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Gabby DiEmma and Erica Fornaro, Editors

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About the Editors

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

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Abstract

The 2025 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 and 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.

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, an independent scientific research institute dedicated to improving the human condition. Our vision is to address the world's most critical problems with technical and science-based solutions in pursuit of a better future. 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 18, 2025, NIJ and the FTCOE held the 2025 NIJ Forensic Science Research and Development (R&D) Symposium. Hundreds of attendees 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 Tracey Johnson, Megan Chambers, Jillian Conte, Gregory Dutton, Tiffany Layne, Danielle McLeod-Henning, Frances Scott, and Rachel Wendt—worked to create a phenomenal research agenda. The full program included 16 presentations and 24 posters from principal investigators and their research partners; these presentations and posters represent accomplishments from NIJ's current R&D portfolio. Most presentations are archived on the FTCOE's website and available to view for free.

Dr. Dutton, Ms. Wendt, Dr. Chambers, and Dr. Layne were moderators. Dr. Dutton moderated Session I, Trace Evidence/Fire Investigation/Physics and Pattern; Ms. Wendt moderated Session II, Forensic Anthropology and Forensic Pathology; Dr. Chambers moderated Session III, Seized Drugs and Toxicology; and Dr. Layne moderated Session IV, Forensic Biology/DNA.

Summary of Oral Presentation and Poster Session Topics

NIJ Forensic Science Research and Development Symposium

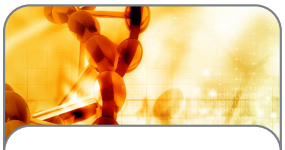
February 18, 2025

Oral Presentation and Poster Session Topics

Session I
Trace Evidence/ Fire Investigation/ Physics and Pattern

Session II
Forensic Anthropology and Forensic Pathology

Session III
Seized Drugs and Toxicology



Session IV
Forensic Biology/DNA

465 Total Attendees
159 In Person
306 Online

33 Community Interactions

3,907 Total Learning Hours

16 Podium Presentations

24 Posters

23 Online Q&A Submissions

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SESSION ABSTRACTS

SESSION I

TRACE EVIDENCE/FIRE INVESTIGATION/ PHYSICS AND PATTERN

Moderated by NIJ Program Manager
Gregory Dutton

Assessment of the Added Value of New Quantitative Methodologies for the Analysis of Surface Soils in Forensic Soil Comparisons

NIJ AWARD #: 15PNIJ-21-GG-02711-SLFO

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Geologic materials, including soil and dust, are ubiquitous and often inadvertently transferred during crime events. Forensic geologists use a range of particle-based analytical approaches to characterize the inorganic fraction of soils, with the resulting data primarily used to form subjective interpretations. Geological materials are also increasingly used to help address provenance questions for investigative leads and intelligence purposes. In many cases, such analyses provide sufficient information to conclude whether there is or is not the possibility the questioned soil originated from the same source as the known. However, there are inevitably cases where the samples being compared lack exclusionary differences or there is too little inorganic material for analysis. In these scenarios, information gleaned from new quantitative methodologies might provide valuable exclusionary differences. In this study, two types of surface soils representing scenarios that would potentially benefit the most from new quantitative methods were collected from across North Carolina. These include surface soils with (1) similar inorganic content but with distinct land use (15 locations), and (2) limited inorganic content but recognizable organic fractions (15 locations). At each location, triplicate samples (1 m apart) were collected from two sites approximately 100 m apart to assess method reproducibility, accuracy, and small-scale variation that might be realistically observed in Q-to-K comparisons (total n≈180). Each sample was subjected to examination using methods currently used in practice (e.g., manual color determination, polarized light microscopy, X-ray diffraction), along with three new quantitative methods: (1) instrumental colorimetry, (2) automated scanning electron microscopy–energy-dispersive X-ray spectroscopy (SEM-EDS) of soil minerals, and (3) DNA metabarcoding of plants, bacteria, arthropods, and fungi. This presentation will outline the study's final findings, comparing the utility of these three new quantitative methods for the differentiation of highly similar soils at various levels, including triplicate samples, paired samples at the same site, samples across sites in the same region (i.e., Coastal Plain, Piedmont, Mountain), and across North Carolina. Some key findings that will be highlighted include (1) the combination of color and mineral grain presence improves the capacity to differentiate soils, whereby 92% of sites could be differentiated by comparing average color with the unique presence or absence of a mineral type among all three subsite samples; (2) Bray-Curtis dissimilarity derived from plant and fungi taxa could be used to differentiate sites within a single location; and (3) a customized SEM-EDS method was developed that provided objective high-throughput mineral classification and quantitation. The results of this study demonstrate improved methods of differentiating soils and provide analytical methods for samples containing insufficient inorganic content for conventional examination.

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The Influence of Soils and Chlorinated and Non-Chlorinated Agitated Water on Surface-Enhanced Raman Spectroscopic Analysis of Artificial Dyes on Hair

NIJ AWARD #S: 15PNIJ-21-GG-04169-RESS, 2020-90663-TX-DU

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* Presenting author

Chlorine, commonly found in pools and tap water, presents an intriguing concern in forensic hair analysis due to its sources and composition. In addition to chlorinated water, hair can be exposed to soils that contain various microorganisms. Current forensic analysis involves optical microscopy, which is subjected to advanced training where multiple experts can deliver opposing conclusions about the same hair sample. Despite challenges in traditional analysis methods, emerging techniques like surface-enhanced Raman spectroscopy (SERS) offer promising solutions, showcasing success even in harsh environments such as prolonged sunlight exposure or stagnant water immersion. This study employs partial least-squares discriminant analysis (PLS-DA) to evaluate SERS efficacy in identifying dyes on hair immersed in chlorinated and distilled moving water for up to 8 weeks. The researchers also coupled PLS-DA and SERS to examine the effect of hair exposure to different soils. The results demonstrated that one semi-permanent colorant overwhelmingly influenced Raman signals in dyed hair exposed to chlorinated and non-chlorinated water over an 8-week period, masking other colorants' spectral signatures. Despite one colorant's dominance, PLS-DA identified underlying colorants and their exposure conditions, suggesting persistent, unique interactions between original colorants and the environment. The researchers found that SERS enabled the correct prediction of 97.9% of spectra for five out of the eight dyes used within the 8 weeks of exposure to different soils. These results highlight high potential for PLS-DA-based identification of dyes on hair using SERS.

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Experimental Study of Heat Transfer and Fire Damage Patterns on Walls for Fire Model Validation

NIJ AWARD #: 15PNIJ-21-GG-04167-RESS

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Fire damage patterns can occur on solid objects, such as the walls of a structure, when they are subjected to fire exposures. This physical evidence may be collected after a fire to support a fire investigation. Discoloration and mass loss fire effects are driven by thermal decomposition (e.g., mass loss) of walls, which in turn is driven by the fire exposure. Heat flux is known to vary spatially and temporally over fire-exposed walls, and it is commonly presumed that regions of greater damage can be related to greater cumulative heat flux. Fire models can be used to predict heat flux and mass loss on fire-exposed walls. Fire investigators can leverage fire models to test hypotheses; however, there is presently no suitable mechanism to relate fire model heat flux and mass loss predictions to the physical evidence (i.e., fire damage patterns). Furthermore, the validation space for predictions of heat flux over fire-exposed walls is not satisfactory. The objective of this study was to develop a comprehensive dataset to address the shortcomings in this validation space and to identify a mechanism to relate fire model predictions to physical evidence. Freestanding walls constructed of gypsum wallboard (GWB) and measuring 1.2 m (4 ft) wide by 2.4 m (8 ft) tall were exposed to fires. Fire sources included a natural gas burner, gasoline and heptane pools, wood cribs, and upholstered furniture. Full-field temporally and spatially varying heat flux was measured using a newly developed software tool that has been released to the public. Fire damage patterns were measured using photography (i.e., discoloration fire effect) and mass loss surveys (i.e., mass loss fire effect). Fire damage patterns attributed to the discoloration fire effect were defined as the line of demarcation separating charred and uncharred regions. The average cumulative heat flux and mass loss ratio coinciding with the lines of demarcation over all experiments were 10.4±1.5 MJ/m² and 14.9±2.1%, respectively. These damage metrics may have utility in predicting char delineation fire damage patterns in GWB using a fire model, with the mass loss ratio metric being the best fit over all exposures considered. Although cumulative heat flux fields were qualitatively consistent with the observed fire damage patterns on a case-by-case basis, no direct correlation was found between cumulative heat flux and mass loss that applied to all fire types considered. Although heat flux drives thermal decomposition of GWB, mass loss is a temperature-dependent phenomenon for which the time history of exposure is relevant. Based on these findings, it is recommended that cumulative heat flux should not be used as the sole metric for predicting fire damage patterns using a fire model.

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Evaluation of the Occurrence and Associative Value of Non-Identifiable Fingermarks on Unfired Ammunition in Handguns for Evidence Supporting Proof of Criminal Possession, Use, and Intent

NIJ AWARD #: 15PNIJ-21-GG-04192-RESS

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This project explores the application of non-identifiable fingermarks (NIFMs) on loaded ammunition to link suspects with firearms. NIFMs are fragmentary, partial fingermarks that are insufficient for identification and that, as a result, have remained unused as a matter of routine, historical practice. Prior NIJ-sponsored research has shown that NIFMs have strong associative value. The goal of the project is to answer the question of how often NIFMs occur on naturally loaded ammunition and what range of associative values can be expected. If NIFMs of high associative value occur commonly on loaded ammunition, this will provide the impetus for a major paradigm change that will use this additional source of evidence. Alternatively, if the value is very limited or rarely occurring, the results will inform researchers, reviewers, and funding agencies of their limited potential benefits. Cyanoacrylate fuming and fluorescent staining using BY-40 were used to process 934 rounds of handgun ammunition collected from 150 handguns. Rounds showing coherent ridge detail with four or more minutiae were photographed (after Porter et al., 2015), using multiple exposures followed by image processing for cylindrical unwrapping. Minutiae were expert evaluated and annotated using the Picture Annotation System (PiAnoS) (University of Lausanne, 2021). Fingermarks judged identifiable were found on only 2.7% of rounds. However, marks with four or more minutiae (sufficient for measurement of associative value using expected score-based likelihood ratios [ESLRs]) were found on 21.1% of the rounds. ESLR measurements for these marks (after Stoney et al., 2020) show a wide range of values, representing strengths of association ranging from an expected random correspondence of 1 in 62 ($\log_{10}$ ESLR of 1.79) for one of the four minutiae marks, to an expected correspondence of 1 in 428 billion ($\log_{10}$ ESLR of 11.63 for 11 minutiae marks). For comparisons, the researchers grouped rounds of ammunition by caliber class (.22, .32, .38, .45) and handgun type (revolver, semi-automatic pistol), where the caliber class "38" included 38 Special, 357 Magnum, 380 Auto, and 9 mm calibers, and the class "45" included 40 S&W, 44 Magnum, 45 Colt, and 45 Auto calibers. Percentages of fingermarks with four or more minutiae on semi-automatic pistol rounds in the caliber classes were .45: 11.1%; .38: 18.9%; .32: 45.6%; and .22: 18.4%. Percentages for revolver rounds were .45: 31.7%; .38: 31.1%; and .22: 8.5%; no .32 caliber revolvers were observed. Among 269 marks with 4 or more minutiae, there were 46.5% with 4, 23.0% with 5, 11.5% with 6, 6.7% with 7, 5.6% with 8, 3.3% with 9, and 3.3% with 10 or more. The observations clearly demonstrate the common occurrence of NIFMs of measurable associative value on loaded handgun ammunition. Although differences among handgun types and caliber classes occur, it is expected that case-specific factors (mostly unknowable in casework and uncontrolled in this study) will have a significant contribution as to whether useful NIFMs will appear on loaded ammunition in any given case. These factors include the condition of the subject's fingers and the ammunition's surface and the dynamics of alternative loading practices.

