalants can be consumed by huffing, bagging, insufflation, or sniffing/snorting.

When an interference message occurs, there is typically a significant response on the Infrared (IR) detector but no response on the Electrochemical fuel Cell (EC) since DFE cannot be oxidized. The Draeger Alcotest 7110 monitors interfering substances by comparing the IR detector to the results from the EC detector (difference  $\geq 10\%$  suggests an interference) and by the EC reaction curve, which will show differences between what is expected for ethanol and what is observed. These mechanisms allow the detectors to distinguish between different types of alcohol. The Intoximeter® DMT dual sensor uses a third mechanism, which is a multiwavelength IR detector. When DFE is present, both instruments will flag it as an interference.

A search of all Toxicology blood results positive for DFE between January 2019 to July 2022 was conducted. Draeger and DMHost software were used to evaluate breath records from both instruments between the same period that had greater than 10% difference between the IR and EC. Once breath tests with interference messages were isolated, toxicology blood results were matched to the subject's corresponding breath test. A confirmed DFE breath and blood case with an accompanying drug recognition report will be discussed to demonstrate the danger of inhalant use while driving.

Between January 2019–July 2022, Toxicology contained 32 positive DFE cases across all case types; 37% ( $n=13$ ) were DUI cases with a median concentration of 13 µg/mL (range: 0.23–42 µg/mL). The percentage of the interference messages identified in all breath alcohol test records from January 2019–July 2022 was 0.04% and 4.7% for Draeger and DMT dual sensor, respectively. From both instruments combined there were 172 (21 Draeger, 151 DMT) interference messages, with only one having confirmed DFE in the blood. The confirmed case from the Draeger showed no response for the EC, but had an ethanol reading of 0.072g/210L on the IR. The difference between the detectors resulted in the certificate of analysis reporting an “interference” message. The Toxicology report displayed a DFE concentration of 30 µg/mL in the blood. The DRE evaluation showed the subject to have constricted pupils, slow reaction to light, flaccid muscle tone, and eyelid tremors. The opinion of the DRE report was the subject was under the influence of inhalants, which was confirmed with the Toxicology report as well as the interference message seen on the Draeger.

In Alabama, when an interference message occurs, officers are instructed to ask the subject a set of questions that will help identify potential health issues (e.g., ketoacidosis) or if the subject has abused inhalants. Our program instructs the officer to collect blood as soon as possible due to inhalants being extremely volatile and they may only be present in the blood 1.5 to 3 hours after use. Trends of DFE use remain constant in Toxicology casework with an average of eight cases per year from 2006–2022. In summary, officers should be encouraged to collect and submit blood to toxicology when a subject is suspected of using inhalant such as DFE.

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**DFE; DUI; Interference**

**L57 A Standardized Method for Analyzing Toxicology Data in Drugged Driving Research**

Ryan Smith, PhD\*, National Transportation Safety Board, Washington, DC; Mary Pat McKay, MD, MPH, National Transportation Safety Board, Washington, DC; Jana Price, PhD, National Transportation Safety Board, Washington, DC

**Learning Objective:** After attending this presentation, attendees will be informed about a recent National Transportation Safety Board (NTSB) report on drug and polycategory drug usage by United States drivers. Specifically, attendees will learn about the challenges inherent in analyzing raw toxicology data for drugged driving research and the development of a standardized method for conducting these analyses. This includes strategies for categorizing drug usage, documentation of metabolite pathways, determining the impairment potential of various analytes, and removing drugs that are likely administered for post-crash medical care. Ultimately, attendees will gain the resources and tools to better conduct their own drugged driving research when working with toxicology data. Attendees will see results of this method applied to toxicology data on over 25,000 drivers and learn about recent NTSB safety recommendations relevant to the forensic science community.

**Impact Statement:** The forensic science community has provided significant and valuable input on recommendations for the toxicological investigation of drug-impaired driving and motor vehicle fatalities.<sup>1</sup> However, similar standards for the mining and analysis of these toxicology data do not readily exist. Working closely with medical doctors and leading forensic toxicologists, the NTSB developed a standardized method for analyzing and reporting toxicology data for drugged driving research with an emphasis on polycategory drug combinations. This presentation will impact the forensic science community by providing an overview of this approach and links to resources that will help others in the forensic science community and beyond to analyze these complex data. Thus, this presentation will provide tools for the analysis of toxicology data in drugged driving research along with current findings on polycategory drugged driving.

Impaired driving continues as a significant and growing public health concern. In 2020, there were 11,654 fatalities in alcohol-impaired driving crashes in the United States, which represents a 14.3% increase year over year. Unfortunately, significantly less is known about the prevalence of other drugs. This is largely due to the lack of consistent and comprehensive drug testing in the toxicological investigation of drug-impaired driving and motor vehicle fatalities, challenges with the submission of these data to relevant state and federal databases (e.g., Fatality Analysis Reporting System [FARS]), and inconsistencies in the analysis and reporting of available toxicology data.<sup>2,3</sup> The National Safety Council's (NSC's) Alcohol, Drugs and Impairment Division provides recommendations on the collection and testing of biological specimens for drugged driving investigations.<sup>1</sup> However, no such standard exists for the interpretation, analysis, and reporting of toxicology data in the driving domain. This presentation seeks to address this gap by highlighting recent efforts by the NTSB in coordination with the medical and forensic toxicology communities.

The goals of the present research study were to: (1) develop a systematic method for mining, analyzing, and reporting toxicology data for drugged driving research; (2) apply this method to high-quality toxicology datasets to evaluate the prevalence of drug and polycategory drug usage by drivers; and (3) discuss NTSB safety recommendations to enhance drugged driving data and traffic safety. Researchers began by evaluating existing approaches to classifying drug and metabolite equivalents into drug categories and worked with leading medical experts and toxicologists to develop a set of drug categories for data analysis. These categories ultimately included: Ethanol Alcohol, Other Volatiles, Potentially Impairing Neuropsychiatric Medications, Cannabinoids, Narcotic Analgesics, Hallucinogens, Inhalants, Dissociative Anesthetics, Sedatives, Stimulants, Synthetic/Designer Drugs, and Other Potentially Impairing Medications.

The team then evaluated approximately 400 analytes to determine their metabolite pathways and impairment potential. Special coding rules were created for many analytes (particularly benzodiazepines and opioids) that may have multiple metabolite pathways and where an analyte may be both a parent drug and a metabolite of another commonly used drug. All analytes were coded up to their most proximate parent drug for analysis. For example, benzoylecgonine would be coded up to cocaine. This also best assured that a drug and its metabolites were not included as "polydrug" for the analysis. Drugs that were not deemed to be potentially impairing for driving performance or are predominantly used in post-crash medical care were removed from the analysis. This resulted in a set of standardized coding rules and protocols for analyzing any toxicology dataset for drugged driving research. The team worked closely with several advanced toxicology laboratories to apply this new method. In order to be included in the study, these laboratories provided results on driver toxicology samples that were consistently and comprehensively tested for drugs (e.g., no stop testing for Blood Alcohol Concentration [BAC]), used blood as the matrix for testing, and tested for NSC Tier I compounds. Data on over 25,000 impaired driving cases were provided by the four laboratories that met these criteria: Orange County Crime Laboratory, The Wisconsin State Laboratory of Hygiene, Office of the Chief Medical Examiner City and County of San Francisco, and New York State Police Forensic Investigation Center.

This presentation will focus on the development of the standardized drug coding and classification method and give links to resources so others can use this method. The presentation will also provide a brief overview of the results from the 25,000 impaired driving cases that were analyzed for this study. It will conclude by discussing recently adopted recommendations on drugged driving by the NTSB.

**References:**

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**Drugs; Driving; Toxicology**



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**L58 Impaired Driving Drug Trends and Stop-Limit Testing Evaluation**

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**Learning Objective:** After attending this presentation, attendees will be able to describe common trends in impaired driving cases and discuss the patterns and frequency with which these drugs are found in combination. Attendees will also be able to evaluate drug positivity at commonly used blood alcohol concentration thresholds used in “stop-limit” testing.

**Impact Statement:** This presentation will impact the forensic science community by highlighting the impact of the practice of stop-limit testing on the collection of accurate data and rates of drug use in impaired driving and by providing a more comprehensive understanding of what drugs are being underreported in cases where stop-limit testing is implemented.

Driving Under the Influence (DUI) involving alcohol and/or drugs is a significant public health threat as reflected by increasing traffic fatalities. Novel Psychoactive Substances (NPS) and polysubstance use and legalization of recreational drugs have all increased the complexity of toxicological testing in these cases. In 2021 the National Safety Council’s Alcohol, Drugs and Impairment Division (NSC-ADID) released the newest iteration of testing recommendations for Tier I and Tier II drugs (including NPS). Tier I encompasses drugs that should be routinely screened for in laboratories in Driving Under the Influence of Drugs (DUID) and motor vehicle fatality cases. In the survey conducted to inform changes to the recommendations, 45% of labs ( $n=65$ ) reported using stop-limit testing, meaning once the concentration of alcohol is above a certain level, additional tests are not performed to look for other impairing substances. We performed this study to determine the rates of positivity of Tier I and Tier II drugs in DUID cases, patterns of combined drug use, and to evaluate the potential impact of stop-limit testing in estimating true rates of drug positivity.

Blood samples submitted for analysis in suspected DUID cases were retested for an expansive menu of drugs, using two workflows performed by Liquid Chromatography/Mass Spectrometry Quadrupole Time-Of-Flight (LC/QTOF) for basic drugs and synthetic cannabinoids, respectively. Samples were analyzed for alcohol and confirmed for Tetrahydrocannabinol (THC) and its metabolites at a reference laboratory. The basic drug panel library contains over 1,000 drugs and metabolites, and the synthetic cannabinoid panel contains over 300 drugs and metabolites. Both libraries encompass all the recommended Tier I and Tier II compounds, and are updated regularly as new drugs are identified.

To date, 2,127 samples have been screened on the basic and synthetic cannabinoid drug panels. Positivity was assessed for Tier I, Tier II, and other NPS. The findings from Tier I include methamphetamine ( $n=334$ ; 15.7%), fentanyl ( $n=309$ ; 14.5%), amphetamine ( $n=298$ ; 14.0%), benzoylecgonine and cocaine ( $n=148$ ; 6.9%). Ethanol was found in 878 (40%) cases with a median concentration of 0.16g/100mL and range from the limit of detection (0.01 g/100mL) to 0.61g/100mL. THC was found in 1,080 (50.7%) cases with a median concentration of 8.1ng/mL and a range from the limit of detection (0.5ng/mL) to 96ng/mL. For Tier II drugs, the top three detected compounds were diphenhydramine, hydroxyzine, and trazodone. Diphenhydramine was seen in 129 cases (6.0%), followed by hydroxyzine ( $n=83$ ; 3.9%), and trazodone was seen in 62 cases (2.9%). When evaluating the NPS positivity, 8-aminoclonazepam ( $n=70$ ; 3.2%) was seen with the highest frequency, followed by fluorofentanyl ( $n=64$ ; 3.0%) and etizolam ( $n=46$ ; 2.1%).

Ethanol was most frequently found in combination with Central Nervous System (CNS) stimulants. THC was most commonly found with CNS stimulants and narcotic analgesics (fentanyl).

Drug positivity was also evaluated at common BAC levels used in stop-limit testing ( $>0.08$ ,  $>0.10$ , and  $>0.15$ g/100 mL). Tier I drugs were found in 15.4% of all cases with a BAC  $>0.08$ g/100 mL. Positivity for Tier I, Tier II, or a combination of the two for cases with 0.08g/100 mL was 17.7% ( $n=386$ ),  $>0.10$ g/100 mL 16.4% ( $n=358$ ) and  $>0.15$  g/100 mL 10.6% ( $n=231$ ).

This study demonstrates that the current Tier I and Tier II recommendations are appropriate and align with trends in DUID cases. Finding Tier I and II drugs in cases with BAC  $>0.08$ , suggests that comprehensive testing of even high BAC cases provides insight into polysubstance-impaired driving and is a best practice.

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**Impaired Driving; Novel Psychoactive Substances; Stop-Limit Testing**

## L59 $\alpha$ -Pyrrolidinoisohexanophenone ( $\alpha$ -PiHP) in Driving Under the Influence (DUI) Cases

Kristin Kahl, MS\*, University of Miami Toxicology Laboratory, Miami, FL; Lisa Reidy, PhD, University of Miami, Miami, FL

**Learning Objective:** After attending this presentation, attendees will better understand the analytical techniques used for detecting  $\alpha$ -PiHP and its incidence and polydrug use in DUI casework.

**Impact Statement:** This presentation will impact the forensic science community by providing regional data on  $\alpha$ -PiHP and its use with other drugs in DUI cases.

Novel Psychoactive Substances (NPS) trends change rapidly, as seen with synthetic cathinones, synthetic cannabinoids, and fentanyl analogs; with this ever-changing landscape, NPS can be difficult to identify, may only be seen regionally or for a short time, and extensive pharmacological information is rarely known. Synthetic cathinones have been reported in Broward and Miami-Dade Counties, Florida, in the past, with Broward County being the national epicenter for  $\alpha$ -Pyrrolidinopentiophenone ( $\alpha$ -PVP), or flakka, in 2014.<sup>1</sup>

$\alpha$ -PiHP is a novel synthetic cathinone recognized internationally in late 2016 and in the United States in late 2018.<sup>2,3</sup>  $\alpha$ -PiHP has consistently been reported in NPS Discovery stimulant trend reports since their inception in 2020 Q1, but with relatively low frequency in toxicology cases.<sup>4</sup> As a group, synthetic cathinones have similar effects to other central nervous stimulants; these effects include increased locomotor activity, blurred vision, elevated heart rate and blood pressure, and altered mental status.<sup>5,6</sup> These effects can impair an individual's ability to operate a motor vehicle safely. This review examines trends in  $\alpha$ -PiHP detection in DUI cases submitted to the University of Miami Toxicology Laboratory (UMTL) from two Southeast Florida counties.

Blood and urine specimens were from subjects involved in suspected DUI cases in Miami-Dade and Broward Counties submitted to the UMTL from July 2021 to July 2022. Blood specimens were first analyzed for ethanol and other volatiles using Headspace/Gas Chromatography/Flame Ionization Detector/Mass Spectrometry (HS/GC/FID/MS). No additional testing was performed if the blood ethanol concentration was  $\geq 0.150$ g/dL. If ethanol was not detected or was  $< 0.150$ g/dL, then drug screening and confirmation/quantitation were performed as applicable. Urine specimens were only analyzed for drugs. Toxicological analyses for blood and urine specimens included Basic Drug Screens (BDS) by Gas Chromatography/Mass Spectrometry (GC/MS) ( $>800$  analytes) and Liquid Chromatography/Quadrupole Time-Of-Flight/Mass Spectrometry (LC/QTOF/MS) ( $>300$  analytes). The detection and confirmation of  $\alpha$ -PiHP were performed using the LC/QTOF and GC/MS methodologies, respectively; the GC/MS methodology can distinguish  $\alpha$ -PiHP from its structural isomer,  $\alpha$ -PHP, by retention time and mass spectral differences. The Limit Of Detection (LOD) was validated per American National Standards Institute/Academy Standards Board (ANSI/ASB) Standard 036 for  $\alpha$ -PiHP, with LODs ranging from 5-50ng/mL, depending on the methodology and matrix.

July 2021 was the first time the laboratory reported  $\alpha$ -PiHP in a suspected DUI case. In the year of this review, five DUI cases (two blood and three urine specimens) were reported as positive for  $\alpha$ -PiHP. Interestingly, all cases were from Broward County. Of the 231 Broward County DUI cases in which blood or urine specimens underwent drug testing,  $\alpha$ -PiHP was reported in 2% of cases. Ethanol was not detected in either of the two blood specimens; however, in four out of the five cases, other drugs or metabolites were detected. Opioids, cannabinoids, and novel benzodiazepines were the most reported other drug groups, each reported in three of the four polydrug use cases; cocaine was also reported in two cases. In one blood specimen,  $\alpha$ -PiHP was the only drug reported; this was the only case listed as a DUI with serious bodily injuries or fatality. Demographic information reveals that all subjects were between 26 and 35 years old, with 80% being male.

The percentage of DUI cases in which the UMTL has reported  $\alpha$ -PiHP is low; however, given the history of NPS stimulant use in Broward County, it is noteworthy that  $\alpha$ -PiHP has only been reported in Broward County DUI cases. A review of the case results also reveals that  $\alpha$ -PiHP is commonly used in combination with other drugs, including other NPS.  $\alpha$ -PiHP has the potential to impair driving ability, making it important to analyze toxicological results alongside human performance data.

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### s; Driving Under the Influence; Novel Psychoactive Substances

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**L60 Blood Alcohol Concentration and Road Accidents—A Significant Underestimation Due to Delay in Blood Sampling: A Four-Year Retrospective Study in Rome**

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**Learning Objective:** After attending this presentation, attendees will understand how the delay in blood sampling can result in underestimation of values of Blood Alcohol Concentration (BAC) at the time of a road accident. This study aims to analyze the correlation between demographic features and BAC, and the delay in blood sampling.

**Impact Statement:** This presentation will impact the forensic science community by presenting the evidence of a critical bias due to the arrival time at the Emergency Department (ED) and the delay in blood sampling that inevitably influences and underestimates the BAC, resulting in possible false negative results (BAC values below the cut-off). A reduction in the delay in obtaining confirmatory blood tests upon arrival to the ED could mitigate this further bias, which is summed to the time between the incident and arrival at the ED, which is unpredictable and cannot be changed. Primary prevention policies, such as a “zero tolerance” approach could mitigate this bias, although it is necessary to implement preventive strategies to reduce Driving Under the Influence (DUI) of alcohol.

