Northeastern Association of Forensic Scientists

Proceedings of the 2023 Annual Meeting

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Forensic Toxicology Abstracts

OSAC/ASB Toxicology Standards Update
Sabra Jones, PhD, D-ABFT, Regional Toxicology Liaison, NHTSA Region 5

Abstract: The Organizational of Scientific Area Committees (OSAC) Toxicology Subcommittee (SC) is focused on standards and guidelines related to the analysis of biological samples for alcohol, drugs, or poisons, and the interpretation of these results. Since its inception in 2015, the OSAC Toxicology SC is comprised of subject matter experts (SME) from state/local/federal forensic organizations, researchers/academicians, research and development partners, and private practitioners. Once developed documents are approved by the OSAC Scientific Area Committees (SAC), these documents are handed off to the Academy Standards Board (ASB) Toxicology Consensus Body (CB). The ASB is a Standards Development Organization which is accredited by the American National Standards Institute. The ASB Toxicology CB is also populated with SMEs, appointed by ASB and are responsible for the technical content of all documents. The ASB ensures that there is a balance of representatives within the CB to ensure that no one group is over represented. The ASB Toxicology CB is also responsible for ensuring that all documents are disseminated to the scientific community and all public comments are adjudicated and resolved. This presentation will provide an update of the OSAC Toxicology SC and ASB CB. A brief description and status update of active documents as well as provide an opportunity to encourage the forensic toxicology community to ask questions, to learn more about these two processes, and encourage all to be involved in these organizations.

Key Words: Organizational Scientific Area Committee-Toxicology Subcommittee, Academy Standards Board Toxicology Consensus Body, Forensic Toxicology Standards

Understanding ANSI/ASB 036: Standard Practices for Method Validation in Forensic Toxicology
Anisha Paul, M.S.F.S., D-ABFT-FT, Vermont Forensic Laboratory

Abstract: The American National Standards Institute (ANSI) published the Standard Practices for Method Validation in Forensic Toxicology in 2019. This document was originally drafted by the Scientific Working Group on Forensic Toxicology (SWGTOX) but when SWGTOX was disbanded in 2014, it passed ownership of all of its documents to the Toxicology Subcommittee of the Organization of Scientific Area Committees (OSAC) who in turn updated and approved the draft version of this document. The document was revised, prepared, and finalized by the Toxicology Consensus Body of the AAFS Standards Board (ASB) (1). The fundamental reason for performing method validation is to ensure accuracy and reliability in toxicology test results. Therefore, the attendees will learn the minimum standards of practice for validating analytical methods used in the field of forensic toxicology. The goal is to help the attendee understand the document and so that they feel comfortable using it in their daily practice.
Reference: (1) Standard Practices for Method Validation in Forensic Toxicology. ASB Standard 036, First Edition. American Academy of Forensic Sciences Standards Board, Washington DC; 2019.

Laboratory Quality Metrics in a Pain Medication Monitoring Laboratory
Gregory McIntire, PhD, D-ABFT, Consultant, and Frank Wallace, West Virginia Forensics Laboratory

Abstract: While most labs understand the need for an in-depth quality program, identifying parameters to monitor and the resulting data is difficult. Thus, this talk focuses on a few aspects of the quality assurance program followed by a successful pain medication monitoring laboratory where both enzyme immunoassay (EIA) and liquid chromatography / tandem mass spectrometry (LC/MSMS) were employed to assess qualitative and quantitative urine drug testing. Both the types of errors and the numbers of errors are discussed along with the impact of automation are discussed. Typical data is presented with instances of failures as well as how to interpret the data collected in pursuit of a "quality result in a timely fashion".

Data Modeling of Urine Drug Testing Results of Drugs of Toxicological Interest
Gregory McIntire, PhD, D-ABFT, Consultant

Abstract: Using a library of over 200,000 patient data points from urine drug testing (UDT), a normalized and transformed representation of that library can be prepared. This representation can be used to estimate "normal" results from results which are outside the distribution of normal results. The representation is "gaussian" and thus yields results from common statistical approaches. Data for alprazolam, buprenorphine, and fentanyl will be presented.

Enhancing High-Resolution Mass Spectrometry Performance for NPS Analysis with Improved Sensitivity and Characterization
Casey Burrows, Sciex

Abstract: The aim of this presentation is to introduce the instrument features on the ZenoTOF 7600 system that provide qualitative flexibility combined with quantitative power for NPS detection and characterization. The presentation will demonstrate that these new technological advancements on the system can be leveraged to provide more confidence in the quantified amounts of drugs and metabolites detected in discarded authentic case samples which is critical when determining the cause of death following an accidental overdose.

Method: Drugs and metabolites were extracted from human whole blood using a liquid-liquid extraction (LLE) procedure. HPLC separation was performed on an ExionLC system using a Phenomenex Kinetex C18 column Mobile phases were ammonium formate and formic acid in methanol and acetonitrile. The flow rate was 0.4 mL/min with a total LC runtime of 15.5 minutes. The injection volume was 10 µL. MS and MS/MS data were collected for each sample using Zeno IDA for optimal sensitivity on the ZenoTOF 7600 system. Fragmentation was performed using both collision-induce dissociation (CID) and electronactivated dissociation (EAD) to compare the generated fragment ions. Data acquisition consisted of a TOF MS scan to collect accurate mass precursor ions from 100 to 700 Da, followed by a TOF MS/MS full scan ranging from 25 to 700 Da to ensure all fragments were captured for identification using a maximum of 16 candidate ions. Data was acquired using SCIEX OS software 2.1.

Results: The use of a hybrid collision cell (which offers an alternative fragmentation capabilities) in combination with the Zeno trap technology (which improves MS/MS duty cycle) was leveraged for the characterization of structurally related isomeric species present at low levels in discarded postmortem case samples. The depth of information extracted from the unique fragmentation capabilities of electron-activated dissociation (EAD) enabled differentiation of structurally related isomeric species that were not previously discernable using convention collision-induced dissociation (CID). In addition, the improved MS/MS sensitivity resulted in confident identification of potent novel synthetic opioids and metabolites at concentration levels that were not previously achievable. Overall, the use of the ZenoTOF 7600 system provided a means to characterize and monitor low-levels of ultra-potent NSO in poly-drug intake scenarios. These advancements are shown to support the case of combined opioid drug toxicity leading to death, which offers a clearer picture for help in determining the cause of death.

Conclusion: A novel fragmentation technique combined with a highly-sensitive QTOF system for the screening and identification of low-level potent NSO and metabolites in discarded postmortem case samples is described. The depth of information extracted from EAD-based MS/MS spectra combined with the improved MS/MS sensitivity were leveraged for characterization of structurally related isomeric species present at low levels. The results demonstrate that the new technological advancements on the ZenoTOF 7600 provide high levels of confidence in the quantified amounts of drugs and metabolites detected in authentic case samples, which is critical when determining the cause of death following an accidental overdose.

Method Development for a Quantitative Panel of Psychoactive Adulterants of Illicit Drugs of Abuse in Biological Matrices

Shayna Kasher, Arcadia University

Learning overview: This presentation will offer a demonstration of the development, validation, and application of an original method to quantitate nine adulterants commonly identified with illicit drugs of abuse. It will also discuss the negative impact of these psychoactive adulterants and why the development of this method was necessary to expand upon existing analytical procedures.

Impact statement: This presentation will impact the forensic science community, as well as the larger community, by describing an efficient LC-MS/MS method to detect and quantitate adulterating substances of common drugs of abuse that may harm drug users.

Abstract: Adulterants are substances that are added to an illicit drug product for their pharmacological effects for the purpose of providing the effect of a higher quality drug 1,2 . The adulteration of illicit drugs of abuse with psychoactive substances, like veterinary or unscheduled prescription drugs, has been commonplace for many years. These adulterants can lead to unpredictable synergistic effects, which can increase the toxicity of the primary drug. Some adulterants can also cause toxic effects 1,3 . Despite this, a comprehensive panel for psychoactive adulterants has not previously been published. A single analytical procedure aimed at isolating common adulterants would enable the comparison and tracking of current trends in adulteration of illicit drugs of abuse. In addition, it provides a standardized method for identifying compounds that increase the toxicity of illicit drug products 1.

The objective of this presentation is to introduce a quantitative panel for the analysis the most prominent psychoactive adulterants: levamisole, xylazine, lidocaine, benzocaine, procaine, phenacetin, quinine, and tramadol and O-desmethyl tramadol. These adulterants were selected for their psychoactive properties and because they have been established in literature as frequent adulterants of drugs of abuse. Comparison of two sample preparation techniques, solid phase extraction and liquid liquid extraction, was completed to study the recovery of the analytes in various extraction conditions. The quantitative panel will also be analyzed in various matrices including blood, serum/plasma, urine, and oral fluid to fill the existing gaps in literature for the analytes in such matrices.

Instrumental analysis was performed with an Agilent 6495 Triple Quadrupole LC/MS system. Chromatographic separation was achieved with an Agilent InfinityLab Poroshell EC-C18 (3.0 x 100 mm x 2.7 µm) analytical column combined with gradient elution with 10 mM ammonium formate and 0.1% formic acid in methanol for an overall run time of 5 minutes. Solid phase extraction was performed with the UCT Clean Screen DAU 3 mL SPE cartridges using 78:20:2 dichloromethane:isopropanol:ammonium hydroxide as the elution solvent. The SPE results were compared to a liquid-liquid extraction for basic analytes that used 80:20 dichloromethane:isopropanol as the extraction solvent. During development of both extractions, factors including the organic solvent and pH were altered to optimize analyte recovery. Recovery studies were performed for both extractions and used to determine that the liquid-liquid extraction performed better for the overall panel of analytes. While SPE provided cleaner samples and better recovery for some analytes, recovery was not consistent among the whole panel of analytes due to their different properties.

Method validation will be completed in accordance with ASB 036 guidelines, including calibration model evaluation, carryover evaluation, matrix matching, bias, precision, determination of the LOD and LOQ, evaluation of interferences, and evaluation for ion suppression. The validated method will then be used to analyze authentic case samples provided by NMS labs. Finally, results from the application of the method to case samples will be discussed with a focus on the impact of psychoactive adulterants on drug users.

Keywords: Adulterants, LC/MS/MS, Method development

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Novel Psychoactive Substances and the U.S. Opioid Epidemic at the Intersection of Forensic Toxicology and Pathology

Austin Harning, Syracuse University Forensic & National Security Services Institute

Abstract: Novel psychoactive substances (NPS), colloquially known as designer or synthetic drugs, are a diverse group of chemicals that mimic the pharmacologic effects of existing illicit substances. They subvert the Controlled Substances Act (1971) through continuously changing the chemical structures of each substance, often quicker than chemists can detect them and the United States Drug Enforcement Administration (DEA) can schedule them. Because of their comparatively easy accessibility in the context of the ongoing Opioid Epidemic, low cost, and perceived safety, they are often seen as "legal" alternatives. Due to their variety and sheer numbers, they pose significant forensic and public health concerns at the individual and societal levels. Because they are continuously changing, little is known about their chemistry, toxicity, and the signs and symptoms associated with their use.

The objective of this study is to discuss the roles of the forensic toxicologist and forensic pathologist in the medicolegal system, and the challenges each party faces in detecting NPS through postmortem toxicological testing. The forensic toxicologist chemically screens and confirms the presence of drugs in specimen samples collected at autopsy. One of the major obstacles they face is the unavailability of toxicologic and analytical methods to accurately screen for and detect them. The process to develop such methods requires time and money, resources that some jurisdictions might have fewer of than others. In addition, NPS are often undetected and underreported; therefore, the true impact these substances have in fatal overdoses and drug-related deaths is currently underrepresented. This complicates the cause of death diagnosis made by the forensic pathologist, since the decedents rarely are free of disease or underlying medical conditions. Additionally, decedents rarely use a pure substance, but a mixture of other substances. Thus, a decision must be made in conjunction with the forensic toxicologists and medicolegal death investigators whether to pursue specific identification of these substances through the death investigation, and ultimately how to sign a death certificate in spite of potentially unknown complications. For these reasons, I believe it is more accurate to recognize this epidemic as a polydrug crisis.

