Northeastern Association
of
Forensic Scientists

Proceedings
of the Northeastern Association of Forensic Scientists
October 2025 Annual Meeting

Contents

Scientific Sessions

Contents

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Forensic Toxicology Abstracts

Evaluation of IMCSzyme and UCT Rapid Hydrolysis Abalonase for the Analysis of Benzodiazepines Using GC/MS

Juliana Kucowski, New Jersey Institute of Technology, Marlene Herrera-Lopez, New Jersey Institute of Technology, Caitlyn Staiger, New Jersey Institute of Technology, Bridget Verdino, M.S., NJSP Office of Forensic Sciences

Benzodiazepines are widely prescribed but frequently misused, making accurate detection a priority in forensic toxicology. Many benzodiazepines undergo glucuronidation during metabolism, which hinders detection of the parent drugs. Enzymatic hydrolysis is therefore required to improve recovery. This study evaluated two ß-glucuronidase enzymes, IMCSzyme and UCT Rapid Hydrolysis Abalonase, within the laboratory's urine extraction method for benzodiazepine detection using solid-phase extraction (SPE) with 10 mL UCT benzodiazepine cartridges. The only deviation from the standard protocol was that the prazepam internal standard was applied directly to the cartridge after letting the urine aspirate. Four forensic urine case samples were processed in duplicate, with aliquots analyzed untreated and after enzymatic hydrolysis. Target analytes included alprazolam, diazepam, nordiazepam, oxazepam, temazepam, 7-aminoclonazepam, 8-aminoclonazepam, and midazolam. Hydrolysis conditions were varied by incubation temperature, time, and reagent volume to assess enzyme performance. Following SPE, eluates were evaporated, reconstituted, and analyzed by gas chromatography–mass spectrometry (GC/MS). Extracted ion chromatograms (EICs) were generated for each analyte and the internal standard, and peaks were integrated to obtain relative areas for comparison. These findings emphasize the importance of optimized enzymatic hydrolysis and confirm that GC/MS with EIC-based peak integration provides reliable detection of benzodiazepines in forensic toxicology casework

The Evaluation of Two Enzymes for Analysis of Benzodiazepines in Urine Using GC/MS

Marlene Herrera-Lopez, New Jersey Institute of Technology, Juliana Kucowski, New Jersey Institute of Technology, Caitlyn Staiger, New Jersey Institute of Technology, Bridget Verdino, M.S., NJSP Office of Forensic Sciences

The New Jersey State Police Office of Forensic Sciences (NJSP OFS) Toxicology Unit currently utilizes a United Chemical Technologies (UCT) Red Albalonase for the hydrolysis of benzodiazepine glucuronides. The current protocol requires the enzyme treated urine samples to be incubated for 3 hours heated, or 18-20 hours at ambient temperature to hydrolyze benzodiazepine glucuronides of interest in 3-5 mL urine samples. Previous research evaluating either IMCSzyme or UCT Rapid Hydrolysis Abalonase suggested that these enzymes were able to hydrolyze samples in as little as 15 minutes, offering a more efficient alternative to the UCT Red Abalonase. This study evaluated the performance of the two new ß-glucuronidase enzymes for the hydrolysis of benzodiazepines in urine to determine the optimal approach for use in the NJSP OFS Toxicology Unit. The investigation focused on three variables: sample volume, hydrolysis time, and recovery efficiency of analytes of interest. Four (4) destroyed urine case samples were used to conduct the research. An aliquot of each case was extracted alongside the enzyme treated samples to get a baseline for the benzodiazepine analytes of interest. Enzymatic hydrolysis was conducted on the destroyed urine case samples and UTAK certified drug-free urine spiked with temazepam glucuronide. Using each enzyme's recommended buffer systems, heated incubation temperatures (UCT: 70 °C; IMCSzyme: 60 °C) and ambient temperature, 3 and 1 mL urine samples were hydrolyzed for 30 and 60 minutes heated, as well as 18-20 hours unheated. Following hydrolysis, analytes were extracted via solid-phase extraction (SPE) using UCT 10 mL benzodiazepine cartridges with prazepam as an internal reference material (IRM). Gas chromatography–mass spectrometry (GC/MS) analysis was performed in full-scan mode for the assessment of analyte recovery by calculating the relative abundance ratio of prazepam to each target benzodiazepine. The results indicated that IMCSzyme consistently provided greater recovery and reproducibility, particularly with 3 mL sample volumes hydrolyzed for 30 minutes.

Expiration, Collection, and Storage: A Multi-factor Investigation of Blood Alcohol Testing

Roth Woolley, B.S., Boston University School of Medicine, Sarah Doughlas, M.S., D-ABFT-FT, Tennessee Bureau of Investigation, Celeste Wareing, M.S., D-ABFT-FT, Boston University School of Medicine

A frequent argument in impaired driving cases involving blood alcohol concentration (BAC) is that ethanol may have been produced post collection via microbial fermentation. Prior studies have addressed individual factors such as tube expiration status, collection method, and blood glucose level. This study, however, aims to assess the validity of these concerns by combining the previously mentioned factors into a single study, specifically within 10 mL BD Vacutainer® Grey Top Tubes. We compared expired and unexpired blood collection tubes, exposing them to a variety of environmental conditions intended to simulate common collection scenarios. These scenarios consisted of following the recommended collection technique, as well as allowing the tubes to sit uncapped for one hour on a laboratory bench, a restroom, and outdoors. Following exposure, the tubes were loaded with 5 mL of donor blood (>300mg/dL glucose) artificially spiked with ethanol and placed into three storage conditions: refrigerated, room temperature, and in a vehicle trunk. All samples were tested for their baseline BAC and again after the two-week storage period. The results of this study illustrate that there is no significant effect on BAC levels when using a vacutainer past its expiration. No significant differences in BAC levels were observed among the various simulated collection environments. The greatest decrease in ethanol concentration occurred in samples stored in the vehicle trunk, followed by those stored at room temperature, with refrigerated samples showing the smallest decrease.

Evaluation of Label Accuracy of Connecticut Cannabidiol (CBD) Products

Marimar Torres De Soto, B.S., University of New Haven, Marisia Fikiet, Ph.D., University of New Haven, Alyssa Marsico, Ph.D., University of New Haven, Amanda L.A Mohr, MSFS., D-ABFT-FT, The Center for Forensic Science Research and Education

As a result of its turbulent history, public perception towards Cannabis has been significantly divided despite its increasing acceptance from both medical and social perspectives. The Farm Bill of 2018 is not only the most recent development, but it is the first to loosen federal restrictions on the plant and its byproducts by legalizing industrial hemp. Industrial hemp is defined as Cannabis plants that are cultivated to possess 0.3% or less of tetrahydrocannabinol (THC) on a dry weight basis with the major cannabinoid present being cannabidiol (CBD). Consequently, hemp is not considered a controlled substance and is not under the same restrictions as marijuana– which is any medication, product, or plant with a THC content higher than 0.3%. For individuals unable to get medical marijuana licenses because of eligibility or location, industrial hemp allows them to obtain affordable non-prescription solutions for their ailments. Unfortunately, the government's previous focus on complete prohibition has severely limited current understanding of the full breadth of Cannabis' physiological impact and analytical potential. This oversight now competes with the ensuing social media fever of Cannabis' advantages, resulting in a lack of quality assurance for commercial methods and the production of items with erroneous CBD and THC amounts. Ramifications for unsuspecting consumers include adverse effects and drug-drug interactions; as well as financial repercussions in failed workplace drug tests (WDTs) despite the claim a consumer product is "THC-free". The following study aims to quantify the CBD and THC levels of local hemp products and evaluate the validity of their product label. The samples will consist of twenty Connecticut CBD oils that will be compared to relevant standards using a non-matrix matched calibration curve. Based on previous methods and extraction protocols, experiments will be conducted on a Shimadzu HPLC 8050C QQQ and an Agilent 1100 LC with Photodiode Array.

Determination of Sodium Nitrite in Blood; A Model for Diagnosing Suicide by Sodium Nitrite

MacKayla Reed, Cedar Crest College, Lawrence Quarino, Ph.D., Cedar Crest College

Sodium nitrite poisoning has become an increasing trend as a method of suicide among people of all ages due to its easy accessibility and can simply be ingested resulting in the formation of methemoglobin which is the cause of death. The chemical changes the structure of red blood cells as iron becomes oxidized to a 3+ state

leading to methemoglobinemia which is the increase of methemoglobin. The change of the red blood cells results in the oxygen to no longer be able to bind to the iron and transported throughout the body which in turn leads to death. Post-mortem determination of suicide by sodium nitrite is usually made indirectly through investigative theory and the detection of methemoglobin in the blood of the decedent. The current study aims to create a procedure where sodium nitrite toxicity can be determined directly by detecting the presence of nitrate which is produced when nitrite is oxidized when hemoglobin converts to methemoglobin. No current methods found directly correlate methemoglobin with sodium nitrite that could be utilized for situations like this. The procedure employs the use of UV/Visible spectroscopy to first determine the presence of methemoglobin and possibly methemoglobin-nitrite followed by hemoglobin precipitation with zinc sulfate. Precipitated hemoglobin that has been washed is then tested with microcrystalline tests using nitron and silver nitrate for both ions of nitrate and nitrite respectively. Nitrate and not nitrite ions were detected in mixtures of hemoglobin with sodium nitrite indicating the possibly of developing a robust assay for the detection of sodium nitrite toxicity.

Ethanol Depletion Dynamics in Non-Recirculating System Breath Alcohol Simulators

Oliver Fogarty-Harnish, Harrisburg University of Science and Technology, Jillian Yeakel, M.S., Harrisburg University of Science and Technology, Ted Pauley, Guth Laboratories Inc., Ed T. Svirbely, Guth Laboratories Inc., Shawn P. Barry, Guth Laboratories Inc.

Breath alcohol simulators are devices that use a heated ethanol/water solution to simulate human breath. Vapor created from heating the solution is commonly used to calibrate alcohol breath testing instruments. When the vapor of the same solution is repeatedly sampled, it causes the ethanol concentration to deplete at a currently unknown rate. The goal of this study was to quantify and better understand the ethanol depletion in non-recirculating system breath alcohol simulators. We observed the effects of differing flow rates through the system using differing initial solution concentrations. Simulators were filled with alcohol reference solutions and air was pumped through the simulator and output to the analyzing instrument repeatedly until significant concentration depletion was observed, measured using infrared spectroscopy. Statistical analysis of our results showed that the depletion of ethanol concentration in breath alcohol simulators was correlated to the total liters of air that passed through the system. It was observed that ethanol depletion rates with different flow rates or times were similar when the total liters of air through the system was similar. When a higher solution concentration was used there was a notable increase in depletion rate of the ethanol. Based upon these findings, we determined that the total ethanol depletion in a non-recirculating system breath alcohol simulator can be described as a percentage of the original concentration being lost when a specific volume of air is passed through the system. We then made a model that can predict ethanol concentration in the solution after repeated testing.

Fit for Purpose: A Primer for Realistic LC-MS/MS Method Development

Dustin Abbott, M.S, D-ABFT-FT, New York State Police

Is your laboratory looking to transition from traditional “gold standard” gas chromatography into the modern world of LC-MS/MS analysis? Maybe you have a few targeted LC methods up and running but your workflow would be a lot more efficient with larger cross-class panels? Perhaps you’ve made the decision to drop your aging ELISA or EMIT instruments and try your hand at chromatographic screening? What’s stopping you?

Method Development.

While simple enough at first glance, practical LC-MS/MS method development involves many considerations: mobile phase and column selection, gradient development, transition optimization and deoptimization, management of overloading, and understanding of ion suppression. Attempts to gather information on these topics often lead the novice LC-MS/MS user down one of two paths: recommendations to purchase expensive cutting-edge instrumentation (LC-QTOF, DART-MS) or the use of complex vendor-curated analytical methods. The aim of this presentation is to show that you don’t need any of that to build functional fit-for-

purpose methods using the reagents and instrumentation you already have in-house. It utilizes a comprehensive chromatographic drugs-of-abuse screening method currently in development by the New York State Police toxicology section as an illustrative example of the major considerations that are part of method development. Topics of discussion will include commonly used mobile and stationary phases, behavior patterns of relevant drug classes in reverse-phase chromatography, when to trust automated MS optimization and when to take matters into your own hands, and how to capitalize on the advantages of LC-MS/MS analysis while mitigating its drawbacks. Additionally, there will be a discussion of troubleshooting techniques for poor chromatography, insufficient peak resolution, identifying capacity overloading vs detector overloading as well as other miscellaneous tips and tricks.

Identification of exogenous GHB in blood and urine using Clean Screen® GHB SPE columns coupled with HILIC separation and tandem MS detection

Luette Muir, M.S., UCT Inc.

Gamma-hydroxybutyrate (GHB) is an endogenous neurotransmitter with depressant effects that can also be abused exogenously. The easily accessible industrial chemicals 1,4-butanediol (1,4-BD) and gamma-butyrolactone (GBL) are precursors that rapidly convert to GHB if ingested. GHB has several isomers that are also endogenous to biological matrices and further complicate accurate identification during toxicological analysis. A quick, efficient solid phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method were developed for quantitation of exogenous GHB in blood and urine.

The extraction used only 100 µL of whole blood or urine. Blood samples were precipitated with acidic acetonitrile/methanol (75:25 v/v) and urine samples were diluted with methanol prior to SPE. Clean Screen® GHB SPE columns (200 mg, 3 mL) were sequentially conditioned with deionized water and methanol prior to sample application. The eluate was collected during sample application. Recoveries were enhanced by adding alkaline methanol to the original sample test tube, applying to the column, and combining eluates. GHB was accurately quantitated and identified using hydrophilic interaction liquid chromatography (HILIC) which chromatographically separated GHB from precursors and isomers. Utilizing negative electrospray ionization with acetate mobile phase additives also improved selectivity for GHB. Negative matrices used during development were confirmed to be free of detectable endogenous GHB.

For both urine and blood samples, the SPE procedure achieved recoveries of 90% to 100%. No ion enhancement was observed, ion suppression did not exceed -11%, and relative standard deviation was ≤ 9%. GBL can convert to GHB during in-source fragmentation or under alkaline conditions; however, no such conversion was observed during extraction or analysis. The developed methods displayed substantially lower matrix effects than blood samples prepared solely by protein precipitation and yielded cleaner extracts than dilute-and-shoot urine samples. Using the developed extraction and analytical method, exogenous GHB can reliably be identified and quantified in blood and urine at concentrations between 1 and 100 µg/mL.

Streamlining the Process for Investigative and Routine Drug Testing Using a Toxicological Database Solution on GC/MS

Jeremy Smith, M.S., Shimadzu Scientific Instruments

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) technology has long been a reliable and ubiquitous tool in the forensic toxicology laboratory. Improvements to the technology and workflows are evolving every year. Presented here is a project featuring insights into a simple yet highly robust workflow for routine toxicology testing and tools for unknown substance analysis. Complete with chromatographic and mass spectrometer parameters, the forensic toxicology database for GC/MS analysis alleviates the guesswork and trials of method development. More than 2,000 relevant toxic substances are included in the database, and this robust solution is designed to streamline analysis by offering simultaneous screening and confirmation determinations. This an impactful solution for forensic toxicologists and novel drug detection groups.

Method development can be a lengthy and difficult process. Optimizing the analytical conditions for targeted compounds is the first of several hurdles for a complete workflow. This requires time, troubleshooting, and technical expertise to arrive at a sound, fit-for-purpose solution. The last component of any analytical procedure is data review and compound identification. Like method development, this process takes time and skill to ensure this critical data is reviewed properly to form accurate conclusions. The database workflow discussed here minimizes the effort for method development while offering added confidence for identification through compound library comparisons.

Seized drug or unknown compound analysis is quickly becoming a routine component for the modern forensic toxicologist. This GCMS database tool offers MS/MS mode analysis to help elucidate possible key chemical structures for unknown compound identification. This added functionality makes the GCMS forensic toxicological database both an attractive and practical solution for routine and investigative analysis.

Shimadzu Corporation. (2025). GC/MS Forensic Toxicological Database Ver. 2 (Application: C146-E388B). Shimadzu Corporation. https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/brochures/24045/c146-e388.pdf
Sakamoto, Y. & Miyagawa, H. (2017). Comprehensive Detection and Structural Elucidation of Synthetic Cathinones Using GC-MS/MS (Technical Report: C146-E327). Shimadzu Corporation. https://www.shimadzu.com/an/sites/shimadzu.com.an/files/pim/pim_document_file/technical/technical_reports/11121/jpo217002.pdf

Bayesian network modelling for assessment of drug-related deaths: a forensic toxicology approach

Priscilla T. Merlino, B.S., Rutgers University, Michelle Carlin, Ph.D., Rutgers University

Post-mortem toxicology is essential in establishing whether drugs contributed to an individual's death. However, interpretation is complicated by numerous factors that are not captured in standard toxicological reference tables. These include the choice of biological samples and sampling sites, post-mortem drug redistribution, chronic or previous drug use, and underlying pathological conditions such as chronic obstructive pulmonary disease, liver disease, pneumonia, and cardiovascular disease. Such variables can significantly influence toxicological outcomes and must be considered in context.

Bayesian statistics have been applied in forensic science to quantify the strength of evidence by integrating prior knowledge with new data to update the probability of competing hypotheses, for example, those proposed by the prosecution (Hp) and the defense (Hd) in casework. A practical implementation of Bayesian principles is the Bayesian network, which is particularly effective in modelling multivariate relationships and conditional dependencies among variables. These networks have been widely used in fields such as medical diagnosis, environmental modelling, gaming, and business risk assessment, and are increasingly valuable in forensic science for interpreting complex evidence.

In this study, a Bayesian network was developed and tested using a dataset of over 8,000 cases, each including a recorded cause of death and, in most instances, an associated toxicology report. The initial phase involved data cleaning and the construction of a baseline model using only three variables: cause of death (as determined by a pathologist or coroner), age, and sex. This model was trained on the full dataset and used to assess the likelihood of a death being drug-related. Results were compared to official classifications on death certificates, and a receiver operating characteristic (ROC) curve was generated. The area under the curve (AUC) was calculated to evaluate performance, with values closer to 1 indicating stronger predictive ability, and 0.5 indicating performance equivalent to random chance. The baseline network gave an AUC value of 0.54.

A second, more detailed network was developed, incorporating the same demographic and pathological variables, along with toxicology data indicating the presence or absence of opioids, hypnotics/sedatives, stimulants, and alcohol. Evaluation of this enhanced model showed an increased AUC of 0.61, indicating improved predictive accuracy. However, the values remain relatively low. These results represent early steps in developing a larger Bayesian network model that will assign probabilities to individual drugs based on their concentrations. This future model will leverage multivariate relationships and dependencies not included in earlier versions, with the goal of further improving interpretive capabilities in forensic toxicology.

Method development for the Determination of Fentanyl and its Cutting Agents in Whole Blood using LC-MS/MS

Katherine Hayner, West Chester University of Pennsylvania, Variar Revathi, West Chester University of Pennsylvania, Lisa Mundy, M.S., Pennsylvania Medical Examiner's Office, Constantinos Pistos, Ph.D., West Chester University of Pennsylvania

The presence of xylazine and medetomidine, both veterinary sedatives, in fentanyl cases as "cutting agents", has seen a dramatic rise in recent years, driving a polydrug overdose epidemic. The aim of this study is to develop a rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method, for the simultaneous determination of fentanyl, xylazine and medetomidine in whole blood. Forensic laboratories may take advantage of the high throughput analysis of LC-MS/MS, instead of using GC-MS, assisting the reduction of backlog cases, at comparable cost, and providing shorter time of analysis. Target precursor and product ions (MRM ion transitions) are optimized by injecting standard reference materials and analyzing them via Flow Injection Analysis (FIA). The detection is achieved using an Agilent Jet Spray Ionization source operating in positive mode. The separation is optimized on a Poroshell (2.7µm, 100 x 2.1mm) analytical column, by examining various isocratic and gradient programs, using ammonium formate 10 mM/formic acid 0.1% (solvent A), and acetonitrile or methanol with 0.1% formic acid (solvent B), and different temperatures of the analytical column. In addition, three different solid phase extraction (SPE) cartridges (Clean Screen DAU, Clean Screen Xcel, Bond Elut Certify, OASIS HLB) have been evaluated for the recovery of the three analytes. The method demonstrates short run time (less than 10 minutes), sufficient separation, and specificity of the analytes which makes it suitable for further development. In this presentation, the method's optimized parameters and representative figures and data are reported.

Utility of Written Expert Opinions in Drug-Impaired Driving Investigation

Kristopher Graf, M.S., NMS Labs

Driving is a complex task. The act of driving requires various aspects of cognitive and psychomotor function including, but not limited to, attention, reaction time, visual orientations, vigilance, and perception. Examples of these while driving include reading and reacting to posted signs and signals and maintaining lane position and appropriate speed for the road. Driving under the influence of any psychoactive substance can adversely affect the skills necessary to operate a motor vehicle. While in-court testimony is one method for providing expert testimony in a legal matter such as driving under the influence, expert opinions are another possibility that many may not be aware of, let alone know the process of how to review provided information and how to author.

This platform presentation will look at a deidentified, adjudicated case where I was involved as a toxicologist requested by the prosecution to provide an expert opinion on the driver's ability to safely operate their motor vehicle. The steps covered through this case will include the following:

- Initial communication with law enforcement pertaining to preliminary results
- Standard analysis, review, and issuance of an NMS toxicology report
- The request for an expert opinion
- Review of the additional information provided including:
- Determination of what is necessary versus extraneous
- Connecting known effects of specific drug use to information from lay witness statements, videos, etc that are provided by the requested agency
- Preparation and issuance of the expert opinion
- Preparation for possible testimony

Upon completion of the presentation, attendees should be more prepared to critically think and provide an expert opinion in the event they are requested to author one, while still following best practice recommendations, such as the American Academy of Forensic Sciences (AAFS) Standards Board (ASB) Best Practice Recommendation 037 regarding Guidelines for Opinions and Testimony in Forensic Toxicology.

Applications of Green Chemistry in Toxicology: An AGREEprep Pilot Study for Method Comparisons

Kira Bochard, MSFS., NMS Labs

As forensic laboratories seek to reduce their environmental impact and adopt more sustainable practices, there is a growing need for tools that objectively evaluate the greenness of analytical methods. The AGREEprep scoring method, developed by Wojnowski, Tobiszewski, and Pena-Pereira, provides a structured framework for assessing sample extraction methods based on the 12 Principles of Green Analytical Chemistry1,2. Originally designed for general analytical and environmental chemistry laboratories, the open-source software utilizes ten scoring criteria to assess the environmental impact of a procedure. These criteria include the use of hazardous materials, sample volume, waste produced, energy consumption, integration and automation of extraction steps, and more1,2. The AGREEprep software generates both qualitative and quantitative metrics that allow for comprehensive method comparisons and is freely available through the American Chemical Society.

Forensic toxicology inherently has unique challenges for sample extraction, including the routine use of hazardous reagents, larger sample volumes, and complex extraction procedures. Taking these limitations into account, we have adapted the AGREEprep scoring criteria to better reflect the priorities and constraints of toxicology workflows. Our modified version incorporates toxicology-specific considerations while maintaining the foundational structure of the original AGREEprep model.