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SESSION ABSTRACTS

SESSION II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Moderated by NIJ Program Manager
Rachel Wendt

Optimizing Bruise Detection in Forensic Imaging: A Comparative Analysis of Object Detection Models

NIJ AWARD #: 15PNIJ-21-GG-04145-SLFO

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Bruise detection is essential in forensic investigations, particularly in cases of physical trauma where precise identification and documentation are crucial for legal and medical purposes. This study evaluates the application of widely used object detection models, including Faster R-CNN, FCOS, RetinaNet, and YOLO, for forensic bruise detection. The researchers address key challenges these models face in real-world forensic imaging and propose mitigation strategies. Additionally, the presenter will briefly explore the interdisciplinary connection between structural health monitoring and forensic science, demonstrating how visual diagnostic techniques from engineering can inform and enhance forensic imaging methodologies. Using a high-resolution, expert-annotated dataset, this research assesses the models based on precision, recall, and overall accuracy. Transfer learning techniques are employed to improve detection performance under typical forensic imaging challenges, such as skin tone variability and inconsistent lighting. A core aspect of this research examines the impact of poor image quality—such as low resolution, blur, and suboptimal lighting—on detection accuracy. The presenter will discuss the limitations of current object detection models under these conditions and offer strategies to improve their effectiveness. Additionally, lightweight deep learning algorithms are adapted for rapid bruise detection, potentially streamlining forensic workflows and enhancing injury assessment reliability. These findings highlight the potential for computer vision-based models to address common challenges in forensic imaging, contributing to more accurate and efficient bruise detection. This advancement could significantly improve injury documentation and post-trauma care, with notable implications for clinical and legal outcomes.

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Using Artificial Intelligence: Deep Learning for Human Decomposition Staging

NIJ AWARD #: 15PNIJ-21-GG-04161-SLFO

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The degree of decomposition is vital for estimating the postmortem interval and identifying human remains. Existing decomposition scoring methods are manual and rely on subjective interpretation made by humans affecting the accuracy of downstream tasks. These labor-intensive methods are not scalable to the emerging large-scale archival collections of human decomposition photographs. The aim of this research is to explore the feasibility of automating two common human decomposition scoring methods proposed by Megyesi et al. (2005) and Gelderman et al. (2019) using artificial intelligence. Two popular deep learning model architectures (Inception V3 and Xception) were trained on a large dataset of human decomposition photographs to classify the stage of decay for different anatomical regions, including the head and neck, torso, and limbs (including the hands and feet). An interrater study using the Fleiss kappa statistic found the reliability of the developed artificial intelligence models to be comparable with that of expert human forensic examiners for stage of decay identification. The Xception model (Boesch, 2024) achieved the best classification performance, with macro-averaged F1 scores of 0.878 for the head, 0.881 for the torso, and 0.702 for the limbs when predicting the Megyesi et al. (2005) stages of decay and 0.872 for the head, 0.875 for the torso, and 0.760 for the limbs when predicting the Gelderman et al. (2019) stages of decay. This work demonstrates the potential of artificial intelligence models trained on a large dataset of human decomposition images to automate stage of decay identification.

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Deep Learning Empowers Fine-Grained Population Affinity Estimation With Craniometric Data

NIJ AWARD #: 15PNIJ-22-GG-04431-RESS

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* Presenting author

Estimating population affinity from skeletal remains is one of the most crucial tasks of forensic anthropology. Craniometric data are the most frequently used data source for this task. Multiple statistical and computational methods have been developed for craniometric population affinity estimation, including but not limited to linear discriminant analysis (LDA), geometric morphometrics, mixture model-based clustering, and artificial intelligence (AI). Although LDA remains the most-used method, AI or machine learning–based methods often outperform traditional statistical methods when determining the accuracy of the predictions. The recent debate on the practice of ancestry estimation in forensic anthropology has demanded methods with good performance with fine-grained population definitions. The researchers hypothesize that deep learning models can be potential candidates for such practices. Deep learning is a recent breakthrough in AI that has surpassed traditional machine learning methods in many areas. The researchers developed several deep feedforward neural network models for population affinity estimation using the Howells Craniometric Data Set, which consists of 82 craniometric measurements from 2,412 human crania from 26 populations collected globally. The average accuracies obtained were 94% for 26-population affinity estimation based on cross-validation and 89% based on test data. The results suggest that deep learning models can help to obtain great accuracy for fine-grained population affinity estimation.

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Is Decedent Residual Odor Detectable by Human Remains Detection (HRD) Canines and Analytical Chemistry?

NIJ AWARD #: 15PNIJ-22-GG-04412-SLFO

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James Ha
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Human remains detection (HRD) canine teams (dog and handler) have recently been used to locate "residual" odor of deceased individuals in the absence of a body. However, no scientific research has addressed the reliability of canine detection of residual odor. The goal of this project is to evaluate the ability of 35 canine teams to detect residual odor in a double-blind, standardized residual odor recognition test (ORT). Residual odor was obtained by placing cleaned gauze underneath three recently deceased donors (targets) and three living participants (distractors) for 10 minutes. The nine ORTs consisted of 18 clean paint cans arranged in three rows of six spaced 5 feet apart. Sixteen cans contained control (blank) gauze, one can contained gauze from a target, and one can contained a distractor sample. Each canine team participated in three trials within a single day. All 35 teams were presented with samples from the same targets and distractors, although their location changed in each trial. All gauze samples used in the trials were tested using analytical chemistry immediately after each trial to detect the metabolites present. Each gauze sample used in the ORT was submitted for headspace solid-phase microextraction (SPME) analysis to identify volatile organic compounds (VOCs). A total of 1,790 spectral features were detected. Partial least-squares discriminant analysis demonstrated that there were distinct metabolic profiles of the target, distractor, and blank gauze. This means that there are distinguishable VOC profiles between residual decedent and living person odor. However, HRD teams were unable to discriminate among blank, distractor, and target odors accurately and consistently. In a total of 105 trials, the target was correctly identified only 30 times (28.57% sensitivity). The positive predictive value (PPV) was 0.13, meaning an alert was correct only 13% of the time. Only one team correctly identified the target sample in all three trials, but that team also identified non-targets as positive, resulting in a team PPV of only 23.3%. Analysis of the video and audio showed a wide range of handling styles across handlers, some of which affected the results. Video and audio also revealed effects of handler bias, cuing, and offered insight into training and handling errors that may be correctable. Although the results indicate that residual odor may be below the limits of detection of standard trained HRD canines, the fact that chemical signatures exist indicates that HRD team performance may improve with appropriate residual training aids.

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SESSION ABSTRACTS

SESSION III
SEIZED DRUGS AND TOXICOLOGY

Moderated by NIJ Program Manager
Megan Chambers

Identifying High-Quality Aptamers for Drug Detection

NIJ AWARD #: 15PNIJ-22-GG-04440-RESS

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Aptamers are single-stranded DNA or RNA oligonucleotides that are isolated via an in vitro method, systematic evolution of ligands by exponential enrichment (SELEX). Due to their high affinity and specificity, aptamers can be used in various detection applications, such as the identification of illicit drugs in seized substances. Because the SELEX process typically provides hundreds to thousands of unique sequences, a high-throughput method is needed to rapidly perform binding characterization screens for forensic applications such as drug detection. The gold standard method used to measure binding affinity is isothermal titration calorimetry (ITC), which can provide accurate information on the thermodynamics of binding. However, it is a low-throughput process. Alternatively, there are two established high-throughput methods for screening aptamers—the exonuclease digestion assay and the strand displacement assay—both of which are fluorescence based. Here, the researchers compare the performance and accuracy of these two methods for determining the binding properties of drug-binding aptamers. Although most of the data between assays are consistent, there are some aptamer-ligand pairs for which the results are discordant. For example, both techniques were in close agreement regarding the binding properties of a heroin-binding aptamer (HM116) and an oxycodone-binding aptamer (OM6), but inconsistent results were obtained for the aptamers XA1 and F27, which respectively bind the synthetic cannabinoid XLR11 and the opioid fentanyl. ITC confirmed that the exonuclease digestion assay is more accurate than the strand displacement assay.

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Caught Green-Handed: The Detection of Potential Cannabis-Use Biomarkers in Fingerprint Residues Using Mass Spectrometry

NIJ AWARD #: 15PNIJ-23-GG-04236-RESS

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Cannabis sativa is the most widely used controlled substance in the United States. Despite its growing legality at the state level, there are instances when it is important to know whether an individual has consumed cannabis, such as in the event of a driving under the influence case or an accidental consumption case. Techniques for the definitive detection of cannabis ingestion can be invasive, requiring the collection of blood or urine. Furthermore, these biological matrices cannot be readily collected in the field, such as at a traffic stop. This research seeks to develop a less invasive and field-deployable method for the determination of cannabis use through the detection of cannabis metabolites in fingerprint residues using high-resolution mass spectrometry. Fingerprint residue samples collected from donors who had consumed cannabis via inhalation or oral administration and donors who had not consumed cannabis were solubilized, and their chemical profiles were analyzed using direct analysis in real time–high-resolution mass spectrometry (DART-HRMS). The mass spectral data from the two experimental groups were compared using machine learning models to identify *m/z* values that can differentiate cannabis use from non-use. Several models with cross-validation accuracies of greater than 85% were created. A list of *m/z* values that were found to be impactful in enabling these models to discriminate between the two experimental groups was revealed. Future work will focus on determining the identity of the *m/z* values that enabled discrimination between the experimental groups to identify potential cannabis consumption–specific biomarkers. These compounds can serve as the basis for a field-deployable test for cannabis use.