Finally, in collaboration with the Onondaga County Medical Examiner's Office (OCMEO), I examined data related to drug-overdose deaths in the county since the start of the coronavirus pandemic. I seek to identify new and emerging drug trends in Onondaga County and Upstate New York to better inform the landscape of drug use in 2024. Ultimately, the role of the medical examiner's office is prevention and protection of public health and safety. We must appreciate the human complexity of this crisis behind the scientific complexity. Our understanding and development of new scientific methods is critical, but meaningless if not from a place of respect, dignity, and empathy for the victims and patients of this epidemic.

Blood Alcohol with Drugs Toxicology

Melanie Monetti, University of New Haven, and Emily Prabila, West Virginia University

Abstract: New Jersey is a stop testing state. This means that no further testing is performed when a blood alcohol concentration (BAC) value greater than 0.100% g/100 mL with uncertainty applied is met. Since no further testing is done, additional drugs and drug classes are unknown. Testing destroyed blood samples with a BAC ≥ 0.100% for drugs gives us insight into whether or not individuals are abusing drugs as well, and if there is a relationship to the amount of ethanol consumed.

The blood samples were provided by New Jersey State Police Office of Forensic Sciences (NJSP OFS) East Regional Laboratory (ERL) and were screened for fourteen common drugs/drug classes using Enzyme-Linked

Immunosorbent Assay (ELISA). The drug assay tests were amphetamines, barbiturates, benzodiazepines, carisoprodol, cocaine, fentanyl, methadone, methamphetamine/MDMA, opiates (general and synthetic), PCP, THC, tramadol, and zolpidem. Data provided from cases with a blood alcohol concentration of 0.099% g/100 mL and lower were also evaluated. The samples used for the study had incident dates from August 2022 through March 2023. The study focused on two hypotheses: the prevalence of drugs would decrease as the BAC level increased and except for THC, samples were unlikely to contain additional drugs when a BAC is greater than 0.100% Ethyl Alcohol.
A total of 238 cases were evaluated, and 198 positive (≥0.100%) BAC samples were tested on ELISA. Forty cases were previously screened due to charge or being below the blood alcohol threshold of 0.100% g/100 mL. 113 cases (48%) of samples screened negative for all drugs. Eighty six cases (36%) of samples screened positive for one drug. Thirty nine cases (16%) screened positive for two or more drugs. Notable results were eighty eight cases (37%) screened positive for THC and thirty two cases (11%), screened positive for Benzodiazepines. Cases that screened positive for benzodiazepines were extracted using SPE and analyzed on a GC/MS. The most common benzodiazepine detected was diazepam and metabolites (42%).
Although THC was the most common drug present, ten of the fourteen drug assays that contributed to the positive drug results.

The Proof is in the Print: The Detection of Cannabis Ingestion Using Fingerprint Residues and High-Resolution Ambient Ionization Mass Spectrometry
Niara Nichols, and Rabi Musah, PhD, University at Albany

Abstract: Cannabis sativa is the most widely used controlled substance in the United States. Despite its growing legality at the state level, there are instances where it is important to know if an individual has consumed Cannabis, such as in the event of a driving while under the influence (DUI) case or accidental consumption case. Techniques for the definitive detection of Cannabis ingestion are invasive, requiring the collection of blood or urine. Further, these samples cannot be collected easily in the field such as at a traffic stop. This research seeks to develop a less invasive and field deployable method for the determination of Cannabis use through the detection of Cannabis metabolites in fingerprint residues using high-resolution mass spectrometry. Fingerprint residue samples collected from donors who had consumed Cannabis via inhalation or the oral route and donors who had not consumed Cannabis were solubilized, and their chemical profiles were analyzed using direct analysis in real time – high-resolution mass spectrometry (DART-HRMS). The mass spectral data from the two experimental groups were compared using machine learning models to identify m/z values that can differentiate Cannabis use from non-use. Several models with cross validation accuracies of greater than 90% were created. A list of m/z values that were found to be impactful in enabling these models to discriminate between the two experimental groups was revealed. Future work will focus on expanding the sample size for a more robust statistical analysis. The identities of the m/z values that enabled discrimination between the experimental groups are being investigated to identify potential Cannabis-consumption specific biomarkers. These compounds can serve as the basis for a field-deployable test for Cannabis use.

Fentanyl and Methamphetamine Epidemics: Are They Connected?
Sharana Cook, and Sherri L. Kacinko, PhD, NMS Labs

Background/Introduction: The opioid epidemic has been affecting the United States to devastating levels over the past 8+ years. Simultaneously, methamphetamine cases have been increasing nationwide, including cases that were contaminated with fentanyl. This research is a study to determine whether these increasing epidemics are connected.

Objective: To analyze screening and confirmation data from 2016-2022 focusing on postmortem samples that requested fentanyl and/or methamphetamine analysis, look for possible trends over that span of time, and present findings.

Method: Samples received at NMS Labs were tested for fentanyl and/or methamphetamine using either ELISA, GCMS, or LC-TOF screening techniques. Samples with positive screening methamphetamine results were sent for confirmation analysis by UFLC-MS/MS with a calibration range of 5-2000ng/mL. Samples with positive screening fentanyl results were sent for confirmation analysis by LC-MS/MS with a calibration range of 0.1-40ng/mL, which was increased to 0.1-80ng/mL in September 2020. Positive confirmations for both analytes were then sorted by state and by geographical region[1]. Percent of positive confirmed cases for each drug, alone and in combination, based on total cases screened per year per region were determined.

Results: In 2016, NMS Labs screened 57,178 cases for fentanyl and 58,685 cases for methamphetamine. By 2022, those numbers more than doubled to 136,007 and 135,946, respectively. The percent of those cases that confirmed positive also increased over the seven-year span, meaning there has been a large increase in the amount of people using these drugs. States in the northeast were found to have the highest percent of positive fentanyl cases and very few methamphetamine cases over the six-year span, while states in the west had the highest percent of methamphetamine cases and few (but steadily increasing) fentanyl cases. In terms of cases that confirmed positive for both analytes, that number is also increasing across the country, with the highest count coming from states in the southeast.

Discussion: Although fentanyl cases have been the focus in the news, methamphetamine cases should not be ignored. Methamphetamine positive cases have been steadily rising since 2016, including those cases that are also positive for fentanyl. Possible limitations of this research include regions/states that are underrepresented in data. (Example: less than 20 samples were received for screening from South Dakota, which may have skewed the data from the central region.) However, looking at the overall regions gives a good estimate as to where the largest drug problems are occurring. Further research could be done to look at average concentrations of these analytes over the six years.

[1]Regions: Northeast = CT, DE, DE, MA, MD, ME, NH, NJ, NY, PA, RI, VT. Southeast = AL, FL, GA, KY, LA, MS, NC, SC, TN, VA. North Central = IL, IN, MI, OH, WI, WV. Central = AR, CO, IA, KS, MN, MO, MT, ND, NE, OK, SD, TX, WY. West = AK, AZ, CA, HI, ID, NM, NV, OR, UT, WA.

Drug Screening and Confirmation by LC/HR-MS

Kaleigh Champagne, State of Connecticut - Department of Emergency Services & Public Protection, Division of Scientific Services

Abstract: A problem commonly encountered in forensic toxicology labs with screening by immunoassay is that it is presumptive only and class-specific, therefore cannot provide information about individual drugs that may be present. To combat this problem as well as increase efficiency within the laboratory a rapid liquid chromatographic method using heated electrospray ionization combined with high resolution accurate mass spectrometry was developed to to qualitatively screen blood and urine specimens for the presence of drugs. In addition, to reduce cost and time, a modified protein precipitation method for blood and standard dilute and shoot for urine were developed. This allows for screening and confirmation of approximately 130 drugs with an instrument run time of 15 minutes.

Drug Trends in Rhode Island: An Opioid Epidemic

Amber Paturzo, MS, Rhode Island Department of Health

Abstract: Rhode Island, like many states, has been fighting an opioid crisis for many years. Due to the ever changing evolution of drugs, substance abuse cases are increasing each year with comparable or more deadly results. In the Forensic Toxicology Laboratory at the Rhode Island Department of Health (RIDOH), postmortem overdose and driving under the influence (DUI) case results are being compiled to document the current drug trends in Rhode Island. From this compilation, using both the Office of the State Medical Examiner (OSME) and the RIDOH Surveillance Website regarding OSME overdose statistics, an average of 96 overdose fatalities in 2020, 108 fatalities in 2021, and 116 fatalities documented in the first two quarters of

2022 were evaluated. Of these postmortem overdoses from 2020 through the first two quarters of 2022, opioids, fentanyl, cocaine, and alcohol were stated as factors that contributed to the cause of death with opioids being the most significant, followed by fentanyl, cocaine, and alcohol across all years. As for DUI cases, the Forensic Toxicology Laboratory has seen ethanol, cannabinoids, benzoylecgonine, fentanyl, and methadone most frequently from January to June 2023 with ethanol being the most recurrent, followed by cannabinoids, benzoylecgonine, fentanyl, and methadone. Many of the DUI cases from January to June 2023 report polysubstance abuse, some up to six analytes, and continue to display an increase so far in 2023. When comparing Forensic Toxicology statistics to Forensic Drug Chemistry statistics from 2020 to 2022, it is apparent that cocaine and fentanyl are still on the rise. With novel drugs emerging, some cases require further investigation. However, when looking at the overall statistics, the data demonstrates that similar drugs are being detected during analysis in both postmortem overdoses and driving under the influence cases.

Analysis of Nitazenes by LC-MS/MS

Lisa Mundy, Philadelphia Medical Examiner's Office, and Lydia Grimaldi, Duquesne University

Abstract: An LC-MS/MS method was developed using a Waters Acquity H class UPLC & QSM paired with an Xevo TQD. The LC column used is a SelectraCore C18, 100x2.1mm, 2.7µm (UCT #SCS27-C181021). This method uses SPE-B fraction extracts (UCT ZSDAU030) that were prepared for GC/MS. After the GC/MS injections, the extracts are further processed for nitazene analysis.
Limits of detection vary between 0.5 ng/mL to 2.5 ng/mL. The SPE method was not optimized for nitazenes, as this project was only to see if the extracts that were previously made for GC/MS could be re-analyzed for nitazenes. The percent recovery for the individual nitazenes varied from 77 to 92%. The method was originally used for isotonitazene, etodesnitazene, protonitazene, etonitazene, clonitazene, metonitazene, flunitazene, and n-pyrrolidino etonitazene. Later, 5-aminoisotonitazene, n-desethylisotonitazene, n-desethyletonitazene, n-desethylmetonitazene, n-desethyl rotonitazene, and n-pyrrolidino protonitazene were added.

"Hang on a Minute"; Developing Improved Separation Methodologies for Weakly Retained Polar Molecules in LC/MS/MS

Briana Alarcon, and William Campbell, Ph.D.; Pennsylvania State University, State College, PA

Abstract: Whether a sample is from an individual under the influence, autopsy, or a victim of assault, it is essential for drug chemists and toxicologists to be able to identify and quantify the substances at hand. The wide array of drugs available, both legal and illegal, street samples or biological samples, present a challenge to forensic toxicologist. This becomes even more difficult when one considers that drugs have a range of chemical properties and required analytical techniques may be variable. Drugs such as cannabinoids, for example, are non-polar while other illicit drugs, like cathinone's, are polar (1). Both are heavily used and abused but have completely different functions and interact differently within their chemical environments. This poses an issue in the forensic science community because creating a single chromatographic reference panel to analyze substances with wide variation in hydrophobicity is challenging.

LC/MS/MS is promising and becoming more common in toxicology labs because of the adaptability in method development. Polar substances present a special difficulty since they may elute too quickly to provide reliable quantitative or qualitative results. This research was aimed at developing methodology using reversed-phase conditions that can effectively separate weakly retained polar molecules for identification and quantification. The ultimate aim will be to expand this methodology to a wider range of compound polarities and ultimately to a full panel of drugs from hydrophilic to hydrophobic analytes. HPLC phase chemistries were investigated to optimize retention of model compounds. Seven Cathinones and five Glucuronide metabolites were chosen as an initial evaluation set of compounds. A standard C18 chemistry using fully porous media with a large pore diameter was initially evaluated. The large pores facilitate use under highly aqueous conditions. A polar embedded amide C18 phase was also evaluated. This was chosen since the amide function enhances retention and chromatographic selectivity under highly aqueous mobile phase conditions.

