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Gabby DiEmma and Erica Fornaro, Editors

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RTI International
3040 East Cornwallis Road
PO Box 12194
Research Triangle Park, NC
27709-2194 USA

Tel: +1.919.541.6000
E-mail: rtipress@rti.org
Website: www.rti.org

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About the Editors

Gabby DiEmma, MS, is a forensic scientist in the Justice Practice Area at RTI International.

Erica Fornaro, BS, is a research public health analyst in the Justice Practice Area at RTI International.

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Abstract

The 2024 National Institute of Justice (NIJ) Forensic Science Research and Development (R&D) Symposium is intended to promote collaboration and enhance knowledge transfer of NIJ-funded research. The NIJ Forensic Science R&D Program funds both basic or applied R&D projects that will (1) increase the body of knowledge to guide and inform forensic science policy and practice or (2) result in the production of useful materials, devices, systems, or methods that have the potential for forensic application. The intent of this program is to direct the findings of basic scientific research; research and development in broader scientific fields applicable to forensic science; and ongoing forensic science research toward the development of highly discriminating, accurate, reliable, cost-effective, and rapid methods for the identification, analysis, and interpretation of physical evidence for criminal justice purposes.

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Lucas Zarwell, MFS, D-ABFT-FT

Introduction

The National Institute of Justice (NIJ) is the federal government's lead agency for forensic science research and development as well as the administration of programs that facilitate training, improve laboratory efficiency, and reduce backlogs. The mission of NIJ's Office of Investigative and Forensic Sciences is to improve the quality and practice of forensic science through innovative solutions that support research and development, testing and evaluation, technology, information exchange, and the development of training resources for the criminal justice community.

Through the research, development, testing, and evaluation process, we provide direct support to crime laboratories and law enforcement agencies to increase their capacity to process high-volume cases and provide needed training in new technologies. With highly qualified personnel and strong ties to the community, NIJ's Office of Investigative and Forensic Sciences plays a leadership role in directing efforts to address the needs of our nation's forensic science community.

RTI International and its academic- and community-based consortium of partnerships work to meet all tasks and objectives for the Forensic Technology Center of Excellence (FTCOE), put forward under the National Institute of Justice (NIJ) Cooperative Agreement No. 15PNIJ-21-GK-02192-MUMU.

The FTCOE is led by RTI International, a global research institute dedicated to improving the human condition by turning knowledge into practice. With a staff of almost 6,000 providing research and technical services to governments and businesses in more than 75 countries, RTI brings a global perspective. The FTCOE builds on RTI's expertise in forensic science, innovation, technology application, economics, DNA analytics, statistics, program evaluation, public health, and information science.

On February 20, 2024, NIJ and the FTCOE held the 2024 NIJ Forensic Science Research and Development (R&D) Symposium. Hundreds of professionals joined us online and in person for this hybrid event to learn about NIJ research awards given to several talented researchers spanning the forensic disciplines.

For more than a decade, NIJ has hosted an annual R&D Symposium to showcase great scientific innovations and promote the transition of research into practice. NIJ supports research to advance efficiency, quality, reliability, and capacity in the criminal justice and forensic science communities; this research focuses on developing new technologies, providing proof for evidence-based practices, and evaluating findings for case investigations and legal proceedings.

This year, members of the NIJ Office of Investigative and Forensic Sciences R&D team—including program managers Gregory Dutton, Danielle McLeod-Henning, Frances Scott, and Tracey Johnson—worked to create a phenomenal research agenda. The full program included 16 presentations and 34 posters from principal investigators and their research partners; these presentations and posters represent accomplishments from NIJ R&D grants awarded during 2015–2022. Most presentations are archived on the FTCOE's website and available to view for free.

Dr. Dutton, Ms. McLeod-Henning, Dr. Scott, and Ms. Johnson were moderators. Dr. Dutton moderated Session I, Physics and Pattern Interpretation/Trace Evidence; Ms. McLeod-Henning moderated Session II, Forensic Anthropology and Forensic Pathology; Dr. Scott moderated Session III, Seized Drugs and Toxicology; Ms. Johnson moderated Session IV, Forensic Biology/DNA.

Summary of Oral Presentation and Poster Session Topics

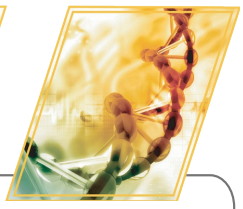
NIJ Forensic Science Research and Development Symposium

February 20, 2024

Oral Presentation and Poster Session Topics


Sessions I & II

Physics and Pattern Interpretation/ Trace Evidence | Forensic Anthropology and Forensic Pathology

Sessions III & IV

Seized Drugs and Toxicology | Forensic Biology/DNA

482 Total Attendees

151 In Person

331 Online

16 Podium Presentations

34 Posters

76 Chat Messages

3,374 Total Learning Hours

35 Online Q&A Submissions

NIJ National Institute of Justice — Advancing Justice Through Science

Forensic Technology Center of Excellence — A program of the National Institute of Justice

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SESSION ABSTRACTS

SESSION I

PHYSICS AND PATTERN INTERPRETATION/TRACE EVIDENCE

Moderated by NIJ Program Manager
Gregory Dutton

Development of Nationwide Reference Population Distributions for Statistically Supported and Objective Testimony in Firearm Evidence Comparisons

NIJ AWARD #: 2016-DNR-6257-3

Xiaoyu Alan Zheng*

Johannes Soons

National Institute of Standards and Technology

* Presenting author

Application and validation of the National Institute of Standards and Technology (NIST) statistical framework requires the use of relevant population distributions. A population distribution describes the frequency distributions of a similarity score for same-source 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 (Ruger, Glock, S&W, and Sig Sauer) with 100+ firearms from each to test the systematic error rates associated with the developed protocols. Each firearm was used to test fire a minimum of two test fires using Remington UMC 9 mm ammunition with brass cases and nickel primers. For each population of firearms, all available known matching scores and 10,000 known nonmatching scores were calculated. These were used to establish the statistical distributions for further analysis of cumulative false positive and cumulative false negative 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. The research also demonstrates opportunities to generalize the reference population through statistical methods such as the score-based likelihood ratio.

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Assessing the Strength of Trace Evidence Fracture Fits Through a Comprehensive, Systematic, and Quantifiable Approach

NIJ AWARD #: 2020-DQ-BX-0012

Tatiana Trejos*,[1]
Zachary Andrews[1]
Meghan Prusinowski[1]
Aldo Romero[1]
Cedric Neumann[2]

[1] West Virginia University

[2] Battelle Memorial Institute

* Presenting author

Criminal activities, such as sexual assaults, kidnappings, and homicides, often lead to fractured materials. The realignment between fragments left at the scene and items recovered from an individual or object of interest could become crucial evidence during an investigation. These fracture fits are often regarded as the highest degree of association of trace materials because of the common belief that fracture edges produce individualizing patterns; there is a need to demonstrate the scientific validity of this assumption. Currently, the examination of fractured edges involves the subjective judgment of the examiner without consensus-based standard methodologies for the identification of distinctive features, a systematic criterion for informing a fit/nonfit decision, or methods for assessing the weight of the evidence. To help reduce these gaps, the overall goal of this research was to develop an effective and practical approach that provides an empirically demonstrable basis to assess the significance of trace evidence fracture fits. In particular, the goals were to develop a systematic method for the comparison of fracture fits of common trace materials such as duct tapes, textiles, and automotive plastics; develop a relevant extensive database of nearly 9,000 samples to evaluate performance rates in this field and assess the probative value of a fracture fit using similarity metrics and score likelihood ratios; and evaluate the utility and reliability of the proposed approach and establish consistency base rates through interlaboratory studies. Partnerships among experienced forensic researchers, computational material science physicists, statisticians, and practitioners were crucial to develop strategies to facilitate the future adoption of the developed approaches within crime laboratories. This study identified material-specific relevant features for duct tapes, textiles, and automotive polymers and developed reporting templates to facilitate thorough and systematic documentation of an analyst's decision-making process and minimize risks of bias. It also established criteria for using quantitative metrics, such as the edge similarity score (ESS) that estimates the quality of a fit and the feature prominence score (FPS) that captures the relative features' importance in each comparison. The method yielded relatively high accuracy (85% to 100%). The auto-populated cell options in the reporting template are provided to characterize the influence of the feature on a decision and, together with the ESS and FPS, offer a means to assess the similarity between two given edges and standardized criteria to support their decision. The method demonstrates that most true nonfit pairs receive low ESS (0%–20%) and low FPS (< −5). True fit pairs generally receive high ESS (80%–100%) and high FPS (> +15). This research specifically addressed several research needs in the field (i.e., quantitative assessment of error rates, scientific foundations, standardization, validation, interpretation, casework review, and proficiency assessment). As a result, this study is anticipated to transform current trace evidence practice by providing—for the first time—harmonized examination protocols and decision thresholds, effective mechanisms to ensure transparent and systematic peer-review process and interlaboratory testing, and a quantitative basis that together substantiate the evidential value of fracture fit conclusions.

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Universal Method for the Detection of Organic and Inorganic Gunshot Residue Based on Fast Fluorescence Mapping and Raman Spectroscopic Identifications

NIJ AWARD #: 15PNIJ-21-GG-04153-RESS

Igor K. Lednev
University at Albany, State University of New Yorky

Gunshot residue (GSR) is an important type of forensic trace evidence produced when a firearm is discharged. GSR can be subdivided into two subclassifications—organic (OGSR) and inorganic (IGSR). Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, also known as SEM-EDS or SEM-EDX, is used to detect and identify GSR particles. The application of this two-step method is limited to IGSR because it relies solely on the detection of heavy metals (lead, barium, and antimony). This is problematic because environmental concerns have led to an increased popularity in heavy metal–free or "green" ammunition. It has been found that in the absence of heavy metals, current elemental analysis techniques are severely hindered when attempting to identify GSR samples accurately. Additionally, the probability of environmental and manufacturing particles being incorrectly assigned as GSR has increased with the onset of green ammunition. OGSR has recently been the focus of many forensic researchers for several reasons. First, the total amount of OGSR generated because of the discharge of a firearm is much larger than the amount of IGSR. Second, OGSR particles are typically much larger than IGSR particles. In addition, the chemical composition of OGSR is quite complex and includes partially burned and unburned smokeless powder, stabilizers, and plasticizers. As a result, it is easier to detect and identify OGSR particles, although new methods are required. This laboratory has developed a new two-step approach for fast OGSR particle detection using fluorescence spectroscopy followed by a confirmatory identification by Raman microspectroscopy. The method uses a single instrument that combines a confocal scanning Raman and a fluorescence microscope working in reflection mode. In the first proof-of-concept study, the presenter used adhesive tape to collect OGSR particles. Most recently, the presenter significantly expanded this emerging methodology by demonstrating the possibility of detecting and identifying GSR particles on original common substrates (e.g., cotton fabric), eliminating the initial GSR particle transfer stage. The presenter will show the results of these recent studies and then discuss challenges and future steps to develop the proposed two-step method for the detection and confirmatory identification of both OGSR and IGSR particles. In addition, the presenter will discuss the preliminary results of using a portable Raman instrument to detect and identify GSR. The latter approach opens the possibility of bringing the technology to the crime scene.

Comprehensive Assessment of Novel Reference Materials and Analytical Methods for the Analysis and Interpretation of Organic and Inorganic Gunshot Residues

NIJ AWARD #: 2020-DQ-BX-0010

Luis E. Arroyo*,[1]
Tatiana Trejos[1]
Kourtney Dalzell[1]
Thomas Ledergerber[1]
Leah Thomas[1]
Madison Lindung[1]
Matt Staymates[2]

[1] West Virginia University

[2] National Institute of Standards and Technology

* Presenting author

Increased gun violence requires an immediate reaction from the criminal justice system to manage workloads and adapt operations. Thus, technological advances that lead to the accurate reconstruction of events, prompt apprehensions, and meaningful data sharing are critical for timely justice and increased public safety. Identifying traces of gunshot residue (GSR) is one of the forensic services of great interest in these investigations. Nonetheless, essential information to make informed decisions about recovery at the crime scene—while safeguarding the integrity of the evidence and evaluating the evidence under competing propositions—is still needed. The complex nature of GSR transfer and persistence introduces challenges and skepticism in its evidential value. For instance, GSR can be transferred in different ways: direct transfer (primary) or indirect transfer (i.e., secondary, tertiary, or quaternary), opening the question in the courtroom about the presence of GSR on a person of interest because of firing a gun or indirect exposure. This is a question most forensic practitioners cannot answer, yet it is of primary interest to the trier of fact. Thus, this study aims to provide solutions to those needs by enhancing current capacity through technology innovation and increasing knowledge of GSR transfer and persistence. The study addresses a primary demand to include organic constituents in the workflow for increased confidence in the results. The main goal is to establish scientific foundations for best practices for the collective recovery, preservation, storage, analysis, and interpretation of inorganic GSR (IGSR) and organic GSR (OGSR). In the first part of this presentation, the researcher will present findings on the behavior and movement of IGSR and OGSR to assist in evidence interpretation using scanning electron microscopy coupled with energy dispersive X-ray spectroscopy (SEM-EDS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. The study encompassed over 650 samples, including 247 collections from human skin after firing a gun and 405 synthetic skin and fabric substrates after depositing a characterized IGSR/OGSR standard. Transfer and persistence experiments were evaluated on different substrates (hands, ears, nostrils, forehead, hair, fabrics, and synthetic skin) at different times after firing (0 to 6 hours) and common post-shooting activities (rubbing hands, handshaking, running, washing hands and fabrics). Ground truth knowledge of particle counts and analyte concentrations were used to calculate the recovery for inorganic and organic constituents from clothing and a synthetic skin membrane (StratM®). In the second part of the presentation, the researcher will discuss the preliminary results of GSR deposition in enclosed environments and evaluate GSR exposure risks on bystanders and passersby. This study uses high-speed and standard videography and laser sheet scattering to investigate visual information about the flow of GSR under various controlled experimental conditions. Also, cost-effective atmospheric samplers and particle counting systems are used to evaluate particle concentrations and distributions in three locations: a shooter, a bystander, and a passerby entering the scene after 10 minutes. The laser-based visualization methods, airborne particle analyzers, and analytical methods offer novel advances to aid in the interpretation of IGSR/OGSR deposition dynamics and exposure risks for nonshooters.

SESSION ABSTRACTS

SESSION II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Moderated by NIJ Program Manager
Danielle McLeod-Henning

Pre-grouping of Commingled Human Skeletal Remains by Elemental Analysis

NIJ AWARD #: 15PNIJ-21-GG-04151-SLFO

Matthieu Baudelet*,[1]
Katie Zejdlik-Passalacqua[2]
Jonathan Bethard[3]

[1] University of Central Florida

[2] W estern Carolina University

[3] University of South Florida

* Presenting author

Although forensic anthropology has developed quantitative tools to recover, analyze, and reassociate individuals in mass graves, challenges still exist for small bones, fragments, or bones that underwent taphonomic changes. When considering the tediousness of the process, additional approaches for sorting skeletal remains in large, mixed assemblages could be a great resource for the community. Several studies have shown that chemical and physical variation exists between individuals' bones because of several factors, such as diet, health, and living environment. As such, chemical analysis of the osseous remains in a mass grave could help anthropologists tackle some of these challenges. To be helpful, this chemical analysis needs to be affordable, approachable, and available outside of research facilities or field-deployable, if possible. In recent decades, portable spectroscopic instruments have seen an increase in use across the field of forensic analysis, from molecular (e.g., Raman, infrared, nuclear magnetic resonance, mass spectrometry) to elemental analysis (e.g., laser-induced breakdown spectroscopy [LIBS] and X-ray fluorescence spectroscopy). Elemental analysis has shown promising results in anthropology, providing information beyond the bone matrix and giving insights on the trace elements profile for each individual. Although X-ray fluorescence has been a strong focus of research, LIBS is a complementary technique that provides additional elemental information while also being field-deployable, easy to use, and visually nondestructive. This National Institute of Justice–funded study explores how the chemical profile of bones may be incorporated into a method for classifying commingled remains using LIBS. The remains of 45 individuals have been analyzed by LIBS after decomposition at the Forensic Osteology Research Station at Western Carolina University. Using data reduction to determine the elemental profile needed for reassociation, supervised discriminant analysis was used to reassociate elemental profiles to their individuals with accuracies reaching 90% to 100%. This presentation will discuss the current challenges in using elemental profiles for reassociation and new paths for better results in chemical reassociation of remains. Recommendations on how to build collaborative approaches between anthropologists and chemists to improve reassociation of commingled remains will also be discussed.