This presentation will introduce the AGREEprep software and explain how the criteria was specifically tailored for forensic toxicology applications. We will also demonstrate how this adapted version can be used to evaluate and compare commonly used methods with sustainability in mind. Examples will be presented to highlight how method improvements, such as reducing solvent volumes, choosing less hazardous reagents, or optimizing instrument parameters, can lead to higher sustainability scores without sacrificing analytical integrity.

The modified AGREEprep scoring tool provides forensic scientists with a practical, standardized method for integrating green chemistry principles into everyday laboratory practice. As sustainability becomes a critical consideration across the scientific community, strategies like this can support laboratories in making more ecologically responsible decisions while maintaining the high standards required for forensic analysis.

References:

1. W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis. AGREEprep - Analytical Greenness Metric for Sample Preparation. TrAC Trends in Analytical Chemistry, 149 (2022), 10.1016/j.trac.2022.116553
2. Francisco Pena-Pereira, Wojciech Wojnowski, and Marek Tobiszewski. AGREE- Analytical GREENness Metric Approach and Software.
   Analytical Chemistry 2020, 92 (14), 10076-10082. DOI: 10.1021/acs.analchem.0c0188
3. Gabriela de Paula Meirelles, André Luis Fabris, Karina Ferreira dos Santos, José Luiz Costa, Mauricio Yonamine. Green Analytical Toxicology for the Determination of Cocaine Metabolites, Journal of Analytical Toxicology, Volume 46, Issue 9, November 2022, Pages 965–978, https://doi.org/10.1093/jat/bkac005

Developing an Optimized Method for the Analysis of Date-Rape Drugs Using High-Performance Liquid Chromatography and Tandem Mass Spectrometry (HPLC-MS/MS)

Caroline Krupka, B.S., Pennsylvania State University, William Campbell, Ph.D., Pennsylvania State University

After attending this presentation, attendees will understand the process of utilizing liquid chromatography and tandem mass spectrometry (LC/MS/MS) to identify date-rape drugs (DRDs) and quantify their concentrations in urine samples.

Drug facilitated sexual assault (DFSA) is the act of committing a sexual offence against a victim who is under the influence of a psychoactive substance, usually defined as a DRD.1 While there are drugs that are commonly used in the context of date-rape, such as benzodiazepines, ketamine, and γ-hydroxybutyric acid (GHB), it's important to note that any drug can be used in DFSA.1 Drugs are often chosen to be used in DFSA

due to their potency and ability to easily dissolved into drinks, usually alcohol, without being detected by the victim.2

Currently, separation techniques such as high-performance liquid chromatography, gas chromatography, or capillary electrophoresis are coupled with spectrophotometric or mass detectors to detect DRDs. However, a major issue in the forensic analysis and detection of DRDs in biological matrices (blood, urine) is their rapid metabolization in the body.3 The majority of DRDs are undetectable in the body within 12-72 hours of dosage.4 Therefore, the methods chosen to determine the presence of DRDs in forensic analysis must be highly sensitive in order to extend the detection window. Optimizing a method on LC/MS/MS for an increased number of compounds will allow for a more comprehensive analysis of the presence of DRDs in clinical cases.

A drug panel consisting of thirty-three (33) potential date-rape drugs (or associated metabolites) were analyzed on five (5) column types (superficially porous C18, biphenyl, phenyl-hexyl, and amide; fully porous C18). All column geometries were 100 x 2.1mm. An evaluation of the most effective mobile phase and gradient conditions, as well as mobile phase modifiers was completed in order to optimize selectivity peak shape and sensitivity. Standard curves were generated for the two (2) phase chemistries that provided the most optimal separation of the 33 compounds, and the optimal column was selected for internal diameter comparison with a 100 x 1.5mm column. The smaller internal diameter acted to further enhance detection limits. As drug tests are most often conducted using urine samples, the optimized method was then repeated using a clean urine matrix to test the accuracy and reliability of the developed LC/MS/MS method.

References:

1. Stelmaszczyk, P.; Gacek, E.; Wietecha-Posłuszny, R. Optimized and Validated DBS/MAE/LC–MS Method for Rapid Determination of Date-Rape Drugs and Cocaine in Human Blood Samples—A New Tool in Forensic Analysis. Separations 2021, 8, 249. https://doi.org/10.3390/separations8120249
2. Bertol, E.; Di Milia, M.G.; Fioravanti, A.; Mari, F.; Palumbo, D.; Pascali, J.P.; Vaiano, F. Proactive drugs in DFSA cases: Toxicological findings in an eight-years study. Forensic Sci. Int. 2018, 291, 207–215.
3. de Costa, Y.R.S.; Lavorato, S.N.; de Baldin, J.J.C.M.C. Violence against women and drug-facilitated sexual assault (DFSA): A review of the main drugs. J. Forensic Leg. Med. 2020, 74, 102020.
4. Drug-Facilitated Sexual Assault, Apr. 2017, www.dea.gov/sites/default/files/2018-07/DFSA_0.PDF.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Trace Evidence/Fire Debris & Explosives Abstracts

A Comparison of Various Adhesives and Their Possible Effects on Subsequent Fourier Transform Infrared (FTIR) Analysis of Fiber and Paint Evidence

Ted R. Schwartz M.S.[1], Ashlyn Renee Bartley M.S.[1], Colin Upton M.S.[2], Brooke Kammrath Ph.D.[1], and Virginia Maxwell, Ph.D.[1]

[1] University of New Haven, West Haven, CT, [2] Westchester County Forensic Laboratory, Valhalla, NY

Forensic analysts at both crime scenes and in a laboratory setting are reliant on a variety of adhesives to collect and to store trace evidence. The adhesives come into direct contact with evidence such as fibers and paint. This contact introduces a possible risk of contamination from the adhesive onto the evidence. In addition, there is the chance of physical alteration of the evidence upon removal from the adhesive. The goal of this project was to investigate if adhesives have a chemical and/or physical impact on the trace evidence, and whether these differences can be observed during laboratory examination.

In this study, six different types of adhesive materials were utilized to test for their potential chemical or physical impacts on trace evidence. The adhesive materials used in this study were forensic fingerprint lifting tape, gelatin lifters, fingerprint hinge lifters, lint rollers, sticky notes, and packing tape. To simulate common trace evidence three diverse types of fiber evidence, as well as three different types of paint evidence were collected. The fibers included nylon, polyester and acrylic; the paints consisted of an automotive clear coat, an exterior house paint and an interior house paint. All adhesive and evidence combinations were stored at four different storage intervals, 1 week, 4 weeks, 14 weeks, and 24 weeks and chemical and physical changes were documented by Fourier Transform Infrared Spectrophotometry (FTIR) using an Attenuated Total Reflectance (ATR) objective.

The results showed that there can indeed be a transfer of adhesive to the trace evidence, and the likelihood and amount of adhesive transferred is dependent on the strength of the adhesive used. There were no differences observed based on the length of time the adhesive was in contact with the samples. The study revealed, however, that even if adhesive transfers to the sample, there are typically clean, non-contaminated areas on the material to obtain a clean FTIR spectrum. Further studies showed that, during typical sample preparation procedures, the adhesive can be successfully removed prior to instrumental analysis, again leading to clean FTIR spectra.

Forensic Discrimination of Black Electrical Tape using Fluorescence Microscopy and Statistical Analysis

Grace Patzer, Cedar Crest College, Lawrence Quarino, PhD, Cedar Crest College

Tape is a common class of evidence submitted to forensic science laboratories. It can serve as a substrate for trace amounts of DNA, fibers, and other materials, and also provide valuable information through analysis of physical fit and manufacturing features. Despite these advantages, many tapes appear indistinguishable at a surface level, which may reduce their perceived evidentiary value in criminal investigations. This study aimed to differentiate fifty unique, unknown samples of black electrical tape using a combination of fluorescence microscopy and statistical analysis of physical features. Samples were subjected to a progressive analytical scheme designed to maximize discrimination. The first method employed was fluorescence microscopy, which assessed the intrinsic fluorescence of the tapes using ultraviolet, blue, and green excitation wavelengths. Both adhesive and non-adhesive sides were quantified by mean gray values, allowing grouping according to fluorescent responses. Tapes that remained indistinguishable were subsequently compared by width and thickness. These measurements were analyzed statistically, providing quantitative data for further individualization. The results indicate that fluorescence microscopy, when paired with simple physical measurements, can enhance the ability of forensic examiners to discriminate between otherwise similar tapes. This approach offers a low-cost, accessible method that strengthens the probative value of tape evidence in trace evidence laboratories. Future work will expand the analytical scheme to include chemical composition analysis using Scanning Electron Microscopy with Energy-Dispersive X-Ray Spectroscopy (SEM-EDX). It is hypothesized that integrating chemical data with fluorescent and physical properties will yield further

discrimination for tape comparison. Overall, this scheme has the potential to increase the value of tape evidence in forensic science and improve casework outcomes.

Developing a Scale to Categorize the Sheddability of Fibers from Fabrics

Flavia Bucci Casari degli Atti di Sassoferrato, MS, Ted R. Schwartz, MS, Henry C. Lee College of Criminal Justice and Forensic Sciences, University of New Haven, West Haven, CT

The purpose of this research project was to examine the sheddability of fibers and to create a scale that can be used by crime laboratories to establish how easily fibers of various textile materials may be transferred. Locard's Exchange Principle states that material from two different surfaces can be exchanged through direct contact with each other. The occurrence of transferred fibers can provide valuable evidence in criminal cases. At present, there is no standardized method for conveying the ease with which fibers can be transferred from a particular garment to other surfaces or items. This lack of communication poses challenges for trace evidence examiners who need to assess the transferred fibers in forensic identifications. The objective of this paper is to propose a comprehensive procedure and a detailed scale that experts can utilize to effectively evaluate and communicate this sheddability factor. The aim of this project is to enhance the accuracy of fiber transfer assessments, ultimately contributing to more reliable evidence interpretation.

A total of 160 garments composed of various fiber types, including acrylic, cotton, polyester, and blends, were tested. A two-inch-by-two-inch piece of Scotch® packing tape was applied to each garment. A 1kg weight was then placed on top of the tape for 30 seconds. Afterward, the tape was lifted and stuck onto a piece of clear transparency film. It was then examined under a stereo microscope to count the transferred fibers. The number of transferred fibers ranged from 0 (a nylon garment) to 248 (a cotton/spandex blend). The average number of fibers transferred was 77. A sheddability scale ranging from 0 to 5 was developed based on the quantity of fibers retrieved from each item.

This procedure was designed to be simple, inexpensive and reproducible, so that it could easily be implemented in a forensic laboratory. This method is cost-effective, utilizes readily available materials, and requires minimal time to perform. It is proposed that forensic laboratories consider adopting a standardized technique such as this. The scale offers a universal framework for assessing and communicating the degrees to which fibers are shed from textile materials, enhancing consistency and clarity in forensic reporting.

Investigating the Capability of HPTLC-MS in the Discrimination of Fluorescent Dyes and Optical Brighteners

Keavy Lynch, BS, Cedar Crest College, Jeanne Berk, PhD, Cedar Crest College, Ethan Groves, PhD, Mictrotrace, Thomas Brettell, PhD, Cedar Crest College, Andra Lewis, PhD, Cedar Crest College

Textile fibers are among the most commonly encountered types of trace evidence and can serve as crucial links between suspects, victims, and specific locations or objects. Examination of fiber characteristics such as color, type of fiber, and applied dye are used to discriminate fibers to determine a possible textile of origin. Therefore, the development and application of accurate methods to analyze these characteristics are necessary for reliable identification and potential source attribution.

Fluorescent dyes have a wide variety of applications, including biological staining, chemical tracing, and dyeing of plastic, paper, fabrics, paints, etc. Further, fluorescent dyes have been gaining popularity in recent years for applications such as “smart textiles” and high-visibility fabrics, including work gear and active wear. Due to their widespread use and popularity, improved methods for their analysis are needed.

High-performance thin layer chromatography paired with mass spectrometry (HPTLC-MS) is a relatively new approach that has been successfully applied to the analysis of some foodstuffs, dyes, and drugs. However, its applicability to fluorescent fiber dyes has not been explored. Additionally, HPTLC-MS has many advantages, such as cost-effectiveness, low solvent use, high throughput capabilities, and high sensitivity.

Therefore, this study aimed to develop and evaluate an HPTLC-MS method for the analysis and characterization of fluorescent dyes and optical brighteners. A total of seven fluorescent dyes and four optical brighteners were selected based on their relevance to textile applications and commercial availability. Standards were prepared in either dimethylformamide, methanol, ethyl acetate and/or toluene depending on polarity of each compound. The standards were applied to silica gel 60 HPTLC plates and developed with 12 distinct mobile phase systems. Of these, three mobile phases yielded optimal chromatographic resolution and narrow bandwidth for each compound. UV-vis spectra were acquired directly from the developed plates, and quantitative analysis was performed at the absorption maxima of each compound. The HPTLC limit of quantitation (LOQ) was determined to be as low as 5 ng for select compounds, with fluorescence detectable visually at levels as low as 100 pg. Mass spectral analysis of dye bands extracted from the HPTLC plates displayed the expected parent ions for most compounds at concentrations down to 25 ng/µL. Further refinement of MS parameters is required to enhance sensitivity and reproducibility.

Overall, HPTLC-MS could provide forensic fiber examiners with an alternative method of analysis that is robust, reproducible, and accurate. This approach would not only improve fiber dye analysis but also benefit a variety of forensic disciplines and other fields in the examination of fluorescent materials.

Forensic Analysis of Soil Trace Persistence: Effects of Organic Matter and Substrate Type

Ashley Buchman, Cedar Crest College, Jennifer Bonetti, PhD, Virginia Department of Forensic Science, Lawrence Quarino, PhD, Cedar Crest College, Andra Lewis, PhD, Cedar Crest College

Soil has long been recognized as a valuable form of trace evidence due to its high transferability, environmental specificity, and compositional variability. However, the interpretive value of soil traces in forensic casework depends on understanding the mechanisms that govern transfer and persistence under relevant environmental and biomechanical conditions. This study systematically investigates the influence of soil organic content and walking surface (pavement vs. grass) on the retention of soil traces on footwear, addressing a documented research gap identified by the OSAC Trace Materials Subcommittee.

Soils were collected from all 13 geologic provinces of New Jersey and categorized as low-, medium-, or high-organic based on percent organic content determined via loss-on-ignition (LOI), with values ranging from 0.17% to 96.01%. Standardized aliquots (1.5 g) of pre-moistened soil were applied to clean, commercially uniform athletic footwear worn by 18 participants. Each individual completed two separate 0.1-mile walking trials—one on a hard, impermeable (pavement) surface and one on a soft, vegetated (grass) surface. Soil remaining on the footwear post-trial was quantified as a percentage of the original mass to assess persistence.

Statistical analyses revealed significant effects of both soil type and surface on trace retention. One-way ANOVAs indicated highly significant differences between soil groups on pavement ($F = 40.73$, $p < 0.001$) and grass ($F = 37.13$, $p < 0.001$), with medium-organic soils exhibiting the highest mean persistence. Two-way ANOVA results confirmed main effects for both soil type and participant weight ($p < 0.01$), as well as a statistically significant interaction between the two variables on grass surfaces ($p = 0.0395$). Linear regression models demonstrated a consistent negative correlation between participant weight and soil retention ($p < 0.05$), except for high-organic soils on grass, where retention increased with body weight.

These findings indicate that substrate texture and soil composition jointly influence trace behavior: mineral-dominant soils persist more readily on hard surfaces, while organic-rich soils exhibit enhanced retention on vegetated terrain. The observed weight-dependent effects further highlight the relevance of individual biomechanical variation in trace analysis.

This research provides a controlled, empirically validated framework for interpreting soil trace evidence on footwear and contributes meaningfully to the broader field of forensic geoscience by quantifying how specific material and contextual variables affect soil trace behavior.

Comparative Analysis of Textile Damage Caused by Canines vs. Firearms and Knives

Tabitha L. Hammond, B.S., Sandra Koch, PhD., Larry Quarino, PhD., and Andra D. Lewis, PhD.

Textile damage analysis is vital in forensic investigations for reconstructing events, assessing applied forces, and verifying witness statements. While traditional studies emphasize firearm- and knife-induced damage using macro- and microscopic methods, canine-inflicted textile damage remains underexplored. This study investigates the distinct characteristics of canine-induced damage across fabric types, comparing them to patterns caused by knives and firearms.

Textile damage incurred during controlled attacks by four trained apprehension canines were compared to damage from a plain-edged knife and 9mm firearm projectiles. Bite samples were collected using a "feed bite" technique designed to minimize claw interference and ensure consistent placement. Denim swatches and long-sleeve cotton or polyester shirts were placed over the right arm of a bite suit and presented during three separate training sessions. Resulting damage was analyzed via photography, stereomicroscopy, and compound microscopy.

Stereomicroscopy was used to examine damaged areas of the samples with both length and width of individual damage measured. Macroscopic analysis revealed punctures in only 14% of denim samples while 86% of the samples exhibited tooth impressions. In contrast, cotton and polyester samples showed punctured damage 100% of the time with additional tooth impressions. These findings highlight material-dependent variation in canine-inflicted damage between woven (denim) and knitted (cotton, polyester) fabrics.

Compound microscopy allowed detailed examination of individual fibers and fiber ends to analyze differences between the fabrics and damage type at the fiber level. The results showed that firearm damage produced rounded, split, flattened, or melted fibers, particularly in polyester and close-range impacts, while knife damage resulted in clean, angled cuts. In contrast, canine damage showed chewed or irregularly flattened fibers, distinct from firearm-induced effects. These results demonstrate the utility of microscopic fiber analysis in distinguishing human- from animal-inflicted textile damage.

Additionally, statistical testing using the Friedman test found no significant differences in damage appearance across fabric types, with p-values of 0.22, 0.37, and 1, all exceeding $\alpha = 0.05$. Similarly, the Wilcoxon Signed-Rank test showed no effect of fabric color, as W-statistics exceeded critical values (cotton: $W = 86 > 83$; polyester: $W = 43 > 40$), indicating that fabric type and color did not significantly influence damage appearance. However, a Kruskal-Wallis test with Dunn's post hoc analysis (critical $Z = \pm 2.94$) revealed significant differences between canine and shooting damage ($Z = 4.74$), but not between canine and stabbing ($Z = 1.55$). Furthermore, a Mann-Whitney U test showed a significant difference between shooting and stabbing damage ($U = 0$, $Z = -3.78$, $p = 0.00016$, $n_1 = 10$, $n_2 = 10$), highlighting that some damage types may not be statistically distinguishable despite visual differences.

This study advances forensic science by focusing on canine textile damage and promoting standardized analysis methods. Cases like the 1980 Azaria Chamberlin case, where fabric evidence proved a dingo attack, show the value of continued research in this area.

The Impact of Firearm Type and Orientation on Gunshot Residue Distribution Patterns on a Shooter

Emily Miller, M.S.F.S., Peter Valentin, PhD., ABC-CC CSCSA

After attending the student forum, attendees will better understand how certain external factors can affect the deposition and distribution of gunshot residue (GSR). They will also recognize the importance of accounting for these variables during evidence sampling and analysis. The information on this poster could influence the forensic science community by encouraging changes to existing GSR collection protocols used by forensic scientists and crime scene investigators. Additionally, it may encourage further research into other external factors to improve GSR collection techniques in real-world scenarios.

When a firearm is discharged, both organic and inorganic GSR are expelled through the openings in the firearm and deposited on nearby surfaces.[1] Primer GSR (pGSR), which is part of the inorganic GSR, is consists of lead, barium, and antimony, whereas organic residue is composed of nitrocellulose and/or nitroglycerin.[2] Nitrites are also classified as inorganic GSR. Although they originate from organic compounds, they are inorganic byproducts formed by the combustion of smokeless powder in a firearm. All these residues are commingled because they are produced as part of the same firing event.[2] Nitrite residues can be detected using various chemical testing methods, including the Modified Greiss Test, the Walker Test, and the Marshall Test.[3] Given the relative abundance of nitrite residue, developing a method for assessing pGSR distribution patterns based on the nitrite distribution would be beneficial. By accurately mapping nitrite distribution, we can identify the locations with the highest likelihood of having pGSR for sampling.

Previous researchers used a Video Spectral Comparator (VSC) because of its ability to select a wavelength and barrier filter combination and take advantage of the inherent fluorescence of the nitrite residue.[4] While multiple studies have confirmed the use of the VSC to analyze GSR on clothing, there appears to be a lack of research focusing on GSR fluorescence on the individual who discharged the firearm. This study addresses these gaps by investigating GSR distribution patterns on 100% cotton, long-sleeve black shirts, and examining how these patterns are affected by the firearm type and its orientation during discharge. After the firearm is discharged, the shirts are doffed and later photographed with the VSC to create a map of GSR distribution. The results indicate the extent to which firearm type and orientation may contribute to any changes in GSR distribution on the shirt of the shooter. These findings could help develop updated GSR collection protocols, potentially reducing false negatives and enhancing the accuracy and reliability of firearm-related forensic examinations. The results are expected to demonstrate that the distribution patterns are significantly influenced by firearm type and orientation, highlighting the need to modify GSR collection protocols when the shooter's position can be determined from case information.

References

1. Wallace, J. S. (2018). *Chemical analysis of firearms, ammunition, and gunshot residue [electronic resource] / James Smyth Wallace*. CRC Press, Taylor and Francis Group.
2. Schwoeble, A. J., & Exline, D. L. (2000). *Current methods in forensic gunshot residue analysis [electronic resource] / A.J. Schwoeble, David L. Exline*. CRC Press.
3. Carneiro, C. R., Lucena, M. A., Santos-Silva, C., & Weber, I. T. (2023). Nontoxic ammunition: Challenges and perspectives for GSR identification. *Wiley Interdisciplinary Reviews: Forensic Science*, *5*(3), e1477.
4. Kersh KL, Childers JM, Justice D, Karim G. Detection of gun-shot residue on dark-colored clothing prior to chemical analysis. J Forensic Sci. 2014;59(3):754–62.

Rapid Spectrophotometric Identification of Peroxide-Based Explosives Using a Modified Iodometric Method

Alana Connor, Cedar Crest College, Marianne Staretz, Ph.D., Cedar Crest College

Peroxide-based explosives (PBEs) have been used in many bombings over the past decade due to their accessibility, high explosive potential, and ease of synthesis from over-the-counter materials. [1] One such example is the coordinated 2019 attacks in Sri Lanka, which resulted in over 250 deaths. [2] Despite this and many other deadly bombings across the world, current detection and quantification methods for PBEs are often limited by complex instrumentation, high cost, or challenges in sample stability.