Superficially porous media were also employed in this study. The advantages of superficially porous media are multifold, but a key issue is increased sensitivity with these materials. These were also investigated using a C18 and an Amide phase. Lastly the column geometry was investigated. Column internal diameter of 2.1mm is common for LC/MS/MS applications. However, using a 1.5mm column diameter further increases the sensitivity of the methodology.

Complete baseline resolution was obtained for all compounds tested with adequate retention to fully identify and quantify the analytes in question. Further, the superficially porous materials have greater efficiency and demonstrated greater sensitivity than the fully porous materials. The 1.5mm columns, in turn demonstrated at least twice the sensitivity of a 2.1mm column of the same phase chemistry. The objective is to provide a more effective analysis method that can incorporate hydrophilic drugs into a larger panel of common drugs for forensic and clinical analysis.

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Forensic Biology/DNA Abstracts

Simplified DE Method for the RapidHIT™ ID to Obtain Investigative Leads from Sexual Assault Evidence

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Abstract: Rapid DNA fully integrates sample analysis workflow from extraction to capillary electrophoresis, generating autosomal STR profiles in as little as 90 minutes without the need for a DNA laboratory. The FBI allows DNA profiles developed from reference samples on rapid DNA instruments to be uploaded to CODIS, but not from crime scene samples. Reference samples collected from arrestees at some booking stations are being uploaded to CODIS and quickly searched against the DNA Index of Special Concern (DISC) established by the FBI for comparison to crime scene profiles from unsolved homicide, sexual assault, kidnapping and terrorism cases. This practice is expanding nationwide because of its ability to link arrestees to unrelated crimes while he/she is still in custody.

Although the FBI does not currently allow DNA profiles generated from crime scene samples on rapid DNA instruments to be uploaded to CODIS, they are working with vendors to enable this capability in early 2025. In the meantime, many law enforcement agencies have been using rapid DNA outside of CODIS to substantially impact criminal investigations, human trafficking, and the identification of human remains. While most of this work has been done with blood and saliva cases, rapid DNA is rarely used for sexual assault cases because rapid DNA instruments do not perform differential extractions. We sought to develop an off-instrument differential extraction method that was compatible with non-technical users and rapid DNA instruments to enable law enforcement to take advantage of the speed of rapid DNA in sexual assault cases.

The goal of the current work was to develop simplified DE methods for the RapidHIT™ ID system for use with semen-containing evidence found in sexual assault investigations. The methods developed are designed to be used for investigative leads in a laboratory or potentially in point-of-collection use environments. The outcome is a simple workflow that utilizes a 1-hour differential lysis to preferentially lyse epithelial cells leaving sperm cells intact. After a few brief washes, the epithelial cell fraction is separated, and the remaining sperm pellet can then be collected with a sterile swab and run on the RapidHIT™ ID system. Using this simplified DE method, single source male DNA profiles can be obtained from as little as 1 µl of semen, with the full sensitivity of the method still being evaluated. Mock mixtures using both buccal and vaginal epithelial cells were evaluated representing possible casework scenarios of vaginal and oral assaults. Successful results were also obtained from volunteer donated 12- and 24-hr post coital samples.

To shed, or not to shed: The impact on DNA recovery from fired cartridge cases.

Jesenia Medina; CT Department of Emergency Services and Public Protection - Division of Scientific Services

Abstract: Recent milestones in DNA recovery from fired cartridge cases called for the implementation of a new DNA collection method at the CT Forensics Laboratory. This study is modeled after the rinse-and-swab collection method detailed by the U.S. Bureau of Alcohol, Tobacco, Firearms and Explosives Laboratory (ATF) in an attempt to obtain interpretable DNA profiles from fired cartridge casings. During the study, touch DNA from known individuals with variable cell deposit (DNA shedding) rates was deposited onto sterile copper and nickel cartridges. The cartridges were then loaded aseptically and fired in an outdoor setting to mimic possible contamination from the environment. DNA collection from the fired cartridges was performed employing ATF's Rinse-and-Swab Method1 using two types of swabs (flock and foam tip swabs) and followed by the CT Laboratory's protocol for DNA extraction. The new recovery method yielded more DNA from individuals known to have higher shedding rates than individuals with lower shedding rates. Additionally, the flock swabs yielded higher signal DNA profiles than the foam tip swabs. The results suggest that the rinse-and-swab procedure may produce quality DNA profiles. However, numerous variables, like the number of cartridges tested together, the shedding rate of the handler, handling time, swab type, and the surroundings play a significant role in the ability to obtain high-yield DNA profiles from fired cartridges.

Keywords: DNA, Forensic Science, Fired cartridge casings

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Robust evaluations by single cell analysis: Highly informative investigative and evaluative results across all mixtures.

Catherine Grgicak, Ph.D., Rutgers University Camden

Abstract: The consistency between DNA evidence and person(s) of interest (PoI) is summarized by the LR: the probability of the data given the PoI contributed divided by the probability given random donors contributed. If there is more than one PoI, or the number of contributors (NoC) cannot easily be determined, then several sets of hypotheses are needed, requiring significant resources to complete the interpretation.

Recent technological developments in laboratory systems offer a way forward, by enabling production of single cell data. The scale of a single cell experiment ranges from tens to millions of cells per sample and is dependent on the isolation and preparation method employed. Smaller scale experiments typically use micromanipulation strategies coupled with tube-based amplification, while large scale experiments exploit the use of barcodes, nanodroplets or picowells, and next generation sequencing (NGS) to parallelize the data generating process. Regardless of scale, there are two features common to all single cell data generating processes: i) that intact cells or nuclei are isolated before the DNA/RNA is extracted, and ii) that the extraction and amplification (or library preparation) occurs in the same vessel to which the cell was added. These two features explain the strength of single cell processing in that: i) by isolating the cell before lysis, the two alleles of any donor are paired and fully resolved from DNA of other interference donors, and ii) by extracting and amplifying the DNA in the same vessel, allele drop-out associated with fractionating the extract into two components – one that is stored and one that is amplified – is abated.

We first use an *in silico* laboratory system, named ReSOLVIt, to automatically optimize the single-cell data generating process by determining what laboratory treatment offers a limit of detection of one copy. We show that laboratory outcomes are consistent with theory. We then experimentally generate 643 scEPGs and stochastically mix them to produce cellular admixtures with up to 5- contributors with 17-75 cells and minor ratios as low as 3.5%.

We then describe the development of a forensically cogent single-cell interpretation strategy that: i) clusters scEPGs into collections, each originating from one genetic source; ii) for each PoI, determines a LR for each cluster of scEPGs; and iii) by averaging the LRs for each PoI across all clusters provides a whole-sample or *sub-source* evaluation. By using Model-Based Clustering (MBC) in step i) and an algorithm, named EESCIt for Evidentiary Evaluation of Single Cells, that computes single-cell LRs in step ii), we show that 99.2% of the comparisons gave log LR >0 for true contributors, and of these all rendered log LR >5, regardless of the number of donors or whether the smallest contributor donated less than 20% of the cells,

For instances where there is no suspect, we demonstrate that the single-cell strategy returns posterior mass distributions that concentrate only on one genotype 85% of the time and that genotype is of the true contributor. This impressive posterior concentration on the true contributor's genotype occurred for all mixtures tested, demonstrating that single cell analysis maximally discriminates hypotheses across all donor numbers and proportions. This shows that forensic single cell DNA data is one of the most robust data types, enabling an expansion to the class of samples for which suspect and no-suspect evaluations bear positive outcomes.

Sex-based targeted recovery of cells in a heterogeneous mixture: separating male and female like-cells

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Abstract: Mixture interpretation remains a central challenge in the forensic DNA field.
Significant levels of research and development has focused on methods and means to improve the interpretation of complex samples including the development of the differential extraction [1] and software solutions such as probabilistic genotyping and statistical and machine learning-based number of contributor prediction. The majority of these solutions seek to ease the complexity mixture interpretation on the "back-end", rather than address the separation of the individual biological components (e.g. cells). With individual components (contributors) of mixtures separated, the resulting profiles are simple to interpret (single-source), require less time and resources, ultimately leading to increased confidence in the conclusions. Currently, there are few methods in use that permit the sex-based separation of like-cell types, e.g. epithelial cell mixtures of males and females. These scenarios are not uncommon in casework, for example in samples collected from bite marks, touched items or cavity swabs from a victim that was assaulted by a vasectomized male. Although Y-STR profiles may be generated, these profiles are not eligible for upload to the DNA database and, therefore, cannot be used effectively when the crime involves unknown perpetrators. This project developed a method to target, isolate, and recover male epithelial cells from a mixture of female like-cells using the DEPArray™ NxT, although any single cell separation method can be used. Mixtures of various dilutions (1:1, 1:10, 1:10, 1:100 and 1:100) of male to female epithelial cells, including samples 10+ years old were selectively labeled with an internally developed protocol using the Abbott Molecular CEPY (DYZ1) Spectrum chromosome enumerating probe which targets the Yq-12 region of the Human Y-chromosome. The DEPArray™ single cell sorter was used to separate and recover male cells from the heterogeneous mixture followed by DNA isolation, amplification (Promega PowerPlex Fusion 6c) and detection via capillary electrophoresis (Thermo Fisher 3500xL Genetic Analyzer). This method has successfully labeled, separated, and led to single source male profiles obtained from mixtures of male and female epithelial cells. These profiles were developed from both samples with single cells and multiple cells, all of which were interpretable using standard interpretation methods.

Effect of Heat-Induced Damage on the Efficiency of Possible DNA Repair Mechanisms.
Sydney Leffler; University of New Haven

Abstract: The effects of DNA degradation via temperature on potential DNA repair mechanisms were assessed. DNA was extracted from known whole blood samples as well as whole blood stains placed onto cotton swatches. The swatches were placed into an oven and subjected to varying temperatures (ranging from 50°C to 200°C) for different periods of time (ranging from 0 to 12 hours) to induce degradation prior to subsequent extraction with QIAGEN© QIAamp DNA Investigator Kit, quantification with Quantifiler™ Trio DNA Quantification Kit, amplification with Globalfiler™ PCR Amplification Kit, and capillary electrophoresis with Applied Biosystems™ 3500xl Genetic Analyzer, Applied Biosystems™ 3500 Series Data Collection Software 4, and GeneMarker®. Half of the samples from each set of swatches were repaired with New England BioLabs© PreCR ® Repair Mix while the others were left without repair. It was found that peak heights increased in response to repair mechanisms while those loci where alleles fully dropped out did not. The repair mixture appeared to repair only those remaining alleles after degradation. It was also seen that there was a higher potential for contamination in samples degraded and repaired for longer periods of time, due to possible drop-in. Data will be presented pertaining to mixtures samples degraded and repaired under these conditions to preliminarily assess its effect on peak height and mixture ratios.

The use of Raman spectroscopy to determine the TSD of bloodstains in crime scene conditions.
Alexis Weber, Igor K. Lednev Ph.D., University at Albany, SUNY/SupreMEtric LLC

Abstract: Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred, and individuals involved. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected,

documented, and processed.

Vibrational spectroscopy paired with chemometrics has shown reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, research conducted with these techniques so far has analyzed the aging of bloodstains, specifically the degradation of hemoglobin, in ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that can estimate the TSD of bloodstains in different environments.

There are infinite varieties of potential environmental conditions. Our goal is to determine how potentially "extreme" conditions affect the aging mechanism of bloodstains, high temperature in particular. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the *ex vivo* aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post deposition. After the spectra were collected, they were loaded into statistical software for preprocessing and modeling. The reproducibility of heated blood analysis and TSD determination model will be discussed.