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Potential Postmortem Microbial Biomarkers of Infant Death Investigation

NIJ AWARD #: 2020-75-CX-0012

Jennifer Pechal*,[1]
Bethany Mikles[1]
M. Eric Benbow[1]
Heather R. Jordan[2]
Carl J. Schmidt[3]

[1] Michigan State University

[2] Mississippi State University

[3] University of Michigan

* Presenting author

The field of forensic science is witnessing a paradigm shift with the emergence of postmortem microbial biomarkers as potential tools in death investigation. This work delves into the practicality of integrating microbial signatures into forensic protocols to enhance the accuracy and reliability of postmortem analyses. This research focuses on elucidating the dynamic interactions between the infant microbiome and decomposition processes, aiming to establish microbial biomarkers as robust indicators of the postmortem interval and other critical factors in death investigation. The researchers conducted a postmortem microbiota survey from a Midwest medical examiner's office to assess the viability of microbial signatures as reliable indicators in a forensic context. To provide robust, variable data, postmortem microbiota were collected from approximately 50 Black and White infants of both sexes and included deaths that were classified as an accident, a homicide, or from natural or unknown causes. Nine individual body sites were targeted for a composite analysis: eyes, ears, nose, mouth, umbilicus, brain, rectum, and cardiac blood. Determining genetic signatures via targeted 16S rRNA sequence analyses and whole genome sequencing will test the utility of the postmortem microbiome to help discern cause and manner of death in infants, especially in cases where no cause of death is apparent. The study findings indicate that the infant postmortem microbiome composition variability was structured by body site, offering valuable insights into the persistence of microbial community structure after death. Microbial community composition appears to correlate with specific circumstances surrounding death. For example, natural and control (e.g., co-sleeping) deaths are highly similar in composition. The practical application of postmortem microbial biomarkers is showcased through case studies and experimental models. By leveraging high-throughput sequencing technologies and advanced bioinformatics tools, the researchers demonstrate the potential for microbial profiling to serve as a supplementary method for forensic practitioners. The noninvasive nature of microbial sampling, coupled with the ability to analyze samples from diverse postmortem environments, underscores the adaptability and practicality of this approach in routine death investigation. Despite promising advances, challenges persist in standardizing protocols, interpreting results, and addressing potential confounding variables. This work highlights the ongoing efforts to establish a robust framework for integrating microbial biomarkers into routine forensic analyses. By aiding in the creation of standardized, best practice recommendations for the analysis of microbiomes in routine case work, the value they add to these cases is highlighted. The presenter discusses the necessity for interdisciplinary collaboration between microbiologists, forensic scientists, and legal professionals to ensure the seamless incorporation of microbial data into the forensic workflow. In conclusion, this research emphasizes the practicality of postmortem microbial biomarkers as valuable

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tools in death investigation. The integration of microbial signatures has the potential to revolutionize forensic science, providing forensic practitioners with additional reliable information to enhance the accuracy and circumstantial analysis of infant deaths. It is conceivable that an individual microbiome profile will have diagnostic significance once more data are obtained. As this field continues to evolve, collaborative efforts are essential to refine methodologies and establish standardized practices for the routine implementation of microbial biomarkers in death investigation.

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Forensic Tool to Identify Fall Characteristics in Infant Skull Fracture

NIJ AWARD #: 2020-75-CX-0014

Brittany Coats*
Yousef Alsanea
Jacob Hirst
Tagrid Ruiz
Ashley Spear
University of Utah
* Presenting author

Early identification of child maltreatment is critical to the prevention of adverse outcomes, but child abuse in young infants (<1 year of age) is still highly underdetected. Skull fractures are common in both accidental and abusive head trauma and provide a unique opportunity to assess the validity of caretaker histories of infant trauma based on fracture initiation sites, fracture lengths, and level of complexity. Skull thickness distribution influences skull fracture patterns, but the effect of age and biological sex in early development on skull thickness distribution has not been reported in detail. This study aimed to develop an imaging pipeline for high-resolution, 3D maps of skull thickness to compare distributions between male and female infants 0–12 months of age and identify appropriate age divisions for sex-based anatomical templates for fracture simulations. Institutional Review Board approval was obtained to review computed tomography images of 281 healthy infant skulls (<12 months of age) from Primary Children's Hospital. Serial stacks of axial, coronal, and sagittal images were segmented and aligned in the 3D space. Identification of suture location and thickness extraction at more than 12,000 sites across the infant skull was performed using custom scripts. To compare skull thicknesses at similar relative locations, despite differences in head shape and size, one subject within predetermined age groups (0–4, 5–8, 9–12 months) was selected as a template. The skull distribution in each subject was then fit to their respective template using an iterative closest point algorithm. Thickness was averaged across 132 discrete regions representing approximately 10–50 locations in four cranial bones (right and left parietal, occipital, and frontal bones). Classification optimization was used to identify natural age divisions for each sex. The effect of age and sex was evaluated for each cranial bone using a two-way ANOVA with repeated measures, correlations of thickness with age by sex, and one-way ANOVAs that controlled for location. Classification analysis indicated five age groups (0–2, 2–5.5, 5.5–8, 8–10, 10–12 months) were optimal within early development of an infant skull. Frontal bones were significantly thicker than all other bones for all age groups and sexes ($p<0.0001$). Female infants had thicker skulls than males between 0–2 months of age ($p<0.03$), but male infants had thicker skulls at all subsequent age groups ($p<0.02$), which was more pronounced with increasing age. The rate of skull thickness growth was continuous for parietal bones, but occipital and frontal bones had periods where growth was temporarily stalled. Symmetry between the left and right parietal bones was moderate at young ages (0–5.5 months) and increased with age. The high-resolution imaging pipeline and skull thickness comparison in this study illustrate distinct changes in skull thickness with age that are dependent on biological sex and cranial bone. This suggests unique skull geometry templates are needed to represent male and female infants at five stages of development in the first year of life to predict skull fracture patterns from head impact accurately.

Determining Fracture Timing From Microscopic Characteristics of Cortical Bone

NIJ AWARD #: 2020-75-CX-0015

Jessica L. Skinner*,[1]
Natalie R. Langley*,[1]
Samuel Fahrenholtz[1]
Yuktha Shanavas[2]
Brian Waletzki[3]
Robert Brown[4]
James Herrick[4]
Peter Goguen[5]
Loukham Shyamsunder[5]
Subramaniam Rajan[5]

[1] Mayo Clinic Arizona

[2] State University of New York Upstate Medical University

[3] Mayo Clinic Rochester

[4] MilliporeSigma

[5] Arizona State University

* Presenting authors

This study investigated whether scanning electron microscopy (SEM) is effective for assessing microscopic surface characteristics of experimentally induced fractures in human bone at various postmortem intervals (PMIs). The researchers hypothesized that microscopic fracture characteristics, including delamination, osteon pullout, and microfractures, may vary as bone elasticity decreases, elucidating perimortem and postmortem events more reliably than macroscopic analyses. Thirty-seven unembalmed, defleshed human femoral shafts from male (n=18) and female (n=2) donors aged 33 to 81 years were fractured at experimentally induced PMIs ranging from 1 to 60 warm weather days (250–40,600 accumulated degree hours, or ADH). Temperature and humidity were controlled using a gravity convection oven to simulate PMIs. The bones were fractured with a drop test frame using a three-point bending set-up. Sensors were used to calculate fracture energy, and high-speed photography was used to document fracture events. SEM micrographs were collected from the primary tension zones of each fracture surface. A region of interest was defined within the center of the primary tension zone, and three microscopic fracture characteristics were scored: percentage of delaminated osteons, percentage of osteon pullout, and number of microfractures. The following variables were recorded for each sample: PMI length in ADH, temperature, humidity, collagen percentage, water loss, fracture energy, age, sex, cause of death, and microscopic fracture feature scores. Bone mineral density (BMD) and cortical bone thickness (CBT) were calculated from computed tomography scans of the bones using regions of interest placed at 90° intervals around a cross-section of the shaft. Multiple linear regression showed that osteon pullout, delamination, and microfractures are strong predictors of ADH (adjusted $R^2 = 0.90$, F-statistic = 95.29 on 3 and 33 DF, $p<0.001$), BMD (adjusted $R^2 = 0.72$, F-statistic = 32.99 on 3 and 33 DF, $p<0.001$), CBT (adjusted $R^2 = 0.70$, F-statistic = 28.52 on 3 and 33 DF, $p<0.001$), and water loss (adjusted $R^2 = 0.71$, F-statistic = 26.23 on 3 and 33 DF, $p<0.001$) but weak predictors of collagen percentage (adjusted $R^2 = 0.10$, F-statistic = 2.31 on 3 and 33 DF, $p = 0.09$). Although BMD, CBT, and water loss play significant roles in microscopic fracture appearance, collagen percentage does not. This may be because the collagen has not begun to degrade significantly prior to 40,600 ADH. Nonetheless, despite collagen retention, postmortem water loss affects elasticity considerably. The hypothesis that microscopic fracture surface characteristics visible on SEM are more predictive of fracture timing than macromorphological characteristics was accepted. Microscopic fracture surface analysis detects the biomechanical effects of decreased elasticity more reliably and with greater sensitivity than macroscopic analysis. In conclusion, SEM analysis of bone fracture surfaces is a promising technique for distinguishing perimortem and postmortem fracture events.

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SESSION ABSTRACTS

SESSION III

SEIZED DRUGS AND TOXICOLOGY

Moderated by NIJ Program Manager
Frances Scott

Non-contact Detection of Fentanyl and Other Synthetic Opioids: Toward a Generalized Approach to the Detection of Dangerous Drug Classes

NIJ AWARD #: 15PNIJ-22-GG-04418-RESS

Lauryn DeGreeff*,[1]
Thouli Jayawardana[1]
Ashley Fulton[2]
Braden Giordano[2]
Stephanie Vaughan[2]

[1] Florida International University

[2] U.S. Naval Research Laboratory

* Presenting author

Field-portable detection of fentanyl has become increasingly imperative in recent years. The opioid epidemic is at its deadliest with the increased fentanyl adulteration of commonly used drugs. Currently, the recommendation for preventative exposure of first responders is to wait for trained personnel to handle suspected scenes. This method is time-consuming and costly. The handheld ion mobility spectrometer (IMS) offers a quick and user-friendly method for the presumptive detection of fentanyl. Through headspace analysis using solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS), N-phenylpropanamide (NPPA) was identified as a target analyte in the vapor identification of fentanyl. A method was developed on the handheld IMS using NPPA as the surrogate vapor and successfully identified clandestine samples containing >6% fentanyl. However, the method was unsuccessful at lower or unknown concentrations. To improve sensitivity metrics, a functionalized silicon nanowire (SiNW) array for the pre-concentration of vaporous compounds was developed. Acrylate-based polymers were screened to determine pre-concentration efficiency using a quartz crystal microbalance. The optimal polymer was selected and deposited onto the SiNW array and incorporated into a miniaturized pre-concentrator for mobile devices. This presentation describes the studies leading up to and including the incorporation of the SiNW array pre-concentrator to the handheld IMS. The SiNW-IMS system was able to accurately identify the target analyte, NPPA, at trace levels. Heavily diluted samples with concentrations less than 5% fentanyl were also accurately identified. Limit of detection studies and testing of street-grade samples are currently being conducted.

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Expert Algorithm for Substance Identification Applied to the Tandem Mass Spectra of Seized Drugs

NIJ AWARD #: 15PNIJ-21-GG-04179-COAP

Glen P. Jackson*,[1]
Alex Adeoye[1]
Emily Ruiz[1]
J. Tyler Davidson[2]

[1] West Virginia University

[2] Sam Houston State University

* Presenting author

In previous work, this research group has demonstrated that the expert algorithm for substance identification (EASI) can be used to model and explain more than 90% of the variance in the branching ratios in replicate electron-ionization mass spectra of cocaine, fentanyl analogues, and cathinones. Ongoing work has also included a foundational relationship with unimolecular reaction (fragmentation) rate theory and is most suitable for distinguishing substances from their spectrally similar analogues. EASI provides superior binary classification rates than existing algorithms for electron-ionization mass spectrometry (EI-MS) data. Here, the algorithm is extended to tandem mass spectra obtained from protonated molecular ions, such as from electrospray ionization (ESI) and direct analysis in real time (DART). In one example, replicate DART-MS/MS spectra were collected for tetrahydrocannabinol (THC) and cannabidiol (CBD) on a triple quadrupole MS at three different collision energies. At each energy, the tandem mass spectra for CBD and THC are visually indistinguishable, so manual classification rates are no better than a coin flip at 50%. After splitting the data into a training set and test set, conventional algorithms that use a consensus spectrum as the exemplar at each collision energy provide classification accuracies of 61%–90%, depending on the collision energy. In contrast, EASI applied to the same dataset provides classification accuracies of 86%–96%. EASI classification rates are also superior to binary classification using the Mahalanobis of each spectrum relative to the training set. Applying EASI to replicate tandem mass spectra from ESI-MS/MS instruments provides superior levels of discrimination, with binary classification rates typically exceeding 99% for structurally related opioids.

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Retinal Cannabinoids: Measures of Function and Impairment

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There is no direct relationship between measures of cannabinoids in fluids and impairment. There needs to be both a measure of presence of cannabinoids and a separate measure of impairment related to the ability to safely drive a motor vehicle or perform tasks. Measures of impairment need to be objective, free of racial bias, and easily performed on the roadside by law enforcement professionals or in the workplace. Saliva is an efficient means to detect cannabinoids; however, the researchers propose a simple retina test of impairment. This research demonstrates the efficacy of using visual retinal dysfunction as an indicator of impairment with cannabis use. Retinal function was assessed using a PicoNeo3 virtual goggle with eye tracking to measure the functional responses related to contrast and temporal processing. The technology has backlit-striped, 10°-sized squares of variable contrast flashed on a fixed, flat virtual reality (VR) screen. The stripes are of low spatial frequencies that undergo counterphase flickering at a high temporal frequency. The contrasts of the squares are variable. Squares are flashed in 19 locations. The presenter is reporting retinal results from one participant, a chronic cannabis user. The researchers collected multiple variables, including the Fitzpatrick Pigment Scale as an additional variable related to race and genetics. There is a substantial difference in retinal pigmentation across different populations, and it is essential that any technology used by law enforcement is not impacted by such variables. The researchers used a validated cannabis use questionnaire to determine categories of marijuana use: early initiators, chronic users, and casual users. Saliva and blood were taken each visit. This study identified cannabinoids, Δ9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-carboxy-THC in the blood of users, and the amount at both baseline and with acute use was related to frequency of use. The volume of cannabinoids detected after consumption was considerably greater and had a relationship to the post-consumption blood sample. The researchers evaluated the retina before dosing and after dosing. The retinal findings were related to the presence of blood cannabinoids at baseline and after dosing. The use of retinal response as a measure of actual impairment from cannabis use is promising, and further research will provide insight into how the retinal response along with the blood or other biomarkers of THC can be used to further differentiate the impairment and differences of chronic heavy recreational users, medicinal users, and casual recreational cannabis users.