The proposed method leverages the redox properties of peroxides, which oxidize iodide to iodine in acidic solution. The liberated iodine forms a triiodide complex that absorbs strongly in the UV-visible spectrum, allowing for sensitive spectrophotometric quantification. [3] The procedure involves acidification with hydrochloric acid (HCl), followed by the addition of potassium iodide (KI). The sample was then placed in a temperature-controlled water bath, and the results were collected at three wavelengths: 352 nm, 420 nm, and 288 nm. Calibration curves were generated using TATP in 7 μM increments from 7 to 35 μM and HMTD in 3 μM increments from 3 to 15 μM. The method demonstrated a limit of detection (LOD) of 0.37 μM for TATP and 1.2 μM for HMTD, while the limit of quantification (LOQ) was 1.1 μM for TATP and 3.5 μM for HMTD. Average relative standard deviations (RSD) were 5.5% for TATP and 5.9% for HMTD, calculated

across all tested concentrations. Kinetic profiles of each peroxide were assessed over a 5-15-minute period at 352 nm: average slopes were 0.03 for TATP, 0.007 for HMTD, and 0.003 for H_2O_2. No signal was observed with non-peroxide explosives, which confirms the method's selectivity.

This modified iodometric technique offers several key advantages compared to other detection methods: it is rapid, requires no advanced instrumentation, and is easily adaptable for use in both traditional laboratory settings and potential field applications. These features make the method highly valuable for forensic laboratories, explosive ordnance disposal (EOD) teams, and investigators working in resource-limited environments or time-sensitive situations.

Overall, this work highlights the utility of classical wet chemistry approaches—when appropriately adapted—for modern forensic applications. By bridging the gap between sensitivity and accessibility, this method contributes to improved screening and analytical capabilities for peroxide explosives, ultimately supporting public safety and enhancing forensic response to chemical threats.

References:

1. Schulte-Ladbeck, R.; Vogel, M.; Karst, U. Recent Methods for the Determination of Peroxide-Based Explosives. *Anal Bioanal Chem* 2006, *386* (3), 559–565. https://doi.org/10.1007/s00216-006-0579-y.
2. Wang, W.; Li, H.; Huang, W.; Chen, C.; Xu, C.; Ruan, H.; Li, B.; Li, H. Recent Development and Trends in the Detection of Peroxide-Based Explosives. *Talanta* 2023, *264*, 124763. https://doi.org/10.1016/J.TALANTA.2023.124763.
3. Alba-Elena, D.; Baeza-Romero, M. T.; Antiñolo, M.; Espildora-García, E. M.; Valero, E. A New Iodometric Microwave-Assisted Method for Peroxide Determination in Secondary Organic Aerosols. *Atmos Environ* 2023, *310*, 119960. https://doi.org/10.1016/J.ATMOSENV.2023.119960.

Investigating the Use of Hypochlorite as a Color Test Reagent for TNT and its Derivatives

Warner, I., Marisco, A.M. (PhD), University of New Haven, Dr. Alyssa Marsico, PhD, University of New Haven

Trinitrotoluene, or TNT, is an explosive compound, which can be detected through rapid quantitative colorimetric testing. However, the current color test for TNT requires a prepared reagent which may not be readily available at a crime scene and there is no presumptive test to indicate to investigators that a substance may be TNT. Without the positive presumptive test, laboratory technicians may never run the confirmatory test, creating the assumption that there is no explosive residue on a questioned item. Previous research performed by a graduate student at the University of New Haven has confirmed that sodium hypochlorite, commonly known as bleach, changes color when it encounters TNT. The current color test for TNT is a solution of potassium hydroxide (KOH) in an alcohol, however this reagent must be prepared and expires after some time. In contrast, bleach is stable and readily available, making it more time and cost effective. If bleach produces a visible color change when reacted with TNT, can it be used to indicate that there was once an explosive at the scene? This research aims to create a more efficient test reagent for TNT, to find the best conditions for the reagent, and identify any compounds that could produce false positives.

The study was conducted in two phases. Firstly, controlled spot plate experiments. In the spot plate tests, TNT, 2,4-dinitrotoluene (DNT), 4-nitrotoluene, toluene, 1-nitronapthalene, and 2-amino-4-nitrophenol were examined. Individual compounds and their mixtures with four concentrations of hypochlorite (2%, 5%, 6.25%, 12.5%) were photographed across three replicate plates under consistent lighting. DNT and TNT were also photographed with each concentration of bleach at 25°C, 40°C, and 50°C. Different concentrations of both DNT (30%, 16%, 10%) and TNT (0.1%, 0.05%, 0.022%, 0.007%, 0.004%, 0.003%) were photographed with each concentration of bleach. Time studies were completed in which each concentration of DNT and TNT were tested with each concentration of bleach and recorded for 2 minutes. RGB values were extracted using Adobe Photoshop's Color Picker tool to quantify color changes and then compared. Control images included empty spot plates and compounds alone in spot plates.

Next, a real-world simulation of a color test was conducted. This test mimicked a criminal attempting to clean up a crime scene. In this experiment, 30% DNT was dried on a glass slide, cleaned with 2% bleach (determined to be the most reactive concentration with DNT), then wiped with acetone to observe for color changes. 3

replicates of each experiment were performed, and both positive and negative controls were tested. Real-world tests were not conducted with TNT.

Results showed consistent and measurable color changes in TNT-bleach mixtures, supporting the potential for sodium hypochlorite to act as an efficient presumptive test for TNT. DNT-bleach mixtures also showed consistent and measurable color changes, however the result was an entirely different color than TNT, so the two compounds can be differentiated in this test. 2% Hypochlorite showed the most noticeable color change in DNT, while 6.25% Hypochlorite showed the most noticeable color change in TNT. The most saturated concentrations of both DNT (30%) and TNT (0.1%) created the most visible color changes. The lowest concentration of DNT that produced a visible color change was 16% and the lowest concentration of TNT that produced a visible color change was 0.004%. The DNT showed the most evident color changes at 25℃ (room temp) and 50℃ and the TNT showed the most evident color change at 25℃ (room temp). Reactions in both the DNT and TNT occurred immediately and increased in intensity until about 25 seconds. Other tested compounds did not show a color change, therefore are not likely to cause possible false negatives. No color change was shown in the real-world application tests, other than in the pre-mixed positive control. While optimal conditions have not been identified yet for DNT or TNT swab tests, there is promise that bleach can be used as a colorimetric test once these conditions have been established.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Criminalistics/Crime Scene & Digital Evidence Abstracts

Effects of Rapid Shearing Depending on Environmental Factors: Acrylic, Modacrylic, and Polypropylene fibers

Charles Jin, M.S., Peter Diaczuk, Ph.D., John Jay College of Criminal Justice.

Textiles can often be found damaged in forensic casework, and analysis of these defects can yield valuable information. Different methods of fiber fracture leave behind varying alterations in structure on individual fiber ends, leading to their possible identification and connection to a case. Interactions between high-speed impacts and synthetic fabrics composed of thermoplastic fibers are classified as rapid shear or high-speed tensile breaks. In these situations, unique fiber end characteristics can form due to excessive heat generation that is unable to dissipate quickly enough to prevent fiber end morphology from changing.

This study aimed to explore how different external conditions could affect the formation of these distinct fiber end characteristics created by high-speed impacts. Fabric samples were shot under unaltered room temperature, water-saturated, and chilled conditions. Stereomicroscopy and polarized light microscopy were used to analyze the fabric defects created by the air rifle pellets. Widened, globular fiber ends were discovered in all the pellet holes examined for the three fiber types and under all environmental conditions. This change in fiber end morphology is characteristic of rapid shear. Additionally, these distinct fiber ends exhibited a loss of birefringence, with reduced retardation and interference colors. This study determined that the various temperature-altering environmental conditions employed did not stop fiber ends from changing and displaying characteristics corresponding to rapid shea

Assessing the Impact of Fire on Bloodstain Pattern Integrity Across Porous Fabrics

Emily Szendrey B.S., Andra Lewis Ph.D., Carol Ritter M.S., Michelle Lambert, M.S.FS

Bloodstain Pattern Analysis (BPA) is a critical tool in understanding and reconstructing bloodshed events at crime scenes; however, its reliability can be significantly compromised by environmental factors such as fire. Previous research examining the effects of fire on bloodstains has primarily focused on chemical enhancement methods and the recovery of DNA, rather than on the analysis of bloodstain patterns. Moreover, no studies to date have systematically investigated the impact of fire on bloodstains deposited on porous fabric substrates. The aim of this study was to evaluate how fire, and the associated conditions of fire and fire suppression, affect bloodstain pattern integrity on various porous fabrics through the measurement of bloodstain size and satellite stain count.

A total of seven bloodstain pattern types were created using human blood on ten commonly encountered fabric types. These samples were then subjected to three controlled fire exposure conditions. The first was direct exposure, in which the fabrics were placed approximately four feet from the ignition site with minimal protection from the fire. The second was proximity exposure, where the fabrics were placed about eight feet from the ignition site, separated by a wall with an open doorway between the fire and fabrics. The third was a flashover simulation, where the fabrics were placed in a flashover container for approximately 40 minutes, during which multiple flashover events occurred, resulting in sustained elevated temperatures and high smoke concentrations within a controlled environment. Pre- and post-exposure photographic documentation was performed using both visible spectrum and infrared imaging to assess the morphological changes in the bloodstain patterns.

This study was focused on analyzing white cotton, white polyester, and black polyester fabrics. These fabrics were chosen as cotton and polyester are among the most common fabrics used in clothing. Additionally, drip stains, drip patterns, and cast-off bloodstain patterns were the only three analyzed in depth. ImageJ software was used to measure changes in stain size and satellite stain count, and results were statistically analyzed using dependent t-tests at a 95% confidence level.

Findings indicate that cast-off patterns exhibited the highest degree of preservation on white polyester fabrics and under flashover conditions. In contrast, patterns on cotton fabrics and those subjected to direct flame were most severely degraded. Satellite stains were more vulnerable to alteration than parent stains, with fire

suppression methods, particularly water application, contributing notably to their distortion. Despite exposure, the overall size of parent stains remained relatively stable across conditions.

These results emphasize the relative resilience of polyester fabrics in fire conditions and highlight the need for forensic investigators to account for both fire damage and suppression techniques when evaluating bloodstain evidence at fire-affected scenes.

Enhancing Forensic Science and Law Enforcement Training: The New Expert Witness and Crime Scene Training Facility at Cedar Crest College

Dr. Lawrence Quarino, PhD., Cedar Crest College

Cedar Crest College has launched a new Expert Witness and Crime Scene Training Facility, designed to meet the growing need for comprehensive, interdisciplinary training in forensic science, courtroom testimony, and crime scene investigation. Supported by a $608,000 grant from the U.S. Department of Justice's Bureau of Justice Assistance, this initiative significantly expands the College's capacity to prepare law enforcement professionals, forensic scientists, first responders, and others who may serve as expert witnesses in legal proceedings.

The facility includes a fully equipped courtroom, a crime scene laboratory, and three dedicated rooms for immersive training in crime scene management and reconstruction. Monthly training will cover a broad spectrum of topics, including planned workshops in professional ethics, courtroom testimony for forensic DNA examiners, forensic genetic genealogy, police de-escalation strategies, fire investigation, fingerprint analysis, bloodstain pattern analysis, and forensic photography. Courtroom testimony is expected to be a component of most of the course offerings. Continuing education for legal professionals is also being considered.

In addition to serving professionals in a variety of disciplines, the Center will support undergraduate and graduate education in forensic science, criminal justice, crime science, pre-law, and social work. The programming will be collaboratively developed by the Center's newly appointed manager, the Director of the Forensic Science Program, and the Assistant Director of the Center, ensuring that training remains current, relevant, and evidence-based. In addition, educational opportunities are planned for secondary education teachers interested in teaching forensic science.

The Center is also the impetus behind the development of a new academic department at Cedar Crest College—the Department of Forensic Science and Justice Studies—which brings together the disciplines of forensic science, criminal justice, and social work. The Center will help meet the continuing education demands of all three disciplines and will serve as the launching point for new academic programs in digital forensics and forensic social work.

This facility positions Cedar Crest College as a regional and national leader in forensic training and expert witness preparation, enhancing the quality and credibility of forensic evidence and testimony in the justice system.

American Board of Criminalistics 2025 Update

Heather L Harris, MFS, JD, ABC-CC, ABC-DA, American Board of Criminalistics and NEAFS Certification Chairperson

This presentation will provide the attendees with information regarding the American Board of Criminalistics and its current certification program for forensic scientists. The ABC certification process has changed significantly over the last year, so information regarding applications, fees, testing, and requirements for certification will be provided.

Forensic Intelligence: A Smart Way to Make Data Work for Us

Joe Treviño, DFS., New York City Police Department

The National Institute of Justice defines forensic intelligence as "using forensic data early in an investigation, when that information can accelerate the process of solving the case […and…] using data across cases to understand crime trends and identify links between cases, such as serial crimes." Historical use of this idea existed as early as the 1970s and 1980s but did not reach our contemporary use until around the late 1990s with robbery patterns. While forensic intelligence can be applied to all forensic science disciplines, we might understand it better through the lens of crime gun evidence and crime gun intelligence via NIBIN. NIBIN Lead Notifications are like investigation capsules, condensed gun crime and crime gun evidence information that relates one shooting incident to another. These might be used to piece together several shooting incidents, but the task can quickly become time consuming as the number of involved firearms grows and the associations become complex.

Even for a simple operation, the workflow needed to maximize efficiency and minimize downtime and errors takes a coordinated effort between crime scene investigators, laboratory personnel, and intelligence analysts. The data from this process must be collected consistently and constantly to support an intelligence framework that is useable for all stakeholders, customers, and end users. Thankfully, most Forensic Science Service Providers (FSSPs) have figured out the analysis workflow balance and are providing timely information to the ATF and law enforcement partners via NIBIN. An FSSP underneath the same organization as the submitting law enforcement agency makes things easier because things get complicated when evidence collection, evidence analysis, data management, and data sharing are handled by many agencies or entities. Complicated doesn't mean impossible, however. A data dashboard on a platform like Microsoft Power BI makes crime gun intelligence and forensic intelligence equitable for all.

This presentation will include examples of what a crime gun intelligence data dashboard can look like (Microsoft Power BI), the data needed to support a working dashboard, where crime laboratories might unlock or start collecting that data, and how a dashboard can be built. This presentation is intended for any and all attendees.

Development of Mass Spectral Approaches for Application in Forensic Entomology and the Analysis of Underutilized Entomological Evidence

Alexa Figueroa, B.S., Louisiana State University, Jennifer Y. Rosati, Ph.D. John Jay College of Criminal Justice, Rabi A. Musah, Ph.D. Louisiana State University

Forensic entomology involves the use of insects to investigate and solve crimes through the utilization of the knowledge of their life cycles, behavior, and ecology. Carrion insects that colonize remains can be utilized to estimate the postmortem interval (PMI). The decomposing remains serve as a feeding, breeding, oviposition (egg laying) medium, and refugia from extreme weather, predation, and resource competition.

Blow flies (Family: Calliphoridae) are capable of detecting remains within minutes of death, often serving as the initial colonizers. A 'back-calculation' method exists in which the PMI can be estimated by correlating the insect species identity, environmental conditions, the stage of decomposition of the remains, and the species-specific insect life cycle timelines. The calculation determines the approximate time since the laying of the eggs, based on the assumption that the eggs were laid within a time period shortly after the death occurred. It has been reported that when multiple species of blow flies are present, *Lucilia sericata* initiates oviposition on the remains, after which other species then begin to lay.

Reported here is a novel rapid method utilizing direct analysis in real time — high resolution mass spectrometry (DART-HRMS) and chemometrics for the rapid identification of blow fly eggs suspended in 70% aqueous ethanol. Application of Support Vector Machine (SVM) to the mass spectral data revealed the species-specific markers for 10 egg species within the genera Calliphora, Chrysomya and Lucilia with accuracies of 96.20%, 96.67% and 98.33% respectively. The presence of these specific markers could facilitate the

development of accurate models for species prediction of unknown entomological evidence based on their chemical profiles.

Additionally, studies were conducted in which the headspace volatiles of *L. sericata* blow flies were monitored by DART-HRMS, using thin film solid phase microextraction coupled with gas chromatography-mass spectrometry, at emergence of the first egg during oviposition. The data showed that there was a subset of compounds whose levels increased during this event. These compounds included those appearing at nominal m/z 63, 69, 73, 74, 81, 89, 89, 109,111,117, 117, 127, 127, 136 and 152. These findings imply the possibility of *L. sericata*-produced oviposition cues, which could be chemical attractants that induce egg-laying behavior in blow flies of other species following oviposition by *L. sericata*.

Validation and Implementation of Cadre TopMatch-3D Scanners for Algorithm-Based Triage

Meghan Davis M.S., Elizabeth Hanley, B.S., Nicole Homburger, B.S., Stephanie Marino, B.S., Jason Berger, M.S., New York City Police Department – Firearms Analysis Section

The New York City Police Department (NYPD) Police Laboratory conducted a validation study to implement Cadre TopMatch-3D High-Capacity Scanner algorithm-based triage (ABT) to group fired cartridge cases. The Laboratory uses Ballistic Evidence Triage to streamline the best representative NIBIN eligible cartridge cases from crime scenes into the NIBIN database. The suitability of the Cadre TopMatch-3D High-Capacity scanners was evaluated through examinations of samples from 15 previously distributed external proficiency tests and samples generated from 57 different firearms from the Laboratory's reference collection by both stereomicroscopic (SBT) and ABT methods. Prior to the examination of sample sets within the study, a calibration curve was created for each scanner to ensure that accuracy and quality of scans are maintained. With these controls in place, the results of this study indicated that using ABT had a specificity ranging from 97% to 100%, depending on the parameters set. Comparatively, the specificity was 84% using the conventional stereomicroscopic-based triage (SBT) method. These findings suggest that ABT utilizing the Cadre 3D scanners is the best practice for conducting triage analysis. To implement ABT into the Laboratory's scope of accreditation and be approved for casework, there are several requirements that must be met. Some of these requirements include competency testing examiners who participated in the study, completion of mock casework, preparation of standard operating procedures and training programs, and completion of a request for changes to the scope of accreditation through ANAB. Upon completion of these requirements, ABT will be fully integrated as the best practice for Ballistic Evidence Triage casework.

The Effects of Over-Lubrication of a Firearm on Discharged Cartridge Cases

Peter Diaczuk, Ph.D., Olsmael Merisier, B.S., John Jay College of Criminal Justice

Forensic firearm examination can be used in forensic investigations to determine if a recovered bullet or cartridge case was fired from a specific firearm. While the use of ballistics evidence has been widely accepted in criminal courts, there are still limitations to the process, starting with how the condition of the firearm before or after the crime could change the marks left behind on the projectiles and cartridge cases. For example, some firearm modifications can alter their marking patterns, preventing the association between a firearm and a crime. This project explored how the modification of firearm barrel lubrication affected the striations left on recovered cartridge cases. This was done by examining recovered cartridge cases fired in dry and lubricated barrel conditions using the comparison microscope. The overall goal was to examine the cartridge cases fired from the same firearm with the same level of lubrication to determine if and how lubrication conditions impact the stria left on those cartridge cases. It was concluded that over-lubricating the breech face led to inconsistencies in the minutia pattern of the cartridge cases. The results from this project could address one of the concerns in firearm examination and help make conclusions made by firearm examiners even more concrete for criminal investigations.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Drug Chemistry Abstracts

Assessing the Greenness of Forensic Chemistry Methods

Thomas A. Brettell, Ph.D., GKE-ABC, Department of Chemical, Physical & Forensic Sciences, 100 College Drive, Allentown, PA 18104

Sample preparation is one of the most energy consuming steps in an analytical procedure due to the large volume of organic solvents, acids, or bases needed to dissolve, extract, or digest samples. Development of solvent-free procedures and or the direct analysis of samples without any chemical treatment is ideal to reduce exposure to toxic and hazardous substances, however not all evidential materials allow this approach.

Green analytical chemistry (GAC) was introduced in 2000 to emphasize the development of environmentally sustainable analytical methods for chemical analysis to reduce or eliminate reagents, solvents, and/or procedures that generate toxic residues and waste (1). In forensic chemistry and toxicological analysis, solventless extraction or reduced solvent usage are desirable. As chemical usage in forensic examinations are always a concern, potential solutions should be sought to reduce the hazardous materials in these methods. Green approaches focus on keeping the ecosystem clean and balanced. The employment of green approaches is relatively new and sparse in forensic science; however, the application of green chemistry and technology has gained momentum elsewhere in industrial areas. The goal of these approaches is to lessen the risks related to chemical usage and reduce the chemicals' undesirable impacts on humans and the environment.

Several metrics exist for measuring and assessing the greenness of analytical procedures. Yin, et al. (2) recently reviewed 15 widely used GAC tools. The purpose of this presentation is to highlight and describe some green analytical tools and metrics that can be used in forensic applications and to bring attention to the need for more sustainable and greener methodologies in the forensic field. A review of the literature will show examples of sustainable analytical methods that have been reported in forensic chemistry and toxicology. As an example, the metrics for the evaluation of the greenness for the analytical methodology of a high-performance thin-layer chromatographic (HPTLC) method used to analyze novel psychoactive substances (NPS) will be presented.

References:

1. S. Armenta, S. Garrigues, M. de la Guardia, Green analytical chemistry, Trends Anal. Chem. 27 (2008) 497–511.
2. L. Yin, L. Yu, Y. Guo, C. Wang, Y. Ge, X. Zheng, N. Zhang, J. You, Y. Zhang, M. Shi, Green analytical chemistry metrics for evaluating the greenness of analytical procedures, J. Pharm. Anal. 14 (2024) 101013, https://doi.org/10.1016/j. jpha.2024.101013.

Rapid Forensic Drug Screening with RADIAN ASAP MS

Pitman, Ciara Ph.D.[1]; Fox, Holly[2]; Swartz, Emily[2]

[1] Waters Corporation, Milford, MA, [2]Cumberland County District Attorney's Office, Carlisle, PA

RADIAN™ ASAP (Rapid Direct Analysis Atmospheric Pressure Solids Analysis Probe) Mass Spectrometry, a class A SWGDRUG technique, provides rapid analysis of substances present in seized drug samples. Utilizing ambient ionization techniques, seized samples can be prepared, detected, and identified within minutes. Solid or liquid suspected drug samples are diluted in a suitable solvent and introduced into the instrument via a glass capillary rod. The sample is ionized at 4 cone voltages simultaneously, resulting in M+H data that is used to aid in identifying controlled substances. Live ID software and SpectralWorks software aids in the identification of multiple components in a single sample. Following an overview of the RADIAN technology, this presentation will discuss how the Cumberland County District Attorney's Office employs the RADIAN for presumptive screening, validation and implementation processes, as well as the associated benefits and challenges.

Distinguishing Positional Isomers of Methylfentanyl

Jennifer G. Skuches, Drug Enforcement Administration (DEA), Northeast Laboratory, New York, NY; and Brianna Singh, Drug Enforcement Administration (DEA), Northeast Laboratory, New York, NY

Learning Overview: After attending this presentation, attendees will be able to assess and identify the different potential methylfentanyl isomers employing DEA's Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) Gas Chromatography-Mass Spectrometry (GC/MS) method.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing an analytical scheme for use in forensic seized drug analysis to distinguish methylfentanyl isomers utilizing DEA's GC/MS GUARDS method.