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Chromatographic Interferences That Can Inflate the Levels of Δ9-THC in Cannabis Samples

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

Walter B. Wilson

National Institute of Standards and Technology

The passage of the Agriculture Improvement Act of 2018 (Farm Bill) legalized hemp plants containing 0.3% or less Δ9-tetrahydrocannabinol (Δ9-THC), leading to a surge in hemp production and availability of hemp-derived finished products. Additionally, hemp manufacturers started to convert cannabidiol-rich hemp flower into other cannabinoids such as Δ8-tetrahydrocannabinol (Δ8-THC) and other THC isomers. For these reasons, forensic laboratories have seen a significant increase in the seizure of cannabis plant samples and cannabis-derived finished products. In response, the National Institute of Standards and Technology (NIST) has developed and evaluated analytical methods to provide forensic scientists with the tools necessary to assign seized samples as hemp or marijuana. The primary technique employed at NIST has included liquid chromatography (LC) coupled to an ultraviolet (UV) detector or photodiode array (PDA) detector. LC is the most widely employed separation technique by the cannabis industry because it permits the determination of total Δ9-THC, which is calculated as the sum of Δ9-THC and its acidic precursor tetrahydrocannabinolic acid (Δ9-THCA). Despite numerous advantages, the LC separation of Δ9-THC is susceptible to chromatographic interferences from chemicals present in the samples or created due to the adulteration of cannabis products (e.g., byproducts of synthetic processes). In this presentation, NIST will highlight examples of these chromatographic interferences, including cannabinolic acid, that form due to improper long-term storage, resulting in the degradation of Δ9-THCA. A second co-elution issue involves the presence of synthetic byproducts in Δ8-THC vape products. Last, NIST will provide examples of how chromatographic methods can be modified to prevent these types of co-elution issues in the future.

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Evaluation of a Quantitative Analysis Method for Tetrahydrocannabinol Isomers in Biological Matrices

NIJ AWARD #: 2020-DQ-BX-0017

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Recently, forensic toxicology laboratories have been grappling with the emergence of tetrahydrocannabinol isomers within biological specimens. Traditional methods for the identification and quantitation of cannabinoids only includes the evaluation of Δ9-tetrahydrocannabinol (Δ9-THC) and its metabolites in biological matrices. Upon analysis of additional tetrahydrocannabinol isomers, laboratories often find co-elution or minimal separation between Δ9-THC; exo-THC; Δ8-THC; Δ10-THC; and Δ6a,10a-THC. These emerging isomers are commonly observed in the seized drug community in manufactured cannabis products (e.g., edibles, electronic cigarette cartridges). Trends within the seized drug community and legislative changes to include tetrahydrocannabinol isomers dictate the need for change within forensic toxicology. Traditional methods require adaptation to the ever-changing climate surrounding tetrahydrocannabinol. Additional method development with subsequent validation to meet ANSI/ASB Standard 036, Standard Practices for Method Validation in Forensic Toxicology, is often required. A dual chromatographic column method was developed and optimized for the separation of tetrahydrocannabinol isomers. An Agilent Technologies 1290 Infinity liquid chromatograph was coupled independently to a 6460 and a 6470 quadrupole mass spectrometer for development and validation. Two independent chromatographic methods were developed using different analytical columns, mobile phase conditions, chromatographic gradients, and flow rates. The qualitative analytical method used an Agilent Technologies Poroshell 120 PFP 3.0 × 100 mm, 2.7 µm column held at 50°C. The quantitative analytical method used an Agilent Technologies Poroshell 120 EC-C18 3.0 × 50 mm, 2.7 µm column held at 50°C. The dual-column methodology was used to enhance the separation of tetrahydrocannabinol isomers. The sample preparation procedure consisted of the supported liquid extraction (SLE) using 0.5 mL of biological specimen. The biological specimen was acidified with 200 µL of formic acid in water before placement onto the SLE cartridge. Specimens were allowed to incubate for 5 minutes before the addition of ethyl acetate (3.0 mL). After elution and collection, *n*-hexane (3.0 mL) was added to each cartridge. Samples were evaporated to dryness at approximately 50°C and reconstituted in 50 µL of methanol. The optimized method was validated for quantitation of Δ9-THC, (±)-11-hydroxy-Δ9-THC (Δ9-OH-THC), (±)-11-nor-9-carboxy-Δ9-THC (Δ9-carboxy-THC), (-)-Δ8-tetrahydrocannabinol (Δ8-THC), and cannabidiol to meet ANSI/ASB Standard 036. All other isomers were validated to meet qualitative identification criteria. When validating, samples were evaluated on both analytical methods to ensure congruence in results. The calibration range was 1/2/5 ng/mL to 100/200/500 ng/mL (Δ9-THC, Δ8-THC/Δ9-OH-THC, cannabidiol/Δ9-carboxy-THC). All compounds were within ±20% for bias and precision when evaluating pooled fortified samples of blank blood, antemortem blood, and postmortem blood. Significant ionization

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suppression (>25%) was noted for antemortem blood, postmortem blood, and urine, requiring additional matrices to be added to the estimated limit of detection and lower limit of quantitation experiments. An extensive validation was performed on the optimized SLE extraction with subsequent analytical analysis using liquid chromatography tandem mass spectrometry. The validated method ensures chromatographic separation between tetrahydrocannabinols, providing enhanced identification of these isobaric compounds.

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SESSION ABSTRACTS

SESSION IV

FORENSIC BIOLOGY/DNA

Moderated by NIJ Program Manager
Tiffany Layne

Trace DNA in Activity-Level Propositions

NIJ AWARD #: 15PNIJ-22-GG-04425-DNAX

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Consider the scene—your DNA is found on a knife handle at the scene of a stabbing. One side presents this as evidence that you stabbed the victim with the knife. The other side, however, argues that you shook hands with the actual perpetrator, and they stabbed the victim with the knife. The critical question becomes not "whose DNA is it?" but rather, "how did the DNA get there?" Evaluations of the evidence given the donor's activities inform such activity-level propositions. The forensic scientist is uniquely qualified to interpret the data that inform activity-level questions and present them to the trier of fact. The fitness of the interpretation depends on the available empirical data, as well as the forensic scientist's education, training, and experience. The goal of this project was to generate empirical data to support activity-level evidence interpretation. The presenters used a well-established protocol—the domesticated fingerprint, a ground-truth sample containing a known quantity of DNA. A transfer vector, the domesticated hand, was included to eliminate the human variable to allow critical evaluation of DNA transfer events. The researchers performed multistep mock crime scene scenarios, including hand-to-hand, hand-to-surface, and surface-to-surface contacts. Samples were collected from each surface in the transfer pathway, as well as from each vector, and the DNA was extracted and quantified. Sampling each surface in the pathway allowed the researchers to track the DNA through the multistep transfer events, accounting for 98%–100% of the DNA originally added as a domesticated fingerprint and increasing confidence in the results. By repeating each pathway 20 times independently, the presenters were able to generate mean DNA recovery values, measure the standard deviation, and run an analysis of variance. The researchers found that the difference in recovery after a direct versus indirect transfer is statistically significant for all scenarios tested. Further, the quantification results were predictive of the DNA profiling success. This is a first step toward developing these empirical data to be useful in the case assessment and interpretation processes that are critical to the use of activity-level propositions. This research suggests that assessments of alternate scenarios provide tremendous value when they are backed by empirical data and can greatly aid the trier of fact. Forensic experts should offer their opinions about activity based on education, training, experience, and empirical data.

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A Comparison of Small-Amplicon Mitogenome Enrichment Methods for Massively Parallel Sequencing of Low- and High-Quality Sample Types

NIJ AWARD #: DJO-NIJ-22-RO-0005

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Massively parallel sequencing (MPS) of mitochondrial DNA (mtDNA) is critical in historical and forensic cases involving degraded remains or distant maternal relatives. Polymerase chain reaction (PCR) enrichment with two long-range (LR) amplicons (~8.5 kb) used for population databasing and reference samples is not amenable to degraded remains. However, hybridization capture of the mitochondrial genome, or mitogenome, capable of targeting DNA fragments less than 100 bp, is time consuming and not necessary for samples with more intact fragments. The commercial development of small-amplicon (~100–250 bp)-based approaches for mitogenome enrichment allows laboratories to implement MPS for mtDNA analysis on a wider scale for a range of sample types and qualities. To evaluate these assays, a study was conducted to compare five small-amplicon mitogenome MPS kits following their respective manufacturers' protocols: Precision ID mtDNA Whole Genome Panel, ForenSeq™ mtDNA Whole Genome Kit, PowerSeq® Whole Mito System, QIASeq Targeted DNA Human Mitochondria Panel, and NimaGen IDseek® Mitochondrial DNA Full Genome Sequencing. A total of 96 samples were processed with each kit, including a variety of substrates and extraction methods, non-human samples, mixtures, serial dilutions, and population samples from a range of haplogroups. The majority of samples also underwent LR PCR enrichment as an assessment of sample quality and to further highlight the need for these small-amplicon kits. Additionally, a subset of samples underwent KAPA library preparation and mitogenome capture as an alternative enrichment method for degraded samples with average fragment sizes of less than 100 bp. All mitogenome sequence data were generated with a MiSeq FGx™ with the exception of the Precision ID data, which were sequenced on the Ion GeneStudio™ S5. Data were analyzed using the manufacturers' recommended software and analysis parameters where applicable; otherwise, CLC Genomics Workbench was the default. Overall performance was evaluated for all enrichment methods based on metrics such as haplotype concordance, heteroplasmy detection, and breadth and depth of coverage. All enrichment methods generated concordant mitogenome haplotypes. The average coverage (i.e., read depth) was largely influenced by the manufacturers' recommended multiplexing levels, ranging from 16 to 96 samples; however, there was minimal impact on the number of bases covered above the kit-specific read depth threshold across the mitogenome. This performance assessment combined with a cost-benefit analysis may assist forensic practitioners looking to implement this technology in their own laboratory. Furthermore, this comprehensive study addresses a majority of the requirements for an internal validation of each commercial mitogenome MPS kit.

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Fragmentomics of Hair DNA

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Rootless hairs are a common source of evidence at crime scenes because most individuals shed around 50–100 hairs daily. The DNA present in these rootless hairs is highly fragmented and degraded but can be recovered and sequenced. Recovered DNA can then be used to generate genotype files for forensic investigative genetic genealogy or for comparison of a sample to known genotypes. From an analysis of a large (N=80) panel of DNA sequence data generated from rootless hair shafts, the researchers have found that sequenced DNA fragments also contain data that are orthogonal to the genetic data. Specifically, patterns of where and how strand breakage occurs in hair DNA allow inference of the epigenetic state of the cells that generated the hair. Using only these fragmentation patterns is sufficient to accurately model the most prevalent type of epigenetic modification, cytosine methylation at CpG dinucleotides, if sequencing depth is sufficient. Modeling CpG methylation this way eliminates the need for specialized assays like bisulfite sequencing, which are not practical for degraded DNA such as that found in rootless hairs. Given the strong associations between CpG methylation and identifying characteristics such as an individual's age, fragmentation patterns observed in hair may help open a new avenue of epigenetic-based forensic identification from shotgun sequencing data.