Denise A. Valenti*,[1]
David Calixte[2]
Trevor Kopp[2]
Rhoda Nankabirwa[2]
Xueling Zou[3]
Holly Kailher[4]

[1] Impairment Measurement Marijuana and Driving (IMMAD) LLC

[2] University of Massachusetts Boston

[3] New England College of Optometry

[4] University of Rhode Island

* Presenting author

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Prevalence of Fentanyl and Its Analogues in a Court-Ordered Mandatory Drug Testing Population

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Katherine Bollinger*
Megan Grabenauer
Nichole D. Bynum
Jeri D. Ropero-Miller
Nicolas Richardson
David Heller
RTI International
* Presenting author

The prevalence of fentanyl and its analogues in the criminal justice system is relatively unknown, although drug testing is frequently conducted in correctional settings. Court-ordered mandatory drug testing (COMDT) using hair is routinely done at large commercial laboratories but does not include testing for fentanyl and its analogues. Limited information is available on prevalence data in the COMDT population to characterize drug use patterns. Phase I of this study analyzed over 400 hair specimens for fentanyl; a selection of fentanyl analogues; and other drugs such as cocaine, methamphetamine, and codeine by liquid chromatography-tandem mass spectrometry (LC-MS/MS). These hair samples were submitted from a COMDT laboratory and previously analyzed at their laboratory from November 2020 through February 2021. Any hair specimens that were positive for opioids on the LC-MS/MS were also analyzed by nontargeted high-resolution mass spectrometry. Hair specimen positivity rates in COMDT were calculated with and without inclusion of fentanyl and fentanyl-related compounds to determine the effect on overall positivity rate when fentanyl targets were included in the hair-testing protocol. Phase II of this study was a retrospective analysis of 5 years of COMDT data from oral fluid and hair collected from 2015 to 2019 in nationally represented COMDT programs. A random, national COMDT sampling of 959,237 oral fluid test results and 65,645 hair test results was analyzed. Specimens in the historical dataset were tested for misused substances by screening with immunoassay and confirmatory testing was performed on a subset of oral fluid and all hair positive specimens. The prevalence of positive drug tests among different demographic groups of the analysis pool and the positivity rates of oral fluid confirmation with and without fentanyl were calculated.

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SESSION ABSTRACTS

SESSION IV

FORENSIC BIOLOGY/DNA

Moderated by NIJ Program Manager
Tracey Johnson

Improved Nucleic Acid Recovery From Trace and Degraded Samples Using Affinity Purification

NIJ AWARD #: 15PNIJ-21-GG-04149-RESS

Arati Iyengar*,[1]
Coral Smith[1]
Michael Rishel[2]
Chrystal Chadwick[2]
John Nelson[2]
Brian Davis[2]

[1] West Virginia University

[2] GE HealthCare Technology & Innovation Center

* Presenting author

Analysis of challenging samples such as trace or degraded DNA is becoming increasingly common in forensic laboratories. Optimization of DNA recovery from these types of samples is essential to downstream profiling success because only nanogram or sub-nanogram quantities of DNA may be available. There are several commercial kits on the market designed and optimized to extract DNA from such samples; however, these products isolate DNA and disregard all other components within the sample. It is well-documented in relevant literature that other components of a biological sample, such as mRNA and proteins, can hold a wealth of information that can provide investigators with insight into the context of a crime. The sensitivity of short tandem repeat (STR) DNA typing kits has dramatically increased in recent years, to the point where a full DNA profile can now be recovered from trace samples containing only a few cells. Because of this, the source of the DNA profile is being questioned in court, and methods have been developed to link a DNA profile recovered from evidence to a specific body fluid stain by profiling the coding single-nucleotide polymorphisms (SNPs) in mRNA. Additionally, genetically variant peptides are useful when DNA profiles are inadequate. This study aims to develop a novel method to recover the entirety of the nucleic acids in the sample while retaining analytes such as proteins and small molecules for additional analysis using a highly efficient nucleic acid binding vector embedded onto the surface of a paramagnetic bead in one efficient, streamlined workflow. This method has demonstrated highly efficient capture (>99%) and elution (>93%) using spiked DNA. Similar efficiencies were obtained with severely degraded (24–500 base pairs) and low template DNA (1 ng–200 pg), which often present significant challenges when using traditional extraction methods. When compared with a popular commercial magnetic bead–based DNA extraction kit, the new method performs equally well, with the added benefit of multi-analyte retention. The researchers have observed highly efficient capture (>95%) and elution (>89%) of spiked RNA and developed protocols for co-elution and differential elution of DNA and RNA. Protocols are being developed for DNA and RNA recovery from multiple body fluids and case type samples, achieving DNA recoveries >1 ng from trace blood, saliva, semen, and thumb touch samples. Multianalyte recovery of DNA and RNA from semen provided successful STR DNA profiling and amplification of a body fluid–specific mRNA marker. Experimentation is underway for blood, saliva, and touch deposits on glass and polypropylene surfaces and after environmental exposure such as heat and ultraviolet light. With continued optimization, the new method presents great potential for successful recovery of multiple analytes from trace biological samples.

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Forensic STR Sequencing Nomenclature Resource

NIJ AWARD #: DJO-NIJ-22-RO-0004

Katherine B. Gettings*,[1]
Lisa Borsuk[1]
Martin Bodner[2]
Jonathan King[3]

[1] National Institute of Standards and Technology

[2] Medical University of Innsbruck

[3] University of North Texas Health Science Center

* Presenting author

This presentation will inform attendees of resources that have been developed via National Institute of Justice funding to support the recently published International Society for Forensic Genetics (ISFG) Short Tandem Repeat (STR) Sequence Nomenclature recommendations. These resources include updates to and expansion of the STRSeq BioProject, tools for STR sequence exploration, and new capabilities for the STRidER allele frequency database. The STRSeq BioProject is a collection of forensic STR sequence GenBank records that was initiated in 2017. Records were added rapidly between 2018 and 2021 based on STR population sample sequencing data generated by the National Institute of Standards and Technology (NIST) Applied Genetics group and partner laboratories. These early records were annotated following the 2016 ISFG DNA Commission Report on STR Sequence Nomenclature Considerations. The recently published 2023 ISFG DNA Commission Report on STR Sequence Nomenclature Recommendations contains new guidance on formatting; thus, all existing STRSeq records (>2,500) are undergoing annotation and metadata updates. In addition, nearly 30 publications of STR sequence population data that have not been considered for STRSeq were identified. From these, an additional >500 GenBank records for new (unique to STRSeq) STR sequences are expected. A suite of tools that are designed to facilitate user access to STR sequences formatted according to the 2023 nomenclature recommendations will also be presented. The Forensic Sequence STRucture Guide, released alongside the 2023 recommendations, is an extensive downloadable Excel file that overlays forensic annotation onto the relevant STR sequences from the GRCh38 Human Genome Reference sequence. Additional annotations include SNPs that users will likely encounter in their STR sequences and overlap between sequence ranges in commercially available STR sequencing kits. Additionally, sequence access and exploration are being improved by updates to the NIST STRBase website, including incorporating STRSeq GenBank records into more easily navigable webpages, developing a string search tool so that users can determine if the sequence(s) of interest are present in STRSeq, and developing an interactive tool for exploring the Forensic Sequence STRucture Guide. Finally, planned updates to the STRidER allele frequency database will be presented, specifically including the capability of serving out sequence-based STR allele frequency data to facilitate the generation of STR sequence-based match statistics and likelihood ratios.

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Comparative Evaluation of Massively Parallel Sequencing STR Kits With the Emphasis on Mixture Deconvolution Utilizing Probabilistic Genotyping

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Elisa Wurmbach*
Vishakha Sharma
Erin Butler

New York City Office of the Chief Medical Examiner

* Presenting author

The technique of individual identification in modern forensics, DNA typing of short tandem repeats (STRs), has brought a standardized, quantitative method with strong statistical underpinnings to the criminal justice system. Although the fundamental principles behind STR typing have not changed, newly developed instrumentation and informative biological markers have the potential to address the limitations of current techniques and improve throughput at lower costs. The forensic community has begun to evaluate massively parallel sequencing (MPS) to overcome these problems. MPS not only adds additional sequencing information but has a nearly unlimited capacity for additional STRs and single-nucleotide polymorphism markers, thereby enhancing individual identification. In addition, amplicons can be designed to be the shortest possible length, making them more useful for degraded samples. One of this project's objectives was to evaluate the recently released ForenSeq™ MainstAY kit. This MPS kit tests for Amelogenin, 26 autosomal STRs, and 25 Y-STRs. So far, the researchers have performed several experiments to analyze different conditions, including benchmark (which is defined as following the recommendations of the manufacturer), sensitivity, degraded DNA, throughput, and two-person mixtures. These experiments were executed using both the recommended micro-flow cell and the standard flow cell. The two flow cells differ in their capacity, sequencing time, and cost. Knowing how they work will provide more flexibility in the experimental design. Although data analysis is still ongoing, it seems that the standard flow cell resulted in higher coverage and showed slightly better outcomes than the micro-flow cell.

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Comparative Assessment of Emerging Technologies for Body Fluid Identification

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Mirna Ghemrawi

Center for Forensic Science Research and Education

Biological fluid detection and identification provides important contextual information to a forensic investigation. Although genetic testing can help establish from whom DNA may have come, only serological testing can provide an indication of the body fluid or tissue from which a DNA profile may have originated. The goal of this project was to compare different approaches to body fluid identification, including DNA methylation, mRNA, and proteomic-based methods. This was performed by preparing identical sets of simulated forensic samples and sending them to three different research groups, each specializing in one of these procedures. The researcher also compared these results with contemporary immunochromatographic procedures. The goal of this research was not to determine which procedure was optimum but instead to produce a time stamp showing the current state of the art and revealing the progress to date. Furthermore, the results from this study provide future directions in terms of developing and further optimizing each process. In this study, a comparative assessment of these emerging "omics"-based technologies for body fluid identification (e.g., epigenome, transcriptome, proteome) was performed on four replicates of 58 blind samples. Peripheral blood, menstrual blood, semen, vaginal fluid, saliva, breast milk, urine, nonhuman samples, and nasal secretions were deposited onto diverse substrates, including denim, leather, and cotton, with volumes as small as 2.5 μL. Some of these samples underwent intentional degradation and inhibition treatments. To assess the performance of each assay, a comprehensive analysis encompassing specificity, sensitivity, and error rates was conducted. The obtained findings also shed light on the capability of contemporary serologic techniques versus emerging technologies. The new omics-based procedures were found to be highly specific with results providing >99.5% specificity and lower error rates than conventional immunochromatographic assays. Additional research and validation studies still remain for these procedures; however, the researcher notes that the technology has been widely heralded and applied in medical diagnostics, and its implementation in forensics is long overdue. The comparative assessment of the strategies discussed in this study provides valuable information to the forensic community, which can aid in the development of new research and facilitate technology transfer.

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POSTER ABSTRACTS

SESSION I

PHYSICS AND PATTERN INTERPRETATION/TRACE EVIDENCE

Physics and Mathematical Models for Error Quantifications in Comparative 3D Microscopy for Physical Match Analysis

NIJ AWARD #: 15PNIJ-21-GG-04141-RESS

Ashraf F. Bastawros*,[1]
HyeokJae Lee[1]
Joshua Berlinski[1]
Ranjan Maitra[1]
William Meeker[1]
Lauren Claytor[2]
John Vanderkolk[3]

[1] Iowa State University

[2] Virginia Department of Forensic Science

[3] Indiana State Police Laboratory (Retired)

* Submitting author

3D microscopy provides a means to analyze distinctive microscopic patterns inherent in fractured surfaces, aiding forensic match analysts in mitigating the subjectivity associated with comparative microscopy in forensic evidence examination. Despite its utility, the repeatability and reproducibility of features generated by 3D microscopy have been insufficiently investigated, with limited understanding of the impacts of the microscope operator or sample alignment on the measurement system. The topography imaging process introduces various sources of variation, encompassing those from the 3D microscope, equipment operator, and physical characteristics of the measured fracture surface. To assess the measurement system's quality, steel rods broken under tensile or bending loading conditions are generated. Five inexperienced microscope operators are trained, and they repetitively image pairs of matching surfaces akin to comparative microscopy. After the third imaging iteration, each operator utilizes a fixture to align the fractures during imaging. The topologies of matching images yield a multivariate similarity measure for the two surfaces, incorporating the aforementioned sources of variation. A gauge repeatability and reproducibility (R&R) model is employed to scrutinize these diverse sources. The model formulation details are presented within the context of the available data for each fracture method. The resultant sources of variation are discussed, highlighting differences for each dataset in the context of physical distinctions between fracture types. Notably, although all matches and nonmatches are correctly classified irrespective of the imaging fixture, gauge R&R reveals that utilizing the fixture enhances the measurement system by minimizing within-operator variability. These findings offer insights into the quality of the dataset acquired by 3D microscopy measurement systems, establishing a valuable framework for training automated fracture matching algorithms. Moreover, they provide guidance on enhancing imaging processes and procedures for various fractured surfaces encountered in crime scenes.

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Physics and Statistical Models for Physical Match Analysis Utilizing 3D Microscopy of Fracture Surfaces

NIJ AWARD #: 2018-R2-CX-0034

Ashraf F. Bastawros*,[1]
HyeokJae Lee[1]
Joshua Berlinski[1]
Ranjan Maitra[1]
William Meeker[1]
Lauren Claytor[2]
John Vanderkolk[3]

[1] Iowa State University

[2] Virginia Department of Forensic Science

[3] Indiana State Police Laboratory (Retired)

* Submitting author

Forensic fracture matching relies on the principle that no two objects break identically because of the variables involved in the fracture process. The development of objective comparisons starts with imaging the topological features of the fracture pairs and performing mathematical and statistical comparisons. The scales of the fracture topologies are rooted in the principles of fracture mechanics. For hardened metals, commonly examined in the forensic laboratory, plastic flow is suppressed in favor of brittle fracture. The fracture or failure commences when the fracture strength is exceeded over a characteristic distance from the crack tip. This fracture characteristic scale is about two grain diameters in size for materials with grain-like structures. Within this scale of observation, the fracture surface roughness exhibits self-affine scaling properties. The fracture surface character becomes more complex and non–self-affine at larger length scales, exhibiting unique roughness characteristics dictated by the material intrinsic properties, microstructure, and exposure history to external forces. The researchers exploited this deviation scale to ascertain the individuality of a pair of fracture fragments. The researchers used 3D microscopy to map the fracture surface's topological details at a scale about 10 times the fracture process scale and employed surface spectral analysis, using the mathematical framework of fast Fourier transforms to identify these critical scales. The researchers applied the statistical correlation analysis and statistical learning tools to develop a classification rule for matching and nonmatching. The study found that this scale is about two grain–sized or micro feature–sized, rendering the required imaging scale to be about 20 times of such scale. Multivariate statistics were employed to develop quantitative topological descriptions, evaluated from 3D spectral analysis of overlapping topographical images, to provide premise of uniqueness for forensic comparisons. The statistical learning tool performance is tested on a robust training dataset and validated on a set of 38 different broken pairs of either knives broken in bending, or stainless-steel rods with similar grain sizes, broken in tension or bending. The generality of the framework under different modes of loading is examined by application to a set of 10 twisted knives to failure. All broken pairs were classified with very high accuracy. The framework lays the foundations for forensic applications with quantitative statistical comparison across a broad range of fractured materials with diverse textures and mechanical properties.