Currently, the United States is experiencing an unprecedented fentanyl epidemic. Through different routes of fentanyl synthesis, modifications can be made to create fentanyl-related compounds (FRCs) that can lead to challenges in their identification. α-Methylfentanyl was first identified in DEA casework in 1981 and closely followed by the identification of 3-methylfentanyl in 1983. Since then, there have been multiple studies conducted to analytically differentiate the various isomers of these FRCs. Over time, the number of structural isomers of methylfentanyl have increased making the identification more complex.

In the Fall of 2024, DEA Forensic Laboratories started observing indications of methylfentanyl in seized drug samples. In these instances, the methylfentanyl was not confirmed nor was the isomer of the methylfentanyl distinguished due to limits in methodology, reference material availability and instrumentation. In addition, these samples had other controlled substances present such as fentanyl and heroin, as well as non-controlled substances, such as acetaminophen, caffeine, and xylazine.

Individual analysis was conducted with 15 methylfentanyl isomers using different instrumental techniques, including DEA's Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) gas chromatography-mass spectrometry (GC/MS) method. Mixes containing the isomers and fentanyl were also tested. Ion fragmentation and retention time differences were key in identification of each of the 15 methylfentanyl isomers. There are a few limitations but with careful analysis, isomer determination can be achieved. This presentation will provide guidance in analyzing unknown seized drug samples containing methylfentanyl isomers using the DEA-developed GUARDS GC/MS method.

Differentiation and Identification of Fentanyl Analogues Using Portable Ion Trap Mass Spectrometry

Mei Yuan, B.S.[1,2]; Ella Galvan, B.S.[1]; Jeff Johnson, Ph.D.[3]; Seth Fisher[3]; Richard Crocombe, Ph.D.[4]; Don Ostrowski[5]; Marisia Fikiet, Ph.D.[1]; Pauline E. Leary, Ph.D,[5]; Brooke W. Kammrath, Ph.D.[1,2]

[1]University of New Haven, West Haven, CT 06516, [2]Henry C. Lee Institute of Forensic Science, West Haven, CT 06516, [3] Astrotech Technologies, Inc., Austin, TX 78758, [4] Crocombe Spectroscopic Consulting, Winchester, MA 01890, [5] Noble, Boston MA 02210

The continually evolving and complex drug landscape presents a formidable challenge for first responders who need reliable tools for their detection and identification. Fentanyl is a potent synthetic opioid analgesic used to manage pain which recently became a significant public health threat due to the illicit drug market. In 2023, overdose deaths exceeded 100,000 in the United States, with over 70% of those involving a synthetic opioid such as fentanyl or tramadol (1). These drugs are being transported to the United States across borders, and thus it has become a national priority to develop tools to help stop their proliferation (2). Portable technologies provide the best potential for addressing this problem, with a specific emphasis on identification of fentanyl and its analogs in the field. This research involves analyzing 250 synthetic opioids and related substances, including 210 fentanyl analogs, to build a comprehensive fentanyl detection library for a portable ion trap mass spectrometer (1st Detect Tracer 1000) designed for rapid identification of drugs and explosives. Ion trap mass spectrometers operate by using electrostatic and radio frequency fields to confine charged particles. This offers high sensitivity, resolution, and the ability to study ion-molecule reactions. It was hypothesized that expanding the instrument's library with a wide range of fentanyl analogs would improve

classification and detection performance in real-world scenarios. Results support this hypothesis, showing improved detection and accuracy with the updated spectral library. To further validate the enhanced library, its detection and identification accuracy were evaluated using mixed samples and adjudicated case samples. This project aims to evaluate and ultimately improve the detection and identification capabilities of portable ion trap mass spectrometry for fentanyl, fentanyl analogs, and other synthetic opioids, thus supporting its use in high-security environments like airports and other border crossings.

References:

1. Centers for Disease Control and Prevention. (n.d.). *Understanding the opioid overdose epidemic*. Centers for Disease Control and Prevention. https://www.cdc.gov/overdose-prevention/about/understanding-the-opioid-overdose-epidemic.html
2. U.S. Customs and Border Protection. (n.d.). *Frontline against fentanyl*. U.S. Department of Homeland Security. https://www.cbp.gov/border-security/frontline-against-fentanyl

The Transacetylation of Controlled Substances in Analytical Matrices

Bailey Davis, MPS; Jaclyn Beshlian, MFS; Anthony Chiusano, BS; Daniel Azevedo, BS; Jennifer Yung, MS, New York City Police Department Police Laboratory, Jamaica, NY, USA

The transfer of acetyl groups from one compound to another is a reaction that has been extensively studied in organic chemistry. Compounds can act as either acetyl group donors or acetyl group acceptors. One compound considered an acetyl acceptor is acetaminophen (paracetamol), while aspirin (acetylsalicylic acid) is an acetyl donor. Both of these substances are commonly observed by the New York City Police Department (NYPD) Police Laboratory as diluents in illicit drug samples. Results obtained from Controlled Substance Analysis Section casework samples in 2023 and 2018 resulted in an in-depth review of the existing literature regarding reactions that involved acetyl donors and acceptors, but research pertaining to the forensic analysis of illicit drugs was sparse.[1,2] As a result, a wealth of experimental data was generated in order to ascertain whether transacetylation was occurring during casework analysis using the Police Laboratory's current procedures. It was found that in the presence of acetaminophen and/or aspirin, two pairs of substances underwent transacetylation, converting one compound into the other depending on whether an acetyl donor or acceptor was present. The first pair was heroin and 6-monoacetylmorphine (6-MAM), and the second was 4-anilino-N-piperidine (4-ANPP) and acetyl fentanyl. The results of this study have profound implications for the analysis of controlled substances and seized drugs in forensic science laboratories nationwide, as they suggest that without proper safeguards to prevent transacetylation, certain substances may be incorrectly reported as present. Although some strategies aimed to minimize transacetylation proved effective, the pursual of analytical solutions to this issue by the NYPD Police Laboratory are ongoing.

References

1. Clark, C. C. (1977). A study of procedures for the identification of heroin. Journal of forensic sciences, 22(2), 418-428.
2. Huizer, H., & Poortman, A. J. (1989). Some aspects of the gas chromatographic (GC) analysis of heroin. United Nations, 1-16.

Analysis of Nitazenes Using Tandem-Column High Performance Liquid Chromatography (TC-HPLC)

Ms. Casey Rech**,** Cedar Crest College, et al.

Synthetic opioids known as 2-benzylbenzimidazoles, also referred to as nitazenes, are a prevalent drug group encountered in forensic drug casework. For this drug class, few chromatographic methods are currently available for analysis. In this study, selected nitazenes were analyzed using an Agilent 1100 series HPLC with an in-series tandem-column coupled to a Diode Array Detector (DAD) to determine if there is greater resolution and detection for these nitazenes compared to known methods and to add this as an analytical technique for this drug class. Three analytical columns, C18-HL, C18-PFP, and Phenyl, were evaluated, all with the same column dimensions and attached in various combinations to determine best resolution.

Gradient conditions and sample preparation were optimized for ideal gaussian peak shape. For each column combination, resolution values were calculated, and limits of detection were determined. Based on these factors, the column pairing that provided the greatest resolution between compounds was C18-HL and Phenyl.

Stability and Degradation of Psilocybin and Psilocin in Mushroom Gummies: Forensic Implications

Ms. Alyssa Ricca, Cedar Crest College, et al.

Psychedelic mushrooms are increasingly being processed into edible forms, such as gummies, for recreational and self-medication purposes. This shift presents new challenges in forensic analysis due to instability of psilocybin and psilocin, as well as the complexity of edible matrices. Psilocybin is both thermolabile and photosensitive, necessitating careful method development and stability testing. The hypothesis of this study is that storage conditions affect the degradation and conversion of these compounds, which may impact quantitation accuracy and evidence interpretation.

Attendees of this presentation will understand how psilocybin and psilocin concentrations change over time under different storage conditions in a gummy matrix, how homogeneously the drugs are distributed within the gummy, and how validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods can be used to analyze these compounds in forensic casework. Due to thermal instability, LC-MS/MS was chosen over gas chromatography, and amber vials were used to limit light exposure. This study provides a validated method for interpreting mushroom gummy results by accounting for storage conditions, time, and psilocybin-to-psilocin conversion.

A LC-MS/MS method was developed and validated for the quantitation of psilocybin and psilocin in mushroom gummies. Deuterated internal standards were spiked into all standards and samples prior to extraction to account for matrix effects and variability. Method validation included calibration curve construction (R^2 = 0.9763 for psilocybin and R^2 = 0.9970 for psilocin), limits of detection and quantitation (LOD = 0.0398 µg/mL and LOQ = 0.1205 µg/mL for psilocybin; LOD = 0.0094 µg/mL and LOQ = 0.0284 µg/mL for psilocin), evaluation of ionization suppression, recovery studies, and stability testing across various storage conditions. Validation also included repeatability and reproducibility studies, with the %RSD of psilocybin and psilocin being 10% and 17% respectively.

Multiple extraction methods were evaluated, including ceramic bead homogenization, dounce homogenization, and QuEChERS, using various solvents. Ceramic bead homogenization with water provided the best recoveries for both analytes: 83.11% for psilocybin and 94.84% for psilocin. Drug distribution within gummies was assessed to determine homogeneity, with sample %RSDs ranging from 8.37% - 19.41% for psilocybin and 2.61% - 14.29% for psilocin. Stability was assessed by analyzing samples at Day 0, 3, 7, 14, 21, and upcoming 30, 45, and 60-day intervals to track trends in analyte degradation or formation over time.

Stability testing revealed that psilocin concentrations increased relative to Day 0 under all conditions, with refrigerated gummies showing the greatest increase (121.4% on Day 14). Psilocybin gummy concentrations initially decreased and then increased, also peaking under refrigerated conditions (72.2% on Day 7). After reaching peak levels, psilocin and psilocybin concentrations declined under all conditions, generally remaining above their Day 0 concentrations, except in the ambient light condition for psilocin. A small psilocin peak was observed forming in psilocybin gummies supporting a possible in-situ dephosphorylation reaction within the matrix. Later time points will also be analyzed and presented.

This presentation provides a framework for understanding chemical changes in psychedelic edibles over time and highlights the need for time-sensitive, validated forensic methods to accurately assess seized samples containing psilocybin and psilocin.

Methods of Separating Isomers of Fentanyl Analogs via LC/MS/MS

David James Cirota, MPS, Pennsylvania State University, University Park, PA; and William Campbell, PhD, Pennsylvania State University, University Park, PA

Fentanyl and its ever-increasing slate of potent analogs present consistent problems for the forensic science community. Analogs can take several isomeric forms, such as positional and geometric isomers. Isomers share masses and frequently have identical mass spectrometric fragmentation, which makes them difficult to distinguish. Therefore, HPLC separation is critical for unique identification. This research will provide tools necessary to separate the isomers for the purpose of profiling complex drug samples containing fentanyl analogs through simple chromatographic methods using tandem mass spectrometry for detection.

This research aimed to develop an HPLC method to separate a broad panel of fentanyl analog isomers with high pH mobile phases. Method development focused on optimizing chromatographic conditions for isomer separation, including column geometry, mobile phase modifiers, pH conditions, and gradients. The base panel included three sets of geometric isomers (Fluorofentanyls, Despropionyl Fluorofentanyls, Fluoroisobutyryl Fentanyls), alongside one set of positional isomers (Methylfentanyls). The final panel was supplemented with other common fentanyl analogs (e.g. Acetylfentanyl), as well as an array of other forensically relevant opioids, stimulants, and cutting agents.

Chromatography utilized high pH stable C18 phases on 100 x 2.1mm columns initially, but the final method was transferred to a 100 x 1.5mm column for enhanced sensitivity. Aqueous mobile phases with ammonium acetate and ammonium hydroxide across a range of pH values were crucial in achieving the best separation. Acetonitrile provided superior separation compared to methanol as an organic modifier.

Compounds were detected using LC/MS/MS in multiple reaction monitoring (MRM) mode for quantitation. Additional analyses for this panel entailed parent ion and neutral loss mode as potential methods to identify unknown fentanyl analogs. Future research will seek to expand this panel to address newly emerging compounds in illicit drug markets.

LC/MS/MS Analysis of Nitazenes

Hannah Kruse, PI: Dr. William Campbell, PhD, Penn State University.

After attending this presentation, attendees will understand HPLC optimization through column chemistry, geometry, and mobile phase conditions. This will be illustrated by a method for nitazene separation and detection by LC/MS/MS.

Nitazenes, or 2-benzylbenzimidazoles, are a new class of opioids that have arrived on the illegal market as a result of bans on fentanyl and its precursors. This impacts the forensic science community heavily, as nitazenes pose issues with limited scheduling history and lack of validated analytical methods[1]. The specific compounds were gathered from the 2024 DEA scheduling 21 U.S.C. § 802(32) including metodesnitazene, metonitazene, flunitazene, N-pyrrolidino etonitazene, clonitazene, isotonitazene, protonitazene, and butonitazene[2].

Initial method development used a superficially porous C18 100 X 2.1mm. We also investigated different column chemistries including biphenyl, phenyl-hexyl, and amide phases. We further transferred the method to a 100 X 1.5 mm column. The smaller column internal diameter accentuates detection sensitivity and provides lower concentration limits. The C18 and amide columns have the best separation. Mobile phase conditions were also investigated. Organic modifiers were acetonitrile or methanol; modifiers for pH control included ammonium acetate / acetic acid or simply formic acid. The ammonium acetate in water in combination with acetonitrile provided the best conditions for both compound peak shape and selectivity. The mass spectrometer was run in multiple reaction monitoring (MRM) mode because of its wider linear range for quantitation and higher selectivity. This nitazene panel was also analyzed using parent ion mode. Six of the eight analytes had a transition ion of m/z = 100. Using parent ion scans provides a potential methodology for identification of unknow nitazenes in drug samples. Next steps in the research will be to integrate these analytes into a broader forensic drug panel including common stimulants, depressants, and hallucinogens.

References

1. Vandeputte, M. M., Krotulski, A. J., Walther, D., Glatfelter, G. C., Papsun, D., Walton, S. E., Logan, B. K., Baumann, M. H., & Stove, C. P. (2022). Pharmacological evaluation and forensic case series of N-pyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole "nitazene" synthetic opioid. *Archives of Toxicology, 96*(6), 1845–1863. https://doi.org/10.1007/s00204-022-03276-4.
2. Schedules of Controlled Substances: Placement of Butonitazene, Flunitazene, and Metodesnitazene Substances in Schedule I. (2024). In *Federal Register*. https://www.federalregister.gov/documents/2024/04/11/2024-07694/schedules-of-controlled-substances-placement-of-butonitazene-flunitazene-and-metodesnitazene.

Cannabis Differentiation with DART-MS: Evaluating the Impact of Polarity, Ionizing Gas, and Chemometric Modeling.

Alina Rovnak, M.S., Adam B. Hall, Ph.D., ABC-FD, Boston University – Chobanian and Avedisian School of Medicine, Biomedical Forensic Sciences Program, Boston, MA

The 2018 Farm Bill amended the Controlled Substances Act (CSA) by removing hemp from the legal definition of marijuana, which federally legalized hemp. This bill classifies hemp as any cannabis or cannabis-derived product with a delta-9-tetrahydrocannabinol (THC) concentration of less than 0.3%. Botanically, hemp and marijuana are identical as they both come from the plant species *Cannabis sativa,* but they differ in their %THC content. Prior to the Farm Bill, laboratories reported the presence of a controlled substance upon detection of cannabinoids, which are found in both hemp and marijuana. Traditional forensic laboratory methods for the analysis of hemp and marijuana include colorimetric screening tests (e.g., Duquenois-Levine), olfactory tests, and macroscopic/microscopic tests. However, these methods are unable to distinguish between hemp and marijuana since they do not measure THC concentration. A definitive distinction requires THC quantification, a more complex and time-consuming analytical process involving extensive sample preparation and often derivatization.

This study proposes a rapid analytical procedure for the differentiation of hemp and marijuana by combining direct analysis in real time–mass spectrometry (DART-MS) with chemometrics analysis. DART is a rapid ambient ionization technique requiring minimal to no sample preparation. In this study, ten hemp and ten marijuana samples were extracted in methanol and analyzed in duplicate using DART-MS. To assess the impact of instrumental parameters, samples were analyzed under different ionization modes (positive and negative) and with two carrier gases (helium and nitrogen). This comparison allowed for the evaluation of ionization efficiency and spectral variation between hemp and marijuana. A training set of the resulting data was created and processed using Mass Mountaineer™, a chemometric-based software specializing in the analysis of mass spectrometry data. Principal component analysis (PCA) and kernel discriminant analysis (KDA) were applied to the training set, reducing the complexity of the data and revealing distinct clustering patterns between hemp and marijuana. To evaluate the model's accuracy, a single-blind study was conducted with ten unknown samples. The results demonstrate that the models can successfully classify unknown samples and rapidly differentiate between hemp and marijuana, further supporting its potential application in forensic science casework.

Drug Analysis Trends in the Mid-Atlantic and Northeast USA: Insights from NPS Discovery (2024–Q3 2025)

Joshua S DeBord, PhD[1], Barry K Logan, PhD[1,2], and Alex J Krotulski, PhD[1]

[1]Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, 206 Welsh Road, Horsham, PA., [2]NMS Labs, 200 Welsh Rd, Horsham, PA

Learning Overview: Attendees will learn how CFSRE's NPS Discovery achieves rapid, backlog-free drug testing and leverages innovative workflows, post-processing, and LIMS integration to provide real-time analytical results and trend analyses to stakeholders. Key findings from 2024–Q3 2025 will be presented, highlighting shifts in the regional drug markets relevant to forensic scientists and toxicologists.

Abstract: Real-time monitoring of drug trends requires efficient analytical workflows, a capable team, and a robust data infrastructure. NPS Discovery at the Center for Forensic Science Research and Education (CFSRE) meets these requirements and often begins testing on a sample the same day it's received. We analyze trends in drug composition and formulation based upon the primary drug component, date received, and geographic region.

Our laboratory performs qualitative analysis by two instrumental techniques: gas-chromatography mass spectrometry (GC-MS) and liquid-chromatography quadrupole-time-of-flight mass spectrometry (LC-QTOF-MS). Our goal is to provide preliminary testing results within five days. An efficient workflow and integrated LIMS enable immediate result-sharing with stakeholders and support dashboards that are automatically updated for real-time visualization.

Data were first grouped for samples that were most recently obtained in the Northeast and Mid-Atlantic (NEMA) regions of the United States and then grouped by the quarter in which the samples were received at our lab. A total of 784 NEMA samples were analyzed in Q2 2025, the primary drug substance in 392 (50%) samples was fentanyl, followed by cocaine with 179 (23%), methamphetamine 41 (5%), heroin 38 (5%), and ketamine 28 (4%). The rates of fentanyl co-occurrences in samples that were primarily heroin, cocaine, or methamphetamine were 68%, 11%, and 7% respectively in Q2 2025. There were 89 observations of novel psychoactive substances (NPS) in Q2 2025, including benzodiazepines, synthetic opioids, and synthetic cannabinoids. The most common were bromazolam (24) and phenazolam (4) among NPS benzodiazepines; carfentanil (16), para-fluorofentanyl (13), and ortho-methylfentanyl (8) among synthetic opioids; and MDMA-4en-PINACA (8) and 5F-ADB (2) among synthetic cannabinoids.

Longitudinal data are continually being reported through Q3 2025. Preliminary Q3 2025 data suggest shifts in the NPS benzodiazepine market, with bromazolam positivity declining from ~90% (Q4 2024) to ~60% (Q3 2025); while observations are limited, phenazolam positivity has risen from negligible levels to become the second-most prevalent NPS benzodiazepine in Q3 2025. Likewise, fentanyl co-occurrence patterns have evolved: xylazine positivity fell from ~80% to <25%, while medetomidine positivity has risen from 0% to over 40%, reflecting an undesired shift in alpha-2 receptor agonists coinciding with localized scheduling of xylazine. The positivity for BTMPS, a substance of unclear nature appearing alongside [or in substitution of] fentanyl since 2024, has declined from 37% in Q1 of 2025 to 22% in Q3 of 2025 suggesting its presence in the supply may be waning.

Things That Make You Go "Hmmm…". Turning Puzzling Encounters into Practical Solutions in Controlled Substance Casework

Matthew Reimer, Stephanie Minero, M.S., ABC-DA, Nassau County Office of the Medical Examiner, Division of Forensic Services

Casework in the controlled substance analysis section of a forensic laboratory can be described as three things: high volume, fast paced, and high stakes. With state and local regulations often requiring short turnaround times to meet the demands of the criminal justice system, a seamless and efficient workflow is critical to the laboratory. As controlled substance casework historically accounts for a substantial percentage of evidence submissions, any ripple in the matrix has far-reaching effects. When unexpected analytical results are encountered, analysis is often significantly delayed or even halted while troubleshooting is performed. While the analyst embarks upon the noble and onerous journey of problem solving, the clock continues to tick. Customers are waiting. The phone is ringing. It's Friday at 4:55pm.

Over the last year, situations were handled during analysis of controlled substance casework that were unanticipated, unexpected, and simply made us go "hmmm…". These included extraneous siloxane peaks permeating GC/MS negative controls in the absence of septum or column bleed, systemic presence of triphenylphosphine oxide in quality control injections, inconsistent detection of pentadecanoic acid in sample preparations, and co-eluting 14-hydroxycodeinone impurity in pharmaceutical oxycodone tablets which nearly precluded identification of the target analyte.

For each puzzling encounter, the information obtained from painstaking troubleshooting and root cause

determination will be presented. Practical solutions and suggestions for improvement will be discussed, including the use of alternate consumables and sample preparations.

Carfentanil and Poly-Drug Adulteration in Counterfeit Tablets and Powder Seized at the Southwest Border

Macenzie Powell, MSFS[1], Mia Borrelli, MSFS[1], Alexandra Kuchinos, MSFS[1], Amanda L.A. Mohr, MSFS[1], Thom Browne, Jr., MA[3], Barry K. Logan, PhD[1, 2]

[1]The Center for Forensic Science Research and Education, Horsham, PA, USA, [2]NMS Labs, Horsham, PA, USA, [3]Colombo Plan, Colombo, Sri Lanka

Counterfeit tablets have been a reoccurring cause of concern throughout the opioid crisis. These tablets often resemble authentic pharmaceutical products, imitating their color, size, shape, and monogramming, and leaving the true contents unknown to the individual. In addition to pre-pressed tablets, powder samples are concerning, as the powder can be used to manufacture counterfeit tablets. The ongoing persistence and widespread prevalence of counterfeit tablets and powders containing illicit drugs emphasizes the need for research to investigate these products in circulation and determine their composition.

Seized samples from the United States Southwest border ports of entry were submitted to the Center for Forensic Science Research and Education (CFSRE) for qualitative and quantitative analysis. A representative sampling of the counterfeit tablets, known as a sub-exhibit, and powder samples were examined using a multi-instrument workflow including microscopic imaging with the MiScope® Megapixel MP3 and Red Green Blue (RGB) color determination with ImageJ software. The samples then underwent qualitative analysis by gas chromatography–mass spectrometry (GC/MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS), as well as quantitative analysis via Waters® Acquity UPLC coupled to a Waters Xevo® TQ-S micro.