**Background:** According to the World Health Organization, alcohol is a significant public health problem since it is considered the leading cause of severe traffic accidents worldwide.<sup>1-4</sup> In Italy, the law prohibits driving to those who have a BAC exceeding 0.5g/L; the legal limit of BAC is zero under 21 years of age, for those who have a license for less than three years, and for those who professionally carry out the activity of transporting people or things.<sup>5</sup> Moreover, law requires BAC assessment at the request of the police for drivers involved in Motor Vehicle Accidents (MVA) and subjected to medical treatment. However, BAC does not often reflect the degree of intoxication at the time of the accident: the unconcealed delay in the time of blood sampling is due both to the time it takes an ambulance to reach a patient and transport him/her to the ED and the delay in blood sampling in the emergency room.

**Methods:** This retrospective study included patients presenting to the ED of Policlinico Universitario A. Gemelli (Rome, Italy) from January 2013 to December 2016 as a driver involved in MVA for the determination of blood alcohol content. Delay in blood sampling and BAC measurement in the ED were recorded for each patient. BAC levels were compared in patients involved in MVA according to age, gender, and time of the accident.

**Results:** The analysis included 398 patients. In 107 out of 398, BAC was higher than the Limit Of Quantification (LOQ) (0.05g/L); of these, 86 had a BAC higher than 0.5g/L and 21 had a BAC between LOQ and 0.5g/L. Moreover, patients involved in road accidents showed positive BAC in more cases both during the night and on the weekend. A significant delay in blood sampling for BAC determination was observed. The median delay was 105min (interquartile range 49–168). The delay between the road accident and blood collection affects the measured BAC value, which could result in being below the law limit even if at the time of accident it was higher. The rate of alcohol metabolism can also affect BAC, resulting in an underestimation of subjects with BAC higher than 0.5g/L; indeed, assuming three alcohol elimination rates respectively of 0.12g/L/h, 0.25g/L/h, and 0.35g/L/h and considering the delay in blood sampling, with an estimated value of 0.12g/L/h, 8 subjects out of 21 exceed the threshold value of 0.5g/L. Moreover, with an estimated value of 0.25g/L/h, 14 exceed the threshold value of 0.5g/L. Finally, with an estimated value of 0.35g/L/h, 17 exceed the threshold value of 0.5g/L.

**Discussion:** This study proved a critical bias due to the delay in blood sampling that inevitably influences and underestimates the BAC getting false negative values below the limit. A back-calculation to extrapolate the measured blood alcohol level to a previous time may be needed, for example, in the case of injured drivers who are taken to the hospital for emergency treatment, when drivers have absconded from the scene of the accident, or in cases where offenders refuse the screening test at the scene of the accident. Therefore, even if results between 0.05g/L and 0.5g/L are below the legal limit, they may have a different meaning due to the difficulties in showing that a defendant was operating a vehicle while intoxicated. A “zero tolerance” policy could help to lessen this bias, even if it is still important to use preventative measures to lessen alcohol-impaired driving (DUI).<sup>6</sup>

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**Blood Alcohol Concentration (BAC); Forensic Toxicology; Alcohol-Related Accidents**

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**L61 Oxycodone Pharmacokinetics in Whole Blood After a Single Dose of Immediate and Controlled Release Formulations**

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**Learning Objective:** After attending this presentation, attendees will be able to describe the metabolic pathways for oxycodone, discuss pharmacokinetic differences between oxycodone formulations, and how genetic disposition can affect metabolism.

**Impact Statement:** This presentation will impact the forensic science community by presenting new data for major and minor metabolites of oxycodone from a controlled study and by discussing how these can (and cannot) be used when interpreting blood concentrations.

Oxycodone is an opioid prescribed for its analgesic effects but also has high abuse potential. Oxycodone is available as Immediate Release (IR) and Controlled Release (CR) formulations. Monitoring blood concentrations of oxycodone and metabolites can be forensically important to establish the time of intake or verify a prescribed dose. However, the few studies involving the pharmacokinetics of oxycodone leave a knowledge gap regarding elimination of minor metabolites, pharmacokinetic differences by formulation, and the impact of CYP2D6 activity on the metabolism and elimination of oxycodone. This study aimed to compare pharmacokinetics of oxycodone and five metabolites by formulation and compare ratio changes over time when taken into consideration the CYP2D6 phenotype. Our hypothesis was that metabolite ratios unrelated to CYP2D6 would better describe the relationship between intake and time.

Subjects ( $n=9$ ) received a single 10mg IR tablet of oxycodone actavis and a few weeks later, a 10mg CR tablet of oxycodone depot actavis. Whole blood was collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 24h. Blood was diluted with acetate buffer pH6 and extracted on Bond Elute Certify columns. Quantitation was performed using a previously validated and published Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method.<sup>1</sup> The limits of quantification were 0.5ng/mL for oxycodone, noroxycodone, 6 $\alpha$ -oxycodol, oxymorphone, and noroxymorphone. The CYP2D6 phenotypes were categorized as Poor Metabolizers (PM), Intermediate Metabolizers (IM), Extensive Metabolizers (EM), and Ultra-rapid Metabolizers (UM) by determining the alleles \*3, \*4, \*5, \*6, and \*41, including copy number variation. Comparisons between IR and CR were performed using two-tailed paired  $t$ -test at a significance level of  $p=0.05$ . Two subjects were PM, two were EM, and five were IM. The CR area under the concentration curve was significantly lower for noroxycodone but not for oxycodone. The mean  $C_{max}$  and  $T_{max}$  for oxycodone were significantly different between IR (31.8ng/mL at 1.2h) and CR (16.0ng/mL at 3.6h) as were they for noroxycodone (IR 13.7ng/mL at 2.2h and for CR 8.9ng/mL at 5.7h). All subjects, except three after the IR dose, were positive for oxycodone and noroxycodone at 24 h. The concentration ratio noroxycodone/oxycodone increased over time after intake for both IR (from 0.4 to 2.7) and CR (from 0.5 to 1.9). However, the ratio was greatly affected by phenotype with noroxycodone/oxycodone  $>1$  at 6, 10, and 24h after intake for IM, EM, and PM, respectively. Noroxymorphone showed mean  $C_{max}$  at 2.6 and 1.8ng/mL,  $\alpha$ -OCL at 2.0 and 1.2ng/mL, and  $\beta$ -oxycodol at 1.9 and 1.2ng/mL for IR and CR, respectively. Concentrations of  $\alpha/\beta$ -oxycodol were significantly higher after IR. Oxymorphone was only detected after IR (0.5-1.0ng/mL). The  $\alpha/\beta$ -oxycodol ratio was also investigated for a relationship with time and increased from 0.6 to 1.5 for IR and from 0.6 to 1.3 for CR but again with differences depending on phenotype. Even though noroxycodone is mainly produced by CYP3A4, the ratio noroxycodone/oxycodone is affected by the CYP2D6 phenotype since it controls the elimination of oxycodone. On the other hand,  $\alpha/\beta$ -oxycodol produced by ketoreductase should not be greatly affected. However, this study's findings suggest that there are differences. Even with the small number of subjects it was clear that the CYP2D6 phenotype affected the metabolism and elimination of oxycodone. The metabolic ratios noroxycodone/oxycodone and  $\alpha/\beta$ -oxycodol showed an increase over time and could possibly be used to estimate the time of intake, but only if the CYP2D6 phenotype is known.

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**Oxycodone; Pharmacokinetics; CYP2D6**

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**L62 The Extraction and Quantification of Toxic Heavy Metals From Calliphoridae Larvae**

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**Learning Objective:** After attending this presentation, attendees will better understand about using necrophagous maggots to extract heavy metals; moreover, attendees will have learned about the different accumulation recorded in maggots feeding on different areas of a corpse as well as the metal accumulation in different parts of the maggots.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees about another application of forensic entomotoxicology. This may encourage the performance of forensic toxicological analyses using non-human substrates, and will even make the community more aware of the role that environmental contaminants may have in forensic investigations.

**Introduction:** Heavy metals are natural non-biodegradable elements and are often found, at low levels, in a wide range of compounds. However, their accumulation in the environment constitutes a health risk for many living organisms; numerous studies have shown how prolonged exposure to heavy metals increases their toxic effects and, therefore, environmental toxicity assessments are often focused on their detection. Arthropods occupy almost all ecosystems, rural and urban, and can therefore be highly exposed to a variety of xenobiotics, including heavy metals present in the environment. Entomological evidence collected from a crime scene can be used as a substrate to extract and quantify toxic substances present in the corpse; this information can help investigators determine the minimum Postmortem Interval (mPMI), cause of death, primary crime scene location, and sources of exposure. The objective of this research study was to design a protocol for the detection and quantification of four common heavy metals, lead, arsenic, chromium, and cadmium, from Calliphoridae larvae collected from three pig carcasses decomposing in an outdoor field during the warm season. An additional goal was to compare the accumulation of heavy metals between specimens collected from different areas and between larval cuticle and gut content.

**Methods:** Larvae were collected from the mouth and the thorax of the carcasses, as well as from the soil under them. Some larvae were dissected to analyze metal accumulation independently in the gut content and in the cuticle, while others were maintained intact to analyze heavy metal accumulation in whole larvae. About 60 samples per pig were analyzed, with an average sample weight of 0.1092g. All samples were digested with nitric acid (HNO<sub>3</sub>) to mobilize heavy metals and were then microwaved and homogenized to facilitate extraction. Heavy metals (Lead [Pb], Arsenic [As], Chromium [Cr], Cadmium [Cd]) were extracted and quantified via Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). Heavy metal accumulation was compared among cuticle, gut content and whole larvae, as well as among different areas (maggots from mouth, from thorax, and from soil under the carcass). Results were also compared with metal concentrations from soil samples and pig tissue samples collected before placing the carcasses in the field.

**Results and conclusions:** The results show lead to be the most abundant heavy metal recorded in larvae (highest concentration 900-1,400ppb in larval gut content), followed by arsenic (highest concentration 90-140ppb in larval gut content). The most abundant metal recorded in pig tissue prior to exposure was chromium (average concentration 63ppb) while the average tissue lead concentration was 10ppb. The results showed differences in the metal concentrations in larvae from different areas of the corpse (highest concentration in larvae from thorax); moreover, higher concentrations of heavy metals were recorded in their gut content than in the cuticle. This study contributes to our understanding of heavy metal accumulation in insect larvae of forensic interest and how accumulation changes depending on the areas where larvae are collected. Future research will apply the same protocol to different insect developmental stages and to different insect species, to assess potentially significant differences in heavy metals accumulation; moreover, studies will be conducted to assess the influence of specific heavy metals on the developmental rate of blow fly species. Since development of Calliphoridae is often used for the estimation of the Time Of Colonization (TOC) or the mPMI, any influence on their growth rate could impact these estimations. For this reason, a better understanding of the extraction and quantification of heavy metals from insect substrates and of the role played by heavy metals in insect development could ultimately help investigators conduct toxicological analyses and refine the estimation of the mPMI.

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**Forensic Entomotoxicology; Heavy Metals; Calliphoridae**



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**L63 A Cost Benefit Analysis of High Resolution Mass Spectrometry (HRMS) -Based Drug Screening in Forensic Toxicology**

*Jessica Ayala, PhD\*, Houston Forensic Science Center, Houston, TX; Sarah Kerrigan, PhD, Sam Houston State University, Huntsville, TX*

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**Learning Objective:** After attending this presentation, attendees will understand the process of Cost Benefit Analysis (CBA) to evaluate the Return On Investment (ROI) for High Resolution Mass Spectrometry (HRMS) -based drug screening within an operational forensic toxicology laboratory as compared to traditional Immunoassay (IA) -based screening techniques.

**Impact Statement:** This presentation will impact the forensic science community by providing insight to weigh the fiscal impacts of adopting HRMS-based drug screening technologies according to laboratory size and throughput.

Recent recommendations and standards related to the scope and sensitivity of testing, in addition to method validation practices, present new challenges to forensic service providers. As a result, operational and financial decisions must be made to maximize cost efficiency. Comprehensive toxicological analysis that offers analytical adaptability and flexibility to incorporate New Psychoactive Substances (NPS) and other emerging drugs of interest is necessary. To assess the value of advanced technologies such as HRMS-based drug screening, CBA can be used to aid forensic toxicologists in financial decisions and resource allocations. Laboratories must effectively distribute resources to optimize the value of expenses and maximize the ROI or minimize the cost per case.

In this study, CBA was utilized to estimate the cost and benefits associated with HRMS-based toxicological drug screening with traditional IA-based methods. Cost data was collected and estimated for consumables, capital and non-capital equipment, chemicals and reagents, personnel costs, and other services. The distribution of expenditures was compared for each technique, and a ROI model was created evaluating the change in cost per case based on the annual number of cases analyzed. The distribution of expenditures between IA and HRMS toxicological drug screening was variable, but the total cost per case was similar for both methods at 5,000 cases per year. HRMS toxicological screening requires a high initial financial investment as indicated by the established ROI model. However, costs were minimized at a moderate laboratory throughput of 5,000 cases per year. Laboratories analyzing 1,000 cases or fewer per year exhibited decreased ROI for HRMS analysis. However, high volume testing (greater than 5,000 cases per year) exhibited lower cost per case as compared to IA testing. Furthermore, perfect economies of scale were realized more rapidly using HRMS technology. Overall, the need to expand the scope of analytical testing depends on the nature of the toxicological investigation and the demands of the consumer. However, this study has shown HRMS-based screening can be efficient and cost-effective for laboratories with moderate annual caseloads.

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**Cost Benefit Analysis (CBA); High Resolution Mass Spectrometry (HRMS); Immunoassay**

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**L64 Assessing Relative Levels of Ionic and Non-Ionic Binding of Drugs and Metabolites in Authentic Hair Reference Material (HRM)**

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**Learning Objective:** After attending this presentation, attendees will understand drug-hair binding interactions.

**Impact Statement:** This presentation will impact the forensic science community by presenting relative levels of ionic and non-ionic binding for Cocaine (COC), Cocaethylene (COCA), Norcocaine (NORCOC), p-Hydroxycocaine (HYCOC), Oxycodone (OXY), Morphine (MOR), 6-Monoacetylmorphine (6-MAM), and Methamphetamine (MET) to the hair matrix and by providing understanding regarding the physicochemical nature of binding between drugs and the hair matrix.

There is currently a lack of comprehensive understanding regarding the mode of binding of drugs to hair. Some general trends found to affect drug-matrix binding include pKa, structure, size, lipophilicity, protein binding capacity, and melanin affinity of the drug. Basic drugs incorporate into the hair matrix better than neutral or acidic drugs, due to hydrogen bonding and non-covalent interactions with eumelanin. Acidic and neutral drugs are hypothesized to produce a hydrogen bond with melanin. Other research groups have examined methods of parent drug binding to the hair matrix; however, published work has not assessed the binding of metabolites to hair.

Authentic HRM containing the drugs of interest was obtained from a commercial source. Samples of 20mg each were weighed into glass test tubes; 250µL of 10X Phosphate Buffer (PBS), pH 12 was added to the test tube. At this pH, all drugs and metabolites of interest were neutral because they are basic drugs with pKa values ranging from 8.61 to 9.87. The test tube was shaken for 2h, evaporated to dryness, and reconstituted in 250µL of Methanol (MeOH), prior to analysis on an Agilent® 1290/6460 Liquid Chromatography/Triple Quadrupole/Mass Spectrometer (LC/QqQ/MS). This was completed in triplicate. To assess ionic binding, this procedure was completed with 250µL of 10X Phosphate Buffer (PBS), pH 6 in triplicate. At this pH, all drugs and metabolites of interest were positively charged because they are basic drugs with pKa values ranging from 8.61 to 9.87.

Relative recovery of each drug and metabolite by buffer pH was evaluated to determine which type of drug-hair matrix interactions were occurring. With the exception of HYCOC and 6-MAM, recovery of drugs and metabolites occurred in both pH 6 and pH 12 buffer, indicating that they participate in both ionic and non-ionic binding with the hair matrix. In contrast, there was no recovery of HYCOC and 6-MAM at pH 6 and 12, respectively, indicating that they participate only in non-ionic (HYCOC) or ionic (6-MAM) interactions. Higher recovery of COC and its metabolites, MOR, and OXYCOD occurred at pH 12, indicating that while these drugs exhibit both types of binding, more non-ionic interactions occur between these drugs and hair than ionic. However, the higher recovery of MET was at pH 6, indicating that MET participates in more ionic interactions with hair than non-ionic. In conclusion, the nature of binding of multiple drugs and metabolites to hair were elucidated.

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**Forensic Hair Analysis; Drug-Hair Binding Studies; Authentic Hair Reference Material**



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**L65 Toxicological Surveillance of Clinical Patients Presenting to the Emergency Department After Suspected Opioid Overdoses: Geographical Trends and Impacts on Forensic Toxicology**

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**Learning Objective:** After attending this presentation, attendees will be able to assess the importance of antemortem toxicological surveillance, as well as the impact of paired clinical observations and forensic toxicology testing.

**Impact Statement:** This presentation will impact the forensic science community by emphasizing the importance of dialog between clinicians and forensic toxicologists to better understand drug trends and effects.

Traditional drugs and Novel Psychoactive Substances (NPS) can lead to adverse events and overdose scenarios where individuals seek clinical treatment. Clinicians examine signs and symptoms of patients presenting to emergency departments to provide proper course of treatment. This can be extremely challenging as unknown drugs and/or new drug combinations may emerge with effects not well characterized or understood. Comprehensive toxicology testing is necessary to determine the different drugs in each case and emerging drug trends that are impacting treatment and outcomes.