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Evaluation of Mobile Technology for Detection of Inorganic and Organic Gunshot Residues in Firearms-Related Investigations

NIJ AWARD #: 2020-DQ-BX-0010

Kourtney Dalzell*
Leah Thomas
Madison Lindung
Tatiana Trejos
Luis Arroyo
West Virginia University
* Submitting author

Firearms-related incidents represent one of the major outcomes of gun violence and a pervasive and ongoing societal issue bringing death and injuries to a significant portion of the population. The investigation of such events is initiated at the scene of the incident. Once specimens are collected from suspects or materials, they are usually sent to laboratories for processing, an inquiry that may take several weeks to be finalized. Therefore, forensic services can greatly benefit from effective real-time screening methods that can be implemented at the crime scene. In recent years, research efforts have boosted mobile devices as practical alternatives, offering rapid screening capabilities to improve workflow in forensic laboratories. Indeed, laboratories rely upon their interpretation using the gold standard in gunshot residue (GSR) detection, scanning electron microscopy–energy dispersive X-ray spectroscopy. However, the surface interrogation of the unknown material in pursuit of particles and later spectroscopic analysis can take 2–8 hours per sample, and no organic information is obtained. This research group proposed using electrochemistry and laser-induced breakdown spectroscopy (LIBS) as complementary methods to bridge these gaps and provide screening methods that may aid in more timely analysis, improved decision-making, and triage from scene to laboratory. In the last decade, this research group has developed LIBS methods for GSR applications as a rapid, reliable technology that can streamline laboratory and crime scene processes. This poster compares a laboratory LIBS unit to a mobile instrument using authentic hand samples from shooters (100 samples) and nonshooters (200 background samples). A significant novelty of the mobile instrument is its enhanced imaging, which allows quick searching and visualization of GSR particles for single micron–sized particle examination. Both instruments obtained accuracy better than 98.8%, demonstrating their suitability for trace inorganic GSR detection from skin specimens. This study evaluates LIBS capabilities for GSR detection in other substrates commonly encountered at a crime scene and is tested in mock crime scene situations. Also, due to their rapid, cost-efficient, and compact platform, electrochemical methods using disposable screen-printed carbon electrodes are proposed for GSR screening at the laboratory and point of care. GSR residues were extracted from typical aluminum/carbon adhesive collection stubs and analyzed via square-wave anodic stripping voltammetry. Both benchtop and mobile electrochemical instruments were compared for the assessment and classification of authentic shooter samples by monitoring a panel of inorganic and organic GSR elements and compounds, including lead, antimony, copper, 2,4-dinitrotoluene, diphenylamine, nitroglycerin, and ethyl centralite. Performance rates obtained by assessing authentic hand samples collected from over 100 known shooter and 200 nonshooter samples compared to their benchtop counterparts will be presented. Results demonstrated the accuracy of the mobile electrochemical and LIBS instruments at 96.5% and 98.9%, respectively, for correctly classifying a sample as positive for GSR. The capabilities and limitations of these devices were further evaluated with mock case scenarios that simulated common firearm-involved situations and assessed the workflow for using the two methods in succession for the screening of GSR. These results highlight the potential for mobile devices as a viable option for rapid and reliable GSR detection.

Assessing the Reliability of Modern µXRF Technology for Expanded Impact on the Forensic Examination and Interpretation of Trace Evidence: Glass Evidence

NIJ AWARD #: 15PNIJ-22-GG-03571-SLFO

Troy Ernst*,[1]
Zachary Andrews[2]
Tatiana Trejos[2]
Cedric Neumann[3]
Ruthmara Corzo[4]

[1] Michigan State Police, Grand Rapids Forensic Laboratory

[2] West Virginia University

[3] Battelle Memorial Institute

[4] National Institute of Standards and Technology

* Submitting author

Glass fragments are among the trace materials most often submitted to forensic laboratories, as their physical and chemical examination can provide valuable information in forensic investigations. These disciplines use state-of-the-art methodologies that hold substantial scientific grounds and consensus-based protocols. However, some challenges come in hand with the advancements of modern technology and the changing manufacture of mass-produced materials. For example, micro-X-ray fluorescence (µXRF) is an elemental analysis technique widely used in forensic laboratories that recently experienced a significant shift in their detection systems (e.g., silicon drift detectors [SDDs] vs. silicon lithium detectors). Among the advantages of SDDs are improved sensitivity and precision, which can lead to superior informing and discriminating power. However, the lagging of research in this area has not caught up with the rapid adoption of the new technology by public laboratories, exposing them to potential increased error risks. Moreover, the manufacture and global marketing of glass has evolved in past years, leaving a void in current literature and datasets based on decades-old collections and instrumentation. Another challenge with µXRF examinations is the subjectivity associated with spectral data interpretation. Thus, the trace community and organizations such as the National Institute of Standards and Technology Organization of Scientific Area Committees and the Department of Justice Forensic Science Technology Working Group have identified these research needs as a high priority. This project's overarching goal is to address these immediate operational needs by developing and validating improved protocols for collecting, examining, and interpreting contemporary glass evidence. To achieve that, this multidisciplinary team (forensic practitioners in a publicly funded laboratory, forensic researchers, and statisticians) proposes to (1) identify the significant sources of variability when using modern µXRF SDDs and provide specific sampling and interpretation recommendations for soda-lime glass casework comparisons; (2) assess the accuracy, discrimination, and informing power of elemental analysis of µXRF SDDs for contemporary broken glass from portable electronic devices; and (3) validate objective and quantitative metrics for µXRF spectral comparison and probabilistic interpretations of glass evidence. The creation of an extensive dataset of over 4,000 µXRF glass spectra is used to test error rates, providing a one-of-a-kind repository that can strengthen the current foundations and modernize standard methods. Findings on datasets of vehicle glass windshields and architectural glass originating from the same source (same windowpane) and different sources are reported in this poster for small full-thickness glasses (~1–2 mm thick) and smaller irregular glasses (~30–50 µm thick) to simulate worst-case scenarios where the sample size is limited. The use of quantitative similarity metrics is also evaluated as a more objective means to compare spectral data. This study is anticipated to lead to best practices for more efficient and objective decision-making processes and increased reliability in the analysis and interpretation of physical evidence and has already provided relevant input to an updated version of the ASTM E2926 standard method. The project deliverables are designed to have maximum impact and become rapidly adopted by forensic laboratories.

Elucidation of the Effect of Heat and Sun Exposures on Hair Colored by Permanent and Semi-Permanent Colorants Using Surface-Enhanced Raman Spectroscopy (SERS)

NIJ AWARD #: 15PNIJ-21-GG-04169-RESS

Dmitry Kurouski*
Aidan P. Holman
Mackenzi Peterson
Texas A&M University
* Submitting author

Confirmatory identification of hair colorants can be used to establish a connection between a suspect and the crime scene or demonstrate the absence of such connections. A growing body of evidence shows that surface-enhanced Raman spectroscopy (SERS) could be a confirmatory, minimally destructive, and fully noninvasive analysis of hair colorants. In SERS, a signal provides information about the chemical structure of both permanent and semi-permanent dyes present on hair and is enhanced a million-fold using noble metal nanostructures. However, it is unclear whether the information of hair colorants can be revealed if hair was contaminated or exposed to harsh environments such as sunlight and heat. This poster will discuss the effect of short- and long-term heat exposure on SERS-based analysis of hair colored with blue and red permanent and semi-permanent dyes. The poster will also discuss the extent to which water and ultraviolet radiation can alter SERS-based accuracy in identification of colorants on hair. The results show that heat, ultraviolet radiation, and water exposure of colored hair causes chemical changes in the dyes, which results in significant changes in the spectroscopic signature of these colorants. Therefore, the effect of environmental factors should be strongly considered upon their SERS-based examination to avoid both false-positive and -negative identification of chemical dyes.

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Assessing the Reliability of Modern µXRF Technology for Expanded Impact on the Forensic Examination and Interpretation of Trace Evidence: Tape Evidence

NIJ AWARD #: 15PNIJ-22-GG-03571-SLFO

Lacey Leatherland*,[1]
Charlotte Vogler[1]
Zachary Andrews[1]
Tatiana Trejos[1]
Ruthmara Corzo[2]
Cedric Neumann[3]
Troy Ernst[4]

[1] West Virginia University

[2] National Institute of Standards and Technology

[3] Battelle Memorial Institute

[4] Michigan State Police, Grand Rapids Forensic Laboratory

* Submitting author

Recent advances in micro-X-ray fluorescence spectroscopy (µXRF) technology provide increased sensitivity and precision compared with other methods of analysis, such as scanning electron microscopy–energy-dispersive spectroscopy (SEM-EDS). At the same time, this new XRF technology is cost-effective and more widely adopted by public laboratories for other trace material analyses. However, µXRF systems equipped with modern silicon drift detectors are underused for tape evidence. Moreover, current analytical standards have not progressed as swiftly as the technology. Thus, organizations such as the National Institute of Standards and Technology Organization of Scientific Area Committees and the American Society of Trace Evidence Examiners have identified the need to develop and normalize objective means of interpreting and comparing spectral data to provide a basis for the development of consensus-based criteria standard methods. This study evaluates the accuracy, discrimination, and informing power of the µXRF elemental analysis of electrical tapes. This is accomplished by modern µXRF silicon drift detector systems to assess the variability within and between rolls of electrical tapes and through an interlaboratory study to evaluate inter-examiner and instrumental variability. The study also reports performance rates of various quantitative spectral comparison methods and further validates them through the examination of a contemporary dataset of 50 tapes originating from different sources, including a variety of manufacturers (3M™, DiversiTech®, Shurtape®, NSI Industries™, Utilitech™, Intertape Polymer Group™, Amazon®); countries of origin (United States, China, Taiwan, Poland); quality grades (high, medium, low); and label brands (Scotch®, Temflex™, Morris Products™, Duck Brand®, Shurtape®, Easy-Wrap™, WarriorWrap®, Utilitech™, Intertape Polymer Group™, 3M™, AmazonCommercial®). In addition, five rolls from this set were selected to analyze intra-roll homogeneity and three rolls to assess variability within a package of multiple rolls. A subset of 10 tapes was distributed for an interlaboratory study within eight participating laboratories. This dataset serves as a repository to provide a more robust foundation for current XRF examinations and provides examiners with a more objective means of spectral interpretation. Spectral data are evaluated using spectral overlay, comparison intervals of integrated peak ratios, and a spectral contrast angle ratio (SCAR). The SCAR method is advantageous because it provides a quantitative metric of the level of similarity considering the within- and between-source variations. SCAR values below 1.5 are typically observed for same-source samples, whereas larger ratios are observed as the spectral differences increase (>1.5 to 60). Recommended comparison criteria are provided based on the lowest combination of false exclusion and false inclusion error rates. Good discrimination power (91%–93%) and accuracy (92%–94%) are observed, depending on the comparison criteria. In this poster, the researchers report and compare various sources of variation in the data (i.e., within roll, between packages, between rolls, inter-examiner/instrumental variation). Finally, the effects of common fingerprint development chemicals on the elemental profiles of electrical tape are presented along with recommendations for proper workflow for tape evidence examinations across multiple disciplines (i.e., trace, DNA, fingerprints).

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Enhancing Fire Pattern Analysis With Experiments on Architectural Finishes Impact and Developing Data-Driven Tools

NIJ AWARD #: 15PNIJ-22-GG-04442-RESS

Shuna Ni*,[1]
Stanislav I. Stoliarov[1]
Pengkun Liu*,[2]
Pingbo Tang[2]

[1] University of Maryland, College Park

[2] Carnegie Mellon University

* Submitting authors

This project studies the effects of architectural finishes on fire patterns and uses the resulting test data in combination with other data to develop data-driven tools for quantitative fire pattern analysis. Interpreting fire patterns on walls and other surfaces is integral to investigating fire incidents. Architectural finishes may potentially influence these patterns. Unfortunately, prior studies of fire patterns did not account for the impact of such finishes on the results of fire pattern analysis or conclusions drawn from this analysis, despite fires frequently occurring in enclosures with finished surfaces. This project seeks to fill this knowledge gap by conducting extensive fire experiments in burn cells with drywalls featuring a range of common architectural finishes, including paints varying in sheen level, color, and chemical composition, and various wallpapers. This project focuses on plume-generated fire patterns. Some tests will also incorporate the effect of sprinkler water. A comparative analysis of all the test results is ongoing, focusing on (1) damage features of a burn cell's fire-exposed surfaces, (2) geometry features of a fire pattern, (3) spatial distribution of fire patterns over the surfaces of a burn cell, and (4) drywall calcination contour maps. Those comparisons have allowed us to (1) identify whether architectural finishes magnify or diminish the degree of fire damage, taking into account both surface damage features and drywall calcination; (2) determine if architectural finishes have a significant impact on the overall geometrical shapes of a fire pattern; and (3) determine if the impact of architectural finishes is reflected in the size of a fire pattern, even if its overall geometrical shape remains consistent. The underlying mechanisms of how architectural finishes influence fire patterns can be accurately described by examining the variances in fire test data (e.g., burning process, temperature, heat flux, and mass loss) and by correlating those data with the properties of architectural finishes such as flammability and thermal inertia. In addition to experiments, the researchers are developing data-driven tools for fire pattern analysis to enhance the traditionally qualitative process, using both experimental data from the previously mentioned tests and other sources and synthesized data. The data-driven tools developed in this project encompass three primary functions: (1) fire damage evaluation models to assess surface fire damage, applicable to both finished and unfinished drywall surfaces; (2) fire pattern classification models to categorize a fire pattern's shape (e.g., triangular, columnar, conical); and (3) spatial feature extraction and reasoning models to extract the spatial relationship among multiple fire patterns in a room. The first and the second functions use 2D images but map results onto a 3D reconstructed fire scene, while the third function extracts spatial information from 3D images. The extraction of a fire pattern from a fire scene is conducted using a human–computer interaction approach with reasoning segmentation via a large language model. The information provided by these data-driven tools offers crucial insights into the development of a fire within a compartment and helps fire investigators determine the origin of a fire.

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Analysis of Small Particles Adhering to the Edges of Duct Tape as a Means to Make Associations in a Way That Is Independent of Manufactured Characteristics

NIJ AWARD #: 2020-MU-CX-0018

David A. Stoney*
Paul L. Stoney
Stoney Forensic, Inc.
* Submitting author

Forensic analysis of duct tape is important for the investigation and prosecution of major crimes, where it is used as blindfolds, bindings, and ligatures. Methods are needed that go beyond comparison of manufactured characteristics of duct tape rolls. Once tape rolls are put into use, exposed adhesive along tape edges presents an ideal opportunity for collection of the very small particles (VSPs) that are ubiquitous in the environment. The researchers have previously reported the development of effective methods for recovering VSPs from the edges of duct tape and the differentiation of environmentally acquired VSPs from the particles present as part of the manufactured adhesive composition. Thirty rolls of partially used silver-colored duct tape were collected from residences within Fairfax County, VA. VSPs were harvested from the tape edges using the previously reported methods. VSPs attributable to the adhesive itself were differentiated after which the acquired VSPs were used, along with previously reported methods of particle combination analysis, to test the ability to (1) discriminate among the 30 tape rolls and (2) link tape segments to rolls. Discrimination among tape rolls was tested by separating the acquired tape edge VSPs from each roll into training and test datasets. Using classification criteria developed from the training datasets, test datasets were correctly classified for 96.7% of the rolls (29 of 30). Linking tape segments to rolls was tested using the same classification criteria. Separate comparisons were made for left edge, right edge, and combined VSP datasets. There were 100% correct associations (30/30) when all three comparisons indicated the same source and 90.9% correct associations (40/44) when two or three comparisons indicated the same source. The correct source was identified by one or more comparisons 53 of 60 times (83.3%). This project has established that once rolls of duct tape have been put into use, the exposed adhesive on the edge of the duct tape roll collects and retains VSPs. These particles, acquired post-manufacture, are a source of individuality for the specific roll of tape, providing a means to distinguish it from the many other rolls of the same make and manufacture. Particle combination analysis of acquired tape edge VSPs allows effective discrimination among duct tape rolls and provides quantitative associations linking tape segments to source rolls. These findings address the requirement that associations be based (in part) on post-manufacture acquired characteristics. The impact is significant due to the frequent occurrence of duct tape evidence in major crimes. This project has also resulted in new methods for (1) the efficient and effective recovery of acquired tape edge particles from duct tape segments and (2) characterization of duct tape adhesives based on small particles included as fillers in their adhesive formulations.