Ongoing analysis of tablets and powders has revealed that the samples contain complex drug mixtures. Notably, a recent batch of counterfeit tablets and a powder sample seized in spring 2025 raised serious concerns. Counterfeit blue M30 tablets and a powder sample were found to contain carfentanil, a synthetic opioid 100X more potent than fentanyl. Fentanyl was also identified in the powder sample, which was quantified at a purity of 16%. The powder sample additionally contained lidocaine and xylazine. Along with carfentanil, all tablets contained acetaminophen and metamizole, both common adulterants. Acetaminophen was quantified at a median of 46 mg per tablet and a mean of 45±1.8 mg per tablet, with a range from 45 to 51 mg per tablet.

These findings highlight the rapidly evolving and unpredictable nature of the counterfeit drug supply. The detection of carfentanil, without fentanyl, in counterfeit tablets, alongside multiple adulterants such as acetaminophen and metamizole, underscores the complexity and variability of these formulations. The powder sample, containing carfentanil in combination with fentanyl, lidocaine, and xylazine, further illustrates the dangers posed by poly-drug adulteration. Together, these results emphasize the urgent need for continued surveillance and comprehensive testing to identify emerging threats and protect public health.

Unpacking the BTMPS Phenomenon: A Snapshot of a New Threat in Counterfeit Fentanyl

Alexandra Kuchinos, MSFS[1*], Mia Borrelli, MSFS[1], Macenzie Powell, MSFS[1], Amanda L.A. Mohr, MSFS[1], Madison Schackmuth, PhD[1], Thom Browne, Jr., MA[3], Barry K. Logan, PhD[1,2]

[1]The Center for Forensic Science Research and Education, Horsham, PA, USA, [2]NMS Labs, Horsham, PA, USA, [3]Colombo Plan, Colombo, Sri Lanka

Abstract: The illicit drug market, driven by the ever-changing composition of counterfeit pills and powders, remains a highly dynamic and unpredictable public health threat—most recently illustrated by the detection of Bis(2,2,6,6-tetramethyl-4-piperidyl)sebacate (BTMPS), a light stabilizer and potent L-type calcium channel blocker, in fentanyl samples.

Seizures of suspected fentanyl powder and tablets were received by the Center for Forensic Science and Research & Education (CFSRE) and analyzed via gas chromatography mass spectrometry (GC/MS) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS). BTMPS, an industrial compound commonly used in pharmaceutical manufacturing and packaging, was detected in five powders and eighteen tablet cases seized from five different Southwest border ports of entry and other western locations. These seizures occurred between June 2024 and June 2025, with the earliest detection reported in late June 2024. Fentanyl was identified in all but one sample, along with various fentanyl precursors, reaction by-products, analogs, and adulterants. Substances related to BTMPS, including Tetramethyl-4-Piperidinol (TMP-OH), Tetramethyl-4-AP (TM-4-AP), and Tetramethylnorfentanyl (TMNF), were also identified in several cases. Quantitative analysis of the tablets showed fentanyl concentrations ranging from 0.49 mg to 3.45 mg, bracketing the 2 mg lethal dose established by the Drug Enforcement Administration. BTMPS was most abundantly detected in powder samples. Using GC/MS peak areas showed that BTMPS concentrations in powder samples increased more than 40-fold between June and September 2024, reached their highest levels from September to October, and then declined by December 2024, where they have since remained.

The reason for the emergence of BTMPS in the fentanyl drug supply is largely unknown, though, early hypotheses proposed that it may be used as a bulking or stabilizing agent, to delay fentanyl withdrawal symptoms, or that its presence may be accidental. However, inconsistent levels of the adulterant across samples made these theories difficult to substantiate. The substituted piperidine group in the chemical structure of BTMPS has prompted speculation about its potential involvement in fentanyl synthesis. This theory is supported by the recent identification of structurally related compounds such as TMP-OH, TM-4-AP, alongside fentanyl-related substances including N-propionyl 4-AP and N-Phenethyl Tetramethyl-4-AP (PE-TM-4-AP).

BTMPS has been observed in samples from various geographic regions, and its rapid and widespread infiltration in the illicit drug supply has made it difficult to trace its origin or predict its future spread. Given the limited understanding of its health risks and its extensive presence worldwide, BTMPS remains a significant concern and should continue to be monitored and leveraged as an intelligence marker in illicit fentanyl synthesis.

Screening for Novel Psychoactive Substances Using Neutral Losses and Common Fragments by LC-QTOF-MS

Angelique B McDowell, BS[1], Barry K Logan, PhD, F-ABFT[1,2], Alex J Krotulski, PhD[1], and Joshua S DeBord, PhD[1]

[1]Center for Forensic Science Research and Education, Fredric Rieders Family Foundation, 206 Welsh Road, Horsham, PA., [2]NMS Labs, 200 Welsh Rd, Horsham, PA

Learning Overview: Attendees of this presentation will gain understanding of the fundamentals of neutral loss (NL) and common fragment (CF) screening as a strategy for detecting unknown novel psychoactive substances (NPS). The presentation will outline the steps taken to utilize high-resolution, quadrupole-time-of-flight mass spectrometry for screening known and unknown nitazene analogs as a model compound class, the ongoing efforts to build a complementary data processing workflow, and the practical challenges encountered throughout the method development process.

Abstract: Screening for novel psychoactive substances (NPS) in forensic chemistry and toxicology has become increasingly challenging due to the shifting dynamics of the recreational drug market. The rapid emergence and disappearance of these substances and the lack of available reference standards complicate traditional screening approaches based on spectral comparisons. This presentation details the ongoing development of a screening method designed to circumvent these limitations by utilizing characteristic neutral losses and common fragments associated with known analogs across a variety of NPS drug classes.

When subjected to collision-induced dissociation, analytes break into fragment ions that are often common among structural analogs. These fragment ions can be used to infer the presence of specific substances, drug classes, or more simply structural moieties. Structural analogs within a drug class produce characteristic

neutral losses during fragmentation, which can be measured using a variety of data acquisition modes (e.g., Data Independent Acquisition [DIA]). A coupled screening method for both common fragments and neutral losses associated with a specific class can not only detect the presence of a suspect analog but can allow for preliminary identification without relying on library spectra or standards, which may not be available.

This presentation outlines the development of a liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) screening method aimed at identifying nitazene analogs in drug material samples. For development purposes, the method targeted 18 known neutral losses and 19 common fragments shared among 40 nitazene analogs. These known neutral losses and com To be included in this preliminary list, each neutral loss or fragment had to be observed in at least three known analogs. This list informed the creation of a DIA method using a SCIEX X500R LC-QTOF-MS.

A sample acquisition method has been developed, and preliminary method comparison has been completed. The barrier to routine implementation and validation of the method is the lack of a post-acquisition processing method to review the identified targets, as routine software for these means has not been commercially distributed. Development of a data processing method remains ongoing, and this preliminary work demonstrates a promising approach for detecting unknown nitazene analogs. While this proof-of-concept study focuses on nitazene analogs, the methodology shows promising applicability to other NPS classes, provided that characteristic fragmentation patterns can be identified. By relying on structural features such as common fragments and neutral losses, this method aims to overcome widespread challenges with NPS detection, enabling early-stage detection and classification of related compounds in complex matrices. Compared to existing targeted methodologies, this approach offers greater agility in responding to rapidly evolving drug trends. Future work will focus on developing a data processing method, method validation, method comparison, and extending the methodology to additional NPS drug classes, contingent upon the successful demonstration of its applicability to nitazene analogs.

Installation and Implementation of the Global Uniform Analysis and Reporting of Drug-related Substances (GUARDS) Gas Chromatography-Mass Spectrometry Method

Mikaela Romanelli, Senior Forensic Chemist, Drug Enforcement Administration (DEA), Northeast Laboratory, New York, NY.

With the landscape of the illicit drug market changing to include an increase of synthetic emerging drugs, innovative comprehensive and reliable analytical methodology is needed to fully capture the complexity of current polydrug samples and aid in the analysis and identification of these newly emerging substances. Differences in federal, state, and local laws impact how seized drug laboratories analyze and report chemical substances. For example, results from CY23 DEA seizures in a New England state show 26% of fentanyl exhibits analyzed contained xylazine. Whereas the National Forensic Laboratory Information System (NFLIS) shows 0% for the same state in CY23. These types of reporting inconsistencies among the various agencies impact law enforcement and public health responses to emerging drug threats, like xylazine.

DEA's Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) gas-chromatography-mass spectrometry (GC-MS) analysis method is addressing these challenges and increasing the validity and reliability of forensic results, while simultaneously generating more comprehensive information. DEA's GUARDS method provides a fast, efficient, cost-effective, and uniform GC-MS method of analysis and reporting for the seized drug community.

As the GUARDS GC-MS methodology is adopted, creating the foundation for an enhanced DEA-sponsored Early Warning System, advanced data analytics modeling opportunities will emerge. In addition, GUARDS supports the TRANQ (Testing, Rapid Analysis, and Narcotic Quality) Research Act of 2023, which requires the National Institute of Standards and Technology (NIST) to support research and other activities related to identifying xylazine, novel synthetic opioids, and other new psychoactive substances.

In this session, the impact of inconsistent drug analysis reporting has to public health and public trust will be discussed. Information will be provided to aid laboratories transitioning to hydrogen carrier gas and GUARDS methodology. In addition, the value and implementation of the GUARDS innovative methodology

in 200+ instruments in DEA labs will be presented, as well as the efforts of other labs, such as U.S. Customs and Border Protection (CBP). Furthermore, new initiatives to increase consistency of reported data to NFLIS will be presented.

Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) Update

Ms. Tiffany Ribadeneyra, Nassau County Office of the Medical Examiner / Division of Forensic Sciences, Dr. Jaclyn Icra, Drug Enforcement Administration, (DEA)

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) was formed in 1997 in a joint effort between the U.S. Drug Enforcement Administration (DEA) Office of acceptance. Considering the formation of the Organization of Scientific Area Committees (OSAC), SWGDRUG continues to work as part of the international community to improve the quality of the forensic examination of seized drugs. In addition, the extensively utilized resources provided on the SWGDRUG website will continue to be updated and available including free spectra libraries and monographs.

This presentation will provide attendees with an update on SWGDRUG activities during the year 2024 and currently in 2025. Proposed revisions to the SWGDRUG Recommendations were recently published and the committee is collecting public comment regarding the addition of Part IIIE: Methods of Analysis/Stop Testing Procedures and Negative/Indicative Results. Additional resources were also published recently, including a new NIST Seized Material Sampling App. Ongoing activities include revising Supplemental Document SD-2: Validation of Analytical Methods. Subcommittees have been devoted to training and outreach as well as developing a new supplemental document regarding inconsistent results encountered in a statistical sampling plan. Lastly, the current state of SWGDRUG and its future initiatives will be reviewed.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Forensic Biology/DNA Abstracts

Assessing RNA Stability in Touch Samples

Cameron Brown of the Forensic and National Security Sciences Institute at Syracuse University

Technological advancements in detection of biological samples commonly found in crime scene evidence, sexual assault kits, etc. have given forensic analysts the ability to detect progressively smaller amounts of DNA various sample types (Jepsen et al, 2024). In these "trace" samples, the DNA and RNA may degrade quite readily depending on various conditions in which the sample is deposited. Currently, there is no known reliable evaluation of RNA degradation in samples deposited via touch. Because there are several types of RNA, each with its own structure and functions in the overall role of RNA, they will likely degrade at different rates. By observing the quality and quantity of the RNA found in these samples, this study aims to establish a reliable method to quantify touch-deposited RNA, as well as how various conditions of the sample may affect this degradation.

In my research, participants deposit their touch samples on the various substrate(s) (types) commonly found at crime scenes. To do so, they must handle the object in either the right or left hand for a specified amount of time, after which the substrates and/or collected samples will be stored at a constant temperature for varying amounts of time. When collected from the substrates, the touch samples will be the source of the RNA. The RNA will be further isolated, extracted, amplified, and analyzed to observe degradation.

The current stage of the project hinges on overall optimization of the collection and extraction of minute amounts of RNA present in these touch samples. Looking forward, the focus will shift to analysis of simulated environmental effects and establishment of an RNA degradation timeline. In another study, similar scientific methodology was used to create predictive modeling for the degradation of DNA in touch samples (Ramsey, 2022). This research project aims to expand upon this method to form an enhanced understanding of touch sample degradation, particularly regarding RNA, as well as the applications and potential limitations of the methodology.

The findings of this research may contribute to scientific knowledge on the topic of individualization and identification using genetic material (other than the sole usage of nuclear or mitochondrial DNA). This will eventually aid in its approval as a reliable practice to use in criminal cases. This application of the method for use in the field will allow crime labs to allocate time and resources more efficiently and be more certain in their results, in turn helping to reduce the amount of backlog and wrongful convictions.

Fluorescence and Raman Spectroscopy for Forensic Skin Cell Analysis

Riley Carter and Igor K. Lednev of the University of Albany, SUNY

Skin is the largest organ of the human body; it is the barrier between our organs and any outside interferences. Because it is accosted daily by things like chemicals and sunlight, it is constantly replenishing itself, forcing new skin to the surface, and shedding the old cells on top. By shedding skin cells every second of the day, we as people are leaving an undeniable footprint of genetic material everywhere we go. Applying this to a forensic setting means that there is a plethora of trace DNA evidence that criminals may forget to think about cleaning up. By developing a method to identify and characterize skin cells in a non-destructive manner, a new avenue for DNA profiling is opened up. By using fluorescence spectroscopy to identify the presence of skin cells, and Raman spectroscopy as a confirmatory test, we can quickly and safely identify skin cells that may be used to generate a DNA profile. The ultimate goal here is to develop a method of identification for the origin site of skin cells, which may be used to corroborate claims for crimes in the future.

Impacts of Mold Growth on DNA Degradation

Caleb Henderson of Lasell University

As DNA analysis has become a mainstay of forensic science, DNA degradation has become a large concern, and a common cause of DNA degradation is mold growth. While there has been research on mold growth's effect on DNA, there has yet to be a study that specifically looks at the effect mold growth has on the ability

to get a DNA profile. This study used blood samples inoculated with Penicillium roqueforti, the DNA was extracted, the D1S80 locus was amplified, then the sample was run through gel electrophoresis against several controls to see the effect the mold had on making a usable profile. Unfortunately, due to limitations in materials used and available, the study was inconclusive. However, this study adds weight to the growing need to study the effects of common crime scene agents on DNA degradation.

Bridging the Gap Between Academics and Practicing Forensic Laboratories

Dr. Amanda Murray of DNA Labs International

Transitioning from an academic environment to a practicing forensic laboratory affords the opportunity to expand on an individual's academic knowledge and budding technical skills. By immersing themselves in a practicing forensic laboratory setting, these individuals will ultimately apply their education and developing technical skills to training, casework, research, validation and/or implementation. The field of forensic science requires endless learning as new methodologies, advancements in science, ground-breaking technologies, and changes within accreditation standards and testimony requirements are developed. These aspects excite those entering the field; however, along with excitement, many may feel apprehensive about applying their academic skills to the various duties which are standard in the forensic science field. This may be partially because applying what is learned in the classroom is often a leap away from doing the real hands-on work needed for live casework and special projects within a practicing forensic laboratory. In this presentation, the differences between academic teaching, training, learning and applications within the academic setting to those that are standard in many practicing forensic laboratories will be discussed. Programs within forensic laboratories such as training for casework and testimony, research, validation, and implementation services will be outlined so both forensic scientist students and those from a non-forensic science background breaking into forensic science can further expand their skills with confidence. Bridging the gap between academics and practicing forensic laboratories is crucial for the foundation and success of those entering the forensic science field. This ensures that a smooth transition into the forensic laboratory is achieved, enables confidence within forensic scientists new to the field, and promotes long-term high-quality work throughout the individual's forensic science career.

Breaking Down Mixtures One Cell at a Time: Optimizing Micromanipulation of Single Cells

Taylor Walther and Dr. Michael Marciano of Syracuse University Forensic and National Security Sciences Institute

Single cell analysis in forensic science is now possible owing to the increased sensitivity of chemistry and instrumentation, and is becoming a strategy for combating the challenging and complex nature of DNA mixture interpretation. Capturing single cells enables deconvolution of a mixture prior to downstream genetic analysis which therefore renders the typical mixture interpretation challenges null, as one cell is only from one contributor. Single cell analysis must begin with the cell capturing, previously investigated methods include both laser capture microdissection (LCM), fluorescence-activated cell sorting (FACS) and the more current DEPArray system. However, these methods can be costly and time consuming, both of which lead to practical implementation challenges within crime laboratories. This project will investigate a less expensive and potentially more rapid single cell capturing method through the use of a simple microscopic technique to capture single cells via pulled capillary tubes and the use of either traditional microscope slides or the Sievewell™ device. The evaluation will be five-fold consisting of (1) visualization quality (2) recovery rate (3) ease of use (4) required resources (5) compatibility with downstream genetic analysis. For this project, single cell capturing of epithelial cells was accomplished using pulled capillary tubes attached to a CellTram manual microinjector with the recovery process visualized microscopically using the Leica THUNDER Imager Model Organism. Preliminary results of this ongoing project indicate cell suspension in PBS using Sievewell™ devices rather than traditional microscope slides produce the most efficient cell capturing strategy. Preliminary results also indicate recovery rates of single cells in as few as only 2-4 minutes. To date, eight single cells were amplified using the PowerPlex® 35GY System and detected on the Promega Spectrum Compact CE System. While in cannot be determined whether sample age is a factor at this point, older cells

(>2 months old) had an average profile completeness of 20.45%, while newer cells (<1 week old) had a 90.34% profile completeness. This method shows significant promise in being able to be used in place of the more costly and time-consuming alternatives.

Single cell Evaluations About *how many* and *who* Donated to Sperm and Other Cell Types

Dr. Catherine Grgicak, Qhawe Bhembe, Anu Khandelwal, and Desmond Lun of Rutgers University Camden

In classic procedures, DNA is extracted while an unknown number of cells from an unknown number of contributors is mixed. What follows is the amplification of targeted sequences, which are usually of the STR varietal. The classic procedure, therefore, carries with it a significant shortcoming; knowledge about what alleles associate with what cell, and hence cell-type, is lost.

A single-cell strategy overcomes this gap by isolating and imaging each cell at the front-end of the pipeline. Here, each cell is sequestered before lysis, and PCR reagents are added directly to the vessel to which the cell was added. What results is a set of single-cell electropherograms (scEPGs) that are subsequently clustered into groups by virtue of their (dis-)similarity to one another. Once clustering is complete, we apply models that assert the probability we observe the data in the cluster under same and different source propositions.

With previous work showing an end-to-end single-cell predictor, named EESCIt™, faithfully clustered epithelial and blood cells according to their contributors, we extend its models to consider sperm (haploid) cells. Unlike diploid cells, sperm are not expected to render equivalent scEPGs across cells and it is for this reason we query EESCIt™'s performance.

To do this we place common measures of validity within data analytics formulations that categorize a novelty's viability to meet forensic aims. The categories of interest are that of *Salience*, *Legitimacy* and *Credibility* (SLC). In this way we build a validation methodology that clearly delineates when to operationalize research.

With *Salience* referring to the applicability of the novelty to meet an actor's needs, we begin by discussing what forensic actor would consider questions about *how many* and *who* donated to the *sperm* relevant. Once established, we query if single-cell haploids can be fairly evaluated over a broad factor space, which establishes a novelty's *Legitimacy*. If so, then we move to *Credibility* which confirms an assessor's refinement and speed/ease.

Using single-cell haploids as a test case, we posit that SLC is a framework under which forensic novelties should be evaluated and we show that in being one of those novelties, single-cell techniques are quickly approaching a technology readiness level justifying translation.

Stutter? That is So Last Amplification: Overcoming Stutter in STR Analysis with Novel Enzyme

Danielle Brownell and Anupama Gopalakrishnan of Promega Corporation

Short tandem repeat (STR) analysis is a fundamental tool in forensic DNA investigations. One of the primary challenges in STR analysis is the presence of stutter artifacts, which arise from polymerase slippage during amplification. These artifacts complicate mixture interpretation and can impact the accuracy of forensic DNA profiling. By focusing on protein engineering and machine learning approaches, we aimed to modify DNA polymerase properties to enhance its fidelity and processivity. This resulted in the development of a novel DNA polymerase that significantly reduces stutter while maintaining the robustness and sensitivity required for forensic applications.

This novel polymerase (Reduced Stutter Polymerase or RSP) has been incorporated into Paradyme™ 27GY STR System, an advanced STR system leveraging eight-color technology for both casework and direct amplification samples. The system amplifies the 20 CODIS core loci along with Penta D, Penta E, and SE33, while also including markers for gender determination, two rapidly mutating Y-STR loci, and two Quality Indicators (QI). By drastically reducing stutter artifacts, Paradyme™ 27GY STR System delivers clearer,

more interpretable DNA profiles without altering standard workflows, offering significant advantages for forensic DNA analysis.

Development of the New EZ2 DNA Investigator Sep&Prep Kit for Automated Processing of Sexual Assault Samples

Amber McManus of QIAGEN

1 in 3 women experience physical or sexual violence in their lifetime. This staggering reality places a growing burden on forensic laboratories, where traditional differential extraction methods are often limited by sample quality, or the number of samples submitted. In many cases, inefficient separation of sperm cells from the non-sperm cells – especially when female cells greatly outnumber male ones – compromises downstream DNA analysis. Extensive manual steps such as multiple wash and centrifugation cycles further slow down the workflow and introduce risk of error. Our new EZ2 DNA Investigator® Sep&Prep Kit streamlines the process with a fully automated solution. It enables separation of sperm cells from non-sperm cells and preparation of the sperm-derived DNA for direct use in downstream applications, such as qPCR, STR or NGS.

Novel Body Fluid Identification Methods: A Comparison of Proteomics, Raman Spectroscopy, mRNA Analysis, and DNA Methylation

Aislynne Torres of Boston University's Graduate Medical Sciences, M.S. in Biomedical Forensic Sciences Program in Affiliation with the Massachusetts State Police Crime Laboratory, Criminalistics Unit

Lateral flow immunochromatographic strip tests are designed to detect the presence of human saliva, semen, blood, and urine. Tests such as the acid phosphatase testing for semen and KM testing for blood are used to screen samples for body fluids. Differential extraction and microscopic examination are used to confirm the presence of human and animal sperm cells. Y-screening uses extraction and PCR methods to detect male DNA. For fecal material, the Edelman's Test is a widely accepted presumptive test for its identification. For saliva, seminal fluid, urine and feces, only screening tests are available. Some laboratory's internal validation studies and several research studies have demonstrated that the strip tests are not specific to human saliva, semen, blood, and urine, as false positives were observed with other body fluids and non-human sources. The Edelman's Test for feces uses hazardous and toxic materials and is rarely used. Many methods are used to test different body fluids. Other methods may be available that will reduce the number of tests performed and provide confirmatory results. The purpose of the presentation is to present a validation plan that explores and compares proteomics with LC-MS/MS, Raman spectroscopy, mRNA analysis, and DNA methylation for the best, confirmatory method for the identification of bodily fluids (blood, semen, saliva, urine, and feces) to be implemented into a validation study. The sensitivity, specificity, and selectivity were assessed for each method along with each method's safety precautions, sample amount required, necessary equipment and materials, method duration, cost, and limitations. While most of these methods are novel, it was revealed any of these methods will be a significantly better alternative to the current immunochromatographic assays. Recommendations for the most feasible method is also provided.