This abstract is submitted on behalf of the ToxIC Fentanyl Study Investigators. Toxicology samples ( $n=532$ ) were received between December 2020 and June 2022 from nine locations across the United States. Samples derived from patients presenting to the emergency department after an apparent opioid overdose and are part of an ongoing collaborative study with the American College of Medical Toxicology (ACMT). Sites are located in the Northeast (PA, NY, NJ), Midwest (MI, MO), and West (CA, OR). Antemortem blood and plasma/serum samples were submitted to the Center for Forensic Science Research and Education (CFSRE) for comprehensive and expanded toxicology testing. All samples were prepared using an acidic and a basic liquid-liquid extraction prior to instrumental analysis. Instrumental analysis was performed via a Shimadzu Nexera® HPLC coupled to a SCIEX™ TripleTOF® 5600+ (Liquid Chromatography/quadrupole Time-Of-Flight/Mass Spectrometry [LC/QTOF/MS]) using SWATH® acquisition. Data were processed against an in-house library containing >1,000 drugs, including a vast majority of NPS and metabolites.

Three hundred one samples were submitted from the Northeast. Traditional opioids were identified in 269 samples (primarily fentanyl, 83%) and NPS opioids were identified in 45 samples (primarily para-fluorofentanyl, 14%). No opioids were detected in 31 samples (10%). Other NPS identified included clonazepam (6%), MDMB-4en-PINACA (4%), and N-piperidinyl etonitazene (1%). One hundred sixty-seven samples were submitted from the Midwest. Traditional opioids were detected in 159 samples (primarily fentanyl, 63%, and tramadol, 9%). No opioids were detected in eight samples. NPS opioids (para-fluorofentanyl, 22%, butyrylfentanyl, 2%, and buprenorphine, 1%) were identified in samples from this region. Clonazepam (6%) was also observed in the Midwest, and a variety of other NPS were identified, including eutylone, BZO-POXIZID, ADB-5Br-INACA, MDMB-5Br-INACA, ADB-HEXINACA, and bromazepam. Sixty-four samples were submitted from the West. Traditional opioids were identified in 57 samples (primarily fentanyl, 63%, and heroin, 25%). NPS opioids were identified in 8 samples (primarily para-fluorofentanyl, 13%). No opioids were detected in 5 samples. Designer benzodiazepines (23%) such as etizolam, bromazepam, flubromazepam, and clonazepam were also detected in this region. Of the 532 samples, no opioids (NPS or traditional) were identified in 44 samples spanning eight of the nine clinical sites. In six of these opioid-negative samples, synthetic cannabinoids were discovered, in some cases being the only drug(s) present. This finding further indicates the respiratory failure and depressant effects that synthetic cannabinoids are precipitating, causing patients to present as if they are suffering from an opioid overdose. Throughout this study, 7 NPS opioids were identified and 26 additional NPS (including synthetic cannabinoids, designer benzodiazepines, and synthetic stimulants). Various traditional drugs (e.g., methamphetamine, heroin) and therapeutic agents (e.g., naloxone) were identified, as well as adulterants and cutting agents (e.g., xylazine, lidocaine).

The wide range of drugs identified in overdose patients underscores the need for collaboration between clinical personnel and forensic toxicologists, as drugs present in antemortem samples may serve as early indicators for medicolegal death investigations and/or drug-impaired driving investigations. Continued surveillance of NPS and other drugs in “opioid-negative” sample populations may lead to important research findings and the ability for clinicians to better understand signs/symptoms and pathologists to better understand autopsy findings. Antemortem toxicology testing results remain a key factor for understanding recreational drug use and emerging trends.

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**Surveillance; Overdose; Toxicology**

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**L66 The 22<sup>nd</sup> Annual Postmortem Pediatric Forensic Toxicology Session**

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**Learning Objective:** After attending this presentation, attendees will better appreciate the challenges unique to toxicological findings in postmortem pediatric cases. Attendees will learn interpretive guidelines for pediatric cases involving forensic toxicology in both general and case-specific senses.

**Impact Statement:** This presentation will impact the forensic science community by further delineating the interpretive aspects of toxicological findings in the pediatric population.

In this 22<sup>nd</sup> Annual Special Session within the Toxicology section, pediatric cases involving toxicological findings are discussed. As a relative dearth of interpretive information exists involving toxicological findings in the pediatric population, this session is a forum to help elucidate and clarify such issues. The format is a short case presentation or issue-specific concern, including pharmacokinetic data and other relevant ancillary information, followed by audience participation to provide interpretive clarity around case-specific impacts of the toxicological findings. This session, attended by various sections of the Academy, allows for various perspectives of case issues that lead to integrative consensus, or differing opinions, as to cause of death in children.

Four cases will be presented that highlight the difficulty in assessing the role of toxicants in each case or the lengths one must go to in some cases to reach some interpretive value. Christopher Milroy, Andrew Baker, Jenny Swatek, Kari Midthun, and Brianna Peterson will be reviewing cases from their experience as forensic pathologists and toxicologists that highlight the issues and confounders in the pediatric population.

Dr. Milroy will be discussing a case involving ketoacidosis in children. As a reminder, ketoacidosis is a state in which serum and urine contain elevated levels of ketone bodies. The three common causes of ketoacidosis include diabetes, alcohol, and starvation. The condition often results from oxidation of free fatty acids and results in an increased anion gap metabolic acidosis. Prolonged ketoacidosis can lead to coma and death.

Dr. Baker will focus on an unresolved case involving an elevated glucose in vitreous humor in an 8-year-old. While toxicologists routinely perform vitreous chemistries, understanding the nuances of findings can be complex and defy normal explanation. This is one such case.

Ms. Jenny Swatek will report on a case involving an uncommonly found substance in children (i.e., glipizide). As an oral antihyperglycemic agent, it can induce a severe hypoglycemia, especially in children, a potentially fatal state. The case serves as a reminder that in children, the scope of testing should be broader than in adults.

Drs. Kari Midthun and Brianna Peterson will be describing two cases involving fentanyl. While fentanyl may be the most commonly found opioid recently in overdose deaths, its presence in children is still somewhat surprising. The presentations will highlight how similar findings can have drastically different explanations and outcomes.

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**Pediatric; Postmortem; Toxicology**

## LW1 The Death Ray: A Historical and Forensic Investigation of an Italian Mystery

*Matteo Borrini, PhD\**; Research Centre in Evolutionary Anthropology and Palaeoecology, School of Natural Sciences and Psychology, Liverpool John Moores University, Liverpool, UNITED KINGDOM; Marco Morocutti, Italian Committee for Examination of Pseudo-Scientific Claims, Padova, ITALY; Luigi Simeoni, Via Campo di Marte, Brescia, ITALY; Lorenzo Paletti, MSc, Vicolo dell'Arciprete, Brescia, ITALY

**Learning Objective:** After attending this presentation, attendees will have a clearer understanding of the potential of applying forensic sciences and facial comparison to historical cases.

**Impact Statement:** This presentation will impact the forensic science community by providing an example of a cold case investigation based on archive documents and craniofacial comparison.

The Death Ray would be a particle beam weapon first theorized in the 1920s and often attributed to famous inventors. The first that claimed to be successful in producing such a futuristic weapon was the American Edwin R. Scott in 1923, followed by Nikola Tesla in the 1930s. The Death Beam also appeared in Tolstoy's 1927 novel, *The Garin Death Ray*, and later in science fiction such as *Flash Gordon* comics and the *Star Wars* saga.

However, the Death Ray appeared as a possible real object during the famous Italian investigation “Armi e Droga” (weapons and drugs) in 1982. The alleged creator was Rolando Pellizza (1938–2022), a businessman already investigated, but found to be unrelated, for kidnapping a member of the Agnelli family, one of Italy's most notorious industrial groups.

During the “Armi and Droga” trial, it was discovered that Rolando Pellizza claimed to have created the Death Beam (that he called “the machine”) and tried to sell it to several governments and private investors. Notably, the United States and Italian and Belgian authorities had been contacted and demonstrated some interest in the product. However, during the demonstrations (usually provided by videos or partial experiments), concerns were raised about “the machine's” true powers. Documents of the American Embassy in Rome and the statement of the Italian Prime Minister at that time, Giulio Andreotti, clarify the sceptic attitude toward the alleged new weapon. In addition, American and Belgian experts performed tests that proved the presence of explosive powders on the target supposedly annihilated by the beam. Also, the president of the Italian National Committee for Nuclear Energy (CNEN), Ezio Clementel, who examined the video recording of the experiments, denied the possibility that “the machine” was using antimatter as its power, contrary to what was stated by its inventor.

Due to the “Armi and Droga” investigation, Rolando Pellizza lived as a fugitive until the accusations fell and returned to Italy in the early 1990s. Despite this, he continued to claim to work on his invention, as detailed in a first biography published in 2011. According to Pellizza's statements, over the years, he discovered different properties of his machine, not limited to the death beam, including matter transmutation and the ability to make living individuals younger. In biographical books, Pellizza claimed he had obtained the know-how to build “the machine” from the physicist Ettore Majorana. The scientist would not have been dead since his disappearance in 1938 but would have lived incognito inside a monastery where Pellizza would have accidentally met him in 1958. To support Pellizza's assertions, in the last edition of the biographical book, some pictures are included allegedly portraying Majorana met by Rolando.

A feature-by-feature comparison of the facial characteristics of the unknown individual and certified portraits of Ettore Majorana has been performed. Some characteristics, including traits linked to the cranial substructure of the face, are not compatible between the two compared faces. The photographic analysis excludes the identification of the person presented in Pellizza's biography as Ettore Majorana, adding new elements of skepticism to the whole Death Ray story.

The authors present an example of a cold case investigation performed on archive documents using craniofacial comparison, demonstrating how science can always work, despite the seductive fictional stories speeded by a pseudoscientific attitude.

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### Ettore Majorana; Facial Comparison; Cold Case Investigation

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## LW2 The Case of the Count's Cranium: (Mis)identification of Revolutionary War Colonel Carl Emil Ulrich von Donop (1732-1777)

*Thomas A. Crist, PhD\**; Utica University, Utica, NY; *Richard F. Velt, PhD*, Monmouth University, West Long Branch, NJ; *Hillary A. DelPrete, PhD*, Monmouth University, West Long Beach, NJ

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**Learning Objective:** By attending this presentation, attendees will learn about a Hessian officer who died at the Battle of Red Bank in New Jersey during the American Revolutionary War and the use of forensic anthropology and archival research to determine if a cranium curated for over 70 years by Rutgers University is in fact his.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating the effective use of non-invasive forensic anthropological methods, combined with archival sleuthing, to address the possible misidentification of the remains of a historically significant individual.

In 1950, the former Speaker of the New Jersey Assembly donated numerous pamphlets, books, souvenirs, and a human cranium to a prominent history professor at Rutgers University. In a subsequent letter to the University Librarian, he wrote that he had obtained the cranium in the 1930s from “a very old doctor” on whose father’s farm the cranium had been unearthed “in the middle 1800s.” The doctor’s father was also a physician who had reportedly used the cranium “principally to adorn a bookshelf” in his office. Admitting that he may have been “inaccurate in this information” but had “no reason to doubt its truth,” the Speaker claimed that the cranium was from none other than Colonel Carl Emil Ulrich von Donop, the Hessian commander of Southern New Jersey who died at the Battle of Red Bank in 1777.

The son of a noble family from Hesse-Kassel in central Germany, the British employed Count von Donop as a colonel and assigned him command of four grenadier battalions and the Hessian Jäger corps when he arrived at Long Island in August 1776. After fighting in several battles, von Donop and his troops settled for the winter in southern New Jersey, just outside of Trenton. His forces, however, were unprepared to assist when General George Washington famously crossed the Delaware River and attacked Trenton over Christmas 1776, reinvigorating the Continental Army and galvanizing the American independence movement. Colonel von Donop was reportedly spending Christmas at a house 18 miles away in Mt. Holly with a “beautiful young widow” (possibly Betsy Ross!), a dalliance that may have cost the British the war.

Ten months later, British General William Howe ordered Colonel von Donop to attack Fort Mercer at Red Bank, located on the New Jersey side of the Delaware River, 15 miles south of Philadelphia. Determined to break the American’s blockade of Philadelphia, on October 22, 1777, von Donop attacked the fort with 1,200 Hessian soldiers. Although outnumbered, the Continental militia successfully defended the fort and won the day. Colonel von Donop was mortally wounded during the battle and died three days later. Buried with his soldiers at the battlefield, his grave was reportedly marked by a large rock inscribed “Here lies buried Colonel Donop.”

Subsequent decades of farming, erosion, and re-enactments of the battle occasionally exposed human bones at Red Bank. Most of the remains likely represented the hundreds of Hessian soldiers buried at the former battleground, with none of them specifically identifiable as Colonel von Donop. His supposed cranium reportedly reappeared on Dr. Henry Clay Clark’s farm property sometime in the 19<sup>th</sup> century, ultimately making its way via the doctor’s son, then a New Jersey Assemblyman, and finally a history professor to the Rutgers University Library in 1950.

Previous anthropological analyses of the cranium failed to conclusively determine if it was Colonel von Donop’s remains. In 2021, the current research team undertook an extensive analysis of the cranium using the most recent forensic anthropological methods and historical and genealogical information. This presentation will reveal once and for all if the “Case of the Count’s Cranium” has been solved.

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**Forensic Anthropology; Taphonomy; American Revolution**

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## LW3 The Somerton Man—Australia’s Coldest Case Solved!

*Colleen M. Fitzpatrick, PhD\*, Identifinders International, LLC, Fountain Valley, CA; Derek Abbott, PhD, University of Adelaide, Adelaide, AUSTRALIA*

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**Learning Objective:** Upon attending this presentation, attendees will gain an understanding of how modern Forensic Genetic Genealogy (FGG) is able to solve decades-old cold cases, both domestically and internationally. The presentation will illustrate how FGG identification can confirm or dispel speculative theories that have been developed over time about such high-profile cases, based primarily on circumstantial evidence.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how FGG can generate investigative leads in even very old cold cases. The presentation will broaden the understanding of how human identification has advanced over recent decades from relatively primitive private investigation techniques on a local level to the advanced research and analysis methods used today on a global basis.

The Somerton Man has been Australia’s most well-known and baffling forensic case, defying analysis since the man was found dead on Somerton Beach, South Australia, on December 1, 1948. The autopsy of the estimated 40–45-year-old “Somerton Man” (SM) indicated his death could not have been natural, with poisoning by a barbiturate or glucoside suspected, although no poison was detected. The case took a puzzling turn when a small scrap of paper was found in the man’s fob pocket with the Persian words “Tamam Shud” (Finished) printed on it. These are the last words of the *Rubaiyat of Omar Khayyam*.

A thorough police investigation produced no identifying information. Somerton Man’s fingerprints, the wide publication of his autopsy photos, and a review of United Kingdom and United States missing persons reports produced nothing. His suitcase, recovered from the nearby Adelaide train station, also revealed no identifying information except for a laundry bag stenciled with the name “Keane” and some items of clothing hand marked “Keane” or “T. Keane” in India ink.

His identity remained a mystery for nearly 75 years until we identified the Somerton Man in July 2022 using FGG as Melbourne resident Carl “Charles” Webb, born November 16, 1905, in Footscray, Victoria, Australia. The identification was made based on DNA obtained from hair shafts found embedded in the man’s plaster death mask held at the South Australia Police Museum. Professor Derek Abbott of the University of Australia was permitted access to the mask and the hair in 2011 by the South Australian Police.

We report details of the genealogical process used to identify the Somerton Man, along with the backstory of what little is known about his life. We dispel popular theories, including the possibility that the Somerton Man may have fathered a son by a nurse whose phone number was penciled on the back page of a copy of the *Rubaiyat*. The book was found to be the source of the scrap of paper in the man’s pocket. We explain how even with the major breakthrough on Somerton Man’s identity, we may never know the full story and why his cause and manner of death may remain undetermined.

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### Somerton; DNA; Genealogy

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## LW4 The Identification of the 1961 Bibb County Teenage Hitchhiker Using Forensic Genetic Genealogy (FGG)

*Colleen M. Fitzpatrick, PhD\*, Identifinders International, LLC, Fountain Valley, CA; Misty Gillis, Identifinders International, LLC, Fountain Valley, CA*

**Learning Objective:** Upon attending this presentation, attendees will gain an understanding of how Forensic Genetic Genealogy (FGG) has been able to identify even decades-old human remains.

**Impact Statement:** This presentation will impact the forensic science community by demonstrating how DNA analysis is doing more and more with less and less, sometimes with spectacular results.

In 1961, not much could be done to identify the teenage hitchhiker who drowned when the car he was riding in collided with a guard rail and plunged into the Cahaba River in rural Bibb Co, AL. Besides the clothes he was wearing, the only items on his person were a Timex® wristwatch, a brown plastic wallet without any identification, a holy medal, and a pack of Pall Mall® cigarettes with a South Carolina tax stamp. He had a tattoo with the inscription RY + LOVE and a blurred picture of himself with a girl inscribed “Think of me always and remember how we used to go places together.” He told the driver of the car who gave him a lift that he was on his way to join the Marines in CA. Unfortunately, not much could be done to identify him except to note that he was a 14–17-year-old Caucasian male, 5'6", 120lbs., with blue eyes and light brown hair. The autopsy also revealed that the unknown boy might have walked with a slight limp. The Bibb Co Teenage Doe was buried by the residents of nearby Centerville under a headstone bearing the inscription “Unknown, Killed in automobile accident, March 27, 1961.”

In 2015, the National Center for Missing and Exploited Children sponsored the boy’s exhumation, hoping that DNA analysis could identify him. A tooth and a piece of tibia bone were sent to the University of North Texas for extraction, but without results. The remains were too compromised to yield DNA.

By 2021, however, new advances in ancient DNA analysis offered hope of a better outcome. The tooth root was re-extracted using ancient DNA protocols to yield nearly 46ng; the tibia bone yielded 5.6ng. Although the DNA was somewhat contaminated with bacteria and also seemed to exhibit inhibition, the extract was much more than enough to make an identification using forensic genetic genealogy.

This presentation describes the DNA identification of the 1961 Bibb County Teen Doe as 15-year-old Danny Armentrout, the youngest of three brothers who were being raised by an abusive stepfather. The middle brother, Donald, left in 1960 to join the military. When he returned for Christmas in 1961, both his younger brother, Danny, and his older brother, David, had left home; he never saw them again. Donald spent decades searching for his brothers, believing that one day he would be able to find David through his social security number. Little did he expect to receive a call 60 years later about the fate of his 15-year-old brother, Danny.