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Adaptive Sampling for the Simultaneous Analysis of STRs, SNPs, and mtDNA in Human Remains Identification

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Short tandem repeat (STR) markers evaluated via capillary electrophoresis (CE) continue to be the gold standard for human remains identification (HRID) in forensic investigations due to their high variability and robust database of comparative samples. However, CE excludes valuable sequence-level information within and around STRs and is not suitable for mitochondrial DNA (mtDNA) or single nucleotide polymorphism (SNP) analysis. The analysis of mtDNA and SNPs is critical in cases where STR analysis fails, such as cases of damaged or 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 forensic laboratories face when analyzing such samples is choosing between depleting sample volumes to repeat 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 HRID that overcomes the most common challenges associated with processing of damaged or degraded remains, bone fragments, aged tissue, and hair samples. Here, the researchers harness the adaptive sampling (formerly read until) capabilities of Oxford Nanopore Technologies (ONT) sequencing to simultaneously analyze STRs, SNPs, and mtDNA for the purpose of HRID. Adaptive sampling uses on-instrument enrichment of target regions of interest, bypassing costly, and often error-inducing, amplification methods. This targeted enrichment approach offers the most efficient DNA-based HRID by allowing simultaneous interrogation of various forensic markers, including STRs, SNPs, and mtDNA, from a single sample while also reducing sample processing costs and turnaround times. The presenter will share the results of this project to date, discuss the pros and cons of using adaptive sampling for HRID, 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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POSTER ABSTRACTS

SESSION I

TRACE EVIDENCE/FIRE INVESTIGATION/ PHYSICS AND PATTERN

Quantitative Matching of Forensic Evidence Fragments of Metals, Ceramics, and Plastics Using Fracture Surface Topography and Statistical Learning

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

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The complex jagged trajectory of fractured surfaces of two pieces of forensic evidence is used to recognize a "match" by using comparative microscopy and tactile pattern analysis. 3D microscopy was used to measure the surface topography of metal, ceramic, and plastic fragment pieces. The measured surface topography is used to establish a quantitative basis for declaring a match, complete with quantified probability and error rates. The comparison scale is configured to capture the transition of fracture surface topography from self-affine to non-self-affine (i.e., surface roughness that is independent of the observation window). At this transitional scale, fracture surfaces display distinctive roughness characteristics, determined by intrinsic material properties, microstructure, and exposure history to environmental and external forces. In the case of the examined class of hardened alloys, which are common in cutlery and tool steel, the identified scale is approximately two times the grain diameter. This scale closely resembles the characteristic distance necessary for the initiation of cleavage fractures in semi-brittle and hardened metallic alloys. Consequently, the imaging scale required is approximately 20 times the grain diameter. Similar trends were also observed in ceramic and plastic fragments. For each pair of fractures, six overlapping images were recorded, with an overlap ratio of 50%. The acquisition of spectral representations for various wavelengths and critical features on the fracture surface was accomplished using the mathematical Fourier transform. Subsequently, quantitative topological descriptions were devised for the image pairs by performing correlation comparisons on two spectral bands encompassing the transitional fracture scale. These frequency bands are bounded by frequencies corresponding to two to four and four to eight times the grain diameter. Consequently, each set of fracture pairs under examination yields a total of 12 correlation values. A statistical learning tool was then formulated, employing multivariate statistical analysis methods to classify the fracture pairs based on this collection of 12 topological descriptors. This classification offers a foundation for establishing the uniqueness of forensic comparisons. The efficacy of the proposed statistical learning methodology was assessed using a robust training dataset and was validated with a set of 38 distinct broken pairs, encompassing knives fractured in bending and stainless-steel rods with comparable grain sizes broken under tension or bending. The versatility of this framework was also examined across various loading conditions by applying it to a set of nine twisted knives until failure. Remarkably, all broken pairs were accurately classified. This framework establishes the groundwork for forensic applications involving quantitative statistical comparisons across a wide spectrum of fractured materials, characterized by diverse textures and mechanical properties (Dawood et al., 2022; Thompson et al., 2020, 2024).

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Application of Particle-Correlated Raman Spectroscopy (PCRS) for the Forensic Examination of Soils

NIJ AWARD #: 2019-DU-BX-0017

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Soil is a valuable type of trace evidence that, when properly recognized, analyzed, and interpreted, has the potential to provide investigative leads and to associate an unknown specimen with a collected known. Although the value of soil evidence is widely recognized by criminalists with a plethora of case examples, criticisms of forensic soil analysis as being subjective, too labor intensive, and too time consuming have resulted in a considerable decline in its use in forensic investigations. As a result, exemplar soil samples are not being collected in the field, which eliminates the possibility of later laboratory analysis. Further, forensic laboratories are not equipped with criminalists who are currently capable of performing a comprehensive forensic soil examination. Thus, the potential of soil traces is not being realized. The purpose of this presentation is to share the results and conclusions of the evaluation of particle-correlated Raman spectroscopy (PCRS) for the analysis of soil minerals for forensic purposes. PCRS is an integrated technique that combines automated image analysis with Raman spectroscopy. Particle imaging determines particle size and shape distributions for each component in a sample, yielding detailed morphological information (e.g., circularity, area). At the same time, Raman spectroscopy can probe the molecular chemistry of specific particles of interest. In forensic soil analysis, PCRS is therefore able to non-destructively identify the types of minerals present and provide morphological information about individual mineral grains. Particle size distributions can be generated for the entire sample or for each mineral present, along with quantitative information on the relative amount of each type of particle. The presenter will report on the results of all aspects of this research, which included (1) method optimization (which involved determining recommended analysis parameters for soil sample preparation, mineral dispersion, imaging, Raman spectroscopy, and data analysis), (2) the evaluation of mineral Raman spectral databases, (3) the comparison of results of PCRS with those of traditional forensic soil analysis by experienced forensic microscopists, (4) the intra- and inter-sample variation and discrimination potential of PCRS using a variety of statistical methods from data collected from triplicate topsoil samples collected from 30 different locations in the Northeast United States, and (5) the analysis of soil collected from mock-evidence items (e.g., shoes and shovels).

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Using Ultrasonic Pulse Velocity to Assess Fire Damage in Drywall

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Traditional techniques for assessing fire damage, such as measuring calcination depth in drywall (gypsum wallboard), often rely on subjective and time-consuming methods. Calcination depth, an indicator of fire severity, correlates with drywall dehydration at high temperatures. Probe devices have been used for measuring calcination; however, inconsistencies persist because the applied force varies between individuals operating the device. This study proposes the use of ultrasonic pulse velocity (UPV) technology, commonly used in civil engineering, as a potential solution. UPV allows for non-destructive, quicker assessments by measuring the travel time of ultrasonic pulses through materials, which correlates with their density and stiffness. Although UPV has been applied to concrete and other materials, its use in drywall fire damage assessments remains underexplored. The study is organized into three tasks. Task 1 was to establish the relationship between UPVs and calcination levels. In this task, drywall samples were exposed to various temperatures for different durations in an oven, ensuring uniform heating and dehydration. The total mass loss of each sample after heating was measured, and thermogravimetric analysis (TGA) tests further characterized the calcination level of each sample. UPV measurements were then taken, and the correlation between UPVs and the calcination levels of drywall was developed. Task 2 focused on developing correlations between the calcination levels of drywall and the total heat exposure and between the total heat exposures of drywall and the UPVs. Drywall samples were exposed to a heat flux of 50 kW/m² for varying durations in a cone calorimeter at one surface. Calcination levels were quantified by measuring total mass loss and by performing TGA tests at different locations along the sample thickness to create a calcination profile. UPV measurements were also taken, and the general correlation between drywall calcination levels and UPVs from the oven tests was verified by the tests from the cone test results. Subsequently, the correlations between drywall calcination levels and total heat exposures and between UPVs and total heat exposures were established. Task 3 performed a sensitivity analysis to ensure the reliability and robustness of the ultrasound-based technique for calcination measurement. This sensitivity analysis examined the effects of drywall density, thickness, moisture content, internal voids, drywall papers, soot deposit, and sample boundary conditions on the UPV test results. Initial tests indicate clear relationships between UPV, calcination level, and total heat exposure. As heat exposure increases and calcination progresses, the UPV decreases, reflecting the material's softening due to gypsum calcination.

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Advancing the Understanding of 3D Imaging for Firearms Identification†

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In forensic firearms identification, one of the newest emerging technologies is 3D imaging technology. The 3D technology allows firearms examiners to virtually compare high-resolution 3D images of the surfaces of bullets or cartridge cases without the effects of variable lighting conditions and shadowing present when using traditional comparison microscopy. As with all new technology, there are challenges associated with implementation and a need to better understand the performance capability and limitations of the systems when used in forensic casework. One such challenge is the demonstrated ability to share images across 3D imaging systems manufactured by different vendors when sharing between crime laboratories. With the adoption of the X3P (XML 3D Surface Profile) file format by the Open Forensic Metrology Consortium, almost all the 3D instruments currently in the market can create scans in the X3P format. In principle, scans obtained using different instruments should be compatible, but this has not been demonstrated. This study focuses on the comparability of images acquired by 3D instruments manufactured by vendors, including Cadre Forensics (TopMatch), Leeds (Evofinder®), and LeadsOnline/ULTRA Forensic Technology (Quantum). This study consisted of two phases: (1) physical and (2) virtual kit comparisons. In Phase 1, each participant performed 10 comparisons using their comparison microscope and current method of comparison. In Phase 2, using software provided by one of the three vendors, the same participants evaluated a virtual test kit consisting of 10 virtual comparisons composed of a combination of scans from the different instruments. The comparisons within each physical and virtual kit (each consisting of three known test fires and one unknown) vary in difficulty and encompass a range of calibers and ammunition types. The preliminary findings of the physical kits and vendor comparisons will be presented.

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Assessing the Reliability of Fire Pattern Indicators in Wildland Fire Investigations: A Field Study†

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Wildland fires pose significant challenges, causing extensive property damage and loss of life. Investigating these incidents requires accurate identification of fire pattern indicators, which are physical markers altered by fire and essential for reconstructing fire origin and spread. Current methodologies, such as those from the National Fire Protection Association (NFPA) 921 (NFPA, 2024) and the National Wildfire Coordinating Group (NWCG) PMS-412 (NWCG, 2016), rely heavily on these indicators. However, the reliability of fire pattern indicators in wildland fire contexts remains underexplored and unvalidated by empirical data. This research seeks to strengthen the scientific basis of fire pattern indicators through a combination of controlled prescribed burns conducted in collaboration with the U.S. Forest Service at the Silas Little Experimental Forest and laboratory-scale experiments. The study evaluates how thermal effects contribute to the formation and reliability of fire pattern indicators under near-ignition conditions. Laboratory-scale experiments were conducted in a 1 m^2 wind tunnel test bed using pine needles as fuel, with staged artifacts such as wood fence posts, pine cones, tin cans, beer bottles, polyvinyl chloride (PVC) plastics, and other supporting elements as experimental materials. These tests investigated fire behavior, focusing on different fire patterns generated with respect to the directionality of the ignition source, as well as the repeatability of observed patterns under controlled airflow velocity and fuel load conditions. The laboratory experimental results demonstrated that fire pattern indicators, such as the angle of deflection and burn height from PVCs, angle of char from the wood fence post, and ash content from the pine cones, are generally reliable for inferring fire ignition direction and spread under consistent conditions. However, certain indicators, such as sooting and staining, exhibited variability influenced by local environmental factors, including airflow velocity and material properties of supporting elements. The field experimental data corroborate the laboratory results, emphasizing the impact of local fire dynamics on indicator reliability. Preliminary conclusions highlight that although fire pattern indicators remain a cornerstone of forensic fire investigation, their reliability can be significantly enhanced by incorporating data on local fire conditions. The findings suggest that indicators should be interpreted in conjunction with comprehensive fire behavior analyses to improve accuracy. This study highlights the importance of establishing standardized protocols that combine laboratory and field data to enhance the scientific accuracy and reliability of wildland fire investigations.