Validation of Evidence Collection Methods: Comparing DNA and Body Fluid Recovery Using M-Vac®, Swabbing, and Scraping-Swabbing

Karla Velazquez-Davis, Maureen Hartnett, and Michelle Levasseur of the Massachusetts State Police Crime Lab, and Beth Saucier Goodspeed

The purpose of this validation was to study the efficiency of DNA and body fluid recovery using the M-Vac® collection system, swabbing and scraping-swabbing methods. The M-Vac® is a wet-vacuum DNA collection system that utilizes a vacuum and a sterile, DNA-free, buffer solution to capture the DNA on a filter. The filter is then removed and prepared for DNA analysis. Performance checks of the filters as well as an initial evaluation of the M-Vac® system were previously completed in the Criminalistics and DNA units. Upon

review of the data collected from the initial comparison of collection techniques, the collection of cuttings for extraction is a sufficient method of collection of biological material. However, when the collection spans a larger area than the recommended maximum sample size for cuttings, which is currently 1" by 1", it is beneficial to have an alternate sampling technique. The swabbing and scraping-swabbing methods are commonly used to recover DNA evidence when the collection of a cutting is not feasible. In this validation, Copan swabs, Puritan swabs and Texwipe swabs were used. The data was compared between all three and the most efficient swab type was then compared to the M-Vac® collection. Three sample sets were processed. Sample set #1 consisted of pieces of cloth and non-absorptive materials ranging from high to low absorbency/retention. This portion of the study focused on identifying which swab resulted in a higher recovery rate. Sample set #2 consisted of items frequently submitted for processing where our current methods yield little to no DNA recovery, i.e. diapers, paper towels, paper, rocks, etc. This portion of the study compared the most efficient swab with M-Vac® collections. Sample set #3 addressed varying methods of collection utilizing the M-Vac® collection system for items considered irregular in shape, i.e. rocks. Overall, results showed that Texwipe swabs consistently outperformed Copan and Puritan swabs across various substrates. M-Vac® generally recovered less DNA than Texwipe swabs but more than Copan and Puritan swabs. M-Vac® collections were successful in recovering DNA from items previously swabbed, indicating its effectiveness post-scraping and for highly absorbent items such as diapers. The method was particularly effective for irregularly shaped items, allowing for better recovery of skin cells. Further studies are being conducted pertaining to different collection mediums and various item types to determine the effectiveness of potential skin cell recovery using the M-Vac® and Texwipe collection methods.

Following a Case from the Crime Scene to the Courtroom

Alyssa Berthiaume, M.S. of the Massachusetts State Police Crime Lab

This presentation will follow a case from the Massachusetts State Police Crime Lab from the crime scene, to lab processing, to court. In July of 2019 this analyst was called to respond to a sexual assault of a young girl where both the preliminary suspect was her father but his father was also potentially involved. Multiple rooms in the residence were examined for the potential presence of blood and semen. Several items of evidence were collected from the scene over two scene responses and submitted to the lab by the investigators. Several rounds of criminalistics testing was completed over the next five years, including serological screening and sperm cell identification. This was conducted on items of evidence including Sexual Assault Evidence Collection Kits, clothing, and wet wipes, but due to the limited amount of results obtained a full STR profile could not be developed. Several samples were eventually sent to a vendor lab to use an expanded YSTR kit to attempt to differentiate between the suspect and his father. This case was eventually resolved in court in the spring of 2025. This case will highlight the importance of body fluid testing in an environment where the majority of labs have switched to direct to DNA testing, collaboration with analysts and attorneys/case officers, and ensuring analysts follow their case from start to finish.

The Exoneration of Leonard Mack: A Case Study in Collaboration Among Stakeholders

Alanna Laureano of Westchester County Department of Labs and Research, Division of Forensic Sciences

The exoneration of Leonard Mack highlights the critical role of evolving forensic biology techniques and interagency collaboration in addressing wrongful convictions. On May 22, 1975, Sharon Freeman and Wanza Jones were attacked in the town of Greenburgh, Westchester County, New York. A rape kit and clothing were collected, but only standard serology testing was available at the time. Leonard Mack was arrested within hours based on eyewitness identification and subsequently convicted of rape and weapon possession, despite asserting his innocence and presenting alibi witnesses. He served over seven years in state prison.

In 2019, Mr. Mack contacted the Innocence Project, and his case was accepted in 2022. The Westchester County District Attorney's Conviction Review Unit (CRU) collaborated with the Innocence Project and the Westchester County Department of Labs & Research, Division of Forensic Sciences (WCFL), to re-examine

the evidence. The CRU and WCFL successfully located items suitable for DNA testing. In 2023, WCFL conducted DNA analysis that produced a profile which did not match Mr. Mack.

This profile was uploaded to CODIS, resulting in hits to a 2004 rape case and a New York State offender profile—ultimately identifying the true perpetrator. Following CODIS confirmation and a confession from the actual assailant, Mr. Leonard Mack was officially exonerated on September 5, 2023—nearly five decades after his wrongful conviction. At the time, this marked the longest-standing wrongful conviction overturned by DNA evidence known to the Innocence Project.

This case underscores the importance of evidence preservation, the advancement of forensic DNA testing, and collaboration among forensic laboratories, prosecutorial review units, and justice advocacy organizations.

Keywords: DNA Exoneration, Post-Conviction Review, CODIS, Collaboration

Identifying the Fallen: The Evolution of DNA Technologies at the Armed Forces DNA Identification Laboratory

Madalynn Martino of the AFMES-AFDIL, SNA International

The Armed Forces DNA Identification Laboratory (AFDIL) was established in 1990 as a division of the Armed Forces Medical Examiner System (AFMES). The AFDIL mission is to utilize DNA methods to identify the remains of US service members from current day operations and past conflicts. AFDIL acts as a mission partner to the Defense POW/MIA Accounting Agency (DPAA) to provide DNA testing for the identification of US service members from World War II, the Korean War, Vietnam and the Cold War. All DNA-supported identifications from these conflicts require the use of family references for DNA comparison due to the lack of a service member direct bloodstain card reference, which was not introduced until 1992.

Over the past 35 years, AFDIL has developed and validated numerous DNA technologies for both skeletal remains and reference samples due to the range of sample types and qualities encountered in casework. These technologies include Capillary Electrophoresis of autosomal STRs, Y-STRs, and Sanger sequencing of the mitochondrial control region. Beyond these more traditional forensic techniques, Next Generation Sequencing (NGS) of the mitogenome and nuclear single nucleotide polymorphisms (SNPs) have also been applied to AFDIL casework. Implementation of these technologies has introduced efficiency gains and addressed challenges such as commingled remains, chemically treated bones, and a lack of suitable family reference samples.

AFDIL continues to make technological advancements as challenges persist. Current plans include addressing excess bacterial contamination found in remains, reducing costs and processing times, and the introduction of a Forensic Investigative Genetic Genealogy (FIGG) approach to generate investigative leads for cases that cannot be solved using traditional methods. Almost 81,000 US service members from past conflicts remain unaccounted for and AFDIL, in partnership with DPAA, will continue to evolve to provide the fullest possible accounting of these service members.

Disclaimer: The opinions or assertations presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, or the Armed Forces Medical Examiner System.

Evaluation of DNA Recovery Success Over Time from Submerged Porcine (*Sus Scrofa*) Femurs in Freshwater and Saltwater Environments

Dr. Ashley Morgan of the University of New Haven

Human remains may be deposited in a body of water due to criminal, accidental or mass casualty incidents. Following deposition, recovery and identification may proceed easily when minimal decomposition has occurred and identifying features and artifacts are mostly intact, including clothing and accessories, fingerprints, dentition, and tattoos or piercings. When such features are lost due to decomposition and

scavenging, identification efforts shift from targeting physical features to focusing on skeletal traits and genetic testing. Unfortunately, with the loss of the protective coverings such as clothing and soft tissue, bone tissue may be subject to water infiltration, surface fracturing, and DNA damage. In such instances, it is imperative to understand the time points in which DNA may be successfully recovered and how different aquatic conditions may impact DNA.

In this study, juvenile porcine (*Sus scrofa*) femurs were submerged for zero to 180 days in freshwater and saltwater environments. Porcine femurs were obtained from a butcher and defleshed prior to the experiment. Aquarium tanks were prepared with a thin layer of aquarium sand, then filled with either freshwater or saltwater. Each tank was equipped with an aquarium filter to mimic faunal filtration and water movement in both saltwater and freshwater environments. A dry femur was placed in a fume hood in a clean tray, which served as the control for the duration of the experiment. At regular intervals, cuttings were collected from the diaphysis of the femur, cleaned with enzymatic detergent, and powdered with a manual mortar and pestle. Samples were extracted using the Prepfiler BTA extraction kit with either the vendor specified protocol, or updated protocol which adjusted the initial lysis solution. All extracts were quantified with a SYBR assay using porcine specific primers. This presentation will discuss DNA recovery success over time for each aquatic condition and differences in DNA recovery observed for the two extraction protocols.

Determining the Effect of Physical Activity on the Amount of Trace DNA Deposited on a Non-Porous Surface

Sarah Marsh and Dr. Ashley Morgan of the University of New Haven

When trying to figure out what may have happened at a crime scene, one of the most commonly analyzed pieces of evidence is trace DNA. The common definition of trace DNA is DNA recovered from a crime scene that has no immediate connection to any other evidentiary item at the scene[1]. Trace DNA recovery may be influenced by surface, skin secretions, as well as individual biological factors[1]. For example, when observing the influence of porosity, porous surfaces retain more DNA than non-porous surfaces[2]; however, when a surface is touched more often than another, the likelihood for that surface to contain more DNA is much higher regardless of its porosity[3]. Nevertheless, very little research has been performed that explores other factors that may influence DNA deposition. The purpose of this study was to further inquire on what may influence trace DNA deposition, specifically how physical activity may impact the quantity and quality when collected from a non-porous surface. Seven individuals provided trace DNA samples after performing increasing levels of jumping-jacks. Three activity levels were tested (10, 20, and 30 jumping jacks). Prior to the collection of the experimental samples, three control samples were collected to establish baseline DNA deposition for each participant. Samples were deposited on pre-cleaned glass microscope slides. They were then collected using a single dry Puritan Purflock Ultra swab (ref 25-3306-U) and stored in a clean 1.5mL microcentrifuge tube. Samples were extracted using the Promega DNA IQ™ System. Real-time PCR quantification was performed on the extracted DNA using the Quantifiler™ Trio DNA Quantification Kit to determine the quantity of trace DNA in each sample. The resulting data was statistically analyzed. A negative relationship was observed between small and large autosomal DNA quantity and physical activity, indicating that as physical activity increased, the amount of DNA recovered for each sample decreased. The results obtained through linear regression analysis showed that a small percentage of the variance in DNA quantity can be explained by either physical activity or participants. Additionally, the data also showed that it was likely that there was a statistically significant relationship between physical activity or participant and trace DNA quantity. Key findings, experiment limitations, and implications for future research will be discussed.

References

1. Burrill J. et al (2019) A review of trace "Touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics*. 39:8-18.
2. Daly D.J, Murphy C, McDermott S.D. (2012) The transfer of touch DNA from hands to glass, fabric, and wood. *Forensic Science International: Genetics*. 06: 41-46.
3. Meakin G. E., et al. (2017) Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives. *Forensic Science International: Genetics*. 29: 38-47.

Northeastern Association of Forensic Scientists

Proceedings of the

October 2025 Annual Meeting

Poster Session Abstracts

P1. Shotgun Pellets Effect of Shot Size and Shape on Pellet Dispersion Jason Schoenfeld, John Jay College of Criminal Justice

Three sets of shotgun shells from different manufacturers and shot sizes had their pellets removed. Three Remington 16 gauge shot size 6 shells, 3 Winchester 12 gauge shot size 8 shells, and 3 Winchester 16 gauge shot size 6 shells were observed. The shells' pellets' respective weights were measured and number of pellets in each counted. 10 sample pellets from each set were separated. Their diameters were measured with a vernier caliper and mass weighed with an analytical balance to compare the weight and size. The pellets observed from each shell varied in diameter and weight by a noticeable amount. The pellets were observed with a visual examination and with the aid of a stereomicroscope to find aspherical pellets. The aspherical pellets diameter was measured and mass measured individually with a vernier caliper and analytical balance. This information was compared to preexisting research on pellet dispersion to approximate how the measured factors here might affect pellet dispersion. The research concludes that shot size and aspherical pellets do affect pellet dispersion. Consequently, these factors could alter the way the dispersion pattern is interpreted when determining firing distance.

P2. Validation of Cadre Top-Match 3D Scanners for use in Ballistic Evidence Triage of Fired Cartridge Cases Stephanie Marino; Nicole Homburger; Meghan Davis; Elizabeth Hanley; Jason Berger, New York City Police Department – Firearms Analysis Section

The New York City Police Department Police Laboratory recently acquired (5) Cadre TopMatch-3D High Capacity Scanners for use in Ballistic Evidence Triage of fired cartridge cases. Traditionally, Ballistic Evidence Triage is performed by examining fired cartridge cases collected at a crime scene using a stereomicroscope, or stereomicroscope based triage (SBT). These cartridge cases are grouped based on shared characteristics, and then a representative sample from each group is uploaded into the NIBIN database. A validation study was conducted to determine the suitability of the Cadre algorithm for algorithm based triage (ABT).

The Cadre TopMatch Scanners provide high definition 3-D scans of the breech face, aperture shearing, and firing pin impression of fired cartridge cases. The Cadre algorithm can then be used to compare the breech face and aperture shearing of scanned cartridge cases and group them based on shared characteristics. For this validation, 44 samples sets were examined consisting of 15 repurposed microscopic examination proficiency tests and 29 sets generated from 57 different firearms from the laboratory's reference collection.

Among four trained examiners, 3,528 triage comparisons were conducted using both SBT and ABT for a total of 7,056 comparisons. There was a SBT false linkage rate of 8.93% and a false non-linkage rate of 3.09%. ABT gave a false linkage rate of 0% to 1.56% and a false non-linkage rate of 2.64% to 8.72%, depending on the parameters set. The results of this study indicated that ABT methods had a specificity from a range of 97% to 100%, depending on the parameters set. Comparatively, specificity was 84% using SBT. These findings suggest that algorithm-based triage utilizing the Cadre 3D scanners is the best practice for conducting Ballistic Evidence Triage analysis.

P3. Comparisons of Terpene Compositions in Cannabis Flowers Between Solvent Extraction and Headspace-Solid Phase Microextraction Dr. Jamie Kim; Grace Poleto; Kaitlyn Ignaszak; Hannah Begner, Department of Chemistry-Buffalo State University, SUNY

The increasing legalization of recreational cannabis across the United States has led to the establishment of cultivators, processors, and dispensaries offering a wide variety of cannabis products. In response, federal and state agencies have enacted policies and regulations mandating laboratory testing of commercial cannabis products. These tests include the quantification of specific cannabinoids, terpene profiling, and the detection of residual pesticides and heavy metals. Terpenes are volatile organic compounds found in cannabis and other plants that contribute to the plant's aroma and play a role in its growth cycle. In recent years, terpenes have gained attention for their potential to interact with cannabinoid receptors through the "entourage effect," in which the combined action of terpenes and cannabinoids may enhance certain pharmacological effects.

This research project focuses on the identification and quantification of terpenes in commercially available cannabis flower strains using gas chromatography–flame ionization detection (GC-FID) and gas chromatography–mass spectrometry (GC-MS). Two extraction methods were employed: liquid extraction using organic solvents and headspace-solid phase microextraction (HS-SPME). In addition, the effects of extraction solvents and extraction time on terpene concentrations for liquid extraction and extraction time and temperature for HS-SPME are presented.

P4. Comparing the Efficacy of DNA Analyses with Kastle-Meyer and Leucomalachite Green Presumptive Blood Tests Using DNA Protective Buffers as Swabbing Solutions Jacqueline Mattox; Kara Jaremko; Deborah Silva, Chemistry Department-Hofstra University; Georgiana Gibson-Daw, Department of Arts and Sciences-Western New England University

Blood identification at crime scenes is an essential process to bolster police investigations and guide forensic processing. Two commonly used presumptive tests for blood detection are Kastle-Meyer (KM) and Leucomalachite Green (LMG). When collecting blood samples, two swabs are typically used; one swab is used for presumptive testing while the other is used for DNA analyses, as the presumptive tests make blood samples no longer suitable for further analysis. The use of two separate swabs can lead to dilemmas when there is a minute quantity of blood at a crime scene. In this scenario, crime scene personnel are faced with two options: perform a presumptive test on the sample in order to confirm the presence of blood or collect the sample for DNA analysis and hope that a feasible human DNA profile is generated.

This study aims to assess the use of a modified sample collection method in obtaining blood samples eligible for presumptive testing and DNA analyses on the same swab. To investigate this method, swabs were moistened with a DNA protective buffer (Tris-EDTA buffer, Tris-acetate-EDTA buffer, Monarch DNA/RNA Protector, or DNA/RNA Shield) prior to sample collection. The KM or LMG test was performed and then samples underwent DNA extraction, quantification, and STR analysis. Another variable tested was duration of storage. Samples were kept in the freezer after performing the modified presumptive testing and prior to DNA processing.

Based on the results, the combination of using TE buffer to moisten the swab and testing with Kastle-Meyer retained higher quantities of DNA with a lower index of degradation when compared to the other options. Additionally, freezing the samples and extracting after one week did not affect the DNA yield, yielding sufficient amounts to produce complete STR profiles. Results also showed that degradation levels were much higher after testing with Leucomalachite Green. However, the use of TAE buffer proved to be more efficient in protecting the DNA, generating less degraded samples which is crucial for STR analysis. Results suggest that using a DNA protective buffer instead of water could be an effective tool to collect bloodstain evidence for presumptive testing and the generation of a usable profile from a singular swab. The successful implementation of this method would allow both presumptive testing and DNA analyses to be carried out for cases where only a limited quantity of blood sample is available at the crime scene.

P5. Optimized Methods for Obtaining DNA from Used Contact Lenses Malena Chacon; Katherinne O'Connor; Kara Jaremko; Deborah Silva, Chemistry Department-Hofstra University; Georgiana Gibson-Daw, Department of Arts and Sciences-Western New England University

In many cases, DNA samples are collected from areas where a donor contacted an object in their environment and transferred biological material. One such object would be a person's contact lenses, which could provide a clean singular sample of the contacts' owner's DNA. The purpose of this experiment therefore is to optimize extraction of nuclear DNA from used contact lenses taking into consideration different collection methods, various periods of time between collection and extraction, and incubation in different solutions. A volunteer wore contact lenses for approximately 12-14 hours, then placed the lenses in a mock crime scene for 24 hours. The lenses were then collected in a sterile manner and processed in various solutions and incubation times, followed by analysis of DNA quantity, quality and STR profiles. The results showed that DNA extraction was optimal from contact lenses stored in centrifuge tubes for 24 hours when the buffers used for incubation prior to extraction were either ATL or ATL/TE. These samples produced sufficient DNA yields to generate full STR profiles. However, samples that had long storage time and/or were stored in paper envelopes produced less DNA quantity, impacting the downstream analysis of STRs. This data provides guidance as to optimal extraction methods for contact lens material that have been left in storage or at a scene, adding this type of sample to the list of evidence that can be collected from crime scenes and provide suitable DNA profiles for the investigation.

P6. Artifact Formation in Methamphetamine in Seized Drug Evidence Analyzed by GC-MS Adam Badinger; Katherine Harkins, NMS Labs

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful instrument in the identification of controlled substances and is the gold standard in the field of forensic chemistry. However, the GC-MS system is susceptible to some limitations such as possible artifact formation; primarily through thermal degradation in the hot injection port when analyzing high concentration samples. In casework, compounds have been observed to appear at trace levels where a relatively large amount of a structurally related compound is present. For methamphetamine, one of the more common drugs of abuse, this artifact formation is frequently observed. Common potential artifacts of Methamphetamine include Amphetamine, Mephentermine, N,N-Dimethylamphetamine (DMA), and N-ethyl-2-phenylethanamine. As some of these compounds have the

potential to be higher scheduled substances, stringent evaluation of these possible artifact scenarios is crucial to ensure there are no misidentifications or false confirmations. Solid and liquid methamphetamine reference standards as well as several case samples were used to evaluate the possible formation of these artifacts during GC-MS analysis. Methamphetamine use does not seem to be slowing down on the drug market any time soon, so it remains vastly important to understand these limitations to ensure accurate results are being provided for legal considerations. This poster aims to expound upon the formation of possible methamphetamine artifacts and when they are most likely to be observed in case samples.

P7. The Effects of Freshwater on DNA Recovery Over Time in Sus scrofa domesticus Femurs Arden Massoia; Ashley Morgan, Ph.D., University of New Haven

Recovering DNA from submerged skeletal remains for the positive identification of individuals is a challenging task further hindered by the absence of current research. Factors like water temperature and depth as well as the presence of coverings on the victim can impact the rate of decomposition which in turn can impact the degree of DNA degradation. The currently available literature suffers from a lack of studies that examine the degree to which the quantity of DNA in submerged skeletal remains changes over time, but a few studies have indicated that the first week is the most successful for recovering DNA. This study examined how DNA quantities change over time in juvenile *Sus scrofa domesticus* femurs submerged in fresh water. Porcine femurs were obtained from a local butcher and defleshed using a combination of maceration and a scalpel. Ten femurs were added to a 20-gallon aquarium tank with aquarium sand substrate on the bottom of the tank and deionized water to represent a freshwater environment. One femur was recovered from the water every 24 hours for one week, then weekly up to one month. Samples were also collected from a dry control bone in concurrence with sampling of the wet bones. Once bones were removed from the water and dried, bone fragments were collected with a Dremel tool and subsequently cleaned and powdered. Genomic DNA was then recovered from the powdered samples by solid-phase extraction through the use of magnetic silica beads (PrepFiler™ BTA Forensic DNA Extraction Kit) and quantified using the QuantStudio™ 5 Real-Time PCR System. Half of the samples underwent the vendor-specified extraction protocol while the other half underwent a modified protocol that substituted Proteinase K with an equivalent volume of dithiothreitol (DTT). This presentation will discuss the differences in DNA recovery between the protocols and among the various time points. These findings will contribute to the body of knowledge that informs DNA extraction procedures and methods used by the teams tasked with identifying victims of criminal activity and natural disasters from submerged skeletal remains.