A memorial service was held in Centerville on December 28, 2021, in which Donald was reunited with his brother so long after his death. Thanks to the efforts of the ancient DNA community and a dedicated forensic genetic genealogist, the 1961 Bibb County Teenage Doe is now resting under a stone that reads: Danny Paul Armentrout, “Danny,” identified on October 30, 2021, Dec. 28, 1945–Mar. 27, 1961.”

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**FGG; Genealogy; DNA**



## LW5 Who Murdered Charles Lindbergh, Jr., and Why?

Lise A. Pearlman, JD\*, Oakland, CA; Peter Speth, MD, Forensic Consultations, Wenonah, NJ; Jamie E. Benvenuti, BA, Bay St. Louis, MS

**Learning Objective:** This presentation informs attendees about a new scientific theory explaining Charles Lindbergh, Jr.'s death, an ensuing cover-up, and an elaborate hoax perpetrated by his father.

**Impact Statement:** This presentation will impact the forensic community by exposing Lindbergh and a leading eugenicist in the toddler's sacrifice for "the needs of science," underscoring the importance of ethical boundaries on scientific research.<sup>1</sup>

On May 12, 1932, remains of an "unknown baby" arrived at the Trenton, NJ, morgue from woods near the Lindberghs' farmhouse. The medical examiner noted the corpse's advanced decomposition, except for the better-preserved, large "square" head and right foot. The fontanel was unclosed. A half-inch round hole was observed behind the right ear. The left leg, one forearm, both hands, genitals, and most internal organs (including kidneys) were missing (leaving the decomposed liver and heart).<sup>2</sup> The Lindberghs' pediatrician couldn't identify the toddler, but the boy's father and nanny did.<sup>3,4</sup> Contrary to homicide protocol, Lindbergh ordered immediate cremation, precluding a full autopsy. Police assumed predators scavenged the corpse. But no investigators reported seeing claw or teeth marks on the corpse or its clothing or, decades later, upon re-examination of remaining digits.<sup>5</sup>

Lindbergh was home the night of March 1, 1932, when his son disappeared. The NJ State Police ignored Scotland Yard's suggestion of infanticide and suspicion of an "inside job" by first responders and the boy's grandmother.<sup>6</sup> Police allowed Lindbergh to head the investigation that focused instead on a kidnap-for-ransom theory. The media immediately sensationalized this theory and the resulting prosecution of Bruno Richard Hauptmann.

In December 1930, Lindbergh had secretly volunteered to assist Nobel-prize-winning surgeon Alexis Carrel at the Rockefeller Institute for Medical Research (RIMR). Lindbergh wanted to save his sister-in-law Elisabeth who had a defective heart valve. Carrel, a leading eugenicist, envisioned pioneering human coronary bypass procedures via carotid artery transplants.<sup>7</sup> Carrel used Lindbergh's mechanical expertise to perfect a perfusion pump for circulating serum in harvested animal and human tissue and organs.<sup>8</sup> Lindbergh also helped refine the centrifuge technique for separating blood to make serum, finishing on February 24, 1932.<sup>9</sup>

In early April 1932, Carrel reported to the RIMR Board a historic month-long experiment: his team, including Lindbergh, successfully perfused a carotid artery segment from an unidentified subject.<sup>10</sup> In 1938, Carrel and Lindbergh co-authored *The Culture of Organs*, which included human vivisection. The book encouraged researchers to continue experiments in this "forbidden field."<sup>11</sup>

In May 1932, the NJ State Police sent detritus found near the corpse to Squibb Biological Laboratories for analysis. The resulting Squibb Report cryptically described items then commonly used in medical research laboratories, including: litmus paper; a tiny (probably mica) disc covered with an unidentified blood-like substance; and red dye.<sup>12</sup> These items duplicated materials referenced in a 1932 centrifuge article by Lindbergh and Dr. Raymond Parker's 1938 *Methods of Tissue Culture* describing Carrel's vivisection experiments.<sup>13,14</sup> Squibb technicians also found black rubber residue under buttons of the toddler's separately recovered onesie, consistent with its removal by surgical gloves.<sup>15</sup>

Recent review of available 1932 reports by first responders and morgue attendees did not reveal any necrophagic activity on the corpse. That peculiarity corresponded with ether employed in Carrel's and other 1930s vivisections. The corpse's face and right foot suggested "chemical interference" by persons possessing specialized preservation skills and knowledge. The absence of kidneys suggested vivisection.<sup>16</sup> The skull hole appeared consistent with drilling before death to insert a shunt, aiming to remove potential hydrocephalic fluid.<sup>17</sup>

Reevaluation of 1932 forensic evidence strongly suggests Lindbergh offered his son for vivisection through novel experimentation in Carrel's laboratory, concealed by an elaborate kidnap-for-ransom hoax. Medical evidence implicates Lindbergh and eugenicist Carrel sacrificing Lindbergh's son for science and precipitating wrongful execution of scapegoat Hauptmann.

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1. The "needs of science" quote is from Lindbergh's lawyer, Henry Breckinridge, Statement to the Police, Sept. 28, 1934, attributing the quote to a stranger who came to his office on March 8, 1932, whom Breckinridge said he believed represented a purported kidnap gang.
2. Medical Examiner Dr. Charles Mitchell, "Report on Unknown Baby," May 12, 1932, NJSPM; retyped copy of Dr. Mitchell's updated May 14, 1932 death report identifying the victim as Charles Lindbergh, Jr., FBI Lindbergh Summary Report, (NY-62-3057) 110 (Autopsy) [www.archive.org](http://www.archive.org). Dr. Mitchell noted at trial that the decomposed liver and heart remained present. (Mitchell testimony, Hauptmann trial, BRHT, reel 1, part 0007, 72 (1501) New Jersey State Police Museum (NJSPM).)
3. "The condition of the body is such that positive identification of the body by me is impossible." Dr. Van Ingen, handwritten note dated May 13, 1932, on stationery of Coroner Walter H. Swayze, NJSPM.
4. Following IDs by Gow and Lindbergh, Dr. Mitchell updated his report to ID the corpse as Charles Lindbergh, Jr. May 14, 1932. See n.2, supra, *FBI Summary Lindbergh Report*, p.110.
5. Affidavit of Dr. William M. Bass, Appendix A, Pearlman, *The Lindbergh Kidnapping Suspect No. 1, The Man Who Got Away* [Regent Press 2020].
6. See, e.g., Lloyd Gardner, *The Case That Never Dies* (New Brunswick, New Jersey: Rutgers Univ. Press, 2004) 25 and n.40; "Kidnapping of Lindbergh Baby was an 'Inside Job', Mrs. Morrow Believes," *The Courier*, (Waterloo, Iowa), March 3, 1932, 1.



7. Alexis Carrel Nobel Lecture, December 11, 1912 *Suture of Blood Vessels and Transplantation of Organs* [www.nobelprize.org](http://www.nobelprize.org)
8. Alexis Carrel and Charles Lindbergh, *The Culture of Organs* (New York: Paul B. Hoeber, 1938), p.10.
9. Charles Lindbergh personal diary entry dated Feb 24, 1932 from Charles Augustus Lindbergh Papers at Yale Archives.
10. "April 1932 Report on Work" Alexis Carrel Papers, Box 17, Sec 19-1, folder 35 Georgetown University Booth Center for Special Collections; A History of the Rockefeller Institute (1901-1953) Origins and Growth (Rockefeller Press 1964) by official RIMR historian George W. Corner, p.233.
11. "Forbidden field", *The Culture of Organs*, n.8, supra, p.219.
12. Report of E.R. Squibb & Sons Biological Laboratories dated May 27, 1932. Original Squibb Report on file at NJSPM, reproduced at Suspect No. 1, supra, n.5, Appendix B.
13. Suspect No. 1, n.5 supra, Act 5, Ch.2 and Appendix B; Lindbergh, "A Method for Washing Corpuscles in Suspension," *Science*, New Series, Vol. 75, No. 1946 (April 15, 1932) pp.415-16.
14. Parker, Raymond C., *Methods of Tissue Culture* (1st edition, Hoeber: 1938).
15. See *The Culture of Organs*, n. 8, supra, p.117, Plate XVI, fig. 22.
16. Declaration of Dr. Peter Speth, dated May 27, 2020 Appendix C to Suspect No. 1, n. 4 supra, pp.469-70.
17. Opinion of Dr. Peter Speth, Suspect No.1, n. 5, supra, pp.404, 532. Concluding the round hole was likely drilled to insert a shunt matched a 1908 experiment performed by a protégé of Dr. Carrel.

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## Lindbergh; Eugenics; Medical Evidence

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**Y1 A Test of a Newly Revised Age-at-Death Method for the Auricular Surface of the Ilium**

*Frankey Leggio\*, Louisiana State University, Bush, LA; Krista Bennett, MA, Louisiana State University, Baton Rouge, LA*

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**Learning Objective:** After attending this presentation, attendees will have a better understanding of the success rate of the age-at-death method developed and how the accuracy of the method is not dependent on an advanced anthropology degree.<sup>1</sup>

**Impact Statement:** This presentation will impact the forensic community by providing validation of the Bennett et al. newly revised method of adult age-at-death estimation using the auricular surface of the ilium.

This project presents a validation study of the aging method using the auricular surface.<sup>1</sup> The method is a newly revised method of the Buckberry and Chamberlain estimation method, with the addition of two traits.<sup>2</sup> Along with transverse organization, surface texture, microporosity, macroporosity, and apical change, retroauricular activity and bone quality were added, making seven scorable categories with a new set of composite scores with nine corresponding age phases using the auricular surface of the ilium.

In the original study, one investigator developed the method on the William Bass Donated Collection from the University of Tennessee consisting of 104 os coxae (50 females, 54 males) of known age. Additionally, three investigators (including the original investigator) with varying levels of experience and degrees (PhD with over eight years of casework, MA with over three years of casework, and PhD student) tested the method on 29 (8 females, 21 males) individuals from the Louisiana State University Forensic Anthropology and Computer Enhancement Services (LSU FACES) Laboratory Donated Skeletal Collection for repeatability.

In this study, the same 29 auricular surfaces from the LSU FACES Donated Skeletal Collection, consisting of left os coxae (right sides were used when taphonomic damage was present on the left), were scored using the newly revised method. This study resulted in a 75.86% accuracy rate. The rate was derived from 22 out of the 29 being accurately assigned to the correct phase age interval using the revised method. For the seven os coxae scored outside of their correct age phase, only two were outliers (over 10 years outside the correct range), with the other five only ranging slightly (less than 1-7 years) outside of the correct age range estimation. The accuracy rate of the original study was 79–93% derived from the scores of three investigators with advanced degrees.

The goal of this project was to validate the method created by Bennett et al. by scoring the same 29 auricular surfaces and having a similar accuracy rating as an undergraduate with prior knowledge of the auricular surface of the ilium and original scoring method (Buckberry and Chamberlain).<sup>1,2</sup> This study shows that the method developed by Bennett et al. has a high accuracy rate among those with or without advanced degrees and validates the method by proving success rate with the same donated skeletal collection.

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- <sup>2</sup> Buckberry, J.L., Chamberlain, A.T. Age estimation from the auricular surface of the ilium: A revised method. *American Journal of Physical Anthropology*. 2002;119(3): 231-239.

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**Age-at-Death; Methods; Auricular Surface**

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**Y2 Growth of the Juvenile Talus: A Pilot Study in Age-at-Death Estimation**

Rebecca Reid, MSc\*, University of Dundee, Dundee, Scotland, UNITED KINGDOM

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**Learning Objective:** After attending this presentation, attendees will understand how measurements of the juvenile talus can be utilized for age-at-death estimations.

**Impact Statement:** This presentation will impact the forensic science community by introducing a juvenile age estimation method using measurements from the talus.

Juvenile growth and its application in age-at-death estimation has been investigated in several regions of the skeleton.<sup>1-4</sup> However, there is a paucity of information relating to growth of the juvenile talus and its utility in juvenile age-at-death estimation. Therefore, the aim of this study was to investigate talus growth and evaluate the use of measurements of the juvenile talus for age-at-death estimation.

Data was collected from 27 documented tali ranging in age from 2–19 years from the Scheuer Collection.<sup>5</sup> The growth of three metrics of the talus (maximum width of the trochlea, body height, and maximum length of the talus) was assessed using polynomial regression to the fifth order. Measurements were adapted for juveniles from the adult literature, therefore intra-observer agreement using intraclass correlation coefficient (ICC) and technical error of measurement (TEM) were calculated.<sup>6,7</sup> Inverse regression was used to produce age-at-death estimation equations from metrics of the talus. The equations were tested on 11 undocumented tali specimens within the Scheuer Collection. The age estimates from the talus were compared to the age estimates from the diaphyseal length of the tibia.<sup>4</sup>

Measurements adapted for the juvenile talus demonstrated excellent intra-observer agreement and acceptable relative TEM levels. Growth of the talar body height and the width of the trochlea could be described as third-order polynomial regression. This regression appeared to demonstrate a period of rapid growth between 0–4 years old consistent with the infant growth spurt and a pubertal growth spurt after 10 years of age. Growth of the length of the talus, however, was expressed as a 2nd order polynomial with steady, consistent growth occurring between 2–19 years.

Statistically significant bilateral asymmetry was observed within body height following a Paired Samples T-Test. As a result, pooled and side-specific age-estimation equations were produced for body height. When the age-at-death estimation equations were tested for group differences using a Related-Samples Sign test, no statistical differences were observed between median age estimates derived from measurements of the talus and median age estimates from the diaphyseal length of the tibia. Additionally, statistically significant correlation between talus and tibia estimates were observed following comparison using Pearson's Correlation Coefficient and Spearman's correlation. This preliminarily indicates that the metric analysis of the talus is a viable option for juvenile age estimation. Further research is required to investigate population specificity and the impact of sex on growth of the talus.

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**Juvenile; Osteometrics; Age Estimation**

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**Y3 Accuracy of DNA Phenotyping Using Next Generation Sequencing**

*Paige Aymar, BA\*, Duquesne University, Pittsburgh, PA; Lisa Ludvico, PhD, Duquesne University, Pittsburgh, PA; Pamela Marshall, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Objective:** This research examines how accurate next generation sequencing instrumentation is at predicting visible external characteristics of a person. The instrumentation being used for this research is the MiSeq® FGx. The three physical features analyzed are hair, eye, and skin color. Skin color is based on biogeographical ancestry from 1,000 genome data while hair and eye color are analyzed using a HirisPlex model, all embedded in the instrument. This research aims to create statistics on the accuracy of prediction.

**Impact Statement:** Forensic science is continuously changing as the years progress. This presentation is essential to the forensic science community because it provides valuable information on the significance of next generation sequencing (massive parallel sequencing) and how useful it can be in creating a DNA profile that includes physical aspects of not only suspects but victims as well. If this new technique is going to be used in forensic labs for identification or convictions, the accuracy of its performance is crucial.

DNA phenotyping is a new and upcoming technique in forensic science that allows facial characteristics of a suspect to be created from DNA evidence left at a crime scene. DNA phenotyping is the science of predicting externally visible characteristics of a person from their DNA sample. By using next generation sequencing (NGS) technology, predictions of the physical characteristics of a person are made. NGS, also known as massive parallel sequencing, has the capability to sequence millions of DNA fragments at a time. Although NGS and DNA phenotyping are quickly advancing in the field of forensic science, there is little research that can be found on how precisely the instrumentation performs.

The question this study aims to resolve is how accurate this technology is at predicting these phenotypic traits. Verogen™, a forensic science company, has created an NGS instrument, MiSeq® FGx, that predicts hair color, eye color, and biogeographical ancestry (skin color) from a DNA sample. The MiSeq® FGx gives exact predictions for hair and eye color based on the HirisPlex model, but it is to be noted that skin color prediction is based on biogeographical ancestry from 1,000 genome data. Before the predictions are examined, the methodology for this research includes the following three main steps: library preparation, cluster generation and sequencing, and data analysis. Library preparation is lab-based and includes the extraction, quantification, and amplification of a DNA sample to prepare it for the MiSeq® FGx. Cluster generation and sequencing are performed by the instrument by setting up a run using the MiSeq® FGx Control Software. The final step, data analysis, examines the sample summary, which includes the samples STRs, SNPs, and phenotypic estimation using the ForenSeq™ Universal Analysis Software (UAS). This research includes a sample size of 12 ( $n=12$ ) to examine the validity of the Verogen™ instrument by demonstrating how accurate and precise the MiSeq® FGx is. The findings will suggest how reliable massive parallel sequencing is at predicting three human phenotypic traits. This research is crucial in aiding in the advancement of DNA profiles by allowing experts to know the significance or insignificance of next generation sequencing and DNA phenotyping during criminal investigations.

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**DNA Phenotyping; Next Generation Sequencing; Forensic Genetics**

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**Y4      Analysis Methods of Ephedra and Related Compounds in Web of Science (WOS) Between 1980-2022: A Systematic Review and Meta-Analysis**

*Nurdan Sandikci, MSc\*, Üsküdar University, Istanbul, Istanbul, TURKEY; Sevil Atasoy, PhD, United Nations International Narcotics Control Board, Vienna, AUSTRIA*

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**WITHDRAWN**

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**Y5 Analysis of the Inorganic Components of Eyeshadows by XRD and SEM-EDS**

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**Learning Objective:** After viewing this research, attendees will have an appreciation for the potential of XRD and SEM-EDS for analysis of cosmetics.

**Impact Statement:** This presentation will impact the forensic science community by providing accessible avenues for the instrumental analysis of the inorganic components of cosmetics, augmenting the more common approaches that focus on microscopical examination of particulates and instrumental analysis of organic constituents.