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Analysis of Oil-Based Ignitable Liquid Residues on Wood and Fabric Debris by Gas Chromatography–Mass Spectrometry and Direct Analysis in Real Time Mass Spectrometry

NIJ AWARD #: 2020-DQ-BX-0003

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Oil- and fat-based ignitable liquids (ILs), such as lighter fluid, torch fuel, charcoal starter fluid, and biodiesel, are more sustainable and environmentally friendly alternatives to petroleum-based products. Despite their growing popularity, research on detecting oil-based IL residues in forensic contexts remains limited. Fatty acid methyl esters (FAMEs), the primary compounds in these liquids, are formed through the transesterification of fatty acids during the refining process. In this study, the researcher analyzed the chemical profiles of oil-based ILs refined from various vegetable oils, in the laboratory and from commercial sources, using gas chromatography–mass spectrometry (GC-MS) and direct analysis in real time mass spectrometry (DART-MS) methods. The researcher systematically examined the GC-MS fragmentation patterns for unsaturated and saturated FAMEs based on databases, such as the National Institute of Standards and Technology (NIST) and Chrombox, and experimental data. Additionally, this study evaluated the impacts of other factors, such as wood and fabric substrates, burning, cooking, and headspace extraction, on the FAME profiles. IL residues on substrates and debris were extracted using the ASTM E1412 (ASTM International, 2022a) activated charcoal method, and the extracts were analyzed via GC-MS and DART-MS. The study included a FAME standard solution, commercial oil-based lighter fluid, and oil-based IL samples prepared in the laboratory using 10 vegetable oils. Results indicated that the variety and relative distribution of FAME compounds in IL products depended on the type of oil. Key FAME compounds—C16:0, C18:0, C18:1, C18:2, and C18:3—were identified in the samples, with their relative quantities varying significantly based on the vegetable oil used. Characteristic fragment ions for FAMEs in the GC-MS spectra, including *m/z* 55, 67, 74, and 79, exhibited unique patterns depending on their saturation levels (i.e., saturated, mono-unsaturated, di-unsaturated, and poly-unsaturated FAMEs). This information, validated by mass spectra from the NIST and Chrombox databases, is particularly valuable for identifying FAMEs in ILs, especially in cases where chromatographic peaks are unresolved or co-eluted, providing an additional layer of specificity in compound identification. After GC-MS analysis, the extracts were further analyzed by DART-MS, where protonated ions of FAMEs were successfully detected, providing additional confirmation, particularly for FAMEs with overlapping GC-MS peaks (e.g., C18:2 and C18:0) or low molecular ion intensities. Although burned and unburned wood and fabric substrates contributed peaks to the GC-MS chromatograms, the extracted ion chromatograms of the four characteristic ions closely matched their counterparts without substrates on the principal component analysis (PCA) score plots. ILs prepared from cooked/waste oil and straight oil yielded similar FAME profiles in GC-MS and DART-MS analyses. Overall, this study offers valuable insights into the analysis of various oil-based IL products through their FAME profiles using the ASTM E1412 and ASTM E1618 (ASTM International, 2022b) methods. The results indicate that DART-MS can effectively detect

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FAME compounds directly from ASTM E1412 extracts without requiring additional extraction or separation, providing complementary data for the identification of oil-based IL products.

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Interoperability of Firearm Toolmark 3D Topography Measurements

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There has been a major paradigm shift in firearm and toolmark analysis toward the use of 3D topography measurements. The new approach bolsters objectivity through SI traceable measurements. Several manufacturers are now offering specialized 3D microscopes for toolmark measurement, which rely on differing measurement principles with their own advantages and challenges. These instruments are currently being used in laboratories for virtual comparison microscopy (VCM). The future goal for these instruments is to generate measurements used to report on the statistical weight of evidence of comparison in casework. For impressed toolmarks, no comprehensive study characterizes the resulting differences in 3D data obtained using different instrument types and their effect on objective similarity scores. This is an important gap in the quest for objective comparison results and quantitative weight of evidence reporting. This gap needs to be addressed to ensure consistency in results among laboratories and to provide associated foundational data for future Daubert hearings. The research evaluated the effect of measurement source variations on similarity metrics. This was accomplished through a round-robin study where each laboratory/instrument measures the same set of 120 cartridge cases fired from four sets of consecutively manufactured firearms. Twelve laboratories participated in this study to generate a total of 18 datasets across 9 different 3D instruments. To quantify the differences between laboratories and technologies, each laboratory's measurements were analyzed using two well-established similarity scores: the normalized areal cross-correlation function ($ACCF_{MAX}$) and the number of congruent matching cells (CMCs). The results were used to generate known matching (KM) and known non-matching (KNM) score distributions, which were used to statistically analyze potential differences between laboratories and systems. Results will facilitate improvements in the consistency of measurement results while providing the foundational research data required to defend the future use and interoperability of 3D measurements in casework.

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POSTER ABSTRACTS

SESSION II

FORENSIC ANTHROPOLOGY AND FORENSIC PATHOLOGY

Improving and Evaluating Computed Tomography and Magnetic Resonance Imaging in the Investigation of Fatalities Involving Suspected Head Trauma

NIJ AWARD #: 2016-DN-BX-0173

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Although autopsy is considered the gold standard for death investigation, research suggests that adding postmortem computed tomography (PMCT) improves injury detection. This study examines whether supplementation with postmortem magnetic resonance imaging (PMMRI) further improves injury detection in cases of suspected head trauma. At a medical examiner's office, PMCT was performed on decedents with unknown cause of death and circumstances unclear or suggestive (but not definitive) for head or neck trauma. Whole-body PMCT was performed (Siemens Definition Edge, 64 slice), followed by PMMRI of the head and neck (Siemens Magnetom Symphony, 1.5T). A general radiologist with postmortem imaging experience performed PMCT interpretation, while a neuroradiologist experienced in emergency radiology performed PMMRI interpretation. The radiologists were blinded to each other's findings and those of the forensic pathologist who investigated the case. Imaging findings were subsequently reviewed by a consensus team that included a forensic pathologist and a radiologist, neither of whom had previous familiarity with the case. The consensus team identified "matched" observations and rated the significance of all findings using a modified Goldman classification scheme. The researchers identified 422 unique findings on PMCT and PMMRI from 94 decedents (56 males, 38 females, ages 14–95). Consensus analysis revealed that more findings were reported at PMCT (359) than at PMMRI (158), but PMMRI detected a greater number of fatal findings (PMMRI 50/64, 78%; PMCT 46/64, 72%). The additional fatal findings detected at PMMRI were most often hemorrhages, infarctions, or encephalopathy characterized as small or diffuse, which are expected to be below the sensitivity of PMCT. Although the complementary nature of autopsy and PMCT is well documented, this study suggests that PMCT and PMMRI are also complementary, with the addition of PMMRI resulting in a 40% increase in the number of fatal findings detected at radiology in medical examiner cases with possible head or neck trauma.

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Pre-Grouping of Commingled Human Skeletal Remains by Elemental Analysis

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

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Sorting skeletal remains from a mixed assemblage is a challenging task for forensic anthropologists investigating modern and archaeological mass graves. Sorting the bones to their respective individual can be a tedious process, especially if the bones are fragmented or have undergone taphonomic changes. This study proposes using laser-induced breakdown spectroscopy (LIBS) as a preliminary sorting technique to aid in the reassociation of skeletal elements. LIBS is a visually non-destructive analytical technique that requires minimal sample preparation and can obtain chemical information from bones in a matter of seconds. Additionally, portable LIBS instruments are commercially available for efficient analysis of full-sized skeletal remains in laboratory and field settings. To test the feasibility of using LIBS for sorting skeletal remains, the study simulated data collection from a mass grave using 62 individuals from the John A. Williams Documented Human Skeletal Collection. LIBS spectra from 1,284 bones provided roughly 8,000 chemical signatures to be used in classification. Machine learning algorithms classified bones to their corresponding individuals with an average of 87% accuracy. In addition, a study on comparison and complementarity of the analysis by five experienced anthropologists was conducted. Anthropologists were provided with an assemblage of 100 skeletal elements and were tasked with sorting remains based on their expertise and training. LIBS profiles were also collected and analyzed in conjunction with human sorting results. This research underscores a potential protocol that connects the strengths of traditional and chemical analyses for an optimized reassociation of commingled remains. Ultimately, the results of this study demonstrate how incorporating LIBS as a complementary technique may expedite the sorting process for skeletal assemblages, which is of high relevance in forensic investigations.

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Initial Assessments of Relic DNA Removal from Host and Environmentally Sourced Microbiome Evidence

NIJ AWARD #: 15PNIJ-23-GG-04205-RESS

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The forensic microbiome plays a significant role in the development and use of postmortem interval (PMI) estimation models and trace evidence analysis models within the criminal justice system. However, these tools exhibit a margin of error that may impact their admissibility, of which the sources of error are not fully known. For these tools to be credible, microbiome samples collected as part of the forensic investigation must accurately represent the microbial community. When collecting forensic microbiome data, various types of DNA are present in the sample, including bacterial DNA, non-microbial DNA (e.g., human DNA), and relic DNA (i.e., free DNA that persists in the environment from dead cells). Because relic DNA can remain in the environment, specifically soils, for long periods of time, it can affect the diversity measurements of the microbiome (Carini et al., 2020; Lennon et al., 2018), but it is unknown to what extent. The presenters hypothesize that the presence of relic DNA in forensic microbiome samples negatively impacts the accuracy of forensic microbiome tools. Therefore, this study aims to assess the impact of relic DNA on forensic microbiome data tools used for PMI estimation and environmental trace evidence source connectivity. To address this, the researchers collected environmental trace evidence plus soil source samples and collected soil adjacent to donors at the University of Tennessee, Knoxville, Forensic Anthropology Center throughout the decomposition process for PMI estimation. The specific sites were chosen due to their variability in geographical distance, foot traffic, and foliage. Samples were split and then processed as paired replicates to create a treatment (i.e., relic DNA removed) group and a control group. Samples underwent microbial DNA extraction and 16S rRNA sequencing to profile the microbiome. Predictive machine learning models were trained and tested on matching trace evidence to the environmental source and predicting PMI using microbiome composition, giving an estimate of model accuracy. To determine the impact of relic DNA on these models, the accuracy of the predictive models was compared when trained and tested to predict samples with and without relic DNA inhibition.