P8. Optimizing Solvent Evaporation with the N-EVAP Nitrogen Evaporator Emma Briggs; Boston University

Sample preparation is a critical step in many analytical workflows, as the quality of subsequent instrumental analysis depends heavily on the effectiveness of this step. Solvent evaporation by nitrogen drying is a common laboratory technique, yet limited data exists on how experimental parameters influence evaporation performance. This study evaluated evaporation rates of various solvents using an N-EVAP nitrogen evaporator (Organomation®, Berlin, Massachusetts) under different conditions. Trials were conducted at ambient temperature and at elevated temperatures using a water bath to assess the effect of heating. The

elevated temperature trials were conducted slightly below each solvent's respective boiling point. Additional experiments examined solvent mixtures commonly used in laboratory settings, the influence of tube type and size (test tubes and microcentrifuge tubes), and the performance of different needle configurations (standard, bent, and pseudo-spiral) across varying flow rates, temperatures, and needle gauges. Results demonstrated that heating substantially increased evaporation rates for all solvents, though the degree of improvement varied. Furthermore, it was observed that even a small increase in bath temperature provided a notable increase in evaporation rate. Following the introduction of bent needle designs, higher nitrogen flow rates were achieved without causing splashing of the sample inside the tube, slightly improving evaporation under ambient conditions but resulting in minimal effect when heat was applied. Additionally, larger tube sizes exhibited significantly increased evaporation rates under heated conditions, compared to smaller tubes. Overall, these findings indicated that controlled, elevated heating and optimized gas delivery improved evaporation efficiency, resulting in reduced sample preparation time and increased laboratory throughput. The ability to modify these parameters to fit specific sample preparation needs makes this applicable across a wide range of laboratory settings.

P9. Evidentiary Evaluation of Single Cells with EESCIt Julianna Jimenez, B.S., Masters Program in Forensic Sciences; Qhawe Bhembe, Ph.D., Center for Computational and Integrative Biology; Desmund S. Lun, Ph.D., Center for Computational and Integrative Biology & Department of Computer Science; Catherine M. Grgicak, Ph.D. Masters Program in Forensic Sciences, Center for Computational and Integrative Biology & Department of Chemistry, Rutgers University – Camden

Determining the number of contributors (NoC) remains a critical step when interpreting electropherograms (EPGs) from forensic evidence. This becomes more difficult to do accurately as NoC increases since allele dropout (due to degradation), allele sharing/overlap or masking of minor contributors by major contributors serve to obfuscate signal from target contributors, often leading to LRs that cannot express discrimination between (non) contribution hypotheses.

Single-cell methods offer a reliable and robust alternative. Here a set of cells is isolated, and the DNA and amplification occurs in the same vessel to which the cell was added. This results in an electropherogram for each cell isolated, and we call each a single-cell electropherogram (scEPG). With the use of EESCItTM (Evidentiary Evaluation of Single Cells), scEPGs can be grouped into clusters based on their similarity to one another other, without referencing a Person of Interest (PoI). With the assumption that each cluster represents a single donor, not only can the total NoC of an admixture be determined, but so can each donor's LR, regardless of how many donors there were.

Building on previous work, we evaluated the performance of a version of EESCItTM that considers extreme stuttering events – a known phenomenon in single-cell analysis. Using 336 admixtures composed of 17 to 75 scEPGs and ranging from two to five contributors, we run each in a model that does not consider rare-extreme stuttering and one that does. Given these admixtures were made to span the variety of mixtures that might be encountered in casework, we determined the proportion of admixtures that provided the correct versus incorrect number of clusters, and found that when EESCItTM models considered extreme signal events, improvements were observed. Specifically, this novel EESCIt only over-clustered in two of the 336 admixtures

(0.6%), and under-clustered in one admixture (0.3%). Notably, the two over-clusters occurred in admixtures containing multiple minor contributors and one major contributor, with clusters containing as few as two cells. This was an improvement from previous work where 16 over-clusters (5%) were reported, occurring in admixtures containing multiple major and minor contributors.

With improved clustering outcomes, we then evaluated the logLR performance by testing the scEPGs against the true contributor(s), H1-true, and a false contributor, H2-true. A positive logLR value is (usually) expected when testing against H1-true, and a negative logLR value when testing against H2-true. Of the 1,234 clusters tested with a true donor, only one cluster (0.08%) was assigned a negative logLR. Notably, this logLR was associated with cells containing low signals, and therefore, poor EPG quality. Of the 336 H2-true tests, none were incorrectly assigned a positive logLR, demonstrating a very high discriminatory power between true and false contributors.

In conclusion, the improvements made to EESCItTM reflect enhanced accuracy and reduced error rates in NoC determination. This translates to high discriminatory power between H1-true and H2-true test, therein demonstrating that the software is robust, accurate, and reliable in interpretation of single cell data including complex, multi-contributor forensic samples while being unaffected by the qualities – i.e., complexity – of the mixtures, themselves.

P10. Determining the Effects of Thermal Decay of Pig Femur Bone Composition through DNA Extraction and ATR FT-IR Spectroscopy Megan Jenkins; Ashley Morgan, Ph.D., University of New Haven

Skeletal remains are often some of, if not the only, evidence left behind in arson cases and thus become incredibly important in investigations. However, as temperatures rise, so do the detrimental effects on the affected bones, making analysis of these samples increasingly difficult. The aim of this study was to expand current knowledge of ATR FT-IR spectroscopy and DNA extraction in such cases and provide previously lacking knowledge on the temperatures at which sufficient analysis can be adequately performed. ATR FT-IR spectroscopy measures a sample's absorption of IR light and provides an understanding of its composition. DNA extraction provides similar information when amplified through PCR quantitation to give an idea of the amount of genetic material present in a sample. It was hypothesized that as temperatures at which bone samples were burned increase, a decrease in the CO/P ratio determined from ATR FT-IR peak intensities as well as a decrease in the quantity of DNA recovered through PCR. Four pig femur bones, each cut into three cross sections, were utilized in this experimental study. Three porcine femur bones were burned at three varying temperatures, with cross-sectional samples from a fourth serving as unburnt controls. Powdered bone samples were collected from each cross section and analyzed through ATR FT-IR spectroscopy and DNA extraction with PCR quantitation. This presentation will discuss the observed effects of thermal exposure on ATR FT-IR spectra and DNA extraction, along with discovered trends and relationships between the two methods. This presentation will also discuss this study's limitations and possible research moving forward.

P11. Beneath the Surface: Effects of Salt Water on Firearms and Ammunition Heather Dover, Ocean County Sheriff's Office

A stolen firearm, that was loaded, was recovered in salt water and submitted to the Forensic Laboratory for identification and operability. Once the firearm was deemed operable, an experiment was done with the live rounds and the results were not what we expected.

P12. The Impact of Temperature, Humidity, Light Exposure, Location, and Human Activity on Latent Fingerprint Persistence: A 2D and 3D Morphometric Examination Emily Kenoyer; Josep De Alcaraz-Fossoul, Ph.D., University of New Haven

Latent fingerprints (LFs) are among the most common recovered form of evidence at crime scene. Because friction ridge patterns (minutiae) are unique to each individual, they have long served as a means for identifying suspects and linking evidence [1]. The initial step in LF analysis involves the detection and visualization of ridges, for which a variety of methods can be utilized depending on the environment and the substrate on which they are imprinted. Once deposited, the topography of LFs begin to degrade and ridge clarity progressively diminishes. This process is driven by multiple factors, including environmental (e.g. temperature, relative humidity, and light exposure), substrate properties (e.g. porosity and roughness), and donor conditions (e.g. biological sex and age) which may influence the initial status of an impression, its evolution, and visualization [2].

In this study, LFs from the thumb and index fingers of the right and left hands were collected from six donors and deposited onto glass microscope slides. These were placed and aged under six distinct conditions to assess the effects of varying temperature, relative humidity, light exposure, location, and human activity, categorized as: low human activity with no light; low human activity with light; high human activity with no light; high human activity with light; outdoor with no light; and outdoor with light. LFs were examined after 48 hours, 4 days, and 30 days. Two distinct methods for evaluating ridge degradation were applied: a traditional 2D-imaging with powdering, photographing, image standardization, and processing with the Universal Latent Workstation (ULW). This computer software generated ridge clarity maps with a color scale, where blue indicated the clearest regions, followed by green, yellow, and red. For this experiment, the blue and green regions were combined to depict areas suitable for identification, referred to as the BlueGreen (BG) metric. In addition, an optical profilometer (OP) captured high-resolution 3D images of the unprocessed LFs by measuring surface roughness, including data on average ridge height (*Sa* metric) and volume (*Vu* metric) [3]. This dual-modality approach would provide a comprehensive assessment of how the factors of interest affected LF degradation.

Preliminary statistical results indicate that temperature played a significant role in LF integrity over time, particularly when comparing outdoor and indoor settings. Outdoor samples experienced a broader temperature range (−0.1 °C to 48.9 °C; mode 16 °C), whereas those indoors remained within a narrower window (18–26 °C; mode ~22 °C). Interestingly, LFs outdoors experienced less degradation. Indirect light exposure also had an effect, as all three locations (low and high traffic indoors as well as outdoors) revealed less degradation when LFs were protected from light. In contrast, humidity and human activity produced minimal effects. Regarding the analytical methods, 3D metrics detected topographical changes as early as 4

days post-deposition, whereas 2D metrics revealed minimal differences for up to 30 days. Collectively, these results demonstrate that temperature and light exposure are primary drivers of LF degradation, and that combining 2D and 3D metrics provides a more comprehensive assessment of ridge degradation over time.

P13. Interpretation and Validation of Single and Mixed Sperm Cell Electropherograms with EESCIt™ Anu Khandelwal, Graduate Program of Forensic Science; Qhawe Bhembe, Ph.D., Center for Computational and Integrative Biology; Desmund S. Lun, Ph.D., Center for Computational and Integrative Biology & Department of Computer Science; Catherine M. Grgicak, Ph.D. Graduate Program of Forensic Sciences, Center for Computational and Integrative Biology & Department of Chemistry, Rutgers University – Camden

Classical, bulk DNA extraction methods extract DNA while the cells are still mixed. In so doing, classical data generating techniques prohibit DNA reports about sperm and, thus, only speak to, who did (not) contribute to the DNA. Single-cell pipelines address this gap by imaging or tagging each cell, isolating them into their own vessels, and applying direct-to-PCR techniques. In this way, we have an association between what DNA was born of what cell, and, thus, cell type. In prior work, evidentiary evaluation of single diploid cells regularly gave informative and robust forensic evaluations for such evidence. We extend this prior work to (haploid) sperm, and query that models addressing haploid realities conform to WoE (i.e., logLR) properties.

To do this, we generated single-cell electropherograms (scEPGs) from 121 sperm cells from single source precursors. We then tested a candidate haploid model by evaluating each of the 121 scEPGs with one true and one false donor, and confirmed that the WoE do not too strongly point in the direction of the counter hypothesis. We found that no scEPGs gave WoE pointing in the wrong direction. For this dataset, the largest WoE for any single haploid was 10 when H_1 was true. When we examined the WoE at the locus level, we found that the WoE properties hold. With this, we conclude that the candidate model well describes haploid data.

Going further, we took the 121 scEPGs and created 33 admixtures with varying contributor ratios ranging from 17-75 scEPGs and 2-5 donors, and used the same performance metrics to confirm that the models work well when in the presence of more than one scEPG and more than one donor.

We found that the haploid-aware model not only faithfully clustered scEPGs into their own donor group, therein giving good estimations about the number of contributors and how many scEPGs were donated by each contributor, but gave WoE that comport with WoE properties.

It is for this reason the haploid-aware model was implemented into EESCIt™, making it the first automated end-to-end system able to legitimately and credibly address questions about: i) the number of sperm contributors; ii) what number of sperm each contributor donated; and iii) who contributed to the mixture of sperm isolated.

P14. Installation and Implementation of the Global Uniform Analysis and Reporting of Drug-related Substances (GUARDS) Method for GC-MS Mikaela Romanelli, Drug Enforcement Administration- Northeast Laboratory

With the landscape of the illicit drug market changing to include an increase of synthetic emerging drugs, innovative comprehensive and reliable analytical methodology is needed to fully capture the complexity of current polydrug samples and aid in the analysis and identification of these newly emerging substances. Differences in federal, state, and local laws impact how seized drug laboratories analyze and report chemical substances. For example, results from CY23 DEA seizures in a New England state show 26% of fentanyl exhibits analyzed contained xylazine. Whereas the National Forensic Laboratory Information System (NFLIS) shows 0% for the same state in CY23. These types of reporting inconsistencies among the various agencies impact law enforcement and public health responses to emerging drug threats, like xylazine.

DEA's Global Uniform Analysis and Reporting of Drug-Related Substances (GUARDS) gas-chromatography-mass spectrometry (GC-MS) analysis method is addressing these challenges and increasing the validity and reliability of forensic results, while simultaneously generating more comprehensive information. DEA's GUARDS method provides a fast, efficient, cost-effective, and uniform GC-MS method of analysis and reporting for the seized drug community. GUARDS provides a standardized method to separate as many compounds as possible (including isomer) to share with the forensic seized-drug community and assist in standardized identification and reporting of compounds in seized drug samples.

As the GUARDS GC-MS methodology is adopted, creating the foundation for an enhanced DEA-sponsored Early Warning System, advanced data analytics modeling opportunities will emerge. In addition, GUARDS supports the TRANQ (Testing, Rapid Analysis, and Narcotic Quality) Research Act of 2023, which requires the National Institute of Standards and Technology (NIST) to support research and other activities related to identifying xylazine, novel synthetic opioids, and other new psychoactive substances.

In this poster, the impact of inconsistent drug analysis reporting has to public health and public trust will be discussed. Information will be provided to aid laboratories transitioning to hydrogen carrier gas and GUARDS methodology. In addition, the value and implementation of the GUARDS innovative methodology in 200+ instruments in DEA labs will be presented, as well as the efforts of other labs, such as U.S. Customs and Border Protection (CBP). Furthermore, new initiatives to increase consistency of reported data to NFLIS will be presented.

P15. The Effect of Skin Tone on the Fluorescence of Biological Fluids Kristyann Haislup; Ralph R. Ristenbatt III, M.S.; Carol Ritter, M.S.; Lawrence Quarino, Ph.D., Cedar Crest College

Forensic nurses frequently employ Alternate Light Sources (ALS) to detect biological fluids—such as semen, urine, and saliva—on the skin of sexual assault victims. ALS enhances the visibility of serological fluids that may otherwise be difficult to detect with the naked eye. However, skin tone may influence which wavelengths of light produce optimal fluorescence. This study sought to determine and quantify the lighting conditions most effective for detecting biological stains across varying skin tones.

Sixteen volunteers spanning the range of skin tones identified in the Fitzpatrick Scale participated in the study. Semen, urine, and saliva samples were self-collected or obtained from a known source early in the morning on the day of examination. Fluids were deposited onto the skin, allowed to dry, and then photographed with the Crime-lite® AUTO (foster + freeman®), utilizing multiple excitation wavelengths (365–640 nm) and emission filters (365–780 nm).

To assess persistence of fluorescence after washing, a bathing simulation was performed in which participants rinsed their arm under running water for one minute with light pressure applied five times for five seconds. Following drying, the same areas were re-imaged under optimal ALS conditions. Image analysis was conducted using Fiji (ImageJ) to quantify fluorescence values, while the Nix QC Color Sensor was employed to obtain RGB values for objective characterization of skin tones of volunteers.

Findings identified the optimal excitation and emission wavelength combinations for each body fluid across different skin tone categories, providing valuable insight into how ALS can be used more effectively in diverse forensic populations.

P16. The Effects of Fingerprint Development Techniques on Forensic Cartridge Case Identifications Sasha Valentino M.S.; Missy Meredith, M.S., Forensic Exploitation Department, Department of Army CID; Sarah Varhola, M.S., Identification Division, Ohio Bureau of Criminal Investigation; Stephanie J. Wetzel, Ph.D., Department of Chemistry and Biochemistry- Duquesne University; Lyndsie Ferrara, Ph.D., Forensic Science and Law Program-Duquesne University

When a fired cartridge case comes into a forensic science laboratory there are various pathways it can go through to be analyzed. The more frequent process of analysis is done by the fingerprint section followed by the firearm section, but some laboratories will analyze them in the opposite way or will not attempt to develop fingerprints. There is no standard that exists among all forensic science laboratories as to which section should analyze the evidence first, if at all. The purpose of this study is to determine what effects certain fingerprint development techniques may have on cartridge cases and if these techniques impact cartridge case comparisons. Brass, steel and aluminum cartridges in a 9mm and .45 caliber were fired using a Taurus 708 and Remington 1911 respectively. The cartridges were processed using cyanoacrylate fuming, gun bluing, basic yellow 40, black powder and a sequence of these techniques. Microscopic markings left on the breech face of the cartridge cases from the firing process were compared using the comparison microscope both before and after processing with each development method and a sequence of them. Markings on the cartridge cases were identified following development with cyanoacrylate fuming, basic yellow 40 and black powder while some markings on the cartridge cases did not contain enough detail for an identification to be made. Cartridge cases that came in contact with gun blue were not able to be identified prior to cleaning. A new set of cartridge cases that were processed using gun blue were cleaned using acetone, an alcohol wipe and soapy water to remove the gun blue that accumulated on the headstamp. In general, performing a cleaning step of either acetone, alcohol wipes or soapy water has proven to be successful. Soapy water proved to be the optimal cleaning technique with the majority of those cases for all of the metal types being identified when compared to the alcohol wipe and acetone. Overall, the majority of fingerprint techniques tested were still able to be

identified. Gun blue was the only one to give problems, but in most issues that was overcome using a cleaning method.

P17. Improving Area of Origin Calculations for Impact Spatter on Rough Surfaces Autumn Reynolds; Carol Ritter, Cedar Crest College; Paul V. Quinn Sr., Cedar Crest College and Kutztown University
Impact spatter bloodstain patterns play a critical role in crime scene reconstruction by helping investigators determine the area of origin of a bloodletting event. Current models for calculating the area of origin rely on stain length and width measurements but assume smooth, non-porous surfaces. In practice, most crime scene surfaces exhibit varying degrees of roughness and absorption, introducing potential errors in origin calculations.

Building on previous research using blood substitutes and animal blood, this study investigates the effect of surface roughness on stain morphology and area of origin calculations using human blood preserved in ACD solution under controlled conditions. Blood was projected onto sandpapers coated with varying grit values coated with polyurethane to simulate surface roughness. Stain measurements were analyzed using ImageJ and processed with established area of origin equations.

Results demonstrate systematic deviations in calculated origins on rougher surfaces, confirming that current models do not fully account for surface interactions. A friction coefficient was derived as an adjustment factor to improve the accuracy of calculations on rough surfaces. This empirical model provides a more realistic representation of blood-surface interactions, leading to more reliable conclusions when analyzing impact spatter patterns in real-world crime scenes.

P18. InnoXtract vs PrepFiler BTA: Extracting DNA from Burned Skeletal Remains Kaitlin Brown; Ashley Morgan, Ph.D., University of New Haven

In cases involving arson or mass casualty incidents, DNA may be exposed to high temperatures undergoing structural alterations and denaturation the longer the heat exposure lasts. In such incidences, there are often very few physically identifiable characteristics of remains and therefore DNA analysis is required to determine the identity of the remains. When DNA is denatured due to thermal alterations, it is often difficult to produce a genetic profile to identify the source of unknown remains. The difficulty comes from the DNA becoming degraded and the risk of contamination. Extraction kits have been developed to specialize in the extraction of DNA from skeletal remains. These extraction kits are frequently designed to work specifically with the degraded fragmented DNA often found in remains exposed to high temperatures and remove contaminants and inhibitors commonly found in bone samples. This project looked at the efficiencies of two different DNA extraction kits, the PrepFiler BTA kit and the InnoXtract Bone kit, to investigate their efficiencies at extracting DNA from thermally altered samples. The first kit used, PrepFiler BTA is a DNA extraction kit designed for use with bone, teeth or adhesive samples and uses silica-coated magnetic beads to extract DNA from powdered bone. The second kit used, InnoXtract Bone kit is a specialized kit for bone samples that uses a similar silica-coated magnetic bead method for DNA extraction. In this study, porcine femurs were used as a human model and given one of three treatments: unburned, burned at 200°C for 15 minutes, or burned at 200°C for 30 minutes. Cuttings of each bone's cross-section were taken and powdered.

Bone powder was extracted with each kit, and all samples were quantified by real-time PCR quantitation using a pig specific primer set. This presentation will discuss differences in DNA recovery due to length of thermal exposure. A comparison of the extraction methods utilized will be discussed and the limitations of this study will be examined.

P19. Influence of Blood on the Persistence of Gunshot Residue Patterns on Frabrics Wendy Bautista B.S.; Carol Ritter, M.S.; Lawrence Quarino, Ph.D.; Paul V. Quinn Sr., Ph.D., Cedar Crest College; Peter Diaczuk, Ph.D., John Jay College of Criminal Justice

Gunshot residue (GSR) patterns on clothing are utilized to determine distance of shooter to victim in cases involving shooting reconstructions. Typically, the analysis of GSR patterns involves using IR cameras and/or chemically enhancing to visualize the GSR pattern on clothing, documenting the general distribution/measuring the diameter of the GSR pattern, and comparing to reference samples created with the same ammunition/firearm. Although this is a very common type of analysis, there remains a significant gap in the literature concerning the interaction between gunshot residue and biological fluids, particularly blood, and how such interactions may influence the persistence and distribution of gunshot residues and patterns. In this study, a total of 60 GSR patterns were analyzed prior to and after the addition of whole blood, applied both vertically and horizontally to mimic position of the gunshot injury, to better understand the effects of blood on GSR patterns. Gunshot residue was created at an indoor firing range (climate controlled, no measurable airflow) using a Ruger P95 9mm Luger handgun and two types of 9mm, 115 grain full metal jacket ammunition, Winchester (ball type powder) and Magtech (flattened ball type powder), positioned 12 inches from three fabric substrates (100% Cotton, 100% Polyester and 65% Polyester/35% Cotton blend). Each GSR pattern was individually packaged flat in cardboard boxes for transport, photographed with white and infrared light using the Crime-Lite Auto (Foster & Freeman), and analyzed using Image J software to obtain particle counts and diameter measurements. 20mL of fresh whole sheep's blood (ACD preservative) was applied to the rear of each GSR pattern to mimic bleeding from a gunshot injury. Thirty samples were positioned vertically, and thirty samples were positioned horizontally, each pattern was photographed with the Crime-Lite Auto at 5min, 24 hours, and 7 days after blood application and analyzed with Image J. Images pre- and post- application of blood were compared and statistics were used to understand the effects of blood on GSR patterns. This study will provide an understanding of the influence of blood on the persistence of GSR particles and effects on the overall GSR pattern when blood and GSR interact on fabric substrates following a blood shedding gunshot injury.

P20. Evaluating the Accuracy of Bloodstain Pattern Analysis (BPA) on Power-Like Surfaces Kaleigh Adams.; Jillian Tite, Cedar Crest College

Bloodstain Pattern Analysis (BPA) is a common forensic tool used to reconstruct crime scenes involving bloodshed. However, most BPA methods have only been validated on smooth, non-porous surfaces, despite the fact that many violent crimes occur outdoors on absorbent, irregular surfaces. This study addresses a major research gap by testing the accuracy and reproducibility of standard BPA techniques on snow-like substrates.