Cosmetics are those products that are applied to the body to enhance appearance. These substances have potential forensic utility due to their potential for transfer between individuals during close contact.

Significant research has already been performed on cosmetics, but that research has often focused on characterization of the organic components by FTIR spectroscopy, Raman spectroscopy, and chromatographic techniques.<sup>1-3</sup> In addition to having been exhaustively evaluated for forensic characterizability, organic components are also susceptible to chemical alteration by weathering and the wearer's biochemistry, which can limit the value of "out of the bottle" studies. Contrastingly, the inorganic components found in cosmetics, particularly mineral particles, are relatively stable over a relatively long time period.

Many inorganic cosmetic ingredients are shared with other forensic traces, such as paint and soil. In these contexts, these ingredients are familiar to forensic chemists. However, published research specific to the analysis of the inorganic components of cosmetics is very limited.

This study attempts to isolate the inorganic particulates in eyeshadow by both density separation and plasma ashing, followed by characterization of the purified material using X-ray diffraction (XRD) and microscopical examination of the particles, including scanning electron microscopy with electron dispersive x-ray spectroscopy (SEM-EDS). Plasma ashing, or plasma cleaning, uses gas or air plasma to remove contaminants and organic components from a sample. The plasma exposes the sample to high energy ions without vigorous heat and leaves only the inorganic components behind, without risking possible heat alteration of the mineral components. Both plasma ashing and density separation allowed for a simple isolation of the inorganics for further consideration. Powder-XRD analysis allowed for the evaluation of the chemical and structural nature of the crystalline components isolated from the sample, with the potential for further elemental characterization by EDS.

Eyeshadow palettes by two different manufacturers were compared. Within each palette, comparison of the diffractograms allowed for the classification of two color groups in each single eyeshadow palette. Further, the two manufacturers yielded classes of visually identifiable diffractograms.

These early results show the promise of XRD analysis for the comparison and characterization of eyeshadows. Further work will include multivariate analysis to determine the extent of the classifiability of these cosmetics and should include more manufacturers and additional types of powder cosmetics.

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**Cosmetics; XRD; SEM-EDS**

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**Y6 Biometrics From Human Hair Using Gas Chromatography-Isotope Ratio Mass Spectrometry of Ethyl Chloroformate Derivatives of Amino Acids**

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**Learning Objective:** After attending this presentation, attendees will understand the benefit of gas chromatography-isotope ratio mass spectrometry (GC-IRMS) over liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) for compound-specific isotope measurements, especially for amino acids found in human hair, nails, and fingerprints.

**Impact Statement:** Although almost no crime laboratory has access to IRMS instrumentation, federal research labs, like the DEA and FBI do, providing one of the only ways to distinguish the origins and history of organic matter that share identical chemistry. However, this proof-of-concept study demonstrates the potential power of IRMS measurements of human hair as an investigative lead in future applications.

If a body is badly decomposed or mutilated, or if the victim's DNA is not in a database, IRMS measurements of the victim's bone, teeth, hair, and nails can be used to determine geographic origins and travel history of the individual, providing an investigative lead to help resolve a crime. This project builds on previous work that demonstrates that compound-specific IRMS (CS-IRMS or CSIA) measurements of amino acids in human hair can also reveal information about an individual's lifestyle habits and traits, like their extent of sun exposure and their biological sex.

CSIA can be accomplished with either LC or GC as the mode of separation before the IRMS analysis. For amino acids found in human hair and nails, derivatization is necessary before GC-IRMS analysis, but not before LC-IRMS analysis. Here, we employ ethyl chloroformate (ECF) as a derivatizing agent for GC-IRMS, which adds an ethoxycarbonyl group to free amines and an ethyl ester to free carboxylic acid groups. The addition of exogenous carbons from the ethyl chloroformate increases the volatility of the derivatives and improves their chromatographic separation. Although derivatization complicates the isotope measurements because of the added carbons from the derivatizing agent, the GC-IRMS instrument is generally more rugged and reliable than its LC-IRMS counterpart.

Previous work analyzed hair samples from 82 donors around the United States using LC-IRMS. LC-IRMS had limited peak capacity, so the analytical method was only able to baseline resolve 9 amino acids. In the prior work, methionine/cystine and isoleucine/leucine were included as coeluting pairs, creating a total of 11 variables for multivariate analysis. Using the newly adapted ECF derivatization procedure, at least 14 amino acids can be baseline resolved. This variable increase incorporates glutamic acid/glutamine, alanine, and proline, which were previously not included. Asparagine and glutamine typically undergo deamidation, during which the amide is removed or converted to another functional group to produce aspartic acid and glutamic acid, respectively. The ECF derivatization method allows both products to be identified in a proportion of ~30%:70% amine:acid, which still enables isotopic analysis of the original glutamine and asparagine residues. In short, GC-IRMS provides many more variables for analysis than the previous LC-IRMS method.

By using ECF derivatives, we can expand upon previous work by increasing the number of variables for multivariate statistical analysis and increasing the sample size to more than 100 donors. This new data provides increased confidence in the determination of biometric data from human hair, including alcohol consumption and age determination, thus furthering the potential of isotope ratio analysis.

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**Amino Acids; GC-IRMS; Hair**



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**Y7 Detection of Ignitable Liquid Residues Using Crime-lite® AUTO**

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**Learning Objective:** This research evaluates the ability to detect ignitable liquid residues on burned substrates using photography with a commercially available imaging system that consists of a combination of alternative wavelengths of light and filters. Attendees will gain appreciation for the challenges of fire investigation and the potential of new technology for the detection of ignitable liquid residues at fire scenes.

**Impact Statement:** This presentation will impact the forensic science community, and specifically fire investigators, by determining the benefits and limitations of an imaging system for the visualization of potential ignitable liquid residues at fire scenes.

It is often difficult for fire investigators to determine if a fire was intentionally set due to challenges such as destruction or rearrangement of the scene during extinguishing efforts. If the use of ignitable liquids is suspected, it may also be difficult for investigators to locate optimum areas for sample collection. Therefore, there is a need for accurate and efficient methods for the visualization of potential ignitable liquid residues at fire scenes.

The Crime-lite® AUTO by Foster + Freeman Ltd. combines the abilities of UV, visible, and infrared light with long pass and band pass filters to visualize and photograph ignitable liquids. Foster + Freeman reports their successful use of the Crime-lite® AUTO to detect several ignitable liquids on various substrates.<sup>1</sup> It is important to note that their tested substrates did not have fire damage prior to being photographed, and there is no information available for the volume of ignitable liquids applied to each substrate. The present research intends to further investigate the use of the Crime-lite® AUTO to visualize and photograph potential ignitable liquid residues in situations simulating those commonly encountered at residential fire scenes. Specifically, the ability to visualize and photograph gasoline, diesel, charcoal lighter fluid, and turpentine on carpet, laminate flooring, hardwood, and oriented strand board was investigated.

In the present research, one cup (237mL) of ignitable liquid was poured over the surface of the substrate and allowed to saturate for one minute. The substrate was ignited with an open flame, allowed to burn for six minutes, and extinguished with one cup (237mL) of water. Immediately upon extinguishment, the burned substrate was photographed in an otherwise dark environment with the Crime-lite® AUTO using all possible combinations of light source and filter available on the instrument. Areas of the burned substrates that gave presumptive positive and negative visualization results were cut and stored in metal, friction-seal paint cans prior to laboratory analysis for the presence of ignitable liquids. All laboratory testing was performed in accordance with published, consensus-based standards using passive headspace concentration onto activated charcoal strips, followed by elution with carbon disulfide, and subsequent analysis by gas chromatography/mass spectrometry (GC/MS).

Regardless of the results of the visualization with the Crime-lite® AUTO, the GC/MS results showed that all the burned substrates in this study were confirmed positive for the presence of ignitable liquid residues. This outcome is likely attributed to the volume of ignitable liquid applied prior to burning, along with the sensitivity of the GC/MS method. However, this study still provides important information regarding the utility of the Crime-lite® AUTO in fire investigations. Specifically, each sample was visualized as presumptive positive under at least one light source and filter combination with the Crime-lite® AUTO, then confirmed positive by GC/MS, thereby indicating that the Crime-lite® AUTO could be a valuable tool in the search for potential ignitable liquid residues. Additionally, samples that were visualized as presumptive negative by the Crime-lite® AUTO but gave positive GC/MS results demonstrate that the presence of heavy charring has an important deleterious effect on fluorescence. Furthermore, when investigating the visualization of the same ignitable liquid on different substrates, there was minimal overlap with respect to which light source and filter combination provided a positive result. This outcome demonstrates that substrate composition has an effect on the visualization of fluorescence. However, when investigating the visualization of different ignitable liquids on the same substrate material, there was greater overlap with respect to which light source and filter combination provided a positive result. This outcome suggests the Crime-lite® AUTO can visualize a broader range of ignitable liquids on some substrate compositions.

**Reference:**

- <sup>1</sup> Foster + Freeman. Use of Crime-lites® for the visualization of Ignitable Liquids in Fire & Arson Investigations. Foster + Freeman Crime-lite® Applications, October 2021.

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**Ignitable Liquid Residue; Crime-lite® AUTO; Fire Investigation**

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**Y8     The Use of Portable Capillary Liquid Chromatography With Dual Silica Hydride Stationary Phases and Dual Ultraviolet Wavelength Detection for the Analysis of Synthetic Cathinone Positional Isomers**

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**Learning Objective:** After attending this presentation, attendees will understand the utility of dual capillary multi-modal liquid chromatography (LC) with dual ultraviolet (UV) wavelength detection for the analysis of emerging drugs, as modeled by the separation of synthetic cathinone positional isomers.

**Impact Statement:** This presentation will impact the forensic science community by providing methodology applicable to both remote and in-lab testing for the analysis of positional isomers of emerging drugs on a portable capillary LC instrument with enhanced orthogonality due to the utility of silica hydride stationary phases. The proposed technique should follow the minimum standards set by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) while being eco-friendly and low-cost.

Raman and infrared (IR) spectroscopy, ion mobility spectrometry (IMS), and gas chromatography/mass spectrometry (GC/MS) are commonly employed for the identification of seized drugs. However, Raman and IR can be problematic for the analysis of mixtures. Ion mobility spectrometry lacks specificity as the separation of ions is dependent on its mobility in a buffer gas. Gas chromatography/mass spectrometry is unable to distinguish between certain positional isomers and diastereomers due to overlapping retention times and identical mass spectra. Gas chromatography with flame ionization detection provides only retention time information. Many isomeric forms of emerging drugs exist, making identification of these drugs difficult for forensic laboratories. In contrast, LC-ultraviolet (UV) separations are amenable to mixtures, can offer increased resolving power over IMS, and increased or complementary detection specificity versus GC detection techniques. In contrast to GC/MS, LC separations can also distinguish between certain positional isomers and diastereomers.

Portable analysis devices allow for expanded testing capabilities in both the laboratory and in the field. These portable devices are useful for the analysis of seized drugs due to their screening and identification purposes. They can provide a quicker, on-site analysis, and by working in conjunction with benchtop systems, can promote efficiency and alleviate backlogs. The use of a low-cost, green, and low-footprint portable capillary LC device occupies 1/5 the bench space and utilizes 1/1,000 the solvent.

The present study employs a portable capillary LC with dual capillary columns in series and dual UV wavelength detection. Stationary phases are utilized that are operable under both complementary reverse phase (RP) and aqueous normal phase (ANP) modes on the same columns using the same solvent reservoir. The dual retention capabilities provide five pairs of relative retention time data. When the mobile phase changes from RP to ANP, the absorbance peaks blueshift to shorter wavelengths and results in two dual UV absorbance ratios providing discriminate detection. Seven synthetic cathinone positional isomers differing in aliphatic and aromatic substitutions are uniquely identified by relative retention times and peak area ratios proportional to absorbance ratios. Based on the results, the relatively low-cost methodology, which offers a high degree of specificity, is valuable for drug analysis, specifically for emerging drugs with isomeric forms.

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**Portable Capillary LC; Synthetic Cathinone Positional Isomers; Dual Mode Separations**

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**Y9      Assessing Genetic Diversity Between Wild and Captive Turkeys (*Meleagris Gallopavo*) in Pennsylvania**

*Savannah Nguyen, BA\*, Duquesne University, Pittsburgh, PA; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA; Lisa Ludvico, PhD, Duquesne University, Pittsburgh, PA*

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**WITHDRAWN**

## **Y10 Comparative Extraction and Identification of Illicit Compounds from Baked Edibles Using Paper Spray Ionization–Tandem Mass Spectrometry and Liquid Chromatography/Triple Quadrupole/Mass Spectrometry**

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**Learning Objective:** Attendees will learn about the applications of paper spray ionization-tandem mass spectrometry and liquid chromatography/triple quadrupole/mass spectrometry in the analysis of baked edibles containing illicit stimulants. Attendees will also learn about the adaptability of the QuEChERS solid phase extraction method in extracting a stimulant from a complex baked edible matrix.

**Impact Statement:** The use of the QuEChERS extraction coupled with paper spray ionization-tandem mass spectrometry and liquid chromatography/triple quadrupole/mass spectrometry will advance the field of drug chemistry because it is an optimized and rapid extraction and detection method for illicit substances in edibles.

Complex baked edible matrices have become more common methods of consumption for illicit substances like MDMA, methamphetamine, and amphetamine, but there is a lack of research regarding the rapid detection of drugs contained in baked edibles.<sup>1,2</sup> The existing research for extracting substances from edibles focuses on cannabis, and the developed extraction methods for this application are very complex and can require multiple extractions to obtain a prepared sample for detection.

To fill this gap in the literature, the QuEChERS (Quick Easy Cheap Effective Rugged Safe) dispersive solid phase extraction method was coupled with paper spray ionization-tandem mass spectrometry (PSI-MS) and liquid chromatography/triple quadrupole/mass spectrometry (LC-QqQ-MS). The QuEChERS method, although not typically used for baked edibles, isolated the illicit compound from a complex matrix to allow for accurate detection of the compound.<sup>3</sup> PSI-MS is a recent ambient ionization method that has produced accurate quantitative and qualitative data for drug detection studies, and it was compared to LC/QqQ/MS, which is the gold standard for drug detection studies.<sup>4,5</sup>

To accomplish this method, cookie dough was spiked with varying amounts of phenethylamine, which is a structural precursor to the stimulants mentioned above. The edibles were baked, and the phenethylamine in each edible was extracted using the QuEChERS method. Several trials were performed to optimize the ratio of acetonitrile, magnesium sulfate, sodium chloride, and lipid sorbent used in the QuEChERS method.<sup>3</sup> For PSI-MS analysis, the QuEChERS supernatants were analyzed using the Thermo Scientific™ LTQ-XL Linear Ion Trap Mass Spectrometer for the presence of phenethylamine, which was confirmed through collision induced dissociation. The same optimized QuEChERS method with an additional filtration step was used to obtain supernatants for LC-QqQ-MS analysis. The Agilent® 1200 Series LC Stack and Agilent® 6460 Triple Quadrupole Mass Spectrometer were used to detect phenethylamine in each sample using an optimized LC/QqQ/MS method.

Preliminary results presented at the AAFFS 2022 conference showed that the QuEChERS-PSI-MS method was optimized to successfully detect phenethylamine in each edible sample. These results were then directly compared to results obtained using the QuEChERS-LC/QqQ/MS method, and PSI-MS and LC/QqQ/MS were evaluated on sensitivity of detection and time-effectiveness. Because these studies were completed using a model phenethylamine compound, future studies can apply these methods to edibles spiked with illicit substances such as MDMA.

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- Liquid Chromatography - Mass Spectrometry (MC-MS-MS). <https://www.eag.com/techniques/mass-spec/lc-ms-ms/> (accessed November 24).

### **Mass Spectrometry; QuEChERS; Edibles**

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**Y11 Development of a Conserved Semenogelin I and II Epitope for Semen Identification with Respect to Allelic Variation**

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**Learning Objective:** Attendees will learn about the RSID-semen antibody-based assay and its techniques for semen identification. The presentation will discuss the effectiveness of the current epitope in the assay and the effectiveness of a hypothesized optimal epitope.

**Impact Statement:** This presentation identifies a peptide fragment that may be the optimal epitope for a semen identification antibody-based assay. This epitope was analyzed against the currently used RSID-semen assay for false negative rates due to allelic variation. The newly identified epitope may result in less false negatives for semen and would have a greater yield in seminal fluid evidence recovery.

Seminal fluid is a source of biological evidence used for forensic DNA analysis. Semen identification may be difficult in many instances, such as sexual assault cases. Preservation of semen is critical in order to perform DNA analysis, a necessity to positively identify a perpetrator. The highly abundant semenogelin proteins are used as a marker in the Rapid Stain Identification Series (RSID) -semen assays. Genetic variation among the semenogelin proteins may cause false negatives in the RSID-semen assay. There are currently no readily available studies analyzing the effects of allelic variation on RSID-semen assays. Therefore, semenogelin I (SEMG1) and semenogelin II (SEMG2) known allelic variants were compiled and computationally analyzed. This was done using publicly available databases of human genome allelic variants. The data was used to identify an optimal peptide fragment (LJG fragment) that was conserved in both SEMG1 and SEMG2, with little known variation. The seminal plasma motility inhibitor fragment (SPMI) is believed to be the current epitope of the RSID-semen assay. SPMI was analyzed for known allelic variants with the LJG fragment. A statistical analysis was done to determine the frequency of allelic variants and compare the effectiveness of semenogelin identification in both peptides. The LJG fragment was cloned and expressed in an *E. coli* system by fusing the desired fragment with maltose-binding protein (MBP) on the amino terminus and a hexahistidine tag on the carboxy terminus. Dual Affinity chromatography was used to purify the expressed LJG fragment. The fragment was then prepared for antibody production of the ideal epitope for SEMG1 and SEMG2 identification.