The Impact of Bone Marrow Transplantation on Forensic Human Identification and Genetic Genealogy Testing

Lisa Sikop, Claire L. Glynn Ph.D., University of New Haven

Abstract: Forensic Investigative Genetic Genealogy (FIGG) has rapidly evolved to become a highly effective investigative tool to assist with identifying perpetrators of violent crimes and unidentified human remains (UHRs). FIGG received global recognition in 2018 when it was announced that the prime suspect in the Golden State Killer investigation was identified using this novel method. FIGG combines advanced DNA sequencing methods, with genetic genealogy analysis and traditional genealogical research methods to generate investigative leads for unsolved crimes. Forensic DNA analysis using Short Tandem Repeat (STR) profiling is a routine method used in forensic investigations to identify unknown persons from evidence samples. The STR profile may be compared to a collected known reference sample from a suspect, or it may also be uploaded to a national DNA database, e.g., CODIS in the United States. If no hits or identifications are reached, a case may become dormant, or until new evidence or suspects are generated. However, the use of FIGG can help further an investigation by utilizing the existing DNA available. FIGG involves the analysis of Single Nucleotide Polymorphisms (SNPs), using Next Generation Sequencing (NGS), with the resulting SNP data uploaded to public genetic genealogy databases to identify genetic relatives of the unknown person. With all scientific methods, it is always necessary to explore potential limitations and/or complexities that may exist which may interfere with results. With Forensic DNA (STR) analysis, it has previously been discussed in the literature that a person who has received a bone marrow transplant may produce a multi-allelic STR profile, as both the bone marrow donor and the recipients DNA is present, which can be misinterpreted as a mixture DNA profile, where in fact it is a chimeric DNA profile. Chimerism is a genetic occurrence in which a single organism contains two sets of DNA for example, bone marrow transplantation recipients. About 5,000 people a year in the U.S. undergo a bone marrow transplant, and this continues to increase. While the impact of chimerism on STR profiles has been studied previously, albeit with little published research, there is no published literature discussing the impact of chimerism on SNP profiles in a forensic context.

Following Institutional Review Board (IRB) approval, a volunteer who received a bone marrow transplant provided buccal samples and blood samples (via fingerprick on to FTA cards), and provided written informed consent. Each sample was extracted using the Qiagen DNA Investigator kit. Each sample was quantified using the Applied Biosystems Quantifiler Trio kit. Each sample was amplified using the Applied Biosystems GlobalFiler PCR Amplification kit. Each sample was separated and analyzed using the Applied Biosystems 3500 Genetic Analyzer. The STR profiles were examined to determine if they were chimeric. Following this analysis, it was seen the genetic profiles of the bone marrow transplant recipient showed a single profile for the FTA card blood samples, while the buccal swab samples also showed a single profile. Although these two samples came from the same individual, the buccal swab sample profile resulted in a completely different profile than the profile developed by the FTA card blood samples. Aliquots of the DNA extracts were then SNP sequenced using the Infinium Global Screening Array. The resulting SNP data files were uploaded to

GEDmatch, in Research mode. It was evaluated whether chimeric samples could be viewed on the database and if the two genetic profiles of the volunteer could be compared and manipulated to represent a single profile of the bone marrow recipient. As this study only involved one donor, limited conclusions can be drawn, but it will continue to be studied with new, incoming volunteers. This study increases the awareness of the impact of bone marrow transplantation on Forensic DNA samples and their resulting STR and SNP profiles and how they may affect forensic human identification. Further research is necessary in this field to understand the effects of bone marrow chimerism and the multi-allelic DNA profiles that may result in DNA analysis. This research serves to bring awareness to the forensic DNA community that there is potential for chimeric/multi-allelic DNA profiles to be present in forensic evidence samples, while it also serves to further understand the limitations or potential complexities that may arise in FIGG investigations.

Unraveling Clues from Cigarette Butts: Analyzing DNA Extracted from Gasoline-Soaked Filters Faith Ruggiero, Bay Path University

Abstract: Perpetrators attempting to start a fire may throw a lit cigarette into gasoline, examples of which include cigarette butts being found in automobile tanks or puddles of gasoline. These cigarette butts do not normally result in a fire but rather are extinguished in the liquid, and as a result gasoline-soaked cigarette butts are found at crime scenes. These cigarette butts have the potential to contain valuable DNA evidence that can assist investigators in identifying the perpetrators. Gasoline is a volatile, flammable mixture of liquid hydrocarbons derived from petroleum, and as a result is a powerful solvent. This has led crime scene responders to question whether usable DNA profiles can be generated after prolonged exposure to gasoline. A study was performed to determine whether gasoline affects the structural integrity of DNA and thus the quality of the DNA profiles obtained from gasoline-soaked cigarette butts. The study examined whether usable DNA can be collected from smoked cigarette butts soaked in gasoline for 2 hours, 1 day, 7 days, and 28 days. DNA was extracted from twelve soaked butts using an EZ1 Biorobot, quantified with the Quantifiler Trio kit, and amplified using the Globalfiler STR Kit. The DNA profiles were generated with a 3500 Genetic Analyzer. DNA quantification indicated slight degradation in only one of the three 1-day samples, while the rest of the samples showed no significant degradation. All twelve samples were amplified and produced complete DNA profiles. This work demonstrates that usable DNA can be extracted from cigarette butts exposed to gasoline and supports crime scene responders in collecting these possibly valuable items of evidence.

Yield of Touch DNA from Primary and Secondary Users on Common Burglary Tools Over Time

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Abstract: Touch DNA analysis can be crucial in connecting a perpetrator to a crime. When multiple individuals touch a surface, there is uncertainty about the duration for which the primary (or initial) DNA profile remains detectable particularly when a secondary (or later) individual touches the same surface. This study aims to determine if, over time, the percentage of the primary user changes significantly and if different substrates factor into DNA detection from the primary user. Ten biologically female participants were designated secondary users, and one biological male was the primary user. Tools with different handled materials- a carbon steel crowbar, a crystalline-handled screwdriver defined as smooth, and a thermoplastic rubber (TPR) handled screwdriver defined as rough were assigned to the participants. The secondary users were assigned to handle a designated batch of the three tools (rough, smooth, and steel crowbar) for two minutes each at time intervals of one week, one month, and two months. Each batch of the tools were touched initially by the primary user (N=90). MicroFLOQ Direct swabs were used to amplify the touch DNA on the tools after each time interval, bypassing the traditional extraction and quantification steps. 70% of samples yielded usable results, denoted by frequency percentages (alleles observed/alleles expected) of at least 20%. Primary and secondary user DNA percentages were calculated across all samples, where secondary user DNA appeared to be the major contributor to the tools according to median values. Kruskal–Wallis one-way analysis of variance was performed on the median primary

user DNA percentages across all samples. The results showed no statistical significance, indicating that the tool type and time interval does not impact the ability to detect primary user DNA.

An Improved Capillary Electrophoresis System for Human Identification

Danielle J. Brownell, Promega Corporation

Abstract: Rapid DNA and Massively Parallel Sequencing (MPS) hold great promise for the forensics community to extend the reach and depth of DNA typing. While both approaches are powerful complements to traditional capillary electrophoresis (CE) STR typing, neither approach is likely to replace CE analysis for the majority of forensic samples. Capillary electrophoresis will very likely remain the "workhorse" of forensic DNA typing for years to come. As such, improving CE technology is critical for the advancement of forensic DNA typing. The Spectrum CE System offers increased spectral capacity, which will allow analysis of currently available multiplexes from a variety of suppliers as well as a new family of 8-color PowerPlex ® STR Systems. The first release of this new class of STR kits is the PowerPlex ® 35GY System. With the inclusion of additional dye channels, smaller, more numerous loci will increase a laboratory's chance of success with degraded or inhibited casework samples. Additionally, the narrower range of product amplicon sizes in 8-color systems can enable more consistent results with variable direct amplification samples. The Spectrum CE System also offers increased workflow flexibility with four continuously accessible four plate positions. This design improves laboratory efficiency by reducing scheduling conflicts, increasing overnight/weekend throughput and reducing the number of instruments needed in the laboratory. The presentation will include an overview of the Spectrum CE System's design, as well as a review of data generated with current 6-color STR multiplexes and a prototype 8-color multiplex in development.

Educational Objectives: After attending this presentation, attendees will understand why CE will remain the method of choice for forensic DNA laboratories and learn about the advantages of using 8 color STR multiplexes and CE system.

Impact Statement: This presentation will impact the forensic science community by suggesting improvements that 8 color STR multiplexes will offer to the forensic DNA workflow.

Keywords: missing persons cases, casework, STRs, increased throughput, 8 color capabilities, capillary electrophoresis, PowerPlex

Comparing Likelihood Ratios of Degraded DNA Mixture Profiles Using DNA-View® Mixture Solution™

Cameron Filipe; Massachusetts State Police Crime Lab/Boston University

Abstract: Interpreting DNA profiles manually can potentially call into question subjectivity between analysts who may interpret specific results differently. There are multiple features of a DNA profile that can complicate interpretation, which include allelic dropout and drop-in, allele sharing, and polymerase chain reaction (PCR) artifacts, as well as degradation of the DNA itself, which can be caused by various environmental factors. Developments in DNA profile interpretation using probabilistic genotyping software have been made in order to assist in the complicated task of deconvoluting and interpreting a challenging mixture. Among these programs is DNA-View® Mixture Solution™, a continuous-model probabilistic genotyping software. Mixture Solution is unique in that it is not based on the Markov-chain Monte Carlo approach used by other programs such as STRmix™ and TrueAllele®. Instead of using an indirect method to compute likelihood ratios like MCMC-based programs, Mixture Solution calculates likelihoods directly from the data. This allows for the hypotheses to be calculated independently, eliminating the need for the analyst to make a guess of the number of contributors to the mixture.

In this research, Mixture Solution was used to provide statistical analyses for DNA mixtures that were subject to various levels of degradation, through the assignment of a likelihood ratio between two given

hypotheses based on the mixture data. The likelihood ratio would either support the hypothesis that the person of interest contributed to the mixture, or support the alternate hypothesis, that the person of interest was not one of the contributors. Three-person mixtures were prepared at four different contributor ratios with varying combinations of three levels of degradation: no degradation, partial degradation, and full degradation, using controlled heating to systematically degrade the DNA template prior to amplification. Using two hypothesis tests, Mixture Solution was used to compute likelihood ratios for each of the mixtures with a variety of defined people of interest.

All likelihood ratios computed that favored the ground truth hypothesis provided "moderate support" or higher. However, when the DNA from a person of interest was degraded, decreases in the likelihood ratio values were observed when compared to the values computed for undegraded DNA from the same person of interest. These decreases occurred primarily as a result of allelic dropout caused by degradation. While allele sharing was also determined to be another major factor in the differences between likelihood ratio values across DNA mixtures with varying levels of degradation, stutter was shown not to impact changes in likelihood ratios. Results showed that Mixture Solution successfully generated appropriate likelihood ratios for 97% of the computations performed for each of the 20 mixtures. Significant levels of dropout resulting from the degradation of the DNA of the person of interest in the remaining 3% of computations pushed the likelihood ratio values into the "uninformative" range.

Expanding Research Data on TPPR of DNA

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Abstract: The sensitivity of forensic deoxyribonucleic acid (DNA) testing has increased tremendously since its inception. The questions for the forensic DNA analysts during expert witnesses' testimony have shifted from: Does the DNA match this individual, to: How did the DNA get there? These questions have introduced the importance of evaluative reporting in casework and can include hierarchy of propositions, transfer, persistence, prevalence, and recovery of DNA. The results of a quality and gap analysis evaluating published research demonstrate the holes in the existing literature, including but not limited to, the use of more recent commercial DNA profiling systems that utilize expanded loci as well as research pertaining to firearms and sexual activity. Subsequently, experiments were conducted to expand upon the existing data to build a database for potential future evaluative reporting in casework. The research focused on evidence routinely encountered in alleged sexual assaults, homicides, and firearm possession. The results of these experiments and the road ahead for evaluative reporting will be discussed in this presentation.

Why Prosecutors and Defense Attorneys Need DNA Specialists, And How Your Career in the Lab Can Have a Second Ac!