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Accounting for Covariates in Forensic Error Rate Assessment and Evidence Interpretation

NIJ AWARD #: 2019-DU-BX-4011

Larry Tang*,[1]
Ngoc Ty Nguyen[1]
Martin Slawski[2]
Emanuela Marasco[2]
Chris Saunders[3]
Semhar Michael[3]
Dannica Ommen[4]
P. Jonathon Phillips[5]

[1] University of Central Florida
[2] George Mason University
[3] South Dakota State University
[4] Iowa State University
[5] National Institute of Standards and Technology
* Submitting author

These error rates reported by recent forensic black box studies are mainly obtained by pooling all the decisions from examiners or computer algorithms with same-source or different-source pairs. These measures report the average error rates across a population of examiners for evidence sources. It would be ideal to account for covariates such as (1) source subjects' covariate information, including their demographics or source images' attributes and quality and (2) examiners' covariate information, such as their training background and demographics. Appropriately accounting for covariates in error rate assessment and evidence interpretation requires sophisticated statistical analyses with modern statistical concepts and methods. In this poster, the researchers will present this National Institute of Justice–funded work on the receiver operating characteristic (ROC) regression framework for error rate quantification by allowing covariates specific to source subjects and examiners. The researchers will discuss statistical techniques by fitting ROC regression in order to relate covariates to error rates quantified by the ROC curve. The resulting covariate-specific ROC curves in face recognition, handwriting, and latent print databases will model the relationship between covariates and decision scores, given the error rates for specific values of covariates. The researchers will also present an R-Shiny app to facilitate the implementation of the developed methods for black box studies.

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Utilizing eDNA From Four Biological Taxa Associated With Geologic Evidence for Sample-to-Sample Comparisons and Study Site Separation

NIJ AWARD #: 2020-R2-CX-0035

Teresa M. Tiedge*
Kelly A. Meiklejohn
North Carolina State University
* Submitting author

Geologic materials, such as soil and dust, are valuable types of trace evidence submitted to crime laboratories. Forensic geologists aim to analyze the inorganic components (e.g., mineral content) and determine their physical properties (e.g., color and pH) for sample-to-sample comparisons or to identify an evidentiary sample's origin. However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information. DNA metabarcoding is a commonly used approach to identify the biological taxa that are present in environmental samples by amplifying and sequencing short, informative regions of the genome and is not restricted by sample amount. The goal of this research was to determine the utility and stability of environmental DNA from four biological taxa associated with soil and dust for sample-to-sample comparisons and sample origin determination. To accomplish this, four taxa—bacteria (16S), fungi (ITS1), arthropods (COI), and plants (ITS2, trnL)—recovered from each sample were characterized (n=1,026) via DNA metabarcoding. An initial soil isolation study was performed to determine the most suitable approach (picking/scraping, swabbing, and sonication) to remove soil from mock evidence for environmental DNA analysis. Following soil removal, DNA was isolated using the PowerSoil® Pro Kit. DNA extracts were amplified using PCR primers specific to 16S, ITS1, ITS2, COI, and trnL. Libraries were then prepared and sequenced on an Illumina® MiniSeq™. Raw sequencing reads were then processed through a bioinformatic pipeline that identifies amplicon sequence variants via DADA2 and searches the amplicon sequence variants against GenBank for taxonomic identification. Picking and scraping of soil produced the highest amount of DNA compared with swabbing ($p = 0.0025$) and sonication ($p = 0.0068$). Although all three methods recovered similar taxonomic assignments, picked and scraped samples tended to cluster together more consistently with the soil reference in multidimensional space and thus was the method chosen for soil isolation in subsequent experiments. Following the soil isolation study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a 1 year period. Mock items included (a) soil scraped from t-shirts, boot soles, and trowels; (b) exposed dust collected from brick pavers using polyurethane swabs; and (c) dry dust from air filters (~1" × 1" area used). DNA was isolated from mock geologic evidence using the PowerSoil® Pro Kit, and libraries were prepared using custom indexed primers and subsequently sequenced using the Illumina® MiSeq™. After sequencing, the bioinformatic pipeline was used to process sequencing reads to characterize the bacterial, fungal, plant, and arthropod communities. Important findings from this research include the following: (1) despite the low DNA concentrations of dust samples, it was still possible to characterize the biological communities in dust; (2) the wet lab workflow successfully recovered taxa associated with mock forensic evidence; and (3) it is apparent that there were changes in the biological communities over time and between locations. This poster also includes 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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Assessing Methods to Enhance and Preserve Proteinaceous Impressions From the Skin of Decedents During the Early Stages of Decomposition While Examining Environmental Variations Across Seasons

NIJ AWARD #: 2019-R2-CX-0070

Jessica Zarate*,[1]
Jane Wankmiller[2]
[1] Madonna University
[2] Northern Michigan University
* Submitting author

Homicides and violent crimes often result in bloodshed; the constant substrate involved in physical altercations in the commission of violent crimes is human skin. Thus, it is likely blood impressions are left on the skin of living victims or decedents during these violent interactions. However, skin is one of the least studied substrates in the impression discipline. In some cases, impressions are clearly visible, but it is much more likely that they are latent and not readily visible. There have been cases where the enhancement of blood impression evidence on human skin was possible, but it is not standard practice, especially when blood impressions are latent. Although visible blood impressions are best enhanced in situ at the scene of the crime, most often these impressions on decedents are not enhanced until the body is moved to the medical facility for autopsy, increasing the possibility of damage to the impression evidence from handling or moving the body. Because it is semi-porous, skin is a difficult substrate to enhance through chemical enhancement methods, which may cause background staining that results in suboptimal visualization of the impressions. In addition to the staining of skin, visualization of impression details may be obstructed by competing background patterns, such as dermal scales, hairs, wrinkles, and variations in skin tones. Two commonly used dye stains, Amido Black and Hungarian Red, have been used to enhance blood impressions on human skin, and a newer method, Zar-Pro™ Fluorescent Blood Lifters, has also been used in preliminary studies to lift and enhance blood impressions from decedent skin effectively. A comparative analysis between methods conducted in collaboration with the Forensic Research Outdoor Station at Northern Michigan University assessed the effectiveness of enhancing semen smears and blood impressions on decedent skin during the early stages of decomposition. All three enhancement methods demonstrated effectiveness in recovering proteinaceous materials with Amido Black and Hungarian Red primarily effective as in situ dye stains. The dye-stained impressions were not reliably lifted using BVDA Gellifters®, thus not removing the substrate variables that can impede visualization of impressions on skin. The Zar-Pro™ Fluorescent Lifters were able to effectively lift and fluorogenically enhance proteinaceous materials in the form of blood impressions and semen smears from decedent skin through 10 days of active decomposition. A statistical assessment of the enhancement methods was conducted among examiners to verify the efficacy of results. During the early stages of decomposition, donor skin will deteriorate; thus recoverable impressions will also be degraded or damaged, yet this degradation is not perilous for the recovery of proteinaceous materials as long as the epidermal skin is still intact. Even during active decomposition, skin—arguably one of the most difficult substrates for impression recovery—can produce viable impressions, and the recoverability of this vital evidence can now be re-evaluated by practitioners in the field.

Recovery and Analysis of Both Volatile and Less-Volatile Compounds From Ignitable Liquid Residues on Substrates/Debris by SPME-DART-MS

NIJ AWARD #: 2020-DQ-BX-0003

Mengliang Zhang*
Shruthi Perna
Victoria Bascou
Ngee Sing Chong
Middle Tennessee State University
* Submitting author

The detection of ignitable liquid residue (ILR) is critical to the arson investigation process, which may potentially identify the cause of a fire. The most commonly used method to analyze ignitable liquids (ILs) is gas chromatography–mass spectrometry (GC-MS), which is used primarily in the detection of the volatile components in ILR. Direct analysis in real-time mass spectrometry (DART-MS) has shown success in profiling the nonvolatile or less-volatile components in IL, which are likely to be contained in the fire debris and could therefore yield corroborating evidence on the use of specific ILs in the investigation. However, the substrates and fire debris tend to cause interference in analysis. In this study, solid-phase microextraction (SPME) was coupled with DART-MS to investigate the matrix effect and optimize the extraction parameters to reduce interference. Gasoline and paint thinner were used as model ILs to evaluate the SPME-DART-MS method. A previous study has shown that fuel additives and polyethylene glycol could be the marker compounds for gasoline and paint thinner, respectively. Two parameters (i.e., temperature and time) associated with SPME were evaluated by using a two-factor central composite design. The full second-order polynomial model that fits the data was constructed, and the optimum condition was reported based on the modeled response surface. The ILRs on wood, paper, sand, fabric, and fire debris were studied to examine their impact on extraction efficiency and analytical interference. The ILs were also mixed with water to simulate wet fire debris that has been exposed to water during fire suppression. The influence of water on the detection of ILR will be discussed. The SPME-DART-MS results indicate that both volatile and less-volatile marker compounds for gasoline and paint thinner were recovered from the substrates and fire debris, and their ion patterns matched well with the gasoline and paint thinner liquid samples analyzed directly by DART-MS. As expected, the effective extraction of those marker compounds required a relatively high temperature (i.e., 150°C and 120°C for gasoline and paint thinner, respectively). In the presence of a matrix, a higher extraction temperature and longer extraction time could benefit the extraction efficiency. The desorption of ILR on the SPME fiber was achieved by inserting the fiber into the DART-MS helium gas stream under 300°C for 1 minute, and no carry-over residues were observed. In conclusion, the SPME-DART-MS has shown promise in ILR detection as an important complementary tool. The chemical information yielded by this method is typically not observed in the current GC-MS-based practice. Since SPME is one of the standard strategies for ILR extraction and DART-MS is becoming more available in forensic laboratories, the implementation of SPME-DART-MS for ILR detection could be achieved without much need for capital expenditure.

POSTER ABSTRACTS

SESSION II
FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

GIS Application for Building a Nationally Representative Forensic Taphonomy Database

NIJ AWARD #: 2020-DQ-BX-0025

Madeline M. Atwell*
Katherine Weisensee
Carl Ehrett
D. Hudson Smith
Patricia Carbajales-Dale
Clemson University
* Submitting author

Estimating the time since death, or the postmortem interval (PMI), poses a significant challenge to medicolegal death investigations when human remains are discovered because of insufficient methodologies. Despite decades of research, existing studies involving PMI estimation are significantly hindered because of reliance on small sample sizes, environmental homogeneity, and inconsistent definitions of the stages of decomposition. These inconsistencies impede the successful identification of unidentified human remains and the reconstruction of events surrounding their death. To transcend enduring methodological issues involving PMI estimation, the researchers developed an ongoing collaborative forensic taphonomy reference database called geoFOR. The geoFOR application is a case entry platform and data repository. The app pairs this comprehensive dataset with ArcGIS and machine learning models to deliver improved PMI estimations. Specifically, the geoFOR app offers forensic practitioners a platform to enter case information, including observations regarding body size; the presence of trauma; and uniform descriptions concerning characteristics of decomposition, insect, and scavenger activity. The app automates weather data collection from the location of discovery using the Global Historical Climatology Network through the National Oceanic and Atmospheric Administration. After case submission, the app delivers a PMI prediction directly to practitioners using a statistically robust regression model. The advanced cross-validated machine learning PMI predictive model results in an R^2 value of 0.8, and users receive a predicted PMI with an 80% confidence interval. The geoFOR database currently contains over 2,600 cases derived from medicolegal death investigations and human decomposition research facilities across the United States and internationally. Data collection is ongoing as new and existing collaborators enter case information. The size and comprehensive nature of the geoFOR dataset allows for the application of machine learning methods for estimating PMI. Estimating PMI using machine learning is a novel concept in the field that can address major limiting gaps in PMI estimation. The impact of the geoFOR application in the advancement of forensic anthropology is two-fold: foremost, it provides the most comprehensive reference database to date, comprising thousands of individuals across varied environments. Many forensic studies are confined to small sample sizes, similar geographic regions, nonhuman proxies (e.g., pig carcasses), and donors of forensic decomposition research facilities, which are not necessarily reflective of the realities of medicolegal forensic cases. Second, the automation of weather data collection and deliverable PMI estimations using machine learning models offer unprecedented data-driven results that can help successfully narrow the search parameters for unknown decedents, which can expedite identification and more accurately inform investigators about the circumstances surrounding their deaths. GeoFOR also follows an Open Science Framework through data sharing that promotes methodological integrity and reproducibility. These principles encourage fairness, equity, and inclusion within the research community.

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Skeletal Blast Trauma: Determining the Effect of Known and Experimental Blast Events on Trauma Patterns, Fracture Behavior, and Blast Scene Recovery Approaches

NIJ AWARD #: 2020-R2-CX-0041

Petra Banks*
Nicholas P. Herrmann
Texas State University
* Submitting author

Skeletal blast trauma is still a relatively unexplored field. Although there are detailed data on the forces and appearance of blast injuries in soft tissue, such as blast lung or inner ear damage, much of the data on skeletal blast trauma is either derived from warfare case studies or from compiled generalized trauma data from military research. Minimal data have been collected regarding civilian skeletal blast trauma. In particular, few studies have compiled these and other skeletal trauma data into comparable datasets. To examine comparable trauma, this study explored the physical distribution of skeletal trauma for blast events, falls from height, aircraft crash trauma, and motor vehicle/pedestrian (MVP) collisions. Analyses were conducted using Chi-squared analysis, comparing trauma presence and absence at the zone, element, region, and axial versus appendicular levels. These data were collected from deidentified decedent case files from medical examiner and coroner offices from across the United States. In addition, blast trauma and aircraft crash trauma data from the Defense POW/MIA Accounting Agency (DPAA) were included to provide a comparison from noncivilian contexts. Hard tissue trauma events were recorded by bone element, limb, and specific bone location using osteological zones described in CORA, a database designed and built by DPAA. Case-specific data were recorded, including a description of the cause of the event, basic demographic information, and a general inventory of the remains to indicate whether the absence of trauma is due to a lack of preservation or a lack of injury. The collected data include 227 individuals from blast events, 70 individuals from aircraft crash events, 42 falls from height, and 50 MVP collisions. Demographically, male individuals predominate the sample of traumatic events ($n_M = 672$, $n_F = 48$). By age, both blasts and aircraft crashes skewed younger, with most cases occurring between age 20 and 25. However, although the rest of aircraft crash cases outside of the 20–25 year cohort were distributed equally, a large portion of blast events outside of this range occurred between the ages of 15 and 20. Both of these younger values are likely from the inclusion of the DPAA data, reflecting the age of military personnel in combat. The ages of individuals in falls and MVPs were statistically normally distributed. At all levels, there were significant differences in trauma frequencies. Axial versus appendicular comparisons resulted in significant results with a p-value of 0.01 between every combination of events. By region, all event types were statistically significant when examined together. When individually compared, there were more regions of dependence (nonsignificance) between blasts, falls, and MVPs, while aircraft crashes and MVPs were significant across all regions. Aircraft crashes and blasts had dependence at hands and thoracic vertebrae, and aircraft crashes and falls had dependence at cervical vertebrae. These trauma distribution data are being used to generate a predictive model of trauma event type using random forest modeling, which will provide investigators with important trauma analysis comparisons to help assess trauma causes.

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Improving Identification of Unknown American Indians and Hispanic/Latinx Americans

NIJ AWARD #: 15PNIJ-21-GG-04139-SLFO

Kelly R. Kamnikar*,[1]
Esteban Rangel[1]
Amaya Abeyta-Brown[1]
Nicollette S. Appel[1, 2]
Heather J.H. Edgar[1, 2]
Natalie Adolphi[2]
Stephen D. Ousley[3]

[1] The University of New Mexico

[2] New Mexico Office of the Medical Investigator

[3] The University of Tennessee

* Submitting author

Stature estimation is a core component of the biological profile in forensic anthropology research and casework. Stature estimates using a mathematical framework are derived from equations that use single or multiple long bone lengths in combination with prediction intervals to create a stature range. The researchers provide mathematical equations for estimating stature for modern American Indians in New Mexico. This research draws on postmortem computed tomography scans from forensic casework (n=222) available from the New Mexico Decedent Image Database. The researchers regressed four long bone length measurements from the humerus, femur, and tibia on measured cadaver length to create 14 combinations of equations. These equations were calculated for the entire sample, by sex, by broad American Indian language group, and age and sex in combination. The most appropriate equations for each group were determined through various methods of model accuracy and efficiency testing. An independent test sample comprising forensic casework from the New Mexico Office of the Medical Investigator demonstrates that the equations created here are accurate and precise, with most overestimating stature by approximately 1 cm. The researchers provide recommendations for the use of these equations in a forensic setting and introduce a downloadable macro sheet for estimating stature available for use by practitioners.