This study showed a more reliable epitope can be produced for the RSID-semen assays and recommends new antigens be produced for SEMG1 and SEMG2 detection. Application of this epitope may increase the accuracy of the RSID-semen assay, improving seminal fluid identification techniques.

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**Semen Identification; Allelic Variation; Evidence Recovery**

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**Y12 eDNA Detection of *Lycorma Delicatula* Using Multiple Types of Forensic Swabs Via STR Analysis**

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**Learning Objective:** Attendees will learn what spotted lanternflies (*Lycorma delicatula*) are, what they are doing to the environment in the United States, and how forensics can be used to proactively detect and manage this invasive insect species.

**Impact Statement:** This presentation will demonstrate the utility of forensic science methods in invasive species management applications. This will further the development of the field of environmental forensics and assist in efforts to stop an invasive insect that is damaging plant life across the United States.

The purpose of this study is to determine the most effective forensic swab for proactively detecting the presence of the invasive spotted lanternfly, *Lycorma delicatula*, early into its invasion of non-native regions so that preventative actions can be taken to stop it from damaging trees and crops across the United States. This objective was addressed by utilizing short tandem repeat (STR) analysis to identify the insect's DNA in environmental samples. STR analysis was applied to this research because of its capacity to identify species-specific DNA in multi-source DNA samples.

It was previously established that *L. delicatula* produces an excretory fluid that contains DNA, which can be collected from tree and leaf surfaces in areas where the insects feed.<sup>1</sup> Environmental DNA (eDNA) was collected from bushes around the campus of Duquesne University in Pittsburgh, PA, using three types of forensic swabs: cotton swabs, Whatman® OmniSwabs, and CytoSoft™ Cytology brushes, which were compared based on their ability to collect intact *L. delicatula* DNA within field-collected eDNA to allow for accurate and early detection of the insect. Cotton swabs were tested using the dry swab technique and the wet swab technique. Bushes growing on the campus were targeted because their woody branches and thin bark are the preferred type of food for *L. delicatula* nymphs until they become strong enough to feed on trees. Wet cotton swabs collect DNA by releasing dried genetic material from substrates using water and absorbing the wet material into the cotton. The other swab types and techniques involved a dry swab collecting dried genetic material.

It was predicted that wet cotton swabs would detect the presence of *L. delicatula* more effectively and earlier than the other swab types and techniques. Reference DNA was also extracted from *L. delicatula* individuals for comparison to eDNA extracted from swabs. DNA was amplified with species-specific primers targeting a 63 bp STR in *L. delicatula* DNA. Amplicons were visualized using gel electrophoresis to confirm the amplification of the target region. Next, the target STR will be detected through capillary electrophoresis (CE) and swab data will be compared to reference data to confirm or deny the presence of *L. delicatula* DNA in the eDNA samples.

Quantitation data obtained thus far show that all swab types can collect eDNA and no single swab type or technique consistently collects more DNA than the others. DNA analysis is still being performed and will be completed by May of 2023. This study integrates the fields of environmental science and forensic science by using DNA collection tools from forensics to perform eDNA analysis methods common in environmental research. The resultant contribution to environmental forensics strengthens the connection between invasive species management and the legal science of forensics. This study is an addition to the growing catalog of research on the invasive spotted lanternfly, and it highlights the need for stronger legal action and management to control invasive species.

**Reference:**

- <sup>1</sup>. Valentin, R. E., Fonseca, D. M., Gable, S., Kyle, K. E., Hamilton, G. C., Nielsen, A. L., & Lockwood, J. L. (2020). Moving eDNA surveys onto land: Strategies for active eDNA aggregation to detect invasive forest insects. *Molecular ecology resources*, 20(3). doi:10.1111/1755-0998.13151.

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**Environmental DNA (eDNA); Environmental Forensics; *Lycorma delicatula***

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**Y13 Examining Differences in Exonerations Across the United States**

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**Learning Objective:** After reviewing this presentation, attendees will have a better understanding of exonerations and how they differ throughout the United States based on factors such as crime type, location, availability of DNA, etc. Attendees will also gain an understanding of how innocence groups can affect the potential correction of a wrongful conviction.

**Impact Statement:** This research will provide a representation of exoneration trends across the United States and allow a better understanding of how innocence groups can aid in correcting wrongful convictions. Data regarding exonerations is important for identifying and correcting previous mistakes, as well as improving conviction integrity in the future.

Since the National Registry of Exonerations (NRE) began methodically tracking exonerations in 1989, there have been 3,249 recorded exonerations.<sup>1</sup> Previous literature regarding exonerations focuses primarily on the causes or contributing factors to wrongful convictions. These factors, as recognized by the NRE, include false or misleading forensic science, inadequate legal defense, perjury/false accusation, official misconduct, false confession, and mistaken witness identification.

A prior study by Dr. Gerald LaPorte examined the role of forensic science in DNA exonerations.<sup>2</sup> The main analyses included the relationship between false or misleading forensic science and the other five factors, as well as the commonly associated forensic methods with wrongful convictions. The first part of this study replicated this work using an updated list of exonerations by the NRE. Analyses were replicated with both DNA and non-DNA exonerations. Results from the replication of DNA exonerations show that the trends continue with the newer set of data, in that false or misleading forensic science is most often associated with mistaken witness identification. In non-DNA exonerations, results showed that false or misleading forensic science is most often seen alongside perjury/false accusation.

The second part of this study examined the role of innocence groups and the trends associated with their involvement in correcting erroneous convictions. Innocence groups, such as the Innocence Project, Centurion, and conviction integrity units (CIUs) are becoming more prominent across the United States. These groups are credited with a portion of those 3,249 exonerations due to legal and/or investigative support provided to wrongfully convicted individuals. Despite their success, there is limited research regarding the effect of innocence groups on exonerations. Specifically, part two of this research investigated how factors such as crime type, location, race, the availability of DNA evidence, etc. influence the involvement of innocence groups. Initial results show that innocence groups are involved in both DNA and non-DNA exonerations, with the majority of success in homicide and sexual assault crimes. The results of this research will provide a representation of exoneration trends and allow a better understanding of how innocence groups can aid in correcting wrongful convictions.

**References:**

- <sup>1</sup>. National Registry of Exonerations, <http://www.law.umich.edu/special/exoneration/Pages/detailist.aspx>.
- <sup>2</sup>. Laporte, Gerald. "Wrongful convictions and DNA exonerations: Understanding the role of forensic science." *National Institute of Justice Journal* 279 (2018): 1-16.

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**Exoneration; Innocence Projects; Conviction Integrity**



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**Y14 Identification of Race Through Chemical Analysis of Latent Fingerprints**

*Brynn Myers, BS\*, University of Central Oklahoma, Edmond, OK; Mark McCoy, EdD, University of Central Oklahoma, Edmond, OK; Caitlin Porterfield, MS, Forensic Science Institute - University of Central Oklahoma, Edmond, OK*

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**Learning Objective:** Attendees will learn about the potential to determine race from latent fingerprint residues using mass spectrometry.

**Impact Statement:** This is a new development that will impact the forensic science community. As characteristics of individuals may aid in identification, and this type of analysis has already been able to determine other types of characteristics, this is the next step.

The permanence and uniqueness of fingerprints and the analysis of physical characteristics associated with fingerprints have allowed for identification of individuals in forensic investigations. However, there is additional information that may be obtained from latent fingerprints. Fingerprint residues are comprised of chemical components—both those that are created and secreted from the body naturally (endogenous) and those from products outside the body that are ingested or applied on the skin (exogenous)—that have previously been used by researchers to identify an individual's age, gender, and even lifestyle. This information may be useful for the inclusion and exclusion of suspects when physical fingerprint characteristics may be unidentifiable or as a means of adding additional information to preexisting physical characteristic analyses. However, there is currently no literature regarding the ability to identify an individual's race through analysis of latent fingerprint residues.

The purpose of this study is to identify a pattern, if any, that may allow investigators to identify an individual's race through the analysis of latent fingerprint residues using mass spectrometry as a medium for analysis of the sweat, oil, and other residues deposited in an individual's fingerprint. A sample of 20 male and 20 female individuals who self-identify in one of five racial backgrounds (Hispanic, Asian, European, African, and European) were asked to deposit a single right index print onto a piece of Mylar® film. Following collection of prints, a portion of the sample was cut from the Mylar® film, digested, and analyzed via mass spectrometry instrumental analysis. The procedure followed during the experimentation—both sample collection and preparation as well as instrumental analysis—were subject to many changes during experimentation and preparation to best aid in this new type of identification. Preliminary results suggest that a pattern may exist that will allow for the identification of a person's racial background.

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**Fingerprints; Chemistry; Mass Spectrometry**

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**Y15 Revisiting the CSI Effect: Has the Popularity of Crime Media Changed Anything?**

*Elizabeth Diltz, BA\*, Duquesne University, Pittsburgh, PA; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA; Bobbi Jo Wagner, JD, Administrative Offices of the Pennsylvania Courts, Glenshaw, PA; Sara Walker, MSFS, Intermountain Forensics, Murray, UT*

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**Learning Objective:** After attending this presentation, attendees will understand more about the CSI Effect, including the history of the phrase, and they will also see if different crime media has any effect on prior research conclusions regarding the existence of the CSI Effect. Additionally, attendees will learn more about the impact the CSI Effect has on criminal justice practitioners.

**Impact Statement:** The results of this research will help the forensic science community understand the impact of crime media on juror decision making. These results will also provide information about criminal justice practitioners' experiences related to the CSI Effect and the possible impact that each type of practitioner experiences.

**CSI:** *Crime Scene Investigation*, *NCIS*, *Forensic Files*, *48 Hours*—these are all television programs with a focus on forensic science and crime investigation. While two of these shows (*Forensic Files* and *48 Hours*) focus on real crime stories, *CSI* and *NCIS* are dramatizations of the world of forensic investigations that have led to the idea of a phenomenon known as the CSI Effect. This phenomenon is commonly defined as jurors being influenced by shows like *CSI* and the portrayal of forensics leading to more acquittals when evidence is lacking. While most of the previous research done into the CSI Effect has shown that there is no support for this idea, there has been little research into the examination of crime media subgenres having different influences on jurors' evidence expectations. Additionally, little research has been done that investigates how the CSI Effect impacts criminal justice practitioners.

This two-part study examined both how crime drama watching versus true crime watching impacted jurors' evidence expectations/verdict decision making as well as how criminal justice practitioners are impacted by the CSI Effect. Part one involved using survey software to compare the hours spent watching crime media of both subgenres, evidence expectations of participants, and verdicts in crime scenarios; this method was modeled after previous research done by the Honorable Donald Shelton.<sup>1</sup> It was hypothesized that there would be no significant difference between those that watch more true crime media over those that watch crime dramas in verdict decision-making similar to the results from the earlier study. Preliminary results of part one line up with the results that were observed in the research done by the Honorable Donald Shelton. Part two involved conducting interviews with lawyers, forensic scientists, and law enforcement officers/crime scene investigators to determine if they experience the CSI Effect, believe in its existence, and if they have any personal stories dealing with the CSI Effect. It was hypothesized that the criminal justice practitioners would say they have experienced the CSI Effect and provide anecdotal evidence to support the CSI Effect. Preliminary results from the interviews reveal that most believe the CSI Effect does exist, but only a few gave anecdotes about their personal experience with it.

The results of this research will help the forensic science community understand the impact of crime media on juror decision making. These results will also provide information about criminal justice practitioners' experiences related to the CSI Effect and the possible impact that each type of practitioner experiences.

**Reference:**

- <sup>1</sup> Shelton, D. E.; Kim, Y. S.; Barak, G. A Study of Juror Expectations and Demands concerning Scientific Evidence: Does the CSI Effect Exist. *Vanderbilt Journal of Entertainment and Technology Law* 2006, 9, 331-368.

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**CSI Effect; Evidence Expectations; Jurors**

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**Y16 Streamlining the Extraction and Quantification of Synthetic Cathinone in Oral Fluid by Means of Solid-Phase Extraction (SPE) and LC/MS/MS Analysis**

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**Learning Objective:** After attending this presentation, attendees will understand the challenges approached while developing and validating the streamlined extraction and quantification methodology of synthetic cathinone in alternative matrices.

**Impact Statement:** This presentation will impact the forensic science community by improving the workflow for substance detection in oral fluid using cathinone as the model substance.

Synthetic cathinone, often referred to as “bath salts,” is characterized as the beta-ketone isomer of amphetamine in a more potent formulation while avoiding several legislative roadblocks with constant structural modifications (i.e., addition of a methyl substituent).

Prior studies into the intoxicating effects of cathinone usage point to similar behaviors exhibited by amphetamine users such as exemplified feelings of euphoria. It is of forensic interest to develop a streamlined methodology for the analysis of substances as there is a major lack in quantification standards and to benefit public health.

The research focused on five substances: 3',4'-Methylenedioxy- $\alpha$ -pyrrolidinobutylphenone (3,4-MDPBP), 2'-Methyl- $\alpha$ -pyrrolidinopropylphenone (2-MePPP), 3-Chlorocathinone (3-CC), 2-fluoroisocathinone (2-FIC), and 5-Chloro-2-thiothione (5-Cl-bk-MPA). Methods were developed using an Agilent® 1200 Rapid Resolution LC with tandem 6460 Triple Quadrupole in the positive ESI mode. MRM transitions were developed through the usage of scan and product ion scan modes. Next, specimens were extracted from synthetic oral fluid using a previously validated method for 729 different NPS, including bath salts using Clean Screen® DAU (200mg; 10mL) cartridges. Triplicated samples were pre-treated either using a crash-and-shoot with cold acetonitrile (-20°C) or the addition of pH 9.0 borate buffer to 0.5ml sample. The goal was to minimize the steps and solvents while not affecting the integrity of oral fluid samples.

Preliminary results from the development of mass transitions yielded feasible data for the downstream progression of all substances, with exception to 5-Cl-bk-MPA, as there still exist ambiguities between sample and blank acetonitrile. Development of a streamlined extraction is still in the process and to be completed in two stages: unaltered from the literature and limiting volumes and/or steps. With this, it is proposed to minimize the pre-treatment steps down to a simple centrifugation with buffer prior to loading to cartridge. Future considerations for analysis closely correspond with the minimization of oral fluid needed to acquire clear, reproducible results in a roadside collection scenario.

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**Synthetic Cathinone; Method Development; Novel Psychoactive Substances**

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**Y17 The Development of a Central Mammalian Fur Database**

*Hailey Adamik, BS\*, Trafford, Pennsylvania*

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**Learning Objective:** This presentation will encompass how a central and accessible mammalian fur database can aid the wildlife forensics community. In addition, it focuses on the various factors that must be considered, including a hair preservation method, the age/sex of the individual, seasonality, and so on.

**Impact Statement:** The wildlife forensic community currently lacks an extensive, widely accessible, central mammalian fur database. Such a database would impact the forensic science community by providing forensic hair examiners with consistent references for aiding in wildlife crimes such as poaching and illegal wildlife trafficking.

Wildlife forensics utilizes techniques and methodologies employed in human forensic science, focusing on crimes against animals, including poaching and trafficking endangered species. Human forensic databases exist for DNA, fingerprints, hair, and spent bullet casings, to name a few. They are essential for reference and identification purposes, as they link the perpetrator to the crime. Although some wildlife databases exist, a large gap remains in the forensic wildlife community. One such gap is a comprehensive and fully accessible digital mammalian fur database.

Knowledge of animal hair morphology is necessary for wildlife crimes, as well as domestic animal abuse, meat adulteration cases, and crime scene investigations. Mammalian species may vary morphologically in fur characteristics regarding age, region, and intraspecific variation. The existing atlases describing mammalian pelage are limited in that many are non-inclusive, non-digital, and not freely or readily available. As a result, the development of a single, digitalized database of mammalian hair would greatly benefit the field of wildlife forensics, as well as other fields of study.

The macroscopic and microscopic analysis of guard hairs using Scanning Electron Microscopy (SEM) and compound microscopes can distinguish morphological variations to assist in species identification. These techniques can provide documentation and visual references regarding cuticle scale pattern, medullary type, pigmentation, and basic form and size of individual mammalian hairs. Resulting data shows that the cuticle patterns of hairs viewed under the SEM differ morphologically among taxonomic families, while combinations of other characteristics, such as medulla type and cross-section shape, aid in identification on the species level. Although DNA plays an immense role in species and individual identification, hair morphology remains an important technique due to cost-efficiency and reproducibility. The implications of a singular, widely accessible fur database include providing forensic hair examiners with consistent references across the globe and allowing for the continuation of future research to aid in wildlife investigations.

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**Wildlife Forensics; Mammalian Hair; Digital Database**

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**Y18 Visual Diagnostics for Cartridge Case Impression Evidence**

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**Learning Objective:** After attending this presentation, attendees will be informed about alternative techniques for visualizing cartridge case impression evidence using computer graphics.

**Impact Statement:** This presentation will be useful primarily for firearm and toolmark examiners and researchers. We provide alternative methods for visualizing and comparing cartridge case impression evidence other than a standard comparison microscope. Our visual diagnostics are also useful for explaining automatic cartridge case comparison algorithms.

Firearm and toolmark examiners commonly use a comparison microscope to assess the similarity between known-source and questioned ballistic evidence. Evidence is placed on stages beneath two compound microscopes that are joined via an optical bridge to allow side-by-side viewing through a single eyepiece. Analyzing evidence in this manner requires the examiner to consider factors such as lighting and the positioning of the evidence under the microscope. Changing these conditions may lead to drastically different visualizations of the evidence and may affect an examiner's analysis.