Melissa Mourges, New York County District Attorney's Office

Abstract: DNA is not just for rapes and homicides anymore! Virtually every criminal case has the potential to yield DNA evidence. Just like sex assaults and murders, guns, burglaries and property crimes increasingly get the "CSI" treatment. Police departments often employ evidence collection technicians, who work out of backpacks and the pockets of cargo pants instead of big Crime Scene Unit trucks, to respond to all manner of crime scenes to take photographs, lift prints, and swab for DNA.

DNA Specialists embedded in prosecutor's offices are especially useful because prosecutors must be conversant in the use of DNA from the very beginning of a case, from literally the moment the crime is discovered. Early in the investigation, prosecutors must understanding what to collect, help triage evidence for testing, determine the order in which various forensic tests should be performed, decide exactly how probative particular tests might be, and calculate what quantum of forensic evidence might constitute probable cause. After that, prosecutors must understand the DNA reports, help determine what evidence should be tested next, what victim, suspect and elimination exemplars should be collected, and what, exactly, statistics of inclusion mean to

the case. DNA Specialists can also provide insights into the investigation of cold cases, including potential testing using familial searching and IGG.

Prosecutors also need help from DNA Specialists to prepare cases for trial. This includes understanding the tests and data behind the reports, dealing with Frye and Daubert challenges, ensuring compliance with discovery obligations, eliciting testimony from analysts at trial, preparing exhibits, accurately stating the weight of statistical evidence, and dealing with defense experts. Prosecutors who handle appeals also need help from DNA specialists to counter issues including confrontation clause violations based on which analysts testified at trial, claims that trial prosecutors overstated the weight of evidence, and arguments that defense lawyers at trial were ineffective for failing to aggressively challenge DNA evidence. Conviction Integrity Units within prosecutors' office also need the help of DNA Specialists to determine what type of post-conviction DNA testing would be possible and probative. And DNA Specialists help prosecutors cope with non-conformities and other problems in the lab.

Defense lawyers can benefit from in-house DNA specialists for many of the same reasons: for help reading and understanding reports and the data behind them, pointing out hits and misses in DNA testing, preparing cross-examination of the States' analysts, and suggesting defense experts for trial. DNA specialists can also help explain forensic results to defendants who may need help deciding whether to accept a plea or take a case to trial.

Prosecutors' offices in New York City employ DNA specialists, and we have found that talented analysts from our local DNA lab are excellent candidates. Our experience can help you successfully pitch this position to your local prosecutors and institutional defenders and demonstrate why adding this expertise to their rosters will enhance public safety, achieve better outcomes in court, and save money. In fact, you might be the perfect candidate for the job!

Northeastern Association of Forensic Scientists

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Forensic Drug Chemistry Abstracts

The ever-increasing drug smuggler: PAPER

Holly Fox; Cumberland County District Attorney's Office Forensic Laboratory

Abstract: Have you ever received paper as an evidence submission and thought, why are they sending this to the seized drug lab? Paper isn't a common type of evidence submitted for drug analysis. However, it is one of the main forms of drug transportation in correction institutions. Correctional institutions are well known for underground drug trade, but the drugs in prison don't always look like drugs. Common forms of street drugs, such as powders, pills, and plant material, are not what's routinely seized in these facilities. Instead, notes, legal mail, books, magazines, and photo albums hold the drugs that are smuggled inside. The majority of the paper submitted is unstained, which can make sampling a challenge. A variety of drugs have been identified, with synthetic cannabinoids being the largest drug class. Other drugs identified include fentanyl, cocaine, and PCP. Drugs are smuggled into the prison in various ways making the extraction process and sampling method another obstacle. When they find items suspected to contain a controlled substance, the items are sent to the Cumberland County District Attorney's Forensic Laboratory to be analyzed. The different types of samples received, how these cases are approached in our laboratory and the instrumentation used for analysis will be discussed. Additionally, the resources used to identify novel psychoactive substances (NPS) will be reviewed. Trend information since the start of the program regarding NPS prevalence, sample type and other findings will be provided.

Progress Towards the Development of a Universal Protocol for Extraction of Cannabinoids from Within Complex Matrices

Benedetta Garosi, B.S., Megan I. Chambers Ph.D., Rabi A. Musah, Ph.D.; University at Albany - SUNY

Abstract: In 2019, the National Institute of Justice highlighted the critical need for the development of standardized protocols for the analysis of cannabis-infused products that have become exceptionally popular since the decriminalization and legalization of marijuana at the state level in the U.S. Although the literature is replete with reports on efforts endeavoring to resolve the challenges associated with analysis of cannabinoids that are infused within foods and beverages, this issue remains. The difficulties are often associated with the less than effective extraction of cannabinoids from complex matrices, and the sample clean-up required to make the extracts suitable for direct interrogation by conventional methods. The processes are nuanced, complex- matrix-dependent, and are generally resource-intensive, time-consuming, and require extensive sample preparation. For example, lipophilic/oily products are extracted with solvents of varying polarity, washed, dried and reconstituted, or subjected to solid-phase extraction (SPE). Products with high sugar and carbohydrate content, such as candies and honey, are subjected to dissolution in water, organic solvent extraction, sonication, and filtration, followed by evaporation of the solvent and derivatization. Solid foods such as brownies and cookies, are ground/homogenized prior to extraction. Aqueous products are degassed (if necessary) by sonication,and extracted with QuEChERS extraction salts. Recently, slight modifications of the recommended two-step sequence used with QueECheERS (i.e., liquid-liquid extraction and dispersive solid-phase extraction clean-up) have been employed for the extraction of cannabinoids from food products. Although many of the protocols involve routine steps, these approaches to sample analysis require a perpetual need for highly specialized and nuanced method development in order to accommodate the ever-changing complex matrices that are encountered by crime labs, often at great expense in terms of time and material resources.

To address the challenge of the need for new testing strategies, this study focused on the development of a more universal extraction protocol that could be applied to multiple matrix types, featuring the Waters © QuEChERS DisQue salts. The approach involves suspension of the cannabinoid-containing sample in water, vortexing of the suspension, sonication (only if suitable), addition of acetonitrile followed by the DisQue salts, and vortexing again before allowing the layers to separate. Rapid analysis of the layers by direct analysis in real time – high- resolution mass spectrometry (DART-HRMS) revealed that the cannabinoids were reliably extracted into the acetonitrile layer with high efficiency. This protocol was successfully applied for the extraction of cannabinoids from a wide range of samples including: (a) gelatin candies;(b) chocolates; (c) marshmallows; (d) beverages such as coffee, sodas, and liqueurs; (f) butters and oils; and (g) personal-care products such as balms

and lotions.

Overall, the development of a more universal, simple, rapid, robust, and cost-effective analytical method for the extraction of cannabinoids can streamline sample analysis by: (1) enabling the preparation protocol to be applied to a broad range of matrix types; (2) saving time; and (3) reducing sample testing backlogs.

DART-HRMS Facilitated Quantification of THC and CBD in Chocolates and Gelatin-Based Fruit Candies

Megan I. Chambers Ph.D., Benedetta Garosi, B.S., Rabi A. Musah, Ph.D.; University at Albany – SUNY

Abstract: Crime laboratories are tasked with analyzing a wide variety of evidence and sample types, which includes cannabinoid-infused food products and beverages derived from Cannabis sativa. Currently, chromatographic methods are traditionally used for quantifying cannabinoids in C. sativa materials. However, clogging of columns and syringes, contamination, and carryover in subsequent runs are all examples of disadvantages of these approaches. Therefore, a method to rapidly analyze and quantify cannabinoids (i.e., Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD)) in complex Cannabis matrices is highly desirable. This study focused on the development of validated protocols for the quantification of THC and CBD in common candy matrix types by an ambient ionization mass spectrometric technique termed direct analysis in real time – high-resolution mass spectrometry (DART-HRMS).

Calibration curves (concentration vs. mass-to-chare ratio (m/z)) were developed using a semi-automated sampling approach and deuterated counterparts of THC and CBD as internal standards. After successful method validation, the following sample types were analyzed to examine the accuracy of the quantification protocols: (1) mock edibles (chocolates and gelatin-based gummy candies) were prepared in-house using cannabinoid standards; (2) blank edibles made without cannabinoids to serve as experimental controls; and (3) commercially available candies infused with THC. Each of the samples were first screened by DART-HRMS to verify the presence of THC or CBD in the cannabinoid-infused edibles, in addition to simultaneously confirming the absence of cannabinoids in the candy blanks. All samples were then extracted using a high-cannabinoid recovery rate extraction procedure and analyzed using the validated method. The CBD percent recoveries for the chocolate and gummies prepared in-house compared well with those of previously reported cannabinoid quantification studies. Furthermore, no analyte signal was detected in the blank chocolate and gummy samples. The THC quantified in the commercial chocolates and fruit chews (i.e., gummies) were similar to those reported on the product labels. In summary, the results demonstrate proof-of-concept for the application of DART-HRMS towards the quantification of THC and CBD in edible matrices. Furthermore, the developed protocol is robust and versatile, and can be readily applied to accommodate the range of increasingly complex and novel matrix types within which cannabinoids are infused, without having to resort to development of nuanced matrix specific analysis approaches.

"Hang on a Minute"; Developing Improved Separation Methodologies for Weakly Retained Polar Molecules in LC/MS/MS

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Abstract: Whether a sample is from an individual under the influence, autopsy, or a victim of assault, it is essential for drug chemists and toxicologists to be able to identify and quantify the substances at hand. The wide array of drugs available, both legal and illegal, street samples or biological samples, present a challenge to forensic toxicologist. This becomes even more difficult when one considers that drugs have a range of chemical properties and required analytical techniques may be variable. Drugs such as cannabinoids, for example, are non-polar while other illicit drugs, like cathinone's, are polar (1). Both are heavily used and abused but have completely different functions and interact differently within their chemical environments. This poses an issue in the forensic science community because creating a single chromatographic reference panel to analyze substances with wide variation in hydrophobicity is challenging.

LC/MS/MS is promising and becoming more common in toxicology labs because of the adaptability in method development. Polar substances present a special difficulty since they may elute too quickly to provide

reliable quantitative or qualitative results. This research was aimed at developing methodology using reversed-phase conditions that can effectively separate weakly retained polar molecules for identification and quantification. The ultimate aim will be to expand this methodology to a wider range of compound polarities and ultimately to a full panel of drugs from hydrophilic to hydrophobic analytes. HPLC phase chemistries were investigated to optimize retention of model compounds. Seven Cathinones and five Glucuronide metabolites were chosen as an initial evaluation set of compounds. A standard C18 chemistry using fully porous media with a large pore diameter was initially evaluated. The large pores facilitate use under highly aqueous conditions. A polar embedded amide C18 phase was also evaluated. This was chosen since the amide function enhances retention and chromatographic selectivity under highly aqueous mobile phase conditions. Superficially porous media were also employed in this study. The advantages of superficially porous media are multifold, but a key issue is increased sensitivity with these materials. These were also investigated using a C18 and an Amide phase. Lastly the column geometry was investigated. Column internal diameter of 2.1mm is common for LC/MS/MS applications. However, using a 1.5mm column diameter further increases the sensitivity of the methodology.

Complete baseline resolution was obtained for all compounds tested with adequate retention to fully identify and quantify the analytes in question. Further, the superficially porous materials have greater efficiency and demonstrated greater sensitivity than the fully porous materials. The 1.5mm columns, in turn demonstrated at least twice the sensitivity of a 2.1mm column of the same phase chemistry. The objective is to provide a more effective analysis method that can incorporate hydrophilic drugs into a larger panel of common drugs for forensic and clinical analysis.