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Expanding and Validating the Microbiome Database for Estimating the Postmortem Interval

NIJ AWARD #S: 2015-DN-BX-K016, 2016-DN-BX-0194, 2019-DU-BX-0025, 15PNIJ-22-GG-04402-MUMU

Jessica L. Metcalf*,[1]
Victoria Nieciecki[1]
Zachary Burcham[2]
Sibyl Bucheli[3]
Aaron Lynne[3]
David O. Carter[4]
Rob Knight[5]

[1] Colorado State University

[2] University of Tennessee Knoxville

[3] Sam Houston State University

[4] Chaminade University Honolulu

[5] University of California San Diego

* Submitting author

Estimating the postmortem interval (PMI) in death investigations is important because it helps with reconstructing death scenes, identifying the deceased, issuing death certificates, and distributing assets defined in wills. However, PMI can be challenging to estimate, especially if no last communications or visual sightings are available. Previously, the researchers used 36 human cadavers at three anthropological facilities over four seasons (three cadavers per facility per season) and sampled skin and cadaver-associated soil daily for 21 days to characterize the decomposer microbial community. The researchers discovered a universal set of key microbial decomposers that assembled despite location, season, or climate. These universal key decomposers underlie an accurate random forest regression model for predicting PMI (calculated as accumulated degree days) from microbiome normalized abundance patterns with species-level taxonomy data (16S rRNA gene amplicon data) of the skin predicting PMI within approximately ±3 calendar days (awards 2015-DN-BX-K016 and 2016-DN-BX-0194). However, several knowledge gaps still exist. First, the model only used forensic facilities in two climates present in the United States (Köppen-Geiger classification: "temperate without a dry season and hot summer" and "arid steppe cold"; Beck et al., 2018). Therefore, a gap in knowledge and data exists for building a model that predicts PMI from cadavers in other climates. Second, the model only includes data from outdoor donor decomposition. Therefore, a knowledge gap exists about whether the model is useful for indoor decomposition scenarios. These knowledge gaps are being addressed through samples collected from donors placed across forensic anthropology facilities in a third major climate type (Köppen-Geiger classification: "cold without a dry season warm summer") across North America (award 15PNIJ-22-GG-04402-MUMU) and in-built structures approximating an indoor death scene (award 2019-DU-BX-0025). These data will clarify whether a universal model for predicting PMI is possible, or if predictions are more accurate with climate/environmental specific models. The researchers will validate and estimate error for PMI prediction by collecting an independent test set of samples collected from donor bodies placed at multiple forensics anthropological facilities (including facilities not represented in the training data set), as well as other forensically relevant opportunities. The researchers synthesize across multiple National Institute of Justice–funded projects to determine next steps for developing a tool useful to forensic science practitioners.

Reference

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Finding the Missing and Unidentified: The Application of Predictive Modeling, Ground-Penetrating Radar, and Small Unmanned Aircraft-Mounted Infrared Imagery for the Detection of Unmarked Graves

NIJ AWARD #: 2020-R2-CX-0043

Mariah E. Moe

Texas State University

The migration crisis along the U.S.–Mexico border has claimed the lives of over 8,000 individuals in the last two decades, and Texas marks the state with the highest number of deceased undocumented migrants found. In many cases, partly because of overwhelming numbers, these individuals are buried in cemeteries throughout the South Texas region without a thorough examination and often without documentation of their burial location. This creates a situation in which the remains of individuals may become lost within cemeteries without the possibility of identification. Finding their graves is the first step toward returning their identity and repatriating them to loved ones. Prior efforts to locate these unmarked graves have been performed by Operation Identification and the Forensic Border Coalition and include interviews, pedestrian surveys, and geophysical prospecting with moderate success. However, a thorough examination of current and alternative geophysical methods followed by establishing the ground truth through excavation has not been conducted. This poster will discuss the results of the geophysical survey and subsequent excavation of a cemetery in South Texas known to bury unidentified migrants who perished in their attempt to cross the U.S.–Mexico border. Two areas in the cemetery were identified by county employees to contain unidentified migrants, one active area in which burials are continuing to occur and the other in which the last burial occurred several years prior. Both areas were surveyed using ground-penetrating radar and drone-based infrared imaging, although only the more recent section was excavated due to budgetary and time constraints. This study revealed that ground-penetrating radar is not an ideal method in the search for unmarked graves given the complex soil composition in South Texas; however, it does have the potential to identify subsurface anomalies associated with older burials where other methods fail. It should not be used as the sole method for determining the presence of burials but can provide an initial picture of the subsurface environment. Infrared imaging, as obtained using a DJI Mavic 2 Enterprise Advanced system, is more adept at detecting faint topsoil and vegetation changes associated with more recent burials but fails to identify older graves with less topographical variation. This poster will discuss the benefits and limitations of the equipment used and the environmental factors to consider when conducting grave searches, particularly in highly active cemeteries. In complex environments, including those in which the burials of interest are suspected to be several years old, more sensitive technology may be required to achieve a satisfactory output.

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Towards an "Eggs-perimental" Approach for Species Determination of Blow Fly Eggs to Facilitate Estimations of Postmortem Interval

NIJ AWARD #: 2020-MU-MU-0016

Rabi Ann Musah*,[1]
Alexa Figueroa[1]
Jennifer Y. Rosati[2]

[1] University at Albany, State University of New York

[2] John Jay College of Criminal Justice

* Submitting author

Medicolegal forensic entomology is the utilization of "carrion insects" that colonize human and animal remains to estimate the time since death, commonly referred to as the postmortem interval (PMI). Blow flies (*Calliphoridae*) usually arrive at the body within a few minutes of death, with the remains serving as a feeding, breeding, and oviposition (egg laying) medium and refuge from extreme weather, predation, and resource competition. Because of the well-established correlation between the species of insects that feed on remains and the different stages of corpse or carrion decomposition, along with the well-known species-specific insect life cycle timelines, it is possible to "back-calculate" the approximate time of death by assessing when the eggs from which the retrieved entomological evidence hatched were laid. Accordingly, because it is presumed that the eggs were laid on the remains shortly after death occurred, the determination of when the eggs were laid serves to approximate when death occurred. Accurate species identification is critical for PMI determination, but conventional methods are often time-consuming and resource-intensive and require specialized expertise and laboratory resources. Typically, the juvenile life stages (eggs, larvae, and pupae) are the specimens collected at the scene, but visually determining species identity is challenging because of their similar appearance across species, particularly for the eggs. For this reason, if the retrieved specimens are viable, it is customary for an experienced entomologist to rear them to adulthood for species identification to be based on their more visually apparent distinguishing morphological features. In the absence of the necessary resources to accomplish this task, entomological evidence often remains underused. Therefore, an alternative rapid method for accurate determination of the species identity of retrieved entomological evidence is greatly needed to facilitate PMI estimation using underused entomological evidence such as eggs. Reported in this poster is the development of a chemometric method that enables determination of the species identity of necrophagous species eggs from within the *Calliphoridae* family through their direct analysis in real-time high-resolution mass spectrometry–derived chemical signatures. Chemometric processing of the 70% aqueous ethanol suspensions of eggs, disaggregated by species identity, exploits intraspecies similarities and interspecies differences to enable accurate species identification of *Calliphora vicina*, *Calliphora vomitoria*, *Cynomya cadaverina*, *Lucilia illustris*, *Lucilia sericata*, and *Phormia regina*. Accordingly, the application of Kernel discriminant analysis to the data enabled species identification with an accuracy of 87.35%. As an extension of this work, solid-phase microextraction–facilitated gas chromatography–mass spectrometry analysis of *Lucilia sericata* eggs over time revealed that they emit a range of volatiles as a function of their development progression. The compounds emitted can potentially provide valuable insights on the age of the evidence, thereby providing more refined and accurate PMI-relevant information. Future investigations aim to establish a comprehensive database containing species-specific chemical signatures for identifying entomological evidence, thus enhancing the evidentiary value of immature life stages such as eggs.

An Examination of Musculoskeletal Markers in Modern Populations for Forensic Analysis and Identification Purposes

NIJ AWARD #: 2020-R2-CX-0042

Emilie L. Wiedenmeyer
Texas State University

Forensic anthropologists provide biological profile estimations (sex, age, and stature) of unknown decedents to narrow down the list of missing persons and accelerate positive identifications. The addition of estimated activity level or occupational type holds promise to enhance the biological profile by further individualizing a decedent's remains. Entheseal changes (i.e., osseous changes occurring in musculoskeletal junctions within the body) have long been used to reconstruct lifeways and demographic indicators of archaeological populations. However, few attempts have been made to apply this knowledge of lifestyle and demographic indicators in a modern forensic medicolegal context to help with identification efforts. This study examined 691 individuals within modern donated human skeletal collections across the United States. Entheseal changes in the shoulder and elbow of each individual were scored following the Coimbra method published by Henderson and colleagues (2016). Various statistical comparisons and multivariate models were used to assess whether entheseal changes of the upper limb reflected biosocial information of the skeletal donors included in the study sample. Mixed factor analysis was used to incorporate all data into a single model to explore general relationships between entheseal scores and known donor data. Additional statistical tests explored more specific questions, such as Pearson's Chi-squared tests to find direct correlations between entheseal scores and reported demographics and random forest models to test the predictive strength of entheseal changes in predicting categories of labor and occupational type. Results indicated that age at death is the most consistent explanatory variable to affect developmental rates of formative and resorptive entheseal changes in the upper limb. However, variables such as biological sex, body mass index, and socioeconomic status were found to have varying effects, often accentuated with increasing age. Formative entheseal changes, such as bony spurs and textural changes, were found in higher frequencies in individuals with increasing body mass indices and those who reported working more manual labor jobs. Additionally, resorptive entheseal changes, such as erosive lesions and increased porosity, were seen in higher numbers within individuals of lower body mass, especially in older female individuals and older individuals working nonmanual labor jobs. Random forest models revealed that entheseal changes are more likely to accurately predict general manual versus nonmanual labor categories as opposed to more informed categories of occupation, rising as high as 74% accuracy when using only entheseal scores and biological sex estimates. However, these results are likely skewed by male dominant manual laborers, as only 5% of women represented in the study reported working in manual labor jobs. Results from this study show promising avenues for entheseal changes in forensic work but also display their possible multifactorial origin, likely affected by many aspects of lived experience. Furthermore, the results of this study were limited because of the lack of Black, Indigenous, and People of Color (BIPOC) representation within the study sample, a common bias of modern donated skeletal collections. Additional research is needed to further

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explore how entheseal changes are distributed among individuals with diverse backgrounds and life histories to better assess potential forensic applications.

Reference

Henderson, C. Y., Mariotti, V., Pany-Kucera, D., Villotte, S., & Wilczak, C. (2016). The new 'Coimbra Method': A biologically appropriate method for recording specific features of fibrocartilaginous entheseal changes. *International Journal of Osteoarchaeology, 26*(5), 925–932. https://doi.org/10.1002/oa.2477

POSTER ABSTRACTS

SESSION III
SEIZED DRUGS AND TOXICOLOGY

Development of a Colorimetric Breath Analyzer for THC

NIJ AWARD #: 15PNIJ-22-GG-04437-RESS

Emanuele Alves*
Wagner Pacheco
Savanah Allinson
Kyrah Golden
Virginia Commonwealth University
* Submitting author

Nationally, This presentation will demonstrate the development of a Δ9-tetrahydrocannabinol (THC) breathalyzer for the detection of recent Δ9-THC use in the field. After visiting this poster, attendees will better understand the mechanism of the colorimetric reaction applied to detect Δ9-THC and the application of this reaction to a support base that will work as a prototype for a colorimetric-based Δ9-THC breathalyzer. This presentation will impact the forensic science community by showing the development of the basic chemical foundations needed for the development of a portable colorimetric device for the on-site detection of Δ9-THC in air exhaled for the early detection of driving under the influence of marijuana. Today, the recreational use of marijuana is legal in 31 U.S. states and the District of Columbia. Several studies show that after legalization, a 6.0% increase in injury crash rates and a 4.0% increase in fatal crash rates were observed. Several factors must be considered to reduce driving under the influence of marijuana, such as preventive, educational, and punitive activities. On-site detection of recent marijuana use is one of the possible measures to be adopted. Alcohol breathalyzers are currently used to help law enforcement perform site evaluations quickly and easily. Thus, a Δ9-THC breathalyzer could bring similar advantages. Unfortunately, this is not yet a reality for Δ9-THC. The current devices are collection devices where the exhaled air is stored and must be further analyzed in a laboratory to allow Δ9-THC identification. In this project, the development of the Δ9-THC breathalyzer is based on the application of an additive manufacturing solid device made by 3D printing. To create this support, a commercial Anycubic® 3D polymerizable resin was mixed with different Fast Blue dyes and evaluated in the presence of several cannabinoids. The Fast Blue dye family is known to produce a colorimetric response in the presence of THC. Several dyes from the Fast Blue family were studied (B, BB, and RR), in concentrations varying from 1% to 6% w/v. The results showed that the Fast Blue B in a concentration of 4% w/v in the solid resin is capable of reacting with 0.01 µg with a linear response ranging from 0.01–0.50 µg of Δ9-THC. Achieving lower concentrations is extremely important because the concentration of Δ9-THC in exhaled air is described to be in the range of 1 ng/30 L of exhaled air. The results obtained in this work are the initial fundamental chemical foundation needed for the construction of a reliable semi-quantitative breathalyzer device to be applied in U.S. monitoring of driving under the influence of drugs.

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Illuminating the Dark: Molecular Networking as a Novel Psychoactive Substance Identification Strategy

NIJ AWARD #: 15PNIJ-21-GG-04171-COAP

Maia Bates*,[1]
Abby Helm[1]
Heather Barkholtz[1, 2]

[1] University of Wisconsin–Madison

[2] Wisconsin State Laboratory of Hygiene

* Submitting author

Due to the ever-changing drug landscape, forensic laboratories are increasingly using untargeted techniques for presumptive substance identification. One of the most promising untargeted techniques is liquid chromatography–high-resolution mass spectrometry (LC-HRMS). Untargeted LC-HRMS technology allows for sensitive and selective detection of a vast number of diverse drugs, toxins, and metabolites extracted from complex biological matrices. Although untargeted LC-HRMS methods have many advantages, no one method that works efficiently for all analytes has been described. This work sought to assess the performance of an untargeted extraction and LC-HRMS method for the National Safety Council's Tier I drugs of abuse in whole blood. An untargeted analyte extraction and data acquisition method was developed and validated for Tier I drugs of abuse and metabolites. Target analytes were extracted from whole blood using 96-well Agilent Captiva EMR-lipid cleanup plates. A Waters Xevo G2-XS quadrupole time-of-flight mass spectrometer with electrospray ionization was used in positive and negative ionization modes for data acquisition. A data-independent acquisition mode, MS^E, was used to collect precursor and product ion data within one run. Peak detection was performed using the Waters UNIFI 3D peak algorithm and *m/z* retention time pairs to match data against an in-house spectral library. Performance of the outlined methods were measured using the following criteria: analyte recovery, limit of detection (LOD), ionization, interferents, carry-over, and precision. A total of 32 analytes were considered and method performance data were consolidated according to drug class and chemical structure. LODs were below recommended screening cut-off concentrations for the majority of analytes; several had sub–nanogram per milliliter LODs. Some exceptions were amphetamine, lorazepam, and oxymorphone, which were not detected at the recommended screening cut-off concentration. Extraction recoveries varied depending on the physical and chemical properties of the analytes and ranged from 9% to greater than 100%. Ionization also varied significantly with an average of 94% and a standard deviation of 43.8%. Endogenous and exogenous interferents were assessed, and none were determined. In addition, carry-over was not observed at the concentrations considered. Cannabinoid compounds posed the greatest challenge in this work; Δ9-tetrahydrocannabinol and the 11-hydroxy metabolite were not ionized sufficiently for detection at the recommended cut-off concentrations. However, the 11-nor-9-carboxy-Δ9-tetrahydrocannabinol analyte was ionized and included in the performance assessment data. The method described in this work is efficient and robust with minimal limitations. However, establishing untargeted LC-HRMS screening assays is a difficult process, and it is imperative that laboratories consider the strengths and limitations of these methods across diverse drug classes and chemical properties. Results from this work will be used to inform future assessment of the National Safety Council's Tier II drugs of abuse.