With recent advances in 3D topographical scanning technology, we can obtain lighting- and position-agnostic measurements of a cartridge case's surface. We digitally capture these measurements in a two-dimensional array called a "surface matrix" in which each element contains a numerical value representing the relative height of the cartridge case surface at a specific location. A surface matrix is therefore a precise, high-resolution representation of a cartridge case surface. Using this digital representation, we introduce a suite of visual and interactive diagnostic tools that are useful for comparing cartridge case impression evidence. In particular, they help diagnose potential issues at each stage of an automatic cartridge case comparison pipeline. For example, these visual diagnostics aid in uncovering similarities and differences in the impressions on two cartridge case breech faces. We show how these visual diagnostics help in explaining the behavior of an automatic cartridge case comparison algorithm. We also implement these visual diagnostics in a web application called *cartridgeInvestigatR* for comparing cartridge case surface matrices. The *cartridgeInvestigatR* interactive web application allows those without programming expertise to interact with and understand algorithms to automatically process and compare cartridge case scans. The visual diagnostics and *cartridgeInvestigatR* application are available to the forensic practitioner and researcher communities as free, open-source software so that the explainability and transparency of forensic comparison techniques can continue to improve.

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**Firearms and Toolmarks; Cartridge Case Comparison; Visual Diagnostics**

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**Y19 America's Court System Through the Lens of a Pandemic: The Past, Present, and Future Adaptations**

*Julianna Firek, BA\*, New Kensington, PA; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA*

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**Learning Objective:** After attending this presentation, attendees will better understand how the court system has adapted and responded to the evolving COVID-19 pandemic.

**Impact Statement:** This presentation impacts the forensic science community by documenting the changes that were made to the judicial system during and throughout the COVID-19 pandemic that affect court operations and testimony procedures.

In March of 2020, the courts shut down, along with the rest of the world. Throughout the course of the pandemic, things were expected to return to “normal” in a year. Now, two years later, we are still facing new variants and high transmission rates like we did during the peak of the pandemic. Initially, after a month of shutdowns and cases being put on pause, the courts decided to continue by any means possible. This was met with various solutions by different courts in response to the constantly changing nature of the pandemic. These changes were highly unprecedented and were being made uncharacteristically fast. Within the court system, prior to the pandemic, the court was extremely slow to make any change. However, the pandemic forced many quick changes along with significant investments into new courtroom technology.

This research aimed to analyze and compare the first-hand accounts of the adaptations that courts made during the pandemic. Interviews were conducted during the summer of 2021 and throughout 2022 with various professionals within the court system—at the county criminal courts—to gather information on their experiences during the pandemic. Interviews were conducted across different jurisdictions to compare the changes each court made. The initial rounds of interviews focused on the advantages and disadvantages of the changes that were made during COVID-19. After two years of shutdowns and changes, the interviews conducted this year were reflective of how the adaptations have progressed and what the “new normal” is beginning to look like. Additionally, this research captures how the literature and news has reported about the court system’s pandemic changes with time. The research has a unique perspective as it began early into the pandemic and is continued into a time in which everyone expected the pandemic to be over. However, new variants and higher infection rates are still prevailing, which causes the courts to continue to respond to change.

The technology that was utilized by some courts broadened the capabilities for a hybrid court proceeding and allowed for optional remote testimony for expert witnesses. Documenting the timeline of COVID-19 and the adaptations that were made is important for future analysis of this unprecedented time. The initial concern of the courts was the safety of the public and of their employees. As the shutdown period progressed and already lengthy backlogs continued to grow, the priority was shifted to combatting this backlog of cases. This research provides a retrospective analysis of courtroom operations that shifted to fully virtual proceedings up through re-opening efforts and hybrid models. The initial round of interviews during Summer of 2021 included negative perceptions of the changes made due to COVID-19. The 2022 interviews include more positive perceptions of these changes. At the forefront of all these changes should be equal access to justice and a fair and speedy public trial for every person. This research focuses on what that type of trial looks like in the light of COVID-19 adaptations.

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**COVID-19; Virtual; Court**

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**Y20 Understanding the Use of Forensic Evidence at Trial: A Case Study of Criminal Trials in Ohio**

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**Learning Objective:** This presentation will provide insight into how forensic evidence is used in trials. Attendees will have a better understanding of how seldom-available forensic evidence collected from a crime scene is analyzed for use at trial.

**Impact Statement:** This presentation will highlight the unrealized potential for forensic evidence to improve the American criminal justice system. Drawing on a novel case study of 55 criminal trial transcripts, we considered how ten different types of forensic evidence were presented to juries. Our findings show that juries are frequently advised of the presence of forensic evidence at a crime scene. However, they are seldom presented with any laboratory analysis of such forensic evidence. We quantify the gap between the presence of forensic evidence that could be analyzed by forensic practitioners and the actual analysis of such evidence for use at trial.

The analysis of forensic evidence theoretically has the potential to provide a more objective means to aid fact-finding in the criminal justice system. This potential can only be realized: (1) if collected forensic evidence is actually analyzed, and (2) if the results of this analysis are effectively communicated to the fact-finders (a judge or jury) and decision makers. Historically, empirical studies on how often forensic evidence was collected and analyzed have shown that for most crimes, with the possible exception of homicide and sexual assault cases, forensic evidence was infrequently collected and even less frequently analyzed during the investigation stage. There was hope that with technological advancements and the rapid growth of public laboratories, this pattern might change. However, a recent study of five jurisdictions across the United States illustrated that forensic evidence was still only used in a small fraction of cases.<sup>1</sup> Equally important, but less frequently studied, is whether the collection and analysis of forensic evidence is presented to fact-finders at trial. There is a wealth of scholarship suggesting that the presence of forensic evidence or a forensic practitioner's testimony can have a significant effect on juror perceptions of innocence versus guilt. But little is known about how often fact-finders are actually presented with either forensic evidence or the testimony of a forensic practitioner.

In our novel case study, we examined felony criminal cases filed in Franklin County, OH. Franklin County is the most densely populated county in the state of Ohio and encompasses the state's capital. It has historically had one of the largest criminal caseloads in the state. We conducted an examination of all 6,285 criminal cases filed in 2018, identifying the 97 cases that had gone to trial by October of 2021. Using a sampling scheme stratified on the severity of the offense and disposition, we collected trial transcripts for 55 cases. For each case, every exhibit admitted to the fact-finders was individually examined to see whether it fell into one of ten types of forensic evidence: DNA, Trace Evidence, Footwear, Latent Prints, Firearm, Toolmark, Geolocation, Bloodstain, Drug Chemistry Evidence, or Forensic Toxicology. Testimony by all witnesses was also examined and cross-referenced with exhibits. Information was also collected on whether the exhibit was referenced in the trial for a purpose beyond identification at trial or establishing chain of custody.

A preliminary analysis of the data suggests among those ten different types only DNA, Firearm- and Latent Print-type exhibits were commonly admitted and used beyond the basic purposes mentioned above. Footwear- and Forensic Toxicology-type exhibits were not admitted at all. Of the exhibits that were: (1) admitted to the jury and (2) in at least one of the ten types, approximately 38% were Firearm type, 34% were DNA type, and 20% were Latent Prints type. Of those exhibits satisfying both (1) and (2), only about 60% were referenced for a purpose beyond the basic two purposes mentioned earlier. In other words, when an exhibit was admitted at trial, the fact-finders did not hear an examiner's analysis of the evidence underlying the exhibit or their conclusions about it around 40% of the time. That number varied by type: only about 23% of DNA evidence was never referenced beyond the two basic purposes mentioned earlier, but that number rose to around 48% for Firearm type and around 57% for Latent Prints type. Complete results with a more detailed breakdown will be presented.

**Reference:**

- <sup>1</sup> James M. Anderson, Carl Matthies, Sarah Greathouse & Amalavoyal Chari, *The Unrealized Promise of Forensic Science - A Study of Its Production and Use*, 26 *Berkeley J. Crim. L.* 121 (2021).

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**Empirical Study; Forensic Testimony; Courtroom**



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**Y21 Internal Validation of a Modified Procedure for the DNA Collection From Fired Cartridge Casings**

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**Learning Objective:** Attendees will learn what BTmix solution is and how it is used with the modified rinse-and-swab procedure. They will learn how to perform this modified procedure and how the DNA profiles obtained compare to the current wet/dry swab procedure. The results from the sensitivity, repeatability, reproducibility, and mock case studies will be presented.

**Impact Statement:** This research will give forensic biologists another reliable method to collect biological material from fired cartridge casings. The modified rinse-and-swab procedure was found to increase the quantity and quality of DNA recovered from fired cartridge casings compared to the current wet/dry swab technique. Overall, the modified method demonstrated higher success rates in yielding a DNA profile compared to the standard method, which can give aid to investigations.

Crimes involving firearms are a significant problem for many law enforcement agencies. Fired cartridge casings and bullets are regularly found at crime scenes, and even after firing, these potentially carry trace amounts of biological material. This biological material could provide important information for investigators and could potentially link an individual to a crime. However, the success rates of obtaining DNA profiles suitable for comparison from fired cartridge casings have been low due to the compromised amounts of DNA recovered and the deleterious effects of the copper contained within the brass of cartridge casings. Previous studies have suggested that copper may inhibit downstream PCR and/or degrade DNA through oxidative damage.<sup>1</sup> To improve recovery, the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF) has recently developed a modified DNA collection process for FCCs that uses a rinse-and-swab method with a BTmix solution, consisting of Bovine Serum Albumin (BSA) and Glycine-Glycine-Histidine (GGH). Previous research has shown that when BSA is added to the DNA extraction, it can increase yields for challenging samples by chelating contaminants and the GGH tripeptide has been shown to reduce DNA damage by complexing with copper.<sup>2</sup>

An internal validation of the ATF modified rinse-and-swab method was conducted at the District of Columbia Department of Forensic Sciences (DC DFS) Forensic Biology Unit (FBU). The method was slightly modified to fit the QIASymphony workflow that was recently validated by the FBU. Studies were conducted to compare the ATF modified rinse-and-swab method to the standard wet/dry swab method and to evaluate the sensitivity, repeatability, and reproducibility of the new method. The modified rinse-and-swab method with the BTmix solution was found to increase the quantity and quality of the DNA recovered from the fired cartridge casings. Overall, this method demonstrated higher success rates in yielding a DNA profile compared to the standard wet/dry swab technique.

**References:**

- <sup>1</sup>. L.I Moreno, B.R McCord. Understanding metal inhibition: the effect of copper (Cu 2+) on DNA containing samples, *Forensic Chem.* 4 (June) (2017) 89-95.
- <sup>2</sup>. T. W. Bille, G. Fahrig, S. M. Weitz, G. A. Peiffer. An improved process for the collection and DNA analysis of fired cartridge cases, *Forensic Science International: Genetics* 46 (2020) 102238.

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**Validation; BTmix Solution with Modified Rinse-and-Swab Procedure; Fired Cartridge Casings**

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**Y22 Splenic Rupture: An Unusual Presentation of Ehlers-Danlos Syndrome With Genetic Confirmation**

*Megan Schultz\*, Final Diagnosis Inc., Brandon, FL; Daniel Schultz, MD, Final Diagnosis Inc., Brandon, FL*

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**Learning Objective:** Attendees will learn about a case report of splenic rupture in a patient with confirmed Ehlers-Danlos syndrome. Attendees will also learn how this splenic injury presentation could be incorrectly ascribed to trauma if not for the recognition of an underlying connective tissues disorder. Attendees will learn about how definitive characterization via genetic testing can help to further clarify the diagnosis of Ehlers-Danlos syndrome.

**Impact Statement:** This presentation will impact the forensic science community because only a handful of reported deaths due to splenic rupture were found in the literature review. Non-recognition of Ehlers-Danlos syndrome could lead to an erroneous consideration of homicidal or accidental manners.

We report the case of a 29-year-old woman with no previous medical history who presented with an acute onset of abdominal pain and weakness. She was found to have diminishing hemoglobin and evidence of abdominal bleeding. She was admitted to the hospital and given blood products, ultimately leading to an open laparotomy. She was found to have lacerations of the spleen and despite aggressive fluid, blood, and resuscitation, she did not survive surgery. The autopsy revealed a hemoperitoneum, very friable organs, and extensive splenic laceration effectively in two pieces. Hemorrhagic crush injuries of the liver and hemorrhage into the hilum of the right lung and mediastinum were also appreciated and attributed to CPR. The remarkable finding throughout was marked tissue fragility, allowing connective tissues to be broken and tissue separated with little effort. Concern for connective tissues disorder led to obtaining a refrigerated skin sample later submitted for fibroblast culture after being transferred to tissue culture media. Multiple genetic abnormalities were found, including likely pathogenic heterozygous COL3A1, clearly pathogenic heterozygous LTBP3, and 2 variants of heterozygous ZNF469, which are of uncertain clinical significance. A single mutation in the COL5A1 gene is clinically confirmed causal of Ehlers-Danlos syndrome type I and II.<sup>1</sup>

The Ehlers-Danlos syndromes (EDS) are a clinically and genetically heterogeneous group of heritable connective tissue disorders (HCTDs) characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Given the heritable nature of this condition, a referral was able to be arranged for their 3-year-old child to be subsequently tested. Only a handful of splenic rupture cases attributed to Ehlers-Danlos syndrome have been described in the literature review. But for the recognition of an underlying connective tissue disorder, her splenic injury and hemorrhage would potentially have been ascribed to trauma. Under the right circumstance, this kind of case could easily escalate to a homicide call. In children who have this disorder, they similarly may appear to be victims of child abuse. Awareness of the variants of Ehlers-Danlos and how to definitively identify features during the autopsy are important.

**Reference:**

- <sup>1</sup>. De Paep A, Nuytinck L, Hausser I, Anton-Lamprecht I, Naeyaert JM. Mutations in the COL5A1 gene are causal in the Ehlers-Danlos syndromes I and II. *Am J Hum Genet.* 1997 Mar;60(3):547-54. PMID: 9042913; PMCID: PMC1712501. <https://pubmed.ncbi.nlm.nih.gov/9042913/>.

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**Ehlers-Danlos Syndrome; Splenic Rupture; Connective Tissue Disorder**

## **Y23 The Effect of Motor Oil on the Attraction of Forensically Important Diptera in Toco, Trinidad**

*Alyssa Teixeira\*, Texas A&M University, Houston, TX; Adrienne Brundage, PhD, Texas A&M University, College Station, TX; Kevin Conway, PhD, Texas A&M University, College Station, TX; Ryan Mohammed, PhD, Auburn University, Auburn, AL*

**Learning Objective:** Attendees will learn about the affect motor oil has on the attraction of forensically important flies and further implications as it relates to anomalies in time of colonization.

**Impact Statement:** This presentation will impact the forensic science community by informing attendees that knowledge of the implications motor oil has on Diptera attraction, succession, and development is pertinent for precise time of colonization calculations in the presence of motor oil regarding criminal investigations.

Medicolegal forensic entomology aims to provide close estimates for postmortem intervals (PMI) in criminal investigations using time of colonization (TOC) calculations. Precise TOC calculations can provide investigators with a narrower time frame allowing them to accurately determine when/if a corpse has been moved or stored, and more importantly, how long the body has been there (postmortem interval). This commonly uses species of Diptera, such as those from the families Calliphoridae, Sarcophagidae, and Muscidae, which can arrive to carrion within seconds after death. Because these forensically important flies colonize in known patterns with respect to the surrounding environment, we can use this data to calculate TOC. Various factors, such as temperature, climate, weather, humidity, elevation, and medium are used to account for these patterns of succession and development. This study is the first step to determining the effect car oil has on species of Diptera in the Neotropics.

In this study, the number of forensically important flies attracted to motor oil was tested. Two traps, one containing a commercially prepared bait (control) and another containing commercially prepared bait and motor oil, were set on the Jammeev Beach Resort property in Toco, Trinidad. Three consecutive trials were conducted, each lasting four days in length. Data and specimens were collected every 24 hours. The control attracted 160 flies, while the motor oil treatment only attracted one. Eight total species were identified: *Lucilia eximia*, *Lucilia cuprina*, *Lucilia sericata*, *Cochliomyia macellaria*, *Chrysomya megacephala*, *Chrysomya rufifacies*, *Sarcophagidae* spp., and *Muscidae* spp. There was a significant difference in the number of flies collected from the control trap vs. the trap containing motor oil ( $p=0.007$ ). A p-value of less than 0.05 indicates the results are replicable with a low error rate.

Knowledge of the implications motor oil has is pertinent if we are to uncover anomalies in Diptera attraction, succession, and development. Extensive research has been done on many of these factors to provide accurate data for use in TOC calculations; making sure to account for changes in colonization that occur in these conditions. Additionally, several studies have revealed that various substances can alter these trends and patterns. Acids, alcohols, and paraphernalia are all examples of substances that can affect succession and developmental rates, altering TOC. While there have been studies conducted regarding the effect of various substances on Diptera colonization, there has been little-to-no research done on how oil, specifically, can alter these factors. This is problematic as oil, especially motor oil, is a main substance found in everyday households, vehicles, and stores. Additionally, in 2020 alone, there were 38,824 fatalities as a result of motor vehicle accidents in the United States. This presents a significant forensic impact regarding incidents involving motor oil.<sup>1-5</sup>

As outlined above, insect development can be used to estimate time of colonization and postmortem intervals; however, in order to calculate an accurate TOC in the presence of motor oil, a known value for the time delay in Diptera colonization is needed. To ascertain this value, further studies are needed, such as longer trials, volatile analysis, and olfactory analysis of Diptera. Determining the exact delay in colonization in the presence of motor oil will allow for more precise TOC estimations in criminal cases.

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### **Attraction; Motor Oil; Diptera**

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**Y24 The Impact of Sunscreen on the Attractancy of Forensically Important Flies in Toco, Trinidad**

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**Learning Objective:** This presentation provides a baseline knowledge about a substance (sunscreen) that repels forensically important flies from decaying matter with statistical significance.

**Impact Statement:** Since it is now known that sunscreen repels forensically important flies from decaying matter, we can do further research to figure out why this substance repels flies. This information is currently unknown. We can also figure out exactly how long the delay in insect colonization is, so forensic entomologists can have a more accurate time of colonization calculation under these conditions.