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Identification of Psilocybin in Microdosing Capsules

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Abstract: Hallucinogens are known for their strong effects on a person's mind. A new form of consuming hallucinogens, called microdosing, is becoming a popular means of self-medication for those with mental illnesses. A common drug often seen in cases involving microdosing is psilocybin. The problem that could potentially arise from microdosing in the realm of forensic science is that such small quantities of these drugs might not be detected by current instrumentation methods. The following is a case-analysis that involved the examination and testing of 11 microdosing capsules using methods regularly encountered in forensic drug chemistry laboratories. This involved the presumptive and confirmatory testing of the capsules using stereomicroscopy, color testing using Ehrlich's reagent, and GC/MS. Additionally, limit of detection studies were performed for both color testing and GC/MS testing. For the color testing limit of detection study, non-drug containing mushroom samples were spiked with a specific amount of psilocybin and psilocin based on the average percent composition found in microdosing capsules. In the end, four levels for psilocybin and four levels for psilocin were created in total. For the GC/MS limit of detection study, five dilutions were performed until a low signal to noise ratio was obtained. This was mainly used to help finalize a methanol extraction protocol that was established specifically for testing the microdosing capsules on the GC-MS instrument. During analysis using stereomicroscopy intrabatch variabilities between the capsules being tested were noticed. More specifically, three of the capsules appeared brown and unpowderized, noticeably different from the other eight capsules which all had a tan coloration and powderized appearance. For color testing, the limit of detection was shown to be above 0.5 mg; for the two highest levels, the color change was noted as inconclusive, while the other lower levels did not show any color change. Eight out of the eleven capsules produced a color change, indicating the presence of psilocybin. The only capsules that produced no color change were those that also had a different appearance noted during stereomicroscopy. GC/MS analysis showed that all capsules except one contained some amount of psilocin above the limit of detection. Since the data produced by this instrument was only qualitative, the area under the curve of each peak was used to compare how much psilocin was present in each capsule when compared to each other. This analysis showed that three of the capsules had less psilocin than the others. These

were the sample capsules described to have a brown and unpowderized appearance during stereomicroscopy. Overall, this case-study has demonstrated that not all capsules contain the same amount of psilocybin, regardless of whether they come from the same batch, further demonstrating that care should be taken in analyzing microdosing capsules.

Connecticut Drug Trends

Breanne Steimle, M.S.F.S.; Connecticut Department of Emergency Services and Public Protection / Division of Scientific Services

Abstract: This presentation will provide attendees with an insight into the types of controlled substances that have been analyzed at the Connecticut State Forensic Laboratory currently in 2023. These drug trends will include those analyzed within the first three quarters of 2023, as well as some comparisons to the previous year, 2022. These trends will include types of packaging that common drugs are found in within the state of Connecticut, as well as the most common substances that are analyzed at the laboratory. Other trends that will also be mentioned are new substances, as well as uncommonly seen substances that have been analyzed at the laboratory in 2023.

Trends in Positivity in Counterfeit Tablets Monogrammed "M 30"

Sarah Shuda, Amanda L. Mohr, MJ Menendez; The Center for Forensic Science Research and Education; Thom Browne Jr., Colombo Plan; Dr. Barry K. Logan; The Center for Forensic Science Research and Education and NMS Labs

Abstract: Fentanyl use has proliferated in the United States leading to an opioid epidemic that has resulted in numerous overdoses and deaths. Fentanyl can be encountered in seized drug evidence in a variety of forms, most frequently as a powder or in a tablet. Counterfeit tablets monogrammed "M 30" which are made to look like authentic oxycodone pharmaceuticals are produced illicitly and contain fentanyl. These tablets are frequently seized at the Southwest border as they are transported to the United States. Through a collaboration with Customs and Border Protection, tablets seized from 2021-2023 were submitted. Representative samples were taken from tablet populations for analysis. Each representative tablet was homogenized individually by grinding. Aliquots were prepared by performing an acid-base liquid/liquid extraction. The sample was partitioned between deionized water and 90:10 dichloromethane:isopropanol. The samples were pH adjusted with 10% hydrochloric acid and ammonium hydroxide to recover both acidic and basic components. The extracts were analyzed by gas chromatography mass spectrometry (GC/MS) on an Agilent 7890 GC with 5975 MSD equipped with a J&W 12m 0.2 mm x 0.33 μm DB1 column. A total of 1030 tablets from 40 seizure dates were analyzed qualitatively. Samples were processed using an in-house database that contained over 1000 compounds including opiates and opioids, fentanyl analogs, adulterants, and other drugs of abuse and pharmaceuticals.

Fentanyl was identified in 97% of samples analyzed. All other controlled substances typically considered drugs of abuse were positive in less than 10% of tablets: para-fluorofentanyl (9.8%), methamphetamine (2.3%), pentobarbital (1.2%). These constituents were found in combination with fentanyl. The pentobarbital and methamphetamine findings were from a single seizure in the first quarter of 2022. Tablets containing para-fluorofentanyl were seized in 2021 (Q2 and Q3) and 2022 (Q1 and Q2) but have not yet been encountered in 2023 seizures.

The adulterants present were typically analgesic compounds. All samples analyzed contained metamizole (dipyrone), a non-opioid analgesic which was banned in the United States, but it is still available in countries in South America, Asia, and Europe, and 96% of samples contained acetaminophen. Xylazine, a veterinary tranquilizer that has increased in prevalence in recent years was identified in 7% of tablets. Xylazine was positive in samples seized in Q2 and Q3 of 2021, Q1 and Q2 of 2022, and Q1 and Q2 of 2023. Additional adulterants caffeine, lidocaine, and levamisole were positive in less than 2% of tablets.

The data indicates that there is some consistency in the qualitative contents of counterfeit tablets monogrammed "M 30" that are seized at the Southwest border. The majority of these tablets consist of fentanyl, metamizole, and acetaminophen. However, there are batches of tablets that contain additional drugs like para-

fluorofentanyl, methamphetamine, and pentobarbital and other potentially toxic adulterants like xylazine that are produced and can enter the drug supply, which can affect public health.

Direct analysis in real time (DART) in combination with trapped ion mobility QTOF mass spectrometry for fast analysis of seized drugs

Matthew Clabaugh; Bruker Applied Markets, Carsten Baessman

Abstract: Forensic analytics are constantly facing growing demands like an increasing number of targets due to new psychoactive substances. The caseloads in these laboratories are increasing. These issues lead to the need of fastened up preparation and analysis time although not lacking the high sensitivity and selectivity needed in this field. The use of Direct-Analysis-in-Real-Time mass spectrometry (DART-MS) is a technique that combines these aspects. The use of trapped ion mobility spectrometry (TIMS) in combination with DART adds another dimension to the analysis, allowing isomeric drugs to be separated and analyzed using collision cross sections (CCS) and Parallel Accumulation–Serial Fragmentation (PASEF).

Advancements in Sample Preparation Techniques for Forensic Chemistry Analysis

Alexandra Kocaj; Nassau County Office of the Medical Examiner / Division of Forensic Services

Abstract: Forensic chemistry plays a pivotal role in criminal investigations, relying heavily on accurate and efficient sample preparation techniques to extract, isolate, and analyze critical evidence. This presentation delves into the development of sample preparation methodologies, focusing on diverse forensic scenarios. Four distinct sample preparation techniques will be discussed, each tailored to the unique challenges posed by various substances encountered in recent forensic investigations.

Firstly, basic extractions will be explored as an effective means of removing glycerin from LSD samples. Glycerin and other sugary products can obscure the detection of crucial compounds due to its often poor chromatography in GCMS analysis. This technique enhances selectivity of the analyte of interest ensuring more accurate analytical results.

A strong acidic extraction method will be examined in the context of complex solids such as THC edibles. The extraction of tetrahydrocannabinol (THC) from edible matrices demands robust methods that can handle diverse matrices while preserving A integrity of the compound for subsequent analysis. This extraction has been crucial due to the complex matrices of the sample types result in chromatography with significant collusion of compounds and carry over when analyzed using methanol dilutions which is eliminated with this extraction.

A weak acid-base extraction approach will be discussed for the extraction of psilocin/ psilocybin from chocolates with mushrooms. This method optimizes the recovery of these compounds while minimizing interference from the complex matrix. This method also allows for the ability to concentrate the extraction to a desired final volume to confirm psilocin when analyzing "micro dosed" chocolates.

Lastly, a basic extraction employing hexanes will be explored for the removal of caffeine from methamphetamine and fentanyl samples. This technique allows for the isolation and analysis of these substances with minimal interference for samples with abundant caffeine and low-level analyte.

The presentation will showcase how these innovative sample preparation techniques are integral in forensic chemistry as well as highlight limitations encountered when performing complex preparations. Advances in sample preparation are pivotal in maintaining the highest standards of scientific rigor and justice within the field of forensic chemistry.

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

Tiffany Ribadeneyra; Nassau County Office of the Medical Examiner, Division of Forensic Services

Abstract: 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 Forensic Sciences and the

Office of National Drug Control Policy (ONDCP). Historically, SWGDRUG recommended minimum standards for the forensic examination of seized drugs and sought their international 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 2022 and currently in 2023. Recent publications include revisions to Part IVA of the SWGDRUG Recommendations: Quality Assurance/General Practices and Supplemental Document SD-5: Reporting Examples. Recent activities include revising Parts IVB of the SWGDRUG Recommendations: Quality Assurance/Validation of Analytical Methods and IIIA: Methods of Analysis/Sampling Seized Drugs for Qualitative Analysis of the recommendations. Subcommittees have been devoted to revising Supplemental Document SD-2: Validation of Analytical Methods, expanding statistical sampling resources, training and outreach. Lastly, the current state of SWGDRUG as well as future initiatives will be reviewed.

Northeastern Association of Forensic Scientists

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Trace, Arson & Explosives Abstracts

The application of particle-correlated Raman spectroscopic analysis of soils to mock-casework scenarios

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Abstract: Soil is a continuous but complex mixture, reaching across geological bodies. It is primarily composed of minerals, organic matter, organisms, gases, and water. Although it is continuous, soil is distinctly variable and differentiable, based on aspects such as geographic location, seasonal factors, and human interference. In combination with its highly transferable nature, the complexity of soil composition makes it a valuable material for forensic trace evidence and object-to-scene association. The development of forensic soil analysis is currently focused on mineralogy, with the traditional tool for mineral identification being the polarized light microscopy (PLM). One development in forensic mineralogy focuses on employing elemental analysis, most often using the scanning electron microscopy equipped with energy dispersive X-ray spectroscopy (SEM-EDX), to simultaneously visualize and analyze mineral grains. Other methods, including several spectroscopies as well as X-ray diffraction (XRD), have also been explored for application to forensic soil analysis. One method with excellent potential for the interrogation of a range of forensically relevant samples is Raman spectroscopy[1]. In particular, Raman spectroscopy has a demonstrated history for use in the field of geology for mineral identification, thus its application to the forensic analysis of soils is a logical extension.

Particle correlated Raman spectroscopy (PCRS) is a novel analytical method which combines automated image analysis with Raman spectroscopy thus providing both microscopic morphological and chemical information about a single sample non-destructively. PCRS provides both qualitative and quantitative information about a sample. When applied to soil minerals, this information includes their identification, microscopic morphological characteristics (e.g., circularity, elongation, brightness), and particle size distributions. This information is valuable for forensic soil comparisons, but more research is needed to understand the significance of an association of these properties given the complexities of transfer and persistence.

In this project, PCRS is used to analyze soil particles collected from simulated evidence samples. Shoes and shovels were used to collect mock-evidence from three different geographical locations – an urban park, a suburban residential area, and a rural woodland area. Known soil samples were also collected from these locations to serve as reference samples for comparison under PCRS analysis. The mock-evidence items were prepared in a method detailed by Stoney et al. for the analysis of very small particles[2]. The collected adhering soil was then cleaned to isolate the mineral grains per the method described by Palenk[3]. The particles in the size range of 90nm-180nm in diameter were then dispersed onto a Raman-inactive microscope slide and analyzed using PCRS. The results were compared to the reference samples that were treated and analyzed with the same method. Source consistency could then be determined using a set of match criteria that includes mineral identity, particle morphology, and composition percentage.

Analysis of the soil samples showed that it was possible to determine the source of soil collected from mock-evidence by using PCRS, when reference samples from suspected sources are available for appropriate comparison.