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Advanced Microfluidic Technology for Automated, Rapid, and Objective Laboratory Screening of Seized Drugs

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Shannon Krauss
RTI International

When samples are seized for subsequent forensic laboratory analysis, a workflow involving multiple laboratory techniques is used to achieve a sufficient level of selectivity for a scientifically supported conclusion. Color testing continues to be reported as the predominant seized drug screening method because of the advantages of low cost, rapid results, and simplicity in which color changes are observed visually. However, limitations can include the subjectivity of color interpretation, the fully manual procedure, the presence of interferents, incorrect results reported, and multiple color tests for classification, which all provide the potential for user error or unreliable results. Because these results ultimately contribute to criminal justice outcomes, it is important to explore methods aimed at increasing confidence in these tests and providing improvements to incorporate unbiased and quantifiable metrics. This presentation will discuss a method for improved automation and objective analysis through digitization to address seized drug color testing challenges. This method uses custom software that was integrated into a small-scale technology platform for sample processing and analysis. This platform enabled the translation of routine color tests (e.g., cobalt thiocyanate, Ehrlich) to capture and record the resultant color changes for downstream objective analysis in an adaptable format. This format allows for the future implementation of additional or alternative tests to address the evolving drug landscapes and regulations.

Confirming the Presence of Novel Psychoactive Substances in Forensic Samples From Medicolegal Death Investigations

NIJ AWARD #: 15PNIJ-22-GG-04434-MUMU

Alex J. Krotulski*,[1]
Sara E. Walton[1]
Melissa F. Fogarty[1]
Joshua DeBord[1]
Donna M. Papsun[2]
Barry K. Logan[1,2]

[1] Center for Forensic Science Research and Education

[2] NMS Labs

* Submitting author

As the recreational drug supply becomes more volatile and dynamic in the United States, it is now critical to conduct comprehensive postmortem toxicology testing in cases where drug overdose is suspected. Ideally, practitioners should seek testing using novel and innovative methods with up-to-date scopes of analysis that include traditional drugs, novel psychoactive substances (NPS), and adulterants. New synthetic drugs continue to appear in medicolegal death investigations, and it is increasingly common to encounter NPS used in combination with traditional drugs (e.g., fentanyl) to elicit more customized pharmacological effects, a phenomenon that has been confirmed though drug material testing and forensic toxicology analysis. The Center for Forensic Science Research and Education (CFSRE)—a nonprofit, federal grant–funded, state-of-the-art forensic laboratory—conducts drug testing and drug market surveillance using liquid chromatography quadrupole time-of-flight mass spectrometry and liquid chromatography tandem quadrupole mass spectrometry. In 2018, the CFSRE launched NPS Discovery—an open access drug early warning system—to develop and provide testing resources focused on new and emerging drugs to forensic practitioners. The primary goal is to disseminate actionable new drug data to various stakeholders in public health and safety. The CFSRE maintains a battery of novel testing workflows to confirm the presence of specific drug substances emerging in forensic casework, including the latest novel benzodiazepines, opioids, stimulants, hallucinogens, and cannabinoids. In 2022, the CFSRE analyzed nearly 2,000 forensic toxicology samples. Fentanyl (63%) was the most commonly detected drug, found in combination with NPS benzodiazepines (etizolam, 24%; flualprazolam, 19%; and bromazolam, 8%). Fluorofentanyl (19%) was the most frequently detected NPS opioid but when excluded, nitazene analogues (e.g., metonitazene, isotonitazene) comprised the most detections. Dimethylpentylone (5%) was the most encountered NPS stimulant. Synthetic cannabinoid positivity was low compared with previous years, and MDMB-4en-PINACA and ADB-BINACA were the two most detected. Xylazine (11%) was commonly detected alongside fentanyl. The year-over-year drug landscape has differed greatly since 2018. Today, apart from fluorofentanyl, fentanyl analogues have been largely eradicated from the recreational opioid supply, replaced by novel nitazene analogues often accompanied by NPS benzodiazepines. The combination of opioids (traditional or novel) with NPS benzodiazepines has increased significantly as "benzo-dope" use is now common but still less than "tranq-dope" (xylazine-fentanyl) use in most jurisdictions. Synthetic cannabinoid–related fatalities decreased in the dataset because of control measures in China; however, deaths involving these drugs continue. Specific and detailed case examples will be included in this poster.

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Developing an Approach to Standardize the Naming of Novel Psychoactive Substances

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Alex J. Krotulski*
Sara E. Walton
Max Denn
Brianna Stang
Barry K. Logan
Center for Forensic Science Research and Education
* Submitting author

Novel psychoactive substances (NPS) continue to appear in forensic casework with increasing regularity as they are mixed with or substituted for traditional drugs or purchased online as legal or alternative "highs." When NPS are detected by forensic laboratories, their name (and associated identity) is reported on the final forensic report. This information is used downstream by various local, state, and federal agencies, including medical examiner and coroner offices when certifying deaths and the Centers for Disease Control and Prevention when consolidating information on drug morbidity and mortality. Accurately reporting and tracking NPS is contingent on the proper use of nomenclature and consistency between laboratories. Mismatches in NPS naming (e.g., N,N-dimethylpentylone vs. dipentylone) can cause unnecessary confusion and mistakes in communication, interpretation, and reporting. A central authority on NPS naming is needed; however, the framework for naming must first be established. NPS nomenclature is complex, and not all substances under the NPS classification are necessarily new. Some are derived from previous pharmaceutical drug discovery patents but repurposed for illicit use, while others are "old" drugs that have resurfaced or are being used in a new or different way. Some drugs are named based on initials of the inventor and numbers based on the series in which they are discovered (e.g., JWH-018 and John W. Huffman). Some drugs are named based on abbreviations of their structure features with numbers (e.g., AP-237 and aryl piperazine). Some drugs are given fabricated names that become common language (e.g., fentanyl, etonitazene, alprazolam). The Center for Forensic Science Research and Education, through its NPS Discovery program and in collaboration with Cayman Chemical, has launched an initiative to help standardize the manner in which NPS are named. The goal is to develop tools and techniques with enhanced workflows to name new and old drugs more accurately and comprehensively. This will allow storage and consolidation of information in a database that is easily accessible and searchable and rapid dissemination of information about the existence of drugs, literature, trends, effects, and more to the forensic science community. Currently, the Center for Forensic Science Research and Education and Cayman Chemical are developing naming resource documents for synthetic cannabinoids and NPS opioids, specifically the nitazene analogues. Synthetic cannabinoid naming is the most structured under the NPS umbrella, using a semi-systematic alpha-numeric scheme that correlates back to the structure. However, with the constant emergence of new synthetic cannabinoids, this process needs to be documented yet flexible to include evolving chemistries. The recent emergence of the synthetic cannabinoids BZO-HEXOXIZID (formerly MDA-19) and CHO-4'Me-5'Br-FUBOXPYRA (formerly CH-FUBBMPDORA) are examples of these naming efforts and ways the naming scheme has helped standardize the language across the forensic science community. For the nitazene analogues, all names are based on the prototypical drug in the series, etonitazene. Modifications to etonitazene are reflected within the name or as prefixes. N-pyrrolidino etonitazene is an example of the naming efforts within this group. The primary outcome of this initiative will allow the forensic science community to further standardize drug naming and avoid unnecessary communication issues between forensic laboratories, reporting entities, and other stakeholders.

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Breath Measurements of Acute Cannabis Use (BACE): Towards Reliable Determination of Recent Use

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Tara M. Lovestead*
Jason A. Widegren
Kavita M. Jeerage
National Institute of Standards and Technology
* Submitting author

The modern alcohol breathalyzer is a reliable indicator of alcohol intoxication based on 100 years of development. The first device, the Drunkometer, used a balloon to capture breath samples and was tested on extremely intoxicated individuals. Two decades later, the "Breathalyzer" quantified ethanol concentration in breath. Today, the National Institute of Standards and Technology (NIST) and other National Metrology Institutes provide aqueous and gaseous reference materials to deliver gases with known ethanol concentrations to evaluate hundreds of devices from dozens of manufacturers approved by the National Highway Traffic Safety Administration for evidentiary purposes. Evolution from benchtop proof of concept to reliable field use was achieved with ethanol, a small water-soluble and volatile compound with well-characterized properties. Ethanol is consumed in large quantities; a standard drink contains 14 grams. Systemic ethanol can be accurately quantified from one exhalation with current sensor technology when device temperature is controlled and protocols to eliminate mouth ethanol contamination are followed. Δ9-tetrahydrocannabinol (THC), the main psychoactive compound found in cannabis, is lipophilic, has low volatility, and is consumed in small quantities, creating challenges for quantification in breath. THC quantification from multiple exhalations has employed sampling devices designed to trap less-volatile compounds, primarily based on interception or impaction. Device processing and high-resolution mass spectrometry are typically performed in an analytical laboratory. Indirect, nonquantitative approaches that rely on machine learning algorithms have also been investigated. No matter the strategy, infrastructure akin to alcohol breathalyzers for evaluation, calibration, and quality control is not yet available. The researchers are focusing on developing reference materials and delivery systems to deliver breath surrogates with known THC quantities to any sampling device or sensor technology to establish ground truth for the device's performance. The three-pronged approach uses (1) vapor pressure measurements of cannabinoids and cannabis-associated compounds, (2) numerical simulation to identify important parameters to control when delivering breath surrogates containing vapor and aerosol reference materials, and (3) limited human subjects studies with different devices to understand the mode of collection for THC and other compounds in breath with both targeted and untargeted analyses. Although the researchers published the first-ever vapor pressure measurements for THC and cannabidiol (CBD) in 2017, the measurement uncertainty was high, and the temperature range was limited. The researchers have since developed dynamic vapor microextraction to rapidly collect vapor samples while controlling the sample temperature, pressure, and composition. Dynamic vapor microextraction performance has been validated with vapor pressure measurements on the reference compound n-eicosane ($C_{20}H_{42}$). The relative standard uncertainty in the resulting vapor pressure data was about 2%, which is state of the art for measurements in the pressure range studied. Vapor pressure data for the cannabis-associated terpene linalool and for the cannabinoids THC, CBD, and cannabinol will be presented as well as numerical simulations of the effect of aerosol diameter and velocity on capture in an impaction filter device. These data are essential to prototyping initial reference materials and delivery systems for establishing ground truth for the performance of any device intended to determine recent cannabis use.

Application of Insights From High-Level Density Functional Theory for the Differentiation of Marijuana and Hemp

NIJ AWARD #: 15PNIJ-22-GG-04423-SLFO

Rabi Ann Musah*
Parandaman Arathala
Megan I. Chambers
Benedetta Garosi

University at Albany, State University of New York

* Submitting author

Marijuana and hemp are two varieties of the same species, *Cannabis sativa*, that differ in terms of the levels of tetrahydrocannabinol (THC) present: marijuana is a Schedule I substance that contains >0.3% THC by dry weight whereas hemp, designated as an agricultural product, has ≤0.3% THC. The criminal justice implications of these designations require that accurate methods for the differentiation of hemp and marijuana be available. Although such approaches exist, there remains a need for the development of rapid alternative methods for hemp and marijuana differentiation that can be used to triage the large influx of samples encountered by forensic laboratories, so the strained resources required for THC quantification can be prioritized for further analysis of only those samples in which marijuana levels of THC are believed to be present. In this regard, analysis of the plant material by direct analysis in real-time high-resolution mass spectrometry (DART-HRMS) has been shown to rapidly furnish a chemical fingerprint profile that readily reveals a characteristic peak at nominal *m*/z 315, which is diagnostic for protonated THC. However, THC has a number of other isomers, most notably cannabidiol or CBD (the cannabinoid most prevalent in hemp); therefore, the observation of *m*/z 315 is not itself indicative of whether the product is marijuana or hemp. Notably, in addition to the peak at *m*/z 315, a peak at nominal *m*/z 629 consistent with the presence of the protonated dimer [2M + H^+] of THC or CBD is also observed. The structures of the possible dimer complex combinations such as CBD••CBD, THC••THC, and CBD••THC were investigated by performing high-level density functional theory calculations. The results show that even though the computations revealed the THC••THC homodimer complex to be more stable than the others, studies on the effect of temperature on the population of the dimer complexes showed that the populations of the THC••THC and CBD••THC dimers decrease as temperature increases and become negligible at ≥200 K, whereas the population of the CBD••CBD dimer increases with temperature. It was thus observed that at the temperature at which DART-HRMS analysis reveals the presence of the protonated dimer (350°C), the peak at *m*/z 629 corresponds to the most stable CBD••CBD protonated homodimer when CBD levels are high (i.e., in hemp). On the other hand, when marijuana samples with high THC content and negligible CBD content were investigated, *m*/z 629 was not observed. Subsequent analysis of marijuana and hemp samples by DART-HRMS, designated by the ≤0.3% THC (hemp) and >0.3% THC (marijuana) thresholds, revealed the predictions observed by density functional theory calculations to be true. The results show that detection of a peak at *m/z* 629 when analyzing *C. sativa* plant material by DART-HRMS could serve as a means to differentiate hemp and marijuana.

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Accurate THC Determinations in Seized Cannabis-Derived Finished Products for Forensic Laboratories

NIJ AWARD #: DJO-NIJ-22-RO-0002

Walter Brent Wilson

National Institute of Standards and Technology

Hemp was removed from the U.S. Drug Enforcement Administration controlled substances list after the 2018 Farm Bill, and hemp has been defined as cannabis containing 0.3% or less of decarboxylated Δ9-tetrahydrocannabinol (Δ9-THC). Forensic laboratories are now required to have access to reliable analytical methods for differentiating between hemp and marijuana in seized cannabis samples. In response, the National Institute of Standards and Technology (NIST) developed a Cannabis Research Program to help provide forensic laboratories the necessary tools to quantitatively measure Δ9-THC in cannabis plant and cannabis-derived finished products. This poster focuses on the optimization of a sample preparation procedure previously published at NIST for the determination of Δ9-THC in commercial hemp oil samples that required 70 minutes. The goal of the new research was to minimize the sample preparation time to make it more desirable to forensic laboratories (<15 minutes) and expand to include cannabis vape cartridges. The new sample procedure was developed and evaluated at NIST for approximately 60 commercial and seized cannabis vape cartridges. Product labels for the seized cannabis samples often included a total THC mass fraction of 80% to 90%; however, in many cases, the actual mass fractions of Δ9-THC were lower.