Forensically important Dipteran species found at crime scenes are among the most telling pieces of evidence for investigators. The larval form of these forensically important flies can be used to calculate a window of time in which the decaying matter was available for insect colonization, and in conjunction with other biological indicators, can be used to determine a time of death in a homicide investigation.

Large amounts of research focus on improving the accuracy of time of colonization calculations, which utilize the species and instar of the larvae and the ambient temperature of the scene to determine how long the insects have been on a cadaver. Various external factors can impact the time insects colonize remains at a crime scene, but little is known about how different substances impact the attractancy of forensically important flies to decomposing matter. Sunscreen is common for everyday use, and many sunscreens contain harmful chemicals such as oxybenzone.

Certain toxins are anecdotally known to delay insect colonization on decaying matter in forensic investigations, so for this experiment, a control fly trap and a sunscreen-treated fly trap were set out for to determine the impact of sunscreen on the attraction of forensically important flies. A *T*-test showed a statistically significant ( $p > 0.0001$ ) decrease in fly colonization due to the presence of sunscreen in the treated trap. This information should be taken into consideration by investigators and forensic entomologists when sunscreen may be present on remains. The presence of sunscreen could potentially affect time of colonization calculations because of the severe delay and decreased magnitude of insect colonization. The exact reason for the significant decrease in insect colonization on the sunscreen-treated traps is unclear, so further research should analyze the change in volatiles in order to determine the possible deterrent and work to determine the length of the delay in insect colonization due to the presence of sunscreen.

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**Diptera; Attraction; Sunscreen**

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**Y25 The Value of the Autopsy: Considerations on Questions Regarding Drug Toxicity**

*Victoria Sanchez, MS\*, Final Diagnosis, Inc., Lithia, FL; Daniel Schultz, MD, Final Diagnosis, Inc., Brandon, FL*

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**Learning Objective:** Attendees will learn why postmortem toxicology should not be interpreted in a vacuum. Attendees will learn how compelling autopsy findings can serve to quell alternative theories as to the cause of death. Attendees will gain appreciation of the challenges of private autopsy practice in collaboration with death investigative agencies.

**Impact Statement:** Give then magnitude of opioid death toxicities in the United States, the resources of death investigative agencies are stretched and, thus, there are often pushes to eliminate the autopsy when interpreting opioid levels and other drugs. This case report will hopefully serve as a reminder as to why autopsies are relevant.

A common request encountered in the private practice of forensic pathology is to evaluate for drug toxicity. The first response should be to gather details, then refer to the jurisdictional medical examiner/coroner to allow the raised concern to be heard. Sometimes the case is accepted, and no further discussions occur. If the case is cleared, further discussions still need to continue as to the merit of such testing. Frequently the requesting party might ask for toxicology to be simply drawn and sent, sans autopsy, as if the presence of a certain level would signal that an overdose had occurred. This is fraught with issues because of the variability in what are toxic and tolerated levels. Furthermore, as common examples, many living individuals who are functional opioid abusers and alcoholics have achieved much higher levels than those commonly seen in autopsied fatal toxicology deaths.

The point of this presentation is to emphasize a step-by-step, multitiered approach to toxicology concerns, whether forensic or private. What is toxic in one may be an “activity of daily living” level in another. Palliative care cases are titrated for effect to reduce pain and suffering, and the balance between comfort and death can be very tenuous.

We present a case of a deceased 49-year-old Caucasian male who was referred for autopsy due to family suspicion of a possible morphine overdose while receiving palliative care following chemotherapy for leukemia. During the postmortem examination, it was discovered that the decedent had sustained a significant non-traumatic subdural hematoma secondary to profound pancytopenia. The National Association of Medical Examiners Autopsy Performance Standards (2020) indicate that “the forensic pathologist shall perform a forensic autopsy when the death is by apparent intoxication by alcohol, drugs, or poison, unless a significant interval has passed, and the medical findings and absence of trauma are well documented.” It is imperative that toxicological levels alone not be the deciding factor in determining cause or contribution of drugs to death. There are many states in the United States that prosecute individuals for fentanyl deaths, and thus, excluding other reasonable causes is critical. This recommendation is also important in private civil matters to consider, emphasized in medical malpractice cases like the one we present, as families often still grasp for answers and may become overwhelmed with suspicion. A competent autopsy and review of circumstances sets the stage for performing or not performing toxicology and enables prudent use of financial resources. This case illustrates but one of the possible injustices of toxicology in a vacuum that could occur, with implications in both medical malpractice and criminal cases.<sup>1-5</sup>

**References:**

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3. Bove, K. E., & Iery, C. (2002). The role of the autopsy in medical malpractice cases, I: a review of 99 appeals court decisions. *Archives of pathology & laboratory medicine*, 126(9), 1023-1031.
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**Overdose; Autopsy; Toxicology**

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**Y26 Validation for Plate Setup on the QIAgility® Instrument Using the Plexor HY Quantitation Kit and GlobalFiler™ Amplification Kit**

*Madison Davenport, BS\*, Marshall University, Dallas, GA; Kelly Beatty, MSFS, Marshall University, Huntington, WV; Jessica Skillman, BS, District of Columbia Department of Forensic Sciences, Washington, DC*

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**Learning Objective:** Attendees will learn how the QIAgility® works for plate setup in preparation for quantitation and amplification using the Plexor® HY quantitation kit and GlobalFiler™ amplification kit, respectively. They will also learn the reliability and reproducibility of the results obtained from the quantitation and amplification steps after plates were prepared on the QIAgility® instrument and how they relate to the results obtained after manual plate setup.

**Impact Statement:** The use of the QIAgility® instrument can become a very beneficial implementation, especially in high-throughput laboratories. Implementing the QIAgility® into everyday casework flow can help analysts have more time to complete other aspects of casework, such as analysis of results. Specifically, this presentation will help the forensic science community understand how the QIAgility® works for preparing the plates and how it compares to the manual method of plate preparation. This allows the community to evaluate the benefits of how this instrument can be implemented into their own laboratories and aid in pushing out reliable results to the community at large.

In the process of DNA analysis, after extraction of the DNA is complete, the samples must then be set up on a plate with buffers and primers to be quantitated, then later set up on a different plate to be amplified.<sup>1,2</sup> The current process requires manual plate setup by laboratory personnel, which can require a significant time commitment. For quantitation, proper pipetting is essential as the standards used for estimating DNA concentration in unknown samples are manually prepared. These standards must pass certain guidelines, developed through internal validation for the quantitation results to be accepted and for samples to move forward for amplification.<sup>1,3</sup>

To set up a plate for amplification, the samples must be normalized to an optimal target range based on the quantitation results. This means different volumes of each sample must be pipetted directly to the amplification plate or be diluted and then pipetted to the amplification plate. All these different volumes and sample transfers increase the possibility for human error. Therefore, by introducing the QIAgility® instrument, a robotic workstation for automated PCR setup, it is hoped that the possibility of human error will be reduced, and more consistency will be seen.<sup>4</sup> Also, by allowing the instrument to perform the plate setup, laboratory personnel will have more time to conduct other tasks.

This validation investigated the repeatability and reproducibility of the results obtained after plate setup was conducted on the QIAgility® instrument for both quantitation and amplification. The results were then compared to those obtained from manual plate setup for each process to determine if they were consistent with the current method used. The results were also analyzed for accuracy and contamination.<sup>5-8</sup> This validation produced results that were reliable and consistent, indicating the QIAgility® is fit for implementation into the lab's workflow.<sup>9</sup> This study can be replicated by other laboratories to implement time-saving technology that will allow their personnel to spend more time on the analysis, interpretation, and review of results.

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7. ANSI/ASB Standard 038, First Edition 2020, Standard for Internal Validation of Forensic DNA Analysis Methods. (Accessed 16 May 2022).
8. DOM04 – Procedures for Validating Technical Procedures.
9. District of Columbia Department of Forensic Sciences Forensic Science Laboratory Quality Assurance Manual (ISO/IEC 17025:2017 Standards).

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**QIAgility®; Validation; Plexor® HY**



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**Y27 Comparing Insanity Defense Evidence: Psychiatric Assessments vs. Neuroimaging**

Kaitlyn Svencer\*, Duquesne University, Pittsburgh, PA; Lyndsie Ferrara, PhD, Duquesne University, Pittsburgh, PA; Jane Moriarty, JD, MA, Thomas R. Kline School of Law at Duquesne University, Pittsburgh, PA

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**Learning Objective:** For this presentation, attendees will learn more information on the insanity defense and neuroimaging and how they play a part in a medical and legal sense. The medical section will include information on the types of brain scans that are used in neuroscience, the different parts of the brain, the definition of neuroscience, the diagnosis of different mental diseases, different traumatic brain injuries, and how psychiatric assessments are completed. The legal section will give a history of the use of the insanity defense, the types of requirements for an insanity defense, mental disorders that have been seen in past insanity defense cases, how neuroscience is seen in the courts, and the admissibility standards under *Daubert* and *Frye*.

**Impact Statement:** This presentation will impact the forensic science community because neuroimaging could impact the psychiatric assessment evidence that is typically used for an insanity defense case, and it could become a more important factor in the courts. The advantages and disadvantages of using neuroscience evidence in an insanity case are unknown and need more research, so this presentation will be the beginning of that.

The insanity defense is the claim that a person is not responsible for a crime that they commit by reason of “mental disease or defect” where they lacked the capacity to understand the criminality of their act.<sup>1</sup> The first time the verdict of not guilty by reason of insanity was used was in the 1800s.<sup>2</sup> In an insanity defense case, the defendant is not convicted of a crime if they lack the mental capacity to understand their actions. Psychiatric assessments are usually used to determine an individual’s mental capacity and are the main types of evidence in an insanity defense case. Additionally, neuroimaging could be used in the court room to address insanity pleas, but further examination of this evidence type is needed. This is relevant because neuroimaging could enhance the psychiatric assessment evidence for an insanity defense case, and it could become a more important factor in the courts.

Neuroimaging is one aspect in neuroscience that attempts to understand how the human brain works in terms of molecules, membranes, cells, development, plasticity, learning, memory, cognition, and behavior.<sup>3</sup> This is usually done through the use of brain scans and images to see the different regions of the brain as well as any deformities that could be present. Brain images serve as tangible proof of an underlying neurological disorder that is not present when hearing from the experts alone.<sup>4</sup> There are many different brain scans that can be performed, but the most common types consist of functional magnetic resonance imaging (fMRI), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computerized tomography (SPECT).

The goal of this research was to compare psychiatric assessments and neuroimaging evidence and investigate how they contribute to insanity defense cases and the admissibility requirements for neuroscience in the court room. This was done through a literature review that researched the use of neuroimaging in insanity defense cases as well as a case study analysis. The case study analysis researched cases that have used neuroimages as evidence alongside psychiatric assessments in insanity defense cases. A comparison was made between the two techniques to determine the advantages and disadvantages of using neuroscience in the courtroom.

**References:**

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**Insanity Defense; Neuroimaging; Psychiatric Assessment**



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**Y28 Determination of Hair Growth Rates in Various Anatomical Regions from a Student Campus Population**

*Samantha Deibel, BS\*, Arcadia University, Chambersburg, PA; Heather Harris, MFS, JD, Arcadia University, Glenside, PA; Sherri Kacinko, PhD, Horsham, PA; Karen Scott, PhD, University of Alabama at Birmingham, Birmingham, AL*

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**Learning Objective:** After attending this presentation, attendees will be informed of new, updated, and robust hair growth rates from various anatomical regions of a study population in their late teens to mid-twenties. Attendees will also learn the hair growth rate trends over each season, various races, and between sexes.

**Impact Statement:** This presentation will impact the forensic science community by providing a wide range of updated hair growth rates that can increase the accuracy of determining the history of drug abuse in toxicology cases. This historical data may prove to be valuable in cases such as DUIs and illicit drug use in clubs and raves. Additionally, this will provide knowledge of hair growth rates from diverse regions on the body. Finally, this will express a need for a larger study to be performed in the future.

Hair is an important piece of evidence in toxicology cases when determining the history of drug abuse in individuals. It can be used as a less invasive alternative collection technique to blood or urine. Additionally, hair has an indefinite storage life, and the drugs within hair are relatively stable. However, with the evolving drug detection evasion techniques mainly for the head hair, secondary hair sites may need to be explored. The growth rates of hair have not been updated since the early 1990s, and it is known that hair from anatomical locations other than the head may not grow at the same rate. There are many factors that affect the growth rate of hair, including age, race, biological sex, anatomic location, and seasonal changes. These factors were investigated using a FireFly hand-held microscope and TrichoSciencePro<sup>®</sup> software from a subset of Arcadia University's campus population following IRB approval.

During the summer and fall seasons, participants ( $n=20$ ) consented to having various areas, including head, auxiliary, arm, pubic, chest, beard, and/or lower leg, cleaned and shaved. The participants were instructed to not shave the area(s) for the duration of the study. After two weeks, the area(s) were photographed and shaved again. This was repeated on one more occasion. Finally, on the fourth session, the area was photographed. Each photograph was analyzed using the TrichoSciencePro<sup>®</sup> software to obtain the hair growth rate from each anatomical area.

Hair growth rates were compared between males ( $n=5$ ), females ( $n=14$ ), and trans male ( $n=1$ ) between the ages of 18 and 28 years. During both seasons, it was discovered that the overall rate for males (0.257 and 0.315mm/day for summer and fall, respectively) and the overall rate for females (0.259 and 0.243mm/day for summer and fall, respectively) were found to be inconsistent with former literature values after performing one sample Students' *T*-tests. Additionally, during the summer, it was found that the lower leg (0.201mm/day), arm (0.157mm/day), and chest (0.187mm/day) for all sexes were inconsistent with previous literature values. During the fall season, the armpit (0.408mm/day), pubic (0.399mm/day), lower leg (0.234mm/day), and arm (0.196mm/day) for all sexes were found to be inconsistent with the previous literature values. After performing a Paired *T*-test to compare the data for the individuals that participated in both seasons ( $n=10$ , females  $n=5$ , and males  $n=5$ ), exactly half have shown the differences between seasons to be statistically significant, meaning that the growth rates are varying with the season changing.

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**Hair Growth Rates; Toxicology; Anatomical Variation**

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**Y29 Evaluation of Novel Psychoactive Substance Drug Loss From Storage in Serum Separator Tubes**

*Devin Kress, BS, Forensic Science\*, Hanover, PA; Melissa Fogarty, MSFS, The Center for Forensic Science Research and Education, Willow Grove, PA; Heather Harris, MFS, JD, Arcadia University, Glenside, PA; Barry Logan, PhD, CFSRE/Fredric Rieders Family Foundation, Willow Grove, PA*

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**Learning Objective:** After attending this presentation, attendees will be able to evaluate if storage of serum in serum separator tubes causes adsorption of novel stimulants into the polymeric gel separator layer.

**Impact Statement:** This presentation will impact the forensic science community by providing information on the appropriate storage conditions for serum samples if there is a need for forensic toxicology testing. The community will be able to determine if the current practice of storing serum in serum separator tubes is acceptable.

**Introduction/background:** Serum separator tubes (SST) are a type of blood collection tube used primarily for clinical testing. These tubes contain a clot activator and a separator gel. The clot activator causes the blood to clot in the tube, and centrifugation allows the polymeric gel to separate the serum and red blood cells due to differences in densities. These tubes are used to collect blood due to their many advantages, including the convenience of simply storing any remaining sample in the vacutainer tube. A disadvantage that comes with storage in SST is that certain drugs are susceptible to being adsorbed into the gel polymer, resulting in a decreased concentration remaining in the serum, possibly to the point where the drug may be below detection limits. This is a concern when the sample is submitted days, weeks, or even months after collection for analysis in a forensic toxicology laboratory. Furthermore, there is little investigation for this effect with novel psychoactive substances (NPS), which are not always targeted in the initial investigation due to the ever-changing selection of which are currently being used on the streets.

**Aim/objective:** The aim of this study is to determine if a subset of trending NPS stimulants will be susceptible to adsorption into the SST polymeric gel during storage and the resulting decrease in concentration.

**Methods:** Samples were prepared by spiking citrated whole blood with known concentration of the following novel stimulant standards: diethylone, tertylone, N-ethyl pentylone, dimethylpentylone, eutylone, pentylone, and hexylone. This drug-fortified blood was aliquoted into 21 5mL Becton Dickinson Vacutainer SST, recalcified with calcium chloride, and centrifuged at 3,000RPM for 10mins to separate the serum; 1.5mLs of the serum from each SST was aliquoted into a correspondingly labeled borosilicate glass tube to be used for the control where no adsorption would occur, and the remaining serum remained in the SST for storage.

Serum aliquots were extracted and quantitatively analyzed via liquid chromatography/tandem mass spectrometry (LC/MS/MS). These aliquots were stored in the refrigerator (approximately 4°C) and were analyzed on days 0, 1, 2, 7, 14, 30, and 60. The resulting concentration from the analysis was then plotted against time for both the SST and borosilicate glass tube storage.

**Results:** All of the compounds had a larger loss of drug in the SST versus the borosilicate glass tubes, and dimethylpentylone and hexylone had the largest decrease over the 60-day time span. For dimethylpentylone, the concentration decreased in the SST by 65%, while in the borosilicate glass tube, the concentration only decreased by 8%. Hexylone had a 63% decrease in the SST and only a 7% decrease in the borosilicate glass. All other compounds had a decrease of 49% or less in the SSTs.

**Discussion:** This study shows that while SST are useful for extended storage of serum samples in the clinical setting, they can produce additional interpretation issues for forensic toxicology cases in terms of quantitation. Without further testing of all NPS, it will be unknown if the concentration is or is not affected by adsorption into the separator gel. Until this testing can be completed, it would be advantageous that if hospitals suspect a novel drug was ingested, to store that serum in a secondary container or to send the SST to the laboratory as soon as possible.

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**Serum Separator Tubes (SST); Novel Psychoactive Substances (NPS); Serum Specimen Storage**

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*The following presenting authors disclose no financial relationships with commercial entities.*

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