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Differentiation of Architectural Paint by Sheen Using Reflectance Spectroscopy

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Abstract: Paint is a form of transfer evidence that is analyzed within the Trace Evidence discipline of Criminalistics. This evidence is common in cases including breaking and entering, burglary, assault, homicide, vandalism, as well as other cases involving contact between a coated surface with another surface. Architectural paint, specifically, is often separated based off the coating's glossiness, or what is termed sheen, which is a commercial measurement of reflectance of light at a specific angle off the surface of the coating. This research aims to show a new method of architectural paint differentiation based on sheen utilizing reflectance spectroscopy and other microscopic and spectroscopic methods in order to add another level of differentiability to this type of evidence.

A total of 140 dried architectural interior paint samples were commercially obtained encompassing paints within the same product line, within the same manufacturer, and between manufacturers. Using an Ocean Optics reflectance probe, percent reflectance measurements between 350-800 nanometers at an angle of 45 degrees were recorded. Principle Component Analysis to Linear Discriminate Analysis was performed on each measurement in order to determine and visualize statistical separation. Utilizing this methodology, within product samples of the same color showed clear separation between sheens when both Raman and IR spectra showed high levels of similarity.

As shown in this study, sheen often was the only distinguishing characteristic as Raman and IR spectra were similar for within product and within manufacturer samples. Therefore, sheen can provide more evidentiary power to architectural paint evidence in trace evidence analysis.

Automation Possibilities for Fire Debris Analysis using ChemStation Macros

Eugene Zegocki, Monroe County Crime Laboratory

Abstract: ChemStation macro language provides ample possibilities for automation tasks. Using macros, it is possible to automate screening, pattern recognition, identification and documentation of GC/MS instrument results. The author designed and tested a set of macros for Agilent GC/MS instruments using ChemStation software for Fire Debris analysis.

A set of macros automatically process sequences containing samples for one or several cases. The program screens GC/MS data and identifies ignitable liquids. In certain cases the program only indicates ignitable liquids and those should be cleared by analyst. If analyst does not agree with the automated identification results, he/she could change suggested results or references manually prior to printout. There are embedded tools for comparison of patterns and peaks, MS library search and searches in folders containing GC/MS data of previously run samples, for example, NCFS samples helping an analyst to confirm or reject suggested result.
There is a predefined directory containing references (previously run ignitable liquids). If an ignitable liquid is identified, the program selects appropriate reference(s), automatically makes extracted ion profiles and/or peak RT and mass spectra comparisons. In the case of low intensity peaks the background could be subtracted, thus making better library hits.

Results are produced in PDF format for final review. The printout includes total ion chromatograms of ASTM test mixture, blanks, items in the case, library searches, extracted ion profiles for identified ignitable liquids and appropriate references and might include all additional manual comparisons.

Data is combined in one PDF file, which is easy to use in a paperless workflow process. Printout could be automatically paginated. Also, obtained results could be automatically inserted into pdf form notes, if analyst use those.

Statistical Analysis of Hair Color for Racial Determination"

Alisha Desai, B.A., Lawrence Quarino, Ph. D., ABC-GKE, Carol Ritter, Jennifer Bonetti, Cedar Crest College, Allentown

Abstract: Although microscopic hair comparison for associative purposes has largely been discredited, hair

evidence may still be helpful as an investigative tool. The goal of this research is to develop a predictive model for the determination of racial origin of hair using quantitative color measurements from photographs of hair taken under a stereomicroscope. Human head hair was obtained from ten European, ten African American, and ten Asian participants (self-reported). Photographs of each of the ten strands from each individual were imported into Adobe Photoshop to obtain the red, green, and blue (RGB) color values of the hair strands. Ten points on ten hair strands were examined to obtain 100 color value points for each individuals. The RGB values were then statistically analyzed to determine whether significant differences existed between the racial groups using nested ANOVA. The p-value for the comparisons between European, African American, and Asian groups were found to be less than 0.05 for red, green, and blue values showing differences between groups. A predictive model using principal component analysis and linear discriminant analysis was developed to discriminate between the racial groups. The results show that European, African American, and Asian hair color offers the potential for hair discrimination based on race due to the results of the proof of concept study.

Investigating the Effect of Collection Mechanisms and of Drying Nanoparticles that have on Gun Shot Residue Enhancement Using Laser Induced Breakdown Spectroscopy

Cameron Dwyer, University of New Haven

Abstract: The objective of this experiment is to see whether the "swab" or "stub" method is optimal for the collection of gunshot residue (GSR). This will be done by using predetermined Laser-Induced Breakdown Spectroscopy (LIBS) conditions to obtain spectra of the two methods, both using and without using Nanoparticle Enhanced Laser-Induced Breakdown Spectroscopy (NELIBS). Using the spectra obtained, a conclusion can be made about whether the "swab" or "stub" is the best method for collection for GSR. In addition, determine whether it is better to use NELIBS or not for analysis of the produced spectra.

Due to its sensitivity and non-destructiveness, Laser-Induced Breakdown Spectroscopy (LIBS) is a highly potential analytical tool for gunshot residue (GSR) analysis. In LIBS, a smaller portion of sample material is shot at by a high-energy laser pulse, creating a plasma that emits light at distinctive wavelengths. By examining the emission spectra, it is possible to decide the sample's elemental makeup. An important type of analysis in forensic investigations involving firearms is the examination of GSR. When a weapon is fired, microscopic particles known as GSR, which might contain the primer, propellant, and bullet components, are emitted. In order to identify the shooter and recreate the crime scene, GSR analysis is utilized to decide whether a firearm was discharged, the distance between the shooter and the target, and other significant facts.

However, the tiny amount of material that may be taken from the crime scene may restrict the sensitivity of detection of LIBS analysis for GSR. Swab or stub collection devices can be used to gather GSR particles, however, the volume of material may not be enough for sensitive LIBS analysis. Additionally, it can be challenging to collect GSR particles from the crime scene because they can be distributed all over the surrounding area.

Nanoparticle enhancement has been proposed as a method to improve the sensitivity and limit of detection of LIBS analysis for GSR. Nanoparticle enhancement involves the use of nanoparticles to amplify the signal intensity of the LIBS emission. The nanoparticles can be designed to bind to the GSR particles, leading to a stronger and more intense emission when the laser pulse interacts with the particles.

Procedural-wise for the effects of collection methods, there will be a training a safety process to understand the workings of the LIBS and what conditions are optimal. Once that has been completed, test runs with salts/elements such as barium, antimony, and lead will be performed to get an understanding of their spectra and characteristics that are portrayed through the LIBS. The next step will be to burn gunpowder to do another test to analyze the chemical makeup of it (using swabs and stubs). The professor then will be going to a gun range to fire approximately 10-20 rounds for stub and swab collection. The stubs will stay the same however, different solvents will be used with the swabs to have a comparison in spectra. Once all the spectra have been collected, an analysis will be done to see which collection method came out the clearest and most accurate. For the effects of nanoparticles on LIBS enhancement, metallic nanoparticles will be used to amplify the signal created by the laser matter. In turn, the sample will breakdown faster and will absorb the laser more causing the nanoparticles to heat the sample. The expected results are that the stub will be the better collection method and the use of nanoparticle enhancement will improve the results/spectra.

The development of an SEM-EDS based analytical routine for automated mineral identification
Jack Hietpas, Ph.D., John Jay College of Criminal Justice; Microtrace, LLC

Abstract: Geological materials, such as soil and surficial materials, are ubiquitous and often inadvertently transferred to people and objects during criminal events. Unfortunately, geological materials are one of the most underutilized and underappreciated forms of trace evidence. When properly analyzed and interpreted, soils and surficial materials can be a powerful form of physical evidence to help establish or refute associations between people, places, and objects. The limited use of this form of physical evidence stems from the need for highly specialized knowledge to analyze and interpret soil evidence. To help overcome this issue, we developed an automated mineral identification routine that leverages scanning electron microscopy - energy dispersive X-ray spectrometry (SEM-EDS) and two open-source software packages. For this research we utilized Desktop-Spectrum Analyzer-II (DTSA-II), a software package that was used to model and synthesize EDS spectra *in silico* from published mineral composition data, to develop a reference database that is easily customizable to accommodate different SEM/EDS configurations. Questioned and known minerals were identified (classified) using spectral matching algorithms written in R, an open-source data analysis language. The analytical method described in this presentation was designed to increase its ability to be potentially "operationalized" in crime laboratories. The method utilizes freely available software and the instrumentation and expertise that already exists in most forensic laboratories.
This presentation discusses the analysis of several hundred mineral samples from Microtrace's geological materials reference collection. These known mineral samples were used to evaluate, test, and optimize instrumental parameters and algorithm search criteria. More specifically, this presentation will cover sample preparation, EDS collection parameters, data post-processing, and the evaluation of multiple search algorithms. The strengths and limitations of this approach will be further explored by characterizing surficial soil samples collected from known locations across the three primary physiographic regions of North Carolina. The proposed method may provide quantitative and objective metrics for forensic soil analysis and its interpretation.

Investigating the Impact of Drying Nanoparticles on Gun Shot Residue Enhancement Using Laser-Induced Breakdown Spectroscopy (LIBS) & Scanning Election Microscpoy (SEM)
Marlee James, Alyssa M. Marisco, University of New Haven

Abstract: Forensic investigations heavily rely on identifying and characterizing gunshot residue (GSR) particles, traditionally employing techniques like scanning electron microscopy (SEM). However, emerging non-destructive methods like Laser-Induced Breakdown Spectroscopy (LIBS) offer rapid analysis potential. This project explores how drying nanoparticles (NPs) affect GSR enhancement using LIBS and SEM. It focuses on NP concentration patterns during drying and their potential to concentrate GSR particles. By comparing LIBS and SEM, the study evaluates sensitivity, selectivity, and concentration capabilities for GSR analysis and examines enhanced GSR particle characteristics. The research advances NP-assisted GSR analysis understanding and highlights LIBS and SEM benefits for forensic investigations.

Nanoparticles, with sizes ranging from 1 to 100 nanometers, possess unique properties. Drying NPs significantly impacts GSR particles, influencing their dispersion, aggregation, and spatial distribution. Depending on factors like NP characteristics and drying conditions, NPs can either disperse GSR particles evenly or aggregate them into clusters. NP drying can also affect GSR particle spatial distribution, potentially enhancing detection and localization using LIBS or SEM.

LIBS is a powerful analytical technique for studying the impact of drying nanoparticles on GSR enhancement. It allows elemental analysis by generating emission spectra with a high-intensity laser pulse. By analyzing emitted light, LIBS identifies characteristic GSR elements like lead, barium, and antimony. NPs present during drying can enhance the LIBS signal by concentrating GSR particles, improving sensitivity and reliability. LIBS also provides spatial mapping of GSR particles on surfaces, which can be influenced by drying nanoparticles, offering valuable information for forensic investigations.

To initiate GSR analysis, specific nanoparticles are selected for their interaction properties with GSR particles. These NPs are applied to the sample surface containing GSR particles. Controlled drying processes concentrate GSR particles, enhancing detectability. LIBS analysis is then conducted on the concentrated GSR

particles. The LIBS apparatus focuses a laser beam on the sample, generating plasma with distinct wavelengths corresponding to GSR elements. Analysis of these lines reveals the GSR particle's elemental composition, assessing LIBS sensitivity and selectivity. SEM analysis follows, where a conductive layer is applied to ensure image accuracy. High-resolution SEM images capture surface distribution and morphology. Results from SEM and LIBS analyses are meticulously compared, yielding insights into GSR particle characteristics, aiding forensic investigations. It's important to note that while this project is currently in progress, we anticipate having conclusive data by late October, which we will present at that time. This research is expected to advance NP-assisted GSR analysis understanding and highlight LIBS and SEM benefits for forensic investigations.

In summary, this research explores the impact of drying nanoparticles on GSR enhancement using LIBS and SEM. NPs play a crucial role in concentrating GSR particles, improving detectability and spatial mapping. LIBS offers powerful elemental analysis and mapping capabilities, while SEM provides high-resolution images. By comparing the two techniques, this study advances our understanding of GSR analysis and its potential benefits for forensic investigations.