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Eggs-ploring the Volatiles Profiles of *L. sericata* Eggs for Postmortem Interval Determination

NIJ AWARD #: 2020-MU-MU-0016

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Blowflies (Calliphoridae) are capable of detecting remains within minutes of death. The decomposing animal tissue serves as an oviposition site and as a food resource for larval development. Leveraging the correlation between insect species identity, environmental conditions, the decomposition stage of the corpse, and the well-known species-specific insect life cycle timelines, a "back calculation" method to estimate the time of death (i.e., postmortem interval [PMI]) exists through evaluation of larval development and eclosion times of insect eggs. Focusing on *Lucilia sericata* (Meigen), the common green bottle fly, the volatiles compound emissions of eggs as a function of level of maturity were monitored in order to enhance the precision of PMI estimation. The headspace volatiles emitted by the eggs were concentrated onto solid phase microextraction (SPME) fibers, which were exposed to the specimens every 2 hours from hour 1 through hour 15, and subsequently analyzed by gas chromatography–mass spectrometry (GC-MS) to tentatively identify compounds. Over 180 compounds were detected in the headspace of the eggs, with their levels varying over the 15-hour time frame during which measurements were made. Among the compound classes observed were alkanes (e.g., hexane, decane, undecane), alkenes (e.g., 2,4-dimethyl-1-heptene), aldehydes (e.g., decanal), alcohols (e.g., 2-ethyl-1-hexanol), and esters (e.g., the 2-ethylhexyl ester of formic acid), among others. A number of emission trends were observed. Some compounds were present throughout (e.g., 3-ethyl benzaldehyde and the 2-ethylhexyl ester of acetic acid). A subset was emitted rhythmically (e.g., mesitylene and 5,6-dimethyldecane). Others were detected for several hours, after which they no longer appeared (e.g., 3,3,5-trimethyl heptane). The results support the hypothesis that the volatiles emission trends of *Lucilia sericata* eggs are a function of level of development. These findings have implications for determination of the exact age of entomological specimens, which can potentially be correlated to more refined assessment of PMI when the evidence is retrieved from decomposing remains.

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Improving Identification of Unknown American Indians and Hispanic/Latinx Americans

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Variation in cranial morphology can be used in forensic casework to estimate population affinity in an unknown individual. Method performance is highest when populations present in forensic casework are represented in reference databanks used for methodological development. Cranial macromorphoscopic (MMS) traits are highly heritable, reflecting neutral traits under selection (Plemons, 2022), and can be used to estimate population affinity in forensic anthropology successfully, serving as a proxy for genetic relatedness. Currently, cranial MMS data for contemporary American Indians (AI) are absent from reference databanks, leading to uncertainty when using these data in population affinity estimates. Here, the researchers present cranial MMS data from modern AI and examine biological distance among other samples. Cranial MMS data were collected from computed tomography (CT) data housed in the New Mexico Decedent Image Database (NMDID) (Hefner, 2018) for individuals with AI (n=839), Hispanic (n=404), White (n=47), Black (n=270), and Asian (n=95) affinities. These scans were collected during postmortem examination at the New Mexico Office of the Medical Investigator from 2010 to 2017 (Berry & Edgar, 2021). Data from the Macromorphoscopic Databank (MaMD) (Michigan State University, n.d.) supplemented the Black (n=49), White (n=274), and Asian (n=230) samples to reach an appropriate analytical sample size. Data for 12 cranial MMS traits were collected following an available protocol for CT data (Stull et al., 2022). Initial data analysis was used to investigate patterns among samples. The Robust Estimator of Grade Differences (RED) was used to assess biological distance and relationships. RED is appropriate for categorical data and avoids issues associated with data compression that may diminish biological relationships (Willermet et al., 2020). A correlation analysis did not reveal any significant positive or negative correlations, but a weak negative correlation was present between interorbital breadth (IOB) and nasal overgrowth (NO). Multiple correspondence analysis identified "population" as a driver of variation in the samples followed by traits in the nasal area (nasal aperture width [NAW], inferior nasal aperture [INS], nasal bone shape [NBS], NO). A biplot separated the White and Asian samples from the AI, Black, and Hispanic samples. RED analysis indicated the greatest dissimilarity among the Asian and AI samples, followed by White and AI. The most similar samples were Hispanic and AI, Hispanic and Black, and AI and Black. These patterns reveal that the AI sample is different from other samples encountered in forensic casework but most like the Hispanic sample. Group separation was most significant using cranial MMS variables of the midfacial and nasal area. The researchers identified several avenues of further exploration, including classification modeling with the AI sample and CT data with MMS traits.

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GIS Application for Building a Nationally Representative Forensic Taphonomy Database†

NIJ AWARD #: 2020-DQ-BX-0025

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Time since death, or postmortem interval (PMI) estimation, remains an enduring challenge to medicolegal death investigations despite decades of research. Existing methods continue to lack the scientific rigor required within the medicolegal sector due to continued reliance on small sample sizes, the lack of environmental heterogeneity, and inconsistent descriptions and methodologies, which impede understanding of the decomposition process (Weisensee et al., 2024). GeoFOR began in 2019 and seeks to inform these longstanding issues by offering data-driven PMI estimations. The geoFOR application serves as a free forensic case entry platform that automates the collection of weather data from the discovery location using the Global Historical Climatology Network (GHCN) and uses machine learning (ML) methods to deliver statistically robust PMI predictions directly to users. Cases entered into geoFOR contribute to a large, ongoing, and collaborative forensic taphonomy database (n=3,217) used to train and update the ML predictive model. ML models, though powerful predictive tools, are often "black boxes" due to their complexity. To ensure ethical and fair application of ML, techniques for "opening the black box" must be combined with the use of ML to make these models transparent and interpretable. The researchers leverage a variety of model explainability techniques in conjunction with the ML model to determine how individual variables of the body and surrounding environment contribute to the model's PMI estimation. This analysis employs permutation importances (Molnar, 2024), SHAP (SHapley Additive exPlanations) values (Lundberg & Lee, 2017), decision tree surrogates (Craven & Shavlik, 1995), and human-in-the-loop interactive tools to extract insights about the ML model of PMI prediction, providing quantitative assessments of how specific variables influence the complex decomposition process. These results demonstrate the power of these explainability tools in interpreting PMI estimates. Feature importance analysis revealed that desiccation is the most critical feature, with a gain value of 205.461. This finding was corroborated by permutation importance analysis, where desiccation showed the highest importance of 0.193, indicating a substantial decrease in model performance when this feature is randomly permuted. SHAP analysis further validated these findings, with desiccation having an average SHAP value of 0.466, the highest among all features. Interestingly, although advanced decomposition characteristics generally showed high importance, the early decomposition feature skin discoloration also emerged as significant, with the fifth-highest permutation importance of 0.032. Among environmental factors, the analysis identified "Precipitation standard deviation days 57–154" as the most important weather covariate, with an average SHAP value of 0.089. To further enhance interpretability, the researchers employed a decision tree surrogate model. Despite its simplicity, this surrogate achieved an R^2 of 0.675, comparable with that of the full model. The surrogate tree's structure reinforced the other findings, with desiccation as the top-level feature, followed by "livor mortis unfixed" and deposition site type at the second level. By providing interpretable

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and justifiable PMI estimations, this research shows how black-box ML models can be made simultaneously to yield forensic insights and to increase their transparency and openness to oversight.

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POSTER ABSTRACTS

SESSION III

SEIZED DRUGS AND TOXICOLOGY

What a Trip! Investigating the Stability of Psilocybin and Psilocin Infused Within Complex Edible Matrices

NIJ AWARD #: 15PNIJ-24-GG-03848-MUMU

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Psilocybin and psilocin, the major and minor components of psychedelic "magic" mushrooms, are Schedule I drugs in the United States. However, the decriminalization of these drugs at the state level, due in part to promising results showing their potential to treat mental health disorders, has led to skyrocketing commercial availability, production, and retail of food and drink products containing "magic" mushrooms or their psychoactive components. Although crime laboratories have well-established protocols for the detection of psilocybin and psilocin by gas chromatography (GC) and liquid chromatography (LC)-mass spectrometry (MS), methods for their detection and quantification when contained within complex edible matrices are sparse. Additionally, there is a lack of knowledge regarding the stability of these mind-altering substances in food and drink products, as well as in solvents used for their extraction before analysis. In this study, the researchers investigated the stability of psilocybin and psilocin as a function of the environment. Specifically, factors such as temperature (e.g., ranging from −80°C to oven-baking temperatures), solvent (e.g., water, acetonitrile, methanol), and pH are considered. The stability of these analytes is monitored by tracking changes in compound levels and degradation patterns as a function of sample processing steps. The results give insight into the optimal conditions for accurate handling (i.e., storage conditions and extraction) of psilocybin- and psilocin-infused edible commodities that enter crime laboratories as evidence. Moreover, the results suggest the possibility of inaccurate labeling of commercially available food and drink products due to structural changes to the psychoactive components that may occur during the manufacturing process.

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Detecting Fentanyl Analogs in Counterfeit Pharmaceuticals by Surface-Enhanced Raman Spectrometry

NIJ AWARD #: 15PNIJ-23-GG-04230-RESS

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There has been increasing concern over the human cost and the analytical challenges resulting from the increasing use and abuse of novel psychoactive substances. In 2023 alone, over 100,000 Americans lost their lives to drug overdoses, with up to 78,000 of these coming from synthetic opioids such as fentanyl. Fully half of the 77 million fentanyl pills seized by the Drug Enforcement Administration in 2023 contained a fatal dose of fentanyl. Clandestine manufacture of dangerous drugs has made the problem worse because simple tests, such as immunoassays, may not detect the wide variety of drug analogs. Thus, there is a need for rapid and efficient methods for detection of these drugs and their analogs. Raman spectroscopy is one such technique that can be useful in field and laboratory applications due to its portability. Unfortunately, Raman spectroscopy is a relatively insensitive technique, particularly given the extreme toxicity of fentanyl. However, the addition of nanoparticles to the analyte solution can greatly enhance Raman sensitivity, permitting detection of subnanogram/mL levels of fentanyl. This technique is known as surface-enhanced Raman spectroscopy (SERS). The goal for this project is to develop SERS methodology on portable Raman instrumentation for detecting fentanyl and fentanyl analogs in counterfeit pharmaceutical tablets. Commercially available silver (Ag) nanoparticles and synthesized gold (Au)/Ag nanostars will be used with portable Raman instruments to analyze suspect tablets. Preliminary data will be shown, demonstrating the detection of characteristic peaks of fentanyl or fentanyl analogs.