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POSTER ABSTRACTS

SESSION IV
FORENSIC BIOLOGY/DNA

Moving Forward With Direct PCR: Touch DNA Samples and CODIS Eligibility

NIJ AWARD #: 2019-DU-BX-0009

Abigail Bathrick*
Anna Salmonsen
Jon M. Davoren
Bode Technology
* Submitting author

After attending this poster presentation, attendees will have a better understanding of how direct polymerase chain reaction (PCR) affects Combined DNA Index System (CODIS) eligibility for touch DNA samples and how direct PCR success rates compare with standard processing. Direct PCR is a DNA processing method in which a sample is added directly to an amplification reaction without prior extraction or quantification. This study examines the CODIS eligibility of GlobalFiler™ (GF) and PowerPlex® Fusion 6C (PPF6C) profiles obtained from touch DNA samples collected from plastic microscope slides, metal tools, handgun grips, vinyl shutters, brass cartridge casings, foam cups, concrete bricks, unfinished wooden tool handles, denim, wool, and polyester with various methods. For GF processing, collection from the nonfabric substrates was performed with Puritan® cotton swabs, Copan microFLOQ® direct swabs, and Whatman nonindicating FTA™ paper, which were either moistened with sterile water or 0.1% Triton™ X-100 or left dry. The GF work was used to identify optimal direct PCR-compatible touch DNA collection methods for each substrate for further testing with PPF6C. Puritan® cotton swabs and Copan microFLOQ® swabs moistened with sterile water or 0.1% Triton-X or left dry were used to collect DNA from the nonfabric substrates for amplification with PPF6C. Fabrics were sampled via cutting for both amplification systems. For each collection method, processing method, nonfabric substrate type, and amplification system, eight replicates were prepared from three donors. One donor was used for each type of fabric. Samples were processed with two methods: (1) standard processing with DNA extraction and quantification and (2) direct PCR. The extracted and direct PCR samples were amplified with GF and PPF6C. No changes were made to the thermal cycling parameters, reaction mixtures, or reaction volumes validated for regular casework processing (25 µL, 29 cycles). Profiles with alleles at a minimum of eight of the original CODIS core loci and match rarities of at least 1 in 10 million were considered CODIS-eligible. CODIS-eligible profiles were tallied across all collection methods for each substrate, processing method, and amplification system, and success rates were determined by calculating the percentage of profiles that were CODIS-eligible for each processing method. For GF, direct PCR produced higher CODIS eligibility success rates than standard processing for touch DNA samples collected from plastic slides, polyester, metal tools, handgun grips, foam cups, and wood tool handles. For PPF6C, direct PCR produced equivalent or higher CODIS eligibility success rates than standard processing for touch DNA samples collected from plastic slides, denim, wool, polyester, metal tools, vinyl shutters, handgun grips, foam cups, and wood tool handles. These results support previous findings that CODIS eligibility for direct PCR profiles is highly dependent on the substrate from which samples are collected and may be affected by the system used for amplification. There are advantages to using PPF6C when certain inhibitors are present and when touch DNA is collected with cotton swabs, whereas GF is advantageous when microFLOQ® swabs are used. Direct PCR results may be further improved through PCR reaction optimization and additional post-PCR cleanup steps.

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Quantifying the Accuracy of Two Innovative Forensic Genetic Identification Techniques

NIJ AWARD #: 2019-DU-BX-0028

Maria Flores

The University of California, Los Angeles

Forensic investigation of DNA samples from multiple contributors has become commonplace. These complex analyses use statistical frameworks accounting for multiple levels of uncertainty in allelic contributions from different individuals, particularly for samples containing few molecules of DNA. These methods have been thoroughly tested along some axes of variation, but less attention has been paid to accuracy across human genetic variation. Here, the researcher quantified the accuracy of DNA mixture analysis over 83 human groups. This research used Forensim, a free open-source R package, to simulate forensic genetic profiles of contributors, generate mixtures of those contributors, and calculate likelihood ratios. This likelihood ratio calculation was performed under the assumption that the genotypes of all non–person of interest contributors in the simulated mixture are known under both the defense and prosecution hypotheses. This research found higher false inclusion rates for mixtures with more contributors and for groups with lower genetic diversity. Even for two-contributor mixtures where one contributor is known and the reference group is correctly specified, false inclusion rates are 10^{-5} or higher for 56 out of 83 groups. This means that some false inclusions may be expected when multiple tests are performed. These false positives could be lessened with more selective and conservative use of DNA mixture analysis.

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py_ped_sim—A Flexible Forward Genetic Simulator for Complex Family Pedigree Analysis

NIJ AWARD #: 2019-DU-BX-0028

Miguel Guardado*,[1]
Ryan Hernandez[1]
Shalom Gutierrez[2]
Joaquín Magaña[2]
Sthen Campana[2]
Kaela Syas[2]
Emily Samperio[2]
Cynthia Perez[2]
Bernice Chavez[2]
Selena Hernandez[2]
Elena I. Zavala[3]
Rori Rohlfs[4]

[1] University of California, San Francisco

[2] San Francisco State University

[3] University of California, Berkeley

[4] University of Oregon

* Submitting author

Large-scale family pedigrees are heavily used across various disciplines, including human genetics and evolution. These family trees are invaluable tools for understanding genetic factors and tracing genetic ancestry over generations. However, there is currently a lack of software available to simulate different pedigree structures with genomes accurately, which limits our understanding of how genetic inheritance functions within various family contexts. To address this gap, the researchers have developed a Python command line–based tool called py_ped_sim that facilitates the simulation of pedigree structure and genomes for pedigrees. This framework represents pedigrees as directed graph data structures, enabling easy conversion between standard pedigree formats and their seamless integration with the forward population genetic simulator, SLiM. Notably, this software allows for the simulation of variable offspring count within a specific set of parents and half-siblings. The researchers validated the accuracy of this software by simulating genomes onto diverse family pedigree structures and observed that the estimated kinship coefficients closely approximated expected values. py_ped_sim is a user-friendly and open-source solution for simulating pedigree structures and conducting pedigree genome simulations. It empowers medical, forensic, and evolutionary genetics researchers to gain deeper insights into the behavior of genetic pedigree analysis. By using py_ped_sim, scientists can comprehensively explore and understand the dynamics of genetic inheritance and relatedness within families.

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DNA Typing Strategies for Identification of Human Remains via Real-Time Nanopore Sequencing

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Katherine McBroom*,[1]
Bupe Kapema[1]
Nicole R. Phillips[1]
Roxanne R. Zascavage[1]
Rupesh Kesharwani[2]
Fritz Sedlazeck[2]
Courtney L. Hall[3]

[1] University of North Texas Health Science Center

[2] Baylor College of Medicine

[3] Johns Hopkins University

* Submitting author

Short tandem repeat (STR) markers evaluated via capillary electrophoresis continue to be the gold standard for human remains identification in forensic investigations because of their high variability and robust database of comparative samples. However, capillary electrophoresis excludes valuable sequence-level information both within and around STRs and is not suitable for mitochondrial DNA (mtDNA) or single-nucleotide polymorphism (SNP) analysis, both of which are valuable in cases where STR analysis fails, such as cases of damaged and degraded remains. Human remains are frequently encountered in forensic laboratories, coming from crime scenes, mass graves, historical samples, mass disasters, and military conflicts. The problem faced by forensic laboratories when analyzing such samples is they must choose between depleting sample volumes by repeating individualizing STR analysis or performing costly, time-consuming, and less discriminatory mtDNA analysis. New DNA sequencing methodologies combined with novel enrichment techniques may provide a more effective platform for human remains identification that overcomes the most common challenges associated with the processing of bone fragments, aged tissue, and hair samples. Using the custom bioinformatic pipeline, STRspy, the researchers designed a streamlined method capable of producing reliable length- and sequence-based STR profiles from data generated on the newest and most affordable next-generation sequencing platform, Oxford Nanopore Technologies' (ONT's) single-molecule sequencer. The researchers combined this process with targeted sample enrichment via RNA probe capture for a robust analysis of forensic markers, including STRs, SNPs, and mtDNA from a single sample. The presenter will discuss the results of the project to date, detail the pros and cons of a bait capture + ONT–centered approach to human remains identification, and provide insight into the adjustments to the platform that are necessary to harness the true potential of ONT sequencing for the identification of human remains.

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Optimizing the Analysis of DNA From Burned Bone Using Ancient DNA Techniques

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Cody E. Parker*,[1]
Erin Rawls[1]
Katelyn L. Bolhofner[1]
Sreetharan Kanthaswamy[1]
Anne C. Stone[1]
Adam Ben Rohrlach[2]
Laura Fulginiti[3]
Giovanna Vidoli[4]
Joanne Devlin[4]

[1] Arizona State University

[2] University of Adelaide and the Max Planck Institute for Evolutionary Anthropology

[3] Maricopa County Office of the Medical Examiner

[4] University of Tennessee

* Submitting author

Identifying human remains using DNA analyses is a vital component of forensic investigation. These highly accurate analyses generally rely on the recovery of high-quality endogenous DNA that may not be available, especially when extracting from highly degraded source material. The decomposition of DNA can alter the amount of DNA retained in source tissue and its base composition and quality, making downstream analysis problematic. As such, the field of ancient DNA analysis has invested heavily in the development of optimized protocols for sampling, extracting, and analyzing DNA recovered from archaeological remains. In a forensic context, the use of these same techniques in modern degraded skeletal samples may increase the likelihood of successful DNA identification. The exposure of tissue to extreme temperatures affects DNA recovery and quality in a similar fashion to that observed in archaeological remains. Additionally, although soft tissue may still be present, many current guidelines recommend removing and discarding this charred tissue because it is hypothesized that the extreme levels of morphological degradation render this substrate unusable for DNA identification. Here, the researchers present a systematic investigation comparing forensic and ancient DNA laboratory protocols: the Dabney 2019 extraction protocol (Dabney & Meyer, 2019) and the Lorielle 2007 protocol (Loreille et al., 2007). This study examines DNA yields across a range of levels of thermal alteration on different skeletal locations and an assessment of DNA preservation in severely charred soft tissues using the QIAGEN DNeasy® blood and tissue extraction kit. Ten donor cadavers were systematically exposed to extreme temperatures (i.e., burned) at the University of Tennessee Anthropology Research Center. From each donor, approximately 10 samples representing all regions of the body (i.e., thorax, long bones) were collected and sent to Arizona State University for processing. Each sample was then visually examined and assigned a burn score on a 1–5 scale, with 1 being the least thermally altered and 5 being the highest based on observed morphological condition. Using both extraction protocols, DNA was isolated from each skeletal sample and from corresponding tissue samples. The resulting DNA extracts were then assessed for total DNA recovery (Qubit™ HS DNA assay and Agilent TapeStation D5000 HS), endogenous DNA content (Quantifilier™ Trio), and short tandem repeat (STR) profile recovery (Promega Powerplex Fusion 6C). Preliminary results indicate that the standard DNA quantification techniques (Qubit™ fluorometry, TapeStation, and Quantifiler™ Trio) are not reliable predictors of actual DNA recovery. However, the detection of any DNA using these metrics does directly correlate to successful STR profile recovery. Additionally, this study found that charred tissue samples consistently returned higher concentrations of both raw and endogenous human DNA and more robust STR profile recovery. This indicates that a re-evaluation of previously established sampling guidelines for severely thermally altered remains recommending the removal of this substrate may be necessary moving forward.

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In terms of the skeletal samples, the complete demineralization protocol developed by Lorielle et al. (2007) generally performed well at lower to medium levels of thermal alteration whereas the ancient DNA Dabney protocol (Dabney & Meyer, 2019) was more suited for STR profile recovery at higher estimated levels of thermal alteration.

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Species Identification in Forensic Casework Using Proteomics

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Glendon Parker*,[1]
Kyle Burk[1]
Nicole Slattengren[2]
Ashley Spicer[2]
Barry Baker[3]

[1] University of California, Davis

[2] California Department of Fish and Wildlife

[3] United States Fish and Wildlife Service

* Submitting author

A significant portion of wildlife crime is focused on the international trafficking of furs from endangered and protected species. Protection for these species includes the international Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) treaty, enforced through the Federal Endangered Species Act, and state-level regulations such as the California Endangered Species Act and the California Fur Ban (FGC §2023). These furs are not amenable to DNA typing because of the harsh chemicals used in fur processing that degrade DNA, rendering the fur unusable for species identification. To address this gap in law enforcement, this project is developing proteomic workflows to identify protein markers for species identification of fur from a single hair shaft. The Proteomic Fur Project is engaged in processing 45 species, 29 of which are relevant to the California Fur Ban, and 16 *Felidae* species that are a focus of the United States Fish and Wildlife Office of Law Enforcement. Two approaches are being used: (1) the measurement of relative reference proteome efficiency in aligning peptide sequences to data and (2) the identification, discovery, and characterization of species-specific peptide biomarkers. One example is muskrat (*Ondatra zibethicus*), a major economic driver of the wild-caught fur trade. Triplicate hair shafts from three individuals were processed (n=9) and submitted to mass spectrometry. Resulting spectra were aligned with peptide sequences from a custom muskrat reference proteome. A total of 17 peptides from 11 proteins were detected and aligned to the muskrat proteome with 100% specificity and sensitivity. The average intensity of the top nine peptides was greater than 109 ions, four orders of magnitude above the detection limit. These candidate peptide biomarkers will help establish sensitive, specific, and robust targeted assays for species identification of degraded samples and therefore represent a powerful new tool for wildlife forensic casework.

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Testing New Methods for Degraded DNA Recovery and Next-Generation Sequencing

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Erin M. Rawls*[,1]
Matthew Emery[1]
Katelyn L. Bolhofner[1]
Amber Coffman[1]
Stevie Winingear[1]
Jane Buikstra[1]
Cody E. Parker[1]
Anne C. Stone[1]
Amanda Wissler[1,2]
Laura Fulginiti[3]
Adam Ben Rohrlach[4]

[1] Arizona State University

[2] McMaster University

[3] Maricopa County Office of the Medical Examiner

[4] University of Adelaide and the Max Planck Institute for Evolutionary Anthropology

* Submitting author

Attendees will learn how technical advances used to isolate and sequence genomic DNA from ancient remains opens new doors for the analysis of modern degraded tissue. The presenter will discuss the efficiency of isolating genomic DNA using ancient DNA and modern forensic extraction methods and their success rates for subsequent short tandem repeat (STR) and mitochondrial genome analyses. Attendees will also learn about DNA damage patterns as assessed using bioinformatic analyses of genomic data revealing how hot desert environments impact DNA preservation in skeletal remains in the American Southwest. According to the National Missing and Unidentified Persons System (NamUs), there are over 600,000 unidentified human remains in the United States as of February 2023. On average, 4,400 are added each year, of which roughly 1,000 remain unidentified. Degradation of these remains presents technical challenges for their identification by researchers and government agencies alike. Techniques used to isolate ancient DNA from archaeological samples could be efficient in cases of highly degraded forensic remains. For example, a method for DNA extraction (using guanidine hydrochloride) was developed by Dabney et al. (2019) to recover DNA fragments as small as 30–50 bp in size. This method has been used to successfully recover analyzable mitochondrial DNA (mtDNA) data from paleoanthropological samples as old as ~400,000 years. For this study, the Maricopa County Office of the Medical Examiner provided 75 skeletal samples representing 42 individuals who have remained unidentified by standard forensic procedures, such as STRs. DNA from bone and teeth samples was extracted using the Dabney protocol and a forensic protocol developed by Loreille and colleagues (Loreille et al., 2007) for degraded samples. The DNA extracted was used to create double- and single-stranded libraries. These libraries were then used for targeted enrichment of the mtDNA genome performed using biotinylated mitochondrial RNA baits synthesized from the H. sapiens Representative Global Diversity Panel (197 mtDNA sequences) (Daicel Arbor Biosciences, Ann Arbor, MI). Single-nucleotide polymorphism (SNP) capture was completed using a custom SNP panel targeting ~4,200 SNPs (Daicel Arbor Biosciences, Ann Arbor, MI). These enriched libraries were then subjected to Illumina® sequencing. The researchers found that the Dabney extraction method resulted in an average 4.4-fold improvement in DNA yield compared with the Loreille extraction method. From the double-stranded DNA libraries, the researchers generated mitochondrial genomes ranging from 0.3–246.8× depth of coverage with average fragment sizes of 89 bp from 62 samples. Sequencing reads were not recovered from 13 samples, likely because of a lack of sufficient DNA. Analyses of the mtDNA sequence data from the single-stranded libraries and the sequencing of the genome-wide SNP enriched libraries are currently underway. Using these data and additional analyses of DNA damage patterns

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and preservation across skeletal elements and environmental contexts, the researchers aim to identify the optimal means of DNA recovery from degraded skeletal tissues.

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