Using sheep's blood, droplets were deposited from a fixed height and known angle onto the surface of a rough, porous powder. A standard pipette ensured consistent droplet size, and each impact was recorded with a high-speed camera. Measurements were taken immediately after impact and again after a set time to account for absorption. Patterns were then analyzed using established BPA equations to assess directionality, angle of impact, and classification. Upon preliminary analysis of the collected data, there is a significant decrease in the diameter of the blood drops observed on the rough, absorbent surface when compared to a nonabsorbent surface such as tile. This noticeable reduction in size suggests that absorbent materials interfere with the natural spread of the bloodstain, leading to potentially misleading measurements. By applying the angle of impact equation to the absorbed droplets and calculating the percent variation between surfaces, the data consistently supports this conclusion.

These findings highlight an important concern: the reliability of bloodstain pattern analysis is notably affected by the nature of the surface onto which the blood is deposited. As such, this underscores a pressing need for further research and development in this area of forensic science. Any conclusions drawn from bloodstains found in environments with rough, absorbent surfaces should be carefully reevaluated and critically challenged to avoid misinterpretation and to enhance the accuracy of forensic reconstructions.

P21. Analysis of Product Removal from Hair Shafts for Forensic Applications Lauren Lozano.; University of the Incarnate Word

The average person loses approximately 100-150 hair strands per day through natural shedding, making it a common source of potential evidence at crime scenes. Hair fibers are an important part of trace evidence in criminal investigations due to their ease of transference and their ability to link suspects, locations, and victims. Due to their composition, they are more resistant to biological and chemical decomposition compared to other biological tissues, allowing hairs to greater withstand harsh conditions encountered at crime scenes. The most commonly requested analysis for hair submitted to a crime laboratory is nuclear DNA analysis, which utilizes solely the hair root. With the exception of microscopic comparison and mitochondrial DNA analysis, the remaining hair shaft has limited use in forensic examinations, which could be overlooking valuable information the shaft might contain. Hair products, such as sprays, waxes, and gels, are commonly applied to the entirety of the hair shaft and could increase the probative value of hair evidence by adding an additional defining feature. In this research, we are using GC-MS to study this potential, aiming to find if the presence of hair products can be detected within a hair sample. Hair shafts were coated with one of four different products and allowed to sit up to 24 hours. To remove the applied product, hairs were soaked in ethanol in a microcentrifuge tube, vortexed, and placed in a heated sonicator. Product extracts in ethanol were then analyzed via GC-MS analysis. For each of the four products used, the pure product was first dissolved in ethanol and ran through GC-MS to give standard spectra for comparison.

P22. Comparing Drug Recognition Expert (DRE) Assessments with Oral Fluid Toxicology Findings Zach Langley.; Amanda L.A. Mohr, M.S., D-ABFT-FT; Barry K. Logan, Ph.D., F-ABFT, Center for Forensic Science Research and Education

Impaired driving investigation is a serious public safety challenge, made more complex by rising

polysubstance use and a rapidly evolving drug landscape. Identifying specific causes of impairment has become increasingly difficult for both law enforcement and toxicologists. To characterize impairment resulting from drugs other than alcohol, the Drug Recognition Expert (DRE) program was created, which has enabled police officers to be better equipped to detect impairment. DREs go through an intensive 3-week classroom-based training, at the conclusion of which they must satisfy a certification test by accurately assessing subjects under the influence of drugs and forming an opinion on what category or categories of drugs the impairment is related to, and their conclusions are verified by toxicology testing.

During certification, oral fluid samples are collected from a community of volunteer subjects in Philadelphia who were regular recreational drug users, using an Immunalysis Quantisal collection device and submitted to the CFSRE for testing. Samples are analyzed using a Sciex® TripleTOF® 5600+ quadrupole time-of-flight mass spectrometer coupled to a Shimadzu® Nexera ultra high-performance liquid chromatograph for basic drugs and emerging substances. Samples were also analyzed for tetrahydrocannabinol (THC) using an Agilent®1200 series liquid chromatograph coupled to an Agilent®6495 tandem mass spectrometer.

To date in 2025, a total of 759 oral fluid samples has been analyzed. The most common drugs findings in the oral fluid were cocaine (79%), medetomidine (77%), and fentanyl (76%). Synthetic cannabinoids were detected slightly more often than delta-9 THC, with 109 cases having synthetic cannabinoids and only 87 having delta-9 THC. With respect to DRE opinions, stimulants and narcotic analgesics were the most frequently called categories by DREs. Toxicology testing identified a narcotic analgesic in 86% of the cases where that category was suspected, while stimulants were correctly identified in 93% of suspected stimulant cases. Opinions involving cannabis and dissociative anesthetics were the least frequently supported by toxicology findings at 35% and 49% of cases, respectively.

With the exceptions of cannabis and dissociative drug categories, the toxicology results showed good agreement with the opinions of the DRE officers. False negatives in the DRE call can be due to tolerance, while false positives in the DRE opinion can be due to overlapping symptoms between multiple categories caused by poly substance use.

The data presented demonstrates strong agreement between DRE opinions and toxicology findings, particularly for stimulants and narcotic analgesics, which were confirmed in the vast majority of cases. Although agreement was lower for cannabis (35%) and dissociative anesthetics (49%), the data suggest that DRE observations still capture important indicators of impairment. These findings support the utility of the DRE assessment as a field tool for identifying, documenting, and assessing drug impairment in the field.

P23. The Effect of Acids on a Fiber's Dye Composition Using HPLC Truc Hoang.; Allison Myers, Ph.D., University of the Incarnate Word

Fiber analysis is a branch of trace evidence that utilizes various techniques, including MSP, TLC, and HPLC, to analyze the color and dye characteristics of a fiber. Additional characteristics can include color, structure, if it was naturally or synthetically produced, and source of origin. In crime labs, forensic scientists utilize fiber analysis to compare an unknown fiber to a known source, which can provide substantial probative evidence

in a case, especially if there is a lack of significant DNA related evidence. For example, the kidnapping of Melissa Brannen in 1991, was traced to the handyman at her apartment complex, where the unique fibers of Melissa's outfit and rabbit hair from her mom's coat were found in the perpetrator's vehicle. This evidence helped lead to the conviction of Caleb Daniel Hughes.

The goal of this experiment was to observe the effects of varying molar concentrations of sulfuric acid, hydrochloric acid, and nitric acid on a fiber's dye composition. HPLC was used to quantitatively analyze the fiber's dye change with hopes to improve upon the more commonly used technique of TLC, which provides very limited quantitative data and cannot account for minor variations in chemical composition or concentration. This experiment was conducted with the intent to mimic acid stains found on a victim and/or perpetrator of an acid attack.

P24. An Internal Validation of STRmix v2.11 in Forensic DNA Analysis: An Application of SWGDAM Guidelines Taylor Buie, B.S..; Ashley Welk, MSFS, Thomas Jefferson University; Coral Smith, MS; Mirna Ghemrawi, Ph.D., The Center for Forensic Science Research and Education

DNA profiling is a cornerstone of forensic science, enabling human identification in criminal cases, missing persons cases, mass disaster investigations, and more. Traditional short tandem repeat (STR) interpretation methods rely on binary inclusion/exclusion criteria informed by analytical and stochastic thresholds. While sufficient for single-source samples, these approaches are limited in cases involving complex mixtures, low-template DNA, or degraded samples where stochastic effects, allele dropout, stutter, and contributor imbalance complicate interpretation. To address these limitations, probabilistic genotyping (PG) software applies statistical models that incorporate peak height data, account for stochastic variation, and generate likelihood ratios (LRs) to quantify the weight of evidence under competing hypotheses.

Continuous PG software such as STRmix™ utilize Bayesian inference to model dropout, drop-in, stutter, and mixture ratios, providing improved accuracy and transparency compared to traditional interpretation methods. Although developmental validation has demonstrated the robustness of STRmix™, internal validation is required by SWGDAM and FBI Quality Assurance Standards (QAS) to ensure reliability under laboratory-specific conditions. This study describes the ongoing internal validation of STRmix™ v2.11 conducted at the Center for Forensic Science Research and Education (CFSRE).

Validation studies were designed to assess sensitivity, precision, reproducibility, and mixture interpretation across defined contributor ratios, low-template samples, and degraded/inhibited DNA. Empirical evaluation of key parameters – including stochastic thresholds, peak height variance, and stutter ratios – supports the establishment of laboratory-specific performance guidelines. This validation examines the performance of STRmix™ using DNA profiles generated with the PowerPlex® Fusion 5C kit. The validation studies performed include assessments of specificity, sensitivity, saturated data, single-source profiles, mixture weights, and the effects of degradation and inhibition. These efforts provide a strong foundation for evaluating STRmix's reliability under varied conditions, while additional sections of the internal validation guidelines as provided by STRmix™ will be validated in future phases to further strengthen the dataset. At the CFSRE, as a teaching facility, integrating STRmix™ into our education program provides researchers and students

with practical exposure to probabilistic genotyping, preparing the next generation of forensic scientists for the responsible application of this software technology.

P25. Comparative Analysis of Microbiome DNA Yields using Automated vs. Manual Extraction Methods from Genital Samples J. The'arra Savage, B.S..; Coral Smith, MS; Mirna Ghemrawi, Ph.D., The Center for Forensic Science Research and Education

Forensic human DNA analysis remains a pivotal component in investigative efforts of sexual assault cases. Although significant advances have been made in STR typing of such sample types, challenges remain when analyzing human DNA, including sample degradation, DNA quantity, and interpretation of complex DNA mixtures. To address these limitations, research has explored the potential to integrate microbial DNA recovery as a complementary forensic tool through characterization of sex-specific differences in microbial profiles.

Although the differences in genital microbial flora between females and males are well documented, male urogenital samples have been less extensively studied due to the low microbial abundance and consequently limited recovery of microbial DNA. It is well established that the method of DNA extraction influences the success of downstream analysis. Manual extraction, though effective, can be time consuming and poses a higher contamination risk. Automated extraction platforms, such as the EZ2 system, allow for high-efficiency extraction with minimal human intervention, which is advantageous when working with challenging sample types such as the male urogenital microbiome. Although advantages of automated extraction systems are well known, limited studies have evaluated automated silica-bead based extraction methods on genital microbiome samples.

This study evaluated manual versus automated (using the EZ2 Connect Fx) extraction protocols of genital microbiome samples using the QIAamp® PowerFecal® Pro DNA kit. Initial testing of male urogenital swabs using manufacturer's protocols for both automated and manual methods did not yield quantifiable results. An optimized protocol was developed to increase recovery of microbial DNA which produced quantifiable DNA from male samples during optimization trials. To further test the optimized protocol, genital swabs were collected from a total of 20 donors (10 biological male, 10 biological female) and processed. A total of four swabs were collected from each donor. Two swabs were processed per extraction for female samples whereas four swabs were processed per extraction for male samples to maximize microbial DNA yield. All swabs underwent individual mechanical lysis using the TissueLyser II. Lysate from all swabs from a single donor was combined and homogenized. The homogenized samples were then evenly aliquoted for DNA extraction using either the automated or manual methods. Total DNA concentrations were quantified using the Qubit dsDNA High Sensitivity Assay and compared to human-specific quantification results obtained with Quantifiler™ Trio kit on the QuantStudio™ 5 Real-Time PCR instrument. Success of each extraction method was assessed through calculation of total DNA concentration versus human-specific DNA concentration. Preliminary analysis indicates comparable DNA recovery from both manual and automated extraction techniques from genital samples. Challenges in extracting quantifiable DNA from low biomass samples such as the penile shaft were overcome by protocol optimization which serves as a step towards utilization of the microbiome for forensic applications.

P26. qPCR-Based Normalization as a Strategy to Improve Sample-to-Sample Read Depth Balance in STR Libraries Produced with CombiSTR® Reverse Complement PCR Eleanor C. Starkey, B.S..; Coral Smith, MS; Mirna Ghemrawi, Ph.D., The Center for Forensic Science Research and Education; Jill van Wolfren, MSc; Joop P.G. Theelen, BASc, NimaGen B.V.

Massively parallel sequencing (MPS) has introduced significant advantages over traditional capillary electrophoresis (CE) for short tandem repeat (STR) typing, including the ability to distinguish isoalleles, improved mixture interpretation, and greater multiplexing capacity. The CombiSTR® assay utilizes reverse complement PCR (RC-PCR) chemistry to co-amplify autosomal and Y-STR loci in a single workflow. RC-PCR represents a recent development in forensic library preparation, allowing adapter and index sequences to be incorporated in one closed-tube reaction. This streamlined approach reduces handling time and the risk of contamination while supporting sequencing efficiency.

A critical component of the MPS workflow is sample normalization, which occurs after library prep and prior to pooling. Imbalanced inputs can lead to over- or under-representation of individual samples in the sequencing run, ultimately impacting data quality and profile recovery. Traditionally, the CombiSTR® protocol has relied on post-extraction DNA input quantities to guide pooling decisions. However, this input-based approach does not account for variability in RC-PCR amplification efficiency, which can differ by sample type and quality. To overcome this limitation, a novel qPCR-based normalization strategy has been introduced to quantify the amount of library available after RC-PCR, with the goal of providing a more accurate and consistent pooling approach.

This study compared input-based pooling with qPCR-based normalization across five experimental studies designed in accordance with SWGDAM recommendations. A total of sixty-two samples were examined in triplicate (n = 186), representing conditions commonly encountered in forensic analysis, including sensitivity dilutions, degraded DNA, inhibitor-challenged extracts, mixtures, and mock case-type evidence. Samples underwent DNA extraction, quantification, RC-PCR amplification, pooling, and cleanup prior to sequencing on the MiSeq FGx system. A pairwise comparison of normalization methods was conducted to assess sequencing balance, read depth distribution, locus recovery, and dropout rates.

Preliminary results indicated that the qPCR-based approach improved consistency in sample-to-sample read depth compared to the input-based method. These findings demonstrate the importance of normalization strategy in MPS workflows and support the potential of qPCR-based pooling to enhance data quality, reliability, and operational efficiency in forensic STR sequencing.

P27. Evaluation of Volatile Compounds During Autolysis in Bovine Hepatic Homogenate Robert H. Powers, Ph.D..; Kaitlyn A. Czifra, Department of Forensic Sciences-University of New Haven

There remains an interest in the potential for volatile compounds that might be detectable in a post-mortem environment to provide useful information with regard to the post-mortem interval (PMI). PMI may be readily divided into two stages, autolysis and putrefaction. Autolysis is a breakdown of cellular membranes and components by endogenous enzymes and non-enzymatic processes, prior to the process of colonization of

the body by bacterial and other invasive organisms, normally initiated by a breach of the GI tract. This post-colonization stage is referred to as "putrefaction."

The pattern of volatile generation during autolysis in a species is presumed to be relatively constant, albeit temperature dependent, and with the degree of genetic or other circumstantial variation being as yet, incompletely explored. In contrast, as a function of the potential variability inherent in the colonizing species, the number of volatile compounds that have been recognized in decompositional samples is quite extensive. An obvious complication associated with experimental observations during putrefaction is that specific compounds identified may be reflective of the biochemistry of the various invasive species, and not necessarily characteristic of the species of the dead body.

We have developed a model system to evaluate the time and temperature-dependance of volatile compounds that may be generated in a hepatic homogenate, in the autolytic post-mortem period. We recognize that this model system would not be expected to be reflective of a "whole body" environmental decompositional scenario. In our system, fresh beef liver is homogenized in DDIW, and subsequently incubated in a temperature-controlled water bath, with aliquots collected at 0, 6, 12 (14), 24 hours post-homogenization. The relatively short incubation period was selected in an effort to mimic autolytic processes, and recognizing that an isothermal decompositional environment is unlikely to be reflective of a "real world" scenarios.

One mL aliquots were collected at 0,6, 14 and 24 hours post-homogenization, and reactions were terminated by the addition of 1 mL 10 mg/mL NaF. Aliquots were vortexed, and reserved at 4o C prior to extraction. Aliquots were extracted by the addition of 1.0 mL EtOAc, and subsequently vortexed, and centrifuged at 2000 x g. for 5 min. The organic supernatant was removed and transferred to autosampler vials, and reserved for GCMS analysis, on a 30 M DB-5 column.

Specific compounds were then identified within 4 categories; (A) Present at all sampling times (Palmitic, Linoleic and Steric Acids); (B) Present at 0 time, but disappearing during the course of incubation (Palmitoleic, Arachidonic and Eicosatrienoic Acids); (C) Not present at 0 time, appearing and subsequently disappearing during the course of incubation (4-Hydroxy Benzene Acetic Acid); and (D) Appearing during the course of incubation and remaining detectable through the final sample time (4-Hydroxybenzaldehyde, Harmane, Norharmane).

Our data suggest the potential for time- and temperature- based evaluation of evolved volatile compounds may provide, in combination with post-mortem environmental conditions, a basis for a chemically-based estimate of PMI.

P28. Determination of Gunshot Residue on Fabric Using Chemical and Photographic Testing
Diana Muñoz Ortiz; Peter Diaczuk, Ph.D., John Jay College of Criminal Justice

In an investigation, forensic scientists use gunshot residue (GSR) to analyze evidence from a crime scene and help investigators piece together events. GSR allows for investigators to estimate the distance between the fired gun and the victim by analyzing the density of gunshot residue around the entry hole. The Modified

Griess Test is a chemical test that is currently used to visualize GSR on various objects such as fabric. The nitrite residue from the fabric is transferred onto treated photographic paper producing visible orange coloration. This coloration helps to determine the size, density, and possible distance of a discharged firearm. Photography can serve as a non-destructive method to visualize GSR. Utilizing several photographic techniques to enhance the contrast between the gunshot residue and the fabric allows for the detection of gunshot residue particles on the fabric. This method of analyzing GSR on the fabric is a quick non-invasive test that requires minimal training compared to using chemical techniques to analyze the fabric. GSR can be visualized by inserting the images of the fabric's entry hole into a software program called ImageJ. ImageJ is a software program that is used to analyze, process, edit, and visualize images by using standard image processing as well as statistical measurements that are selected by the user. Adjusting the image's contrast and hues and programming the settings to count certain particles based on the size and shape, the number of particles around the entry hole can be used to measure the area of gunshot residue. This non-invasive technique allows for forensic scientists to perform a preliminary test without destroying evidence.

P29. Bullet Ricochet and Tunnelling of Wood Substrates Peter Diaczuk, Ph.D.; Abdelrahman Khalifa, MS, John Jay College of Criminal Justice-CUNY

With the overall rise in shooting incidents throughout the country, most notably in urban areas, the capabilities and limitations of current ballistic forensic examinations have become more apparent. An underrepresented, yet increasingly more common occurrence in shooting incidents is the ricochet phenomenon which has led to ricochet-related injuries, with some cases proving fatal. Although there are processes and generally accepted standards for bullet trajectory analysis, ricochet analysis typically requires outside input from an expert within the field. Additionally, current research regarding ricochet on wood surfaces remains less thoroughly investigated than ricochet analysis on other yielding surfaces. The goal of this research was to trigonometrically determine the ricochet angle at corresponding angles of incidence in addition to the critical angles of five wooden substrates. In addition, statistical
analysis and data visualization were performed to analyze the tunneling phenomenon observed in the wood substrates. The data was collected using 9 mm Luger 124-grain Full Metal Jacket PMC Ammunition. The wooden substrates utilized in this research experiment were Whitewood, Southern Yellow Pine, European Spruce, Western Red Cedar, and Douglas Fir. The ricochet angles remained larger than the angles of incidence for the wood substrates except in two instances. Tunneling was exhibited by all five wooden substrates, at or within a small range encompassing the critical angle.

P30. Enhancing Single-Cell Forensic DNA Analysis: Evaluating the Promega PowerPlex® 35GY System for Human Identification Maya Medina, Michael Marciano

New technologies in single-cell genomics are driving significant advancements in the tools available for DNA analysis and the resolution of DNA data. With all of these advances it becomes increasingly important to evaluate and validate these methods for consistency and reliability. One such advancement is the development of the Promega PowerPlex® 35GY System which improves upon the earlier PowerPlex® Fusion 6C Kit by introducing two additional dye channels. In the 6C system, larger autosomal loci are concentrated within six channels, limiting the number of loci that can be analyzed at once. The 35GY system allows for the

analysis of an additional 8 loci. The expanded panel in the PowerPlex® 35GY System increases discriminatory power, improves mixture deconvolution, and is advantageous in single-cell applications where maximizing genetic information from limited input is necessary. This study aims to evaluate the effectiveness of the PowerPlex® 35GY System in single-cell analysis and to compare performance across different single cell collection methods. The single cell samples used in this study were collected using two different collection methods: DEPArray™ single cell collection and SIEVEWELL™ collection. DNA was extracted using the DEPArray™ LysePrep Kit and protocol, amplified with the PowerPlex 35GY using half and full reaction volumes, and analyzed on the Spectrum Compact CE system. Single cell samples were tested and analyzed to compare reproducibility and profile quality. This study, still in progress, has shown that profile completeness averages over 70% (approximately 37/ 53 expected alleles), a preference for SIEVEWELL™ collection of cells and there were no significant observed differences between half and full reactions.

Affiliations of authors: Forensic & National Security Sciences Institute, Syracuse University, Syracuse, New York, US

Northeastern Association of Forensic Scientists
Proceedings of the
October 2025 Annual Meeting

Author Index

The presenting author index can provide a quick reference to find when and in what section presenting authors were scheduled to present at the 2025 Annual Meeting. The reference table below assists you in finding the section in which the presentation was given. Letters correspond to the scientific discipline/section in which the presentation was made while the number corresponds to the page number of the abstract in this booklet.

A Forensic Toxicology
B Trace Evidence/Fire Debris & Explosives
C Criminalistics/Crime Scene & Digital Evidence
D Drug Chemistry
E Forensic Biology/DNA
F Poster Session

Author Last Name

L

Langley, Zach F57
Laureano, Alanna E41
Lozano, Lauren F57
Lynch, Keavy B13

M

Marino, Stephanie F45
Marisco Warner I. B17
Marsh, Sarah E43
Martino, Madalynn E42
Massoia, Arden F48
Mattox, Jacqueline F46
McDowell, Angelique B. D33
McManus, Amber E40
Medina, Maya F63
Merlino, Priscilla T. A7
Miller, Emily B15
Minero, Stephanie D31
Morgan, Ashley E42
Muir, Luette A6
Muñoz Ortiz, Diana F62
Murray, Amanda E38

P

Patzer, Grace B12
Pitman, Ciara D25
Powell, Macenzie D32
Powers, Robert H. F61

Q

Quarino, Lawrence C21

R

Rech, Casey D27
Reed, MacKayla A4
Reimer, Matthew D31
Reynolds, Autumn F55
Ribadeneyra, Tiffany D35
Ricca, Alyssa D28
Romanelli, Mikaela D34, F53
Rovnak, Alina D30

S

Savage, J. The'arra F60
Schoenfeld, Jason F45
Schwartz, Ted R. B12
Skuches, Jennifer G. D26
Smith, Jeremy A6
Starkey, Eleanor C. F61
Szendrey, Emily C20

T

Torres, Aislynne E40
Torres De Soto, Marimar A4
Treviño, Joe C22

V

Valentino, Sasha F54
Velazquez-Davis, Karla E40

W

Walther, Taylor E38
Woolley, Roth A4

Y

Yuan, Mei D26

Northeastern Association of Forensic Scientists
Proceedings of the
October 2025 Annual Meeting