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Chiral Separation and Quantification of Methamphetamine in Whole Blood

NIJ AWARD #: 15PNIJ-23-GG-04216-MUMU

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Impaired driving is a major concern for roadway users and contributes to a significant portion of fatal and non-fatal collisions. One particular substance of concern is methamphetamine because stimulant use can lead to more aggressive and reckless driving behaviors. However, the detection of methamphetamine poses difficult questions regarding recreational versus pharmaceutical use. Methamphetamine has two enantiomers; the S-(+)-form is a controlled substance, whereas the R-(-)-version is available as an over-the-counter nasal decongestant. This poses a problem because many forensic and clinical toxicology laboratories do not separate the enantiomers. As a result of this absence, defense attorneys have used this to cast reasonable doubt on the interpretation of methamphetamine test results. The researchers report an enantiomeric-specific simultaneous separation and quantification method in whole blood to better interpret results. Ultra-high-performance liquid chromatography (UHPLC) chiral columns were identified as an avenue of separation and quantification. Multiple columns have been tested, including Agilent InfinityLab Poroshell 120 Chiral-V, a glycopeptide-based chiral column, and Phenomenex Lux 3µM AMP Chiral Column, a polysaccharide-based column. Multiple conditions and mobile phases were tested to optimize enantiomeric-specific quantification of methamphetamine and other common stimulants. This was done to improve workflow and decrease the resources required for quantifying common stimulants. Common challenges with chiral columns such as peak broadening, reduced column lifetime, and lower column pressure limits were addressed through mobile phase, flow rate, and temperature adjustment. Method validation demonstrated excellent model fit (R^2>0.99), with low limits of detection (LOD) and limits of quantification (LOQ). Additionally, robust intra- and inter-day precision, accuracy, and recovery in spiked matrices were demonstrated. This method provides for accurate enantiomeric-specific separation of methamphetamine, providing impactful tools for criminal justice and public health.

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Multimodal Raman Spectroscopy and Mass Spectrometry Analysis of Synthetic Drugs in Blood Plasma Utilizing Nanoparticle-Decorated Porous Substrates

NIJ AWARD #: 15PNIJ-23-GG-04235-RESS

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The United States faces soaring challenges in public health due to the significant increase in the manufacture, smuggling, and use of controlled substances. Currently, there is no routine way to perform high-throughput toxicology drug assays without resorting to complex and expensive robotic sample handling. Therefore, reliable and fast analyses will result in more rapid turnaround times and lower the cost of toxicology analyses. Surface-enhanced Raman spectroscopy (SERS) has shown tremendous promise for analyzing drugs in human biofluids as a part of forensic toxicology. The concentration of intact drugs in blood plasma is exceedingly low; thus, ultrasensitive SERS is extremely useful. However, SERS cannot differentiate between homologs, regioisomers, and diastereomers. Mass spectrometry (MS) techniques can differentiate drugs with atomic resolutions, but this measurement approach has low sensitivity. Herein, the researchers present the first of its kind, the fabrication of silver nanoparticle (Ag NP)-decorated micropillar arrays (nanotechnology-based device), which are a SERS substrate and a substrate-supported electrospray ionization (ssESI) MS sample preparation/ionization platform. The researchers fabricated porous polydimethylsiloxane (PDMS) micropillars whose surface is modified with short-chain polyethylene glycols (PEGs). The porosity (10–200 μm) allows drug preconcentration within the micropillar as a form of solid-state microextraction, which enhances the sensitivity in the MS analysis. The decoration of porous PDMS micropillars with plasmonic Ag NPs allows ultrasensitive SERS analysis. Furthermore, the presence of PEGs helps to negate fouling effects, which reduce background noise in the SERS measurements. Using the porous PDMS micropillars, several synthetic drugs, including designer benzodiazepines, fentanyl analogs, and non-fentanyl synthetic opioids, are analyzed simultaneously by SERS and ESI-MS at a concentration of parts per trillion (ppt) in blood plasma. Most importantly, this fabrication strategy serves as a high-throughput analytical detection tool as different drug types or analogues of a particular drug are detected on each individual micropillar by SERS and ESI-MS analyses. Together, this research has the unique potential to detect, quantify, and identify most potent drugs from human biofluids with minimum sample preparation. Therefore, the methodology can be adopted by forensic toxicology laboratories around the country.

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Potency Testing of Synthetic THC Isomer-Infused Edibles Using Ultra-High-Performance Liquid Chromatography Diode Array Detector With Optional Electrospray Ionization Time-of-Flight Mass Spectrometry

NIJ AWARD #: 15PNIJ-23-GG-04234-RESS

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The 2018 Farm Bill excluded hemp from the statutory definition of cannabis with a Δ9-THC concentration not more than 0.3% (w/w). Since then, synthetic THC isomers, which are psychotropic and often infused in edibles, have been sold across the United States under the premise that the 2018 Farm Bill legalized them. The reasoning for these legal arguments is based on their natural presence in hemp, although in amounts too small to produce psychotropic effects, as well as the ability to derive these compounds from the cannabidiol (CBD) legally extracted from hemp. This study developed an ultra-high-performance liquid chromatography diode array detector (UHPLC-DAD) method for potency testing of 14 neutral cannabinoids, including six synthetic THC isomers (Δ9,11-, Δ9-, Δ8-, [6aR,9S]-Δ10-, Δ6a,10a-, and [6aR,9R]-Δ10-THC), four natural THC isomers (cannabichromene [CBC], CBD, cannabicyclol [CBL], and cannabicitran [CBT]), and four other neutral cannabinoids that are often found in hemp-derived products (cannabidivarin [CBDV], cannabigerol [CBG], cannabinol [CBN], and tetrahydrocannabivarin [THCV]), in synthetic THC isomer-infused edibles. Acidic cannabinoids were absent in the samples due to decarboxylation by synthetic conditions of THC isomers. A systematic separation optimization led to a baseline separation of the 14 neutral cannabinoids within 13.5 minutes using an Agilent Poroshell 120 EC-C18 150 mm × 2.1 mm × 1.9 µm column and a mobile phase consisting of 75% (v/v) acetonitrile and 25% (v/v) aqueous solution of 0.02% (v/v) formic acid at 0.4 mL/min, excluding CBL, (6aR,9S)-Δ10-, Δ6a,10a-, and (6aR,9R)-Δ10-THC, which were further baseline separated within 33 minutes using a Restek Raptor ARC-18 150 mm × 2.1 mm × 1.8 µm column and a mobile phase consisting of 70% (v/v) organic solvent (65/35 [v/v] acetonitrile/methanol) and 30% (v/v) aqueous solution of 0.02% (v/v) formic acid at 0.3 mL/min. To the presenter's knowledge, this is the first ever successful LC separation of (6aR,9S)-Δ10-, Δ6a,10a-, and (6aR,9R)-Δ10-THC. A systematic detection optimization showed that multiple reaction monitoring (MRM) tandem mass spectrometry could not definitively distinguish the 10 THC isomers, making DAD a better detection method due to its wide availability. The method was validated according to the ISO 17025 guidelines and met the requirements. The method was then applied to the analysis of four gummy samples, which were first uniformly dispersed in water under pulverization, then extracted by methanol under vortexing and ultrasonication, together with two tincture and four vaping oil samples, which were directly extracted by methanol under vortexing and ultrasonication. A cannabinoid not naturally present in hemp, 0.3% (w/w) abnormal cannabidiol (ACBD), was spiked into each sample in triplicate, and extraction recovery was tracked in real time. Extraction recoveries of 99.5% to 104.7% with relative standard deviations (RSDs) of 0.4% to 3.3% were obtained for the four gummy and two tincture samples at 500 µg/mL and 90.1% to 115.6% with RSD of 3.6% to 9.9% for the four vaping oil samples at 50 µg/mL. The linear calibration

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range was between 0.04 and 50 µg/mL for each cannabinoid, equivalent to 0.008% to 10% (w/w) for the gummy and tincture samples at 500 µg/mL, but 0.08% to 100% (w/w) for the vaping oil samples at 50 µg/ mL. Electrospray ionization time-of-flight mass spectrometry (ESI/TOFMS) confirmed a good method specificity (i.e., without any false-positive identification of individual cannabinoids).

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Enhancing Field Detection of Fentanyl: A Novel Pre-Concentrator for Ion Mobility Spectrometry Using Silicon Nanowires†

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Identifying dangerous drugs in field settings is crucial for public safety and law enforcement. Ion mobility spectrometry (IMS) plays a vital role in the rapid detection of drugs. Although swab sampling is commonly used for IMS, it poses risks, especially with potent drugs like fentanyl, where even nanogram levels can be lethal. Vapor sampling offers a safer alternative, and recent research has optimized a handheld IMS to detect fentanyl by targeting N-phenylpropanamide (NPPA) as a vapor surrogate. However, challenges remain in detecting trace levels of fentanyl and fentanyl in mixtures due to its low vapor pressure. To address this, the current research focuses on developing a novel pre-concentrator with silicon nanowires (SiNWs) and an acrylate-based polymer to enhance IMS detection capabilities. NPPA, identified as a key vapor component in fentanyl's headspace, serves as a vapor surrogate for IMS detection. Building on previous work at the Naval Research Laboratory, which developed a library of acrylate-coated SiNWs for pre-concentration, this research used selected polymers from this library. These polymers were screened for NPPA pre-concentration using a quartz crystal microbalance (QCM). To validate the efficiency of the optimal coating, filter paper coated with the optimal polymer was fixed under the lid of a Teflon jar containing 100 mg of reference-grade fentanyl and sampled for 1 week. The samples were analyzed via thermal desorption–gas chromatography–mass spectrometry. The efficiency of the optimal coating was further validated on fentanyl samples from the Kentucky State Police crime laboratory. Preliminary results indicate that ethylene glycol methyl ether acrylate (EGMEA) is the most effective polymer for selectively collecting NPPA vapor, based on screening five polymers using QCM. In validation studies conducted at Florida International University, EGMEA-coated filter paper successfully captured NPPA from a 100 mg fentanyl sample headspace over 1 week. Further validation with confiscated samples from the Kentucky State Police crime laboratory, which contained fentanyl, confirmed that EGMEA is a suitable pre-concentrating material and that NPPA is a viable target vapor for IMS detection. The ongoing phase of this research focuses on optimizing a SiNW array coated with EGMEA for integration into a microchip compatible with handheld IMS devices. This innovation is expected to improve trace fentanyl detection by selectively capturing NPPA vapor, addressing the urgent need for enhanced sensitivity in non-contact field detection of fentanyl. The technology could be expanded to other classes of dangerous drugs, providing a versatile solution to drug detection challenges in forensic and law enforcement applications.

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Navigating the Unknown: A Comparative Analysis of Targeted and Nontargeted Approaches for Detecting New Psychoactive Substances in Human Matrices

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The proliferation of new psychoactive substances (NPS) has resulted in unique detection and analytical challenges due to their diverse chemical structures and rapid emergence in the market. Targeted analysis, typically done with low-resolution mass spectrometry (LRMS), provides high specificity through predetermined compounds, whereas nontargeted analysis using high-resolution mass spectrometry (HRMS) offers a broader, more comprehensive screening of known and unknown substances. This study evaluated the comparative efficacy of these two approaches in detecting a panel of 40 NPS in human biological matrices. The liquid chromatography (LC)-MS platforms compared include LC-QqQ-MS (triple quadrupole MS), LC-QTOF-MS (quadrupole time-of-flight MS), and LC-Q-Orbitrap-MS. The primary objective was to systematically compare the relative performance of targeted and nontargeted MS-based analysis for NPS detection, focusing on their ability to accurately identify compounds in complex human matrices. By assessing the analytical outcomes, the researchers seek to establish a framework that informs best practices for forensic and clinical settings, where timely and reliable identification of these substances is crucial. Drug-free human urine, whole blood, and oral fluid (OF) samples were prepared by spiking with a mixture of 40 NPS, including various isomers and metabolites of different NPS classes. Urine samples were processed using a dilute and shoot method, whereas blood and OF underwent crash and shoot preparation. Samples were analyzed using LC coupled with LRMS and HRMS systems in targeted and nontargeted acquisition modes. Data processing and compound identification were facilitated by software tools designed for qualitative and quantitative analysis, as well as for fragmentation assessment. Additionally, a combination of an in-house database and online spectral databases was used for accurate compound identification. To assess and compare the performance of the methods, a scoring system based on performance for selected figures of merit (e.g., sensitivity, selectivity, linearity, precision, and matrix effects) was employed. Comparison of LC-QqQ-MS and LC-QTOF-MS for targeted screening of the 40 NPS compounds indicated better overall performance with the LRMS platform for all three specimen matrices. Generally, the highest scores for both platforms were obtained with urine, followed by whole blood and OF. Neither approach performed particularly well for targeted analysis of NPS positional isomers. For nontargeted analysis, the researchers evaluated two acquisition modes typically available for LC-QTOF-MS systems; Auto MS/MS and All Ions. The Auto MS/MS mode showed higher sensitivity and selectivity for target analytes, whereas the All-Ions mode offered wider coverage and improved detection of unknown compounds. Additionally, All-Ions fragmentation proved to be more resistant to matrix effects and interferences, resulting in more reliable identification of NPS across various sample types. Current work is underway to extend these observations to the LC-Q-Orbitrap-MS platform. This comparative evaluation emphasizes the unique strengths of targeted and nontargeted approaches in detecting NPS. Targeted

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analysis provides reliable qualitative identification of known substances, with potential for quantification that aids clinical and forensic decision making. Conversely, nontargeted analysis is crucial for uncovering unidentified compounds, helping to identify emerging threats and ensuring proactive public health responses. By integrating these methodologies, forensic toxicology can enhance its effectiveness in navigating the complexities of NPS detection.

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Rapid Response to Novel Psychoactive Substances (NPS) Identified in U.S. Recreational Drug Markets

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Novel psychoactive substances (NPS) continue to emerge and contribute to fatal and non-fatal overdoses across the United States in similar manners to traditional drugs of abuse; however, NPS are often outside the scope of forensic laboratory testing. The Center for Forensic Science Research and Education (CFSRE) houses NPS Discovery, an NIJ-funded open-access drug early warning system focused on the identification of NPS. NPS Discovery streamlines the reporting of information regarding NPS to stakeholders, including public health and safety officials, law enforcement, first responders, clinicians, medical examiners and coroners, and forensic and clinical laboratory personnel. The presenters' laboratory employs comprehensive drug testing using gas chromatography–mass spectrometry (GC-MS), liquid chromatography–quadrupole time-of-flight–mass spectrometry (LC-QTOF-MS), and liquid chromatography–tandem quadrupole–mass spectrometry (LC-QqQ-MS) to characterize new drugs found in drug materials and biological specimens. When newly identified NPS reach thresholds of concern, CFSRE scientists develop timely public alerts to notify stakeholders of new information and potential causes for significant public harm. In 2023 and 2024, CFSRE's NPS Discovery program disseminated public alerts for a synthetic opioid (N-pyrrolidino protonitazene), a synthetic hallucinogen (2F-2oxo-PCE), and a novel adulterant (medetomidine). The public alerts included background information on the specific substance, available scientific information (e.g., chemical structure, pharmacological data, legality, chemistry or toxicological data, geographic data, and demographic data), and recommendations for responding to community impacts. In August 2023, a public alert was issued for the synthetic opioid N-pyrrolidino protonitazene due to concerning impacts in drug markets in the United States and United Kingdom. N-Pyrrolidino protonitazene is structurally similar to protonitazene and N-pyrrolidino etonitazene with potency approximately 25 times greater than that of fentanyl. At the time of reporting, N-pyrrolidino protonitazene had been identified in 20 medicolegal death investigations (mean blood concentration: 6.9 ng/mL, range: 0.1 to 55 ng/mL) after the first identification in January 2023. N-Pyrrolidino protonitazene was often found alongside additional NPS (70% co-positivity), including other nitazene analogues and designer benzodiazepines. In May 2024, a public alert was issued after increased proliferation of the synthetic hallucinogen 2F-2oxo-PCE in drug markets across North America. 2F-2oxo-PCE bears structural resemblance to ketamine and has two positional isomers (3F-2oxo-PCE and 4F-2oxo-PCE), providing analytical difficulties. 2F-2oxo-PCE was identified in drug materials and biological specimens alongside traditional stimulants (e.g., methamphetamine, cocaine), NPS (e.g., bromazolam, metonitazene, MDMB-4en-PINACA, N,N-dimethylpentylone), and fentanyl. Most recently, a public alert was issued for the novel adulterant medetomidine, an α2 agonist appearing alongside fentanyl and heroin. Medetomidine exists in two enantiomeric forms, dexmedetomidine and levomedetomidine, for use in veterinary (racemic) and human (dex-only)

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medicine. Medetomidine is significantly more potent than xylazine, causing high public health concern because medetomidine has been identified as a contributor in multiple mass overdose events in large metropolitan markets. The timely dissemination of information related to NPS identified in human exposure events, through biological specimen or drug material testing, allows clinicians, forensic scientists, and medical examiners and coroners to be aware of dangers the drug poses, and provides public access to information regarding the chemistry, pharmacology, and toxicology for these new drugs.

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POSTER ABSTRACTS

SESSION IV

FORENSIC BIOLOGY/DNA

Transfer, Persistence, and DNA Source Attribution of Trace Biological Material in Digital Penetration Assault Cases†

NIJ AWARD #: 15PNIJ-21-GG-04147-RESS

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Sexual assault is commonly thought of as penile penetration of the vagina, without consent from the victim. Only in 2011 was the Uniform Crime Report definition of rape updated from an 80-year-old definition to include the following definition of rape: "penetration, no matter how slight, of the vagina or anus with any body part or object, or oral penetration by a sex organ of another person, without the consent of the victim." Penetration with any body part—specifically digital penetration—is the subject of the current work. Digital penetration cases (e.g., digital penetration of a female by a male) are challenging due to the presence of trace amounts of biological material present from both individuals. Male skin epithelial cells may be present among an overwhelming majority of vaginal epithelial or skin epithelial cells. Not only is the amount of male epithelial cells a challenge; it is also the nature of the epithelial cells themselves. Previous studies involving digital penetration have focused on male profile recovery from samples collected from female victims or volunteers. However, there is another biological transfer scenario in digital penetration cases involving transfer of female biological material (i.e., skin and vaginal secretions) to male suspects. One would expect transfer of female biological material to male hands or fingers and under fingernails (which may provide a better "protective" environment for trace biological material that may not be washed or wiped away as easily as material on skin surfaces). The interval in which the female biological material is detected is likely short, and in many cases, a suspect may not be identified for some time after an assault or incident. However, this will be of use for cases in which a perpetrator is identified quickly, such as domestic violence cases. The goal of the current work is to develop a full rapid digital penetration evidence processing workflow that will assess not only the ability to recover probative DNA profiles in digital penetration samples but to uniquely provide critical contextual information by means of mRNA body fluid identification (BFID) that will provide support for determining the nature or circumstances of the digital assault. Here, the researchers present the results from the first four multi–time point donor-provided digital penetration sample sets collected 1–24 hours after penetration, with each set containing over 100 samples from the male (pre- and post-fingernail/hand surface samples) and female (pre- and post-internal/external vaginal samples) study participants. Using the developed co-extraction workflow, successful profiling results have been obtained for DNA (e.g., male DNA from the internal/external vaginal swabs) and mRNA (e.g., vaginal secretions and female DNA from the fingernail swabs) profiling. The presenter will also show initial results from the use of a BFID and association assay to allow for determination of the presence of same-fluid admixtures (e.g., skin-skin mixtures). With much work still to be done, the researchers are hopeful that the results of the current work will provide the forensic community with valuable information for the routine analysis of digital penetration samples.

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Applications of the Genital Microbiome in Detecting Sexual Contact

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The goal of this project was to examine the potential of the genital microbiome as a method to detect sexual contact between individuals. Prior police department reports have noted that as many as 60% of sexual assault kits collected present no detectable male DNA from commonly collected samples such as semen, saliva, or epithelial skin cells. Recent studies have demonstrated that there are significant differences between the male and female genital microbiome. These differences could be exploited to detect contact; however, little is known about the genital microbiome composition for the male sex and its ability to transfer between individuals. To examine this issue, heterosexual couples were recruited and asked to provide samples of their genital area pre- and post-sexual intercourse. The respective microbial profiles from each sample were then sequenced using shotgun metagenomic sequencing. The results demonstrated a clear transfer from the female vaginal and labial microbiome to the male penile microbiome with lower, less detectable levels of transfer from male to female. Microbial diversity between the labial and vaginal cavity was observed and can help in assessing where to target when collecting a genital swab. Strain analysis demonstrated the potential to differentiate and track bacterial transfer across specific individuals based on sequence-specific markers with the bacterial genomes.

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Assessment of Promega's PowerSeq 46GY Through Testing of the Standard and the Micro Flow Cells

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The analysis of short tandem repeats (STRs) serves as the foundation for human identification in contemporary forensic testing. The standard procedure employs capillary electrophoresis (CE) to separate amplicons based on their length and fluorescent labeling. Although the fundamental principles of STR typing remain unchanged, advances in instrumentation and informative biological markers may overcome limitations of existing techniques while enhancing throughput and reducing costs. Massively parallel sequencing (MPS) not only adds additional sequencing information but also offers a virtually unlimited capacity for incorporating additional STRs and single-nucleotide polymorphism (SNP) markers, thereby improving individual identification. Furthermore, amplicons can be designed to be of minimal length, which enhances their utility for degraded samples. The forensic community has begun to evaluate MPS to overcome these problems. One of this project's objectives is to assess Promega's PowerSeq 46GY, an MPS-STR kit that targets amelogenin, 22 autosomal STRs (aSTRs), and 23 Y-STRs in a multiplex reaction. To date, the researchers have conducted several experiments to test various conditions, including benchmark, which is defined as adhering to the manufacturer's recommendations; sensitivity; different sample numbers per run; different degrees of DNA degradation; and two-person mixtures at ratios ranging from 1:1 to 1:100. Promega recommended the use of the standard flow cell. However, these experiments were executed using the recommended standard flow cell and the micro flow cell. The micro flow cell has a lower capacity and a shorter sequencing time at lower costs. The researchers were interested in whether the coverage obtained from micro flow cells would be sufficient for certain runs that contain DNA of good quality, whereas a higher coverage could be beneficial for higher sample numbers, limited DNA input, or mixtures with high ratios. This knowledge will offer greater flexibility to forensic practitioners in their experimental design. Data analysis was performed using MixtureAce™, a software tool from ArmedXpert™ (NicheVision Forensics). The configurations for MixtureAce™ were tailored for the PowerSeq 46GY kit, including the thresholds of artifacts such as various stutter products and sequence errors, in addition to an analytical threshold set at 200 reads. Preliminary results confirm that the standard flow cell achieved on average a 4.5-fold higher coverage compared with the micro flow cell.

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