"Fireworks in the Professional Field"

Paul Nichols, Sr., NRA Instructor and Chief Range Safety Officer, ATF Pyrotechnics Licensed, Massachusetts State Licensed Lead Pyrotechnician, FCC Licensed Extra Class Amateur Radio Operator, Former Board Member of Gun Owners Action League

Evaluating Chemical Attribution Signatures of Gasoline Using DART-MS and Chemometrics

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Abstract: Gasoline is the most common ignitable liquid utilized as an accelerant in arson cases due to its wide availability and efficacy in starting fires. Therefore, rapid and reliable techniques are necessary for the analysis of gasoline from fire debris evidence in forensic arson investigations. However, while fire debris analysts can be confident in the identification of gasoline, no one within the forensic science community has shown convincing data leading towards source origin determination. Gasoline additives serve as chemical attribution signatures (CAS) and are often referred to as biomarkers during environmental remediation effects. Source attribution using CAS can be an asset as the identification of a gasoline's source would be a powerful investigative tool for law enforcement and other agencies conducting arson investigations. Direct analysis in real time-mass spectrometry (DART-MS) has been employed for the analysis of gasoline samples from different source locations and has been shown to be effective for gasoline source attribution for different brands. DART-MS has been shown to be useful for the ionization of higher molecular weight non-hydrocarbon additives, which correspond to different CAS in gasoline.

Twenty-one gasoline samples were collected across Massachusetts, New Hampshire, Rhode Island, Connecticut, New York, New Jersey and Pennsylvania representing many of the states within the NEAFS region. DART-MS data were collected for each gasoline sample in replicates of ten. The data was grouped based on geographic location and evaluated by Principal Component Analysis (PCA) using Mass Mountaineer Software. DART-MS data across geographical groups was found to have varying levels of similarity and difference through visual examination of the mass spectra. PCA exhibited distinct groups of individual gasoline samples tested across the geographical regions, with three out of six geographical groups showing no overlap between gasoline classifications. The PCA and comparison of DART-MS data provides evidence of successful differentiation between Mobil gasoline samples across Massachusetts, New Hampshire, and Connecticut. As gasoline stations receive new shipments over time, future research aims to conduct a time course study to determine if additives in gasoline samples from the same source differ over time as mixing occurs within the underground storage tanks.

Northeastern Association of Forensic Scientists

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Criminalistics, Crime Scene & Digital Evidence Abstracts

The Key to Successful Research Collaborations

Laura Tramontin, Henry Maynard, American Society of Crime Laboratory Directors, Forensic Research Committee

Abstract: Over the last few years, the American Society of Crime Laboratory Directors (ASCLD) Forensic Research Committee (FRC) has created and launched tools to help advance forensic science research and further research collaborations within the forensic science community. From this presentation, participants will learn about forensic science research needs, the Laboratories and Educators Alliance Program (LEAP) which enables research partnerships, repositories for forensic research, evaluation, and validation efforts, executive research summaries, the research collaboration hub, Lighting Talks, and more! This presentation is for individuals who are looking to become more engaged in forensic science research and want to learn more about the opportunities that the ASCLD FRC is creating to benefit the forensic science community. For more information about these initiatives or other ASCLD FRC Initiatives please review the ASCLD FRC Website: https://www.ascld.org/forensic-research-committee/

The goal of the Laboratories and Educators Alliance Program (LEAP) is to facilitate partnerships between academia and forensic science laboratories. This joint effort between the American Society of Crime Lab Directors (ASCLD) and the Council of Forensic Science Educators (COFSE) promotes strategic partnerships between forensic science laboratories and academia, ensuring high-quality academic research is aligned to address critical challenges within the forensic science community. Benefits and Potential Opportunities through LEAP Partnerships include: Laboratory Recruitment, Internship Opportunities, Subject Matter Expert (SME) Information Exchanges, Curriculum Assistance, Practitioners as guest Instructors/Speakers, Collaboration on Research Project Design/Planning, Support for Testing & Evaluating Methods, Statistical Consulting/Support, Joint Presentations/Publications, etc.

The FRC Collaboration Hub connects researchers and practitioners to promote active engagement and participation to support forensic science research projects. The FRC Collaboration Hub provides a "one-stop-shop" for researchers to solicit participation in specific projects and for practitioners to contribute their knowledge and experience to support research projects. Through the FRC Collaboration Hub:

Forensic science practitioners can quickly and easily identify research projects related to their field of expertise and connect with the researchers to contribute to the success of the research while also advancing their field. Practitioners gain additional professional development opportunities while supporting and engaging in research projects.

Academic researchers can broaden their outreach and participant solicitation efforts by posting in the FRC Collaboration Hub. Researchers gain access to a large network of forensic science laboratories and practitioners which helps ensure the success of their research project.

The FRC Evaluation/Validation Repository promotes transparency, information sharing, and synergy between forensic science laboratories, researchers, and other stakeholders within the criminal justice system. The Evaluation/Validation Repository provides a centralized location for evaluation and validation plans, methods, results, reports and data to be stored. This provides accessibility for other forensic science laboratories and stakeholders to coordinate, collaborate, and build upon existing efforts to break down operational silos and strengthen forensic science practices as an entire community. By working together, the community can better characterize the performance and increase the robustness of studies assessing the validity and reliability of technologies or analytical methods used in practice.

Determining Fluid Dynamic Properties of the Interaction of Blood on Surfaces of Different Roughness Values at Varying Angles of Impact

Autumn Reynolds, Dr. Paul V. Quinn Sr., Cedar Crest College

Bloodstain patterns are used within crime scene reconstruction to illustrate past events that occurred at the scene. Impact spatter is one type of bloodstain pattern created when a source of liquid blood receives a force resulting in the dispersion of smaller stains onto nearby surfaces at various angles. These angles can be combined to locate the area of origin of the spatter; however, discrepancies exist between current models and the data gathered from impact spatter patterns at crime scenes. One such discrepancy could be due to the interaction of the blood with various types of surfaces since current models only exist for smooth, non-porous surfaces. Most surfaces found

at crime scenes are not smooth and non-porous but rather have variations of roughness and absorption characteristics. The lack of applicable models for the wide variety of rough surfaces at a crime scene may possibly result in forensic reports with inconclusive or weakly supported conclusions. This research project creates an empirical model to account for a variation of roughness in various surfaces to increase the accuracy of conclusions made when analyzing impact spatter at a crime scene. The roughness of various surfaces was measured and the flow of blood on those surfaces was observed at varying angles. These empirical results are used to derive a roughness factor which is an adjustment applied to previous models for smooth surfaces by accounting for roughness. This will allow for a more accurate description of the interaction between the blood and the surface on which it flows, and therefore, more accurate conclusions when analyzing impact spatter.

Influence of Handwashing and Handedness on Latent Fingermark Aging as Analyzed by 2D and 3D Imaging

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Abstract: Latent fingermark aging studies aim to determine the 'who' and the 'when' from crime evidence. Understanding how fingermarks age under different influential factors, such as donor and environmental conditions, is essential for establishing time-since-deposition at the scene. [1] The 3D evaluation of fingermark aging processes with an optical profiler (OP) has already been proven and demonstrated to be a formidable contactless method. This has been achieved by using micrometer variations of the ridge height and volume. [2] Additionally, the concurrent use of traditional 2D enhancing methods with 3D imaging, such as color contrast and visual quality metrics, has shown potential to advance knowledge in this forensic subdiscipline. Previous studies have focused on the influence of biological sex, substrate type, temperature over an extended period of time [2,3] as well as the ability to develop and recover fingermarks from various environments [4,5] . However, these have provided limited data on how other factors, such as handedness and handwashing, may affect natural aging. To fill the gap of knowledge on how latent fingermarks age in response to different environmental factors, impressions from four donors were aged on glass microscope slides at room temperature in complete darkness. A selection of fingermarks was analyzed every 48 hours over a period of 192 hours by 3D and 2D imaging. The former was performed with an OP on untreated impressions while the latter used a Canon camera after powdering with titanium dioxide (TiO 2) powder. The metrics evaluated in 2D are the visual quality score (QS), the color contrast (MI and IA), and the ULW color-coded ridge clarity map (BG). For 3D-OP, the metrics evaluated are average surface height (Sa), the squared height of the ridges(Sq), and the volume 'up' and volume 'net', which detect the volume of the area of the surface. ANOVA tests were conducted to assess the effects of environmental factors and the reliability of 2D and 3Dmetrics. Preliminary results revealed no statistical differences in fingermark aging between washed andunwashed hands. However, handedness was a significant factor where fingermarks from the dominant hand degraded at a faster rate than their nondominant. Research, such as this one, is of paramount importance for time-since-deposition determinations in the future.

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Towards Development of a Mass Spectral Approach to Facilitate More "Eggs-act" PMI Determination Using Entomological Samples

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Abstract: Medico-legal forensic entomology focuses on utilizing carrion insects that colonize human and animal remains to estimate the time established since death, also known as postmortem interval (PMI). Blow flies within the Calliphoridae family are often the earliest colonizers to arrive, as they can detect remains within minutes of death. The insects use the remains as a feeding, breeding, and oviposition medium. Knowledge of their species-specific life cycle timelines is well understood and as a consequence, it is possible based on the age of the retrieved evidence, to approximate PMI by estimating when the eggs from which the maggots hatched were laid. In this regard, knowledge of the species identity of the insects is essential because insect development timelines vary as a function of species. However, accurate and rapid species identification remains a prevailing challenge because the immature life stages that are most often collected (i.e., eggs, larvae, and pupae) are visually similar across species.

Traditional methods for species identification of necrophagous insects are time-consuming, and the accuracy of the results can be influenced by factors such as the manner of death, temperature, and weather conditions. If the specimens are viable, an experienced entomologist can rear them to adulthood to make a species identification based on the visually apparent gross morphological features. Here, we present an alternative rapid method that utilizes mass spectrometry and chemometrics to determine species identity, which can be used facilitate estimation of PMI, and demonstrate its application to necrophagous insect eggs. When 70% ethanol suspensions of insect eggs representing Calliphora vicina, Ca. vomitoria, Cynomya cadaverina, Lucilia illustris, Lu. sericata, and Phormia regina were analyzed by direct analysis in real time – high-resolution mass spectrometry (DART-HRMS), intraspecies similarities and inter-species differences were observed. Chemometric processing such as Kernel Discriminant Analysis of the mass spectral data, revealed clustering as a function of species identity, and enabled species identification of eggs with an accuracy of 87.35%. Furthermore, analysis of the volatiles emitted by the eggs of one species, Lucilia sericata, as a function of time, was conducted by concentrating the volatiles on solid phase microextraction (SPME) fibers and analyzing the adsorbed compounds by gas chromatography-mass spectrometry (GC-MS). The results showed that as the eggs developed, a range of volatiles were emitted. This finding suggests that the emission of particular compounds might be correlated with the age of the eggs, and this observation could offer valuable insights to investigators regarding the age of the evidence, and by extension, a more precise PMI interval. Future investigations aim to establish statistical models that are able to accurately identify entomological evidence based upon species-specific chemical signatures, thereby increasing the evidentiary value of immature insect life stages.

Biological Stain Identification in Binary Mixtures by Raman Spectroscopy Coupled with Chemometrics for Forensic Applications

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Abstract: During a crime scene investigation, any source of DNA material can be vital to the case. For this reason, the collection of biological stain samples is carefully performed and identified before it can be used for DNA profiling. However, the presumptive and confirmatory tests of most current techniques are destructive, consuming a substantial amount of sample and time, and some are even target-specific, which could lead to an incorrect result. Here, we present the integration of Raman spectroscopy coupled with statistical analysis to identify biological stains, more specifically, binary mixtures of body fluids. These complex mixtures of body fluids can be challenging to identify, especially during the presumptive test analysis phase, due to the biological target specificity in these tests. On the other hand, Raman spectroscopy can be used to obtain a spectral signature of the biological stain. With the help of visual interpretation and statistical analysis, we can differentiate between a sample containing one single fluid and a mixture sample, and possibly the type of mixture. This could save time and money during an investigation and preserve valuable trace evidence needed for the case. In this project, we evaluate this combined method of Raman spectroscopy and statistical analysis to study complex samples involving dry mixtures of blood, vaginal fluid, and semen. Raman spectra were collected from thoroughly mixed samples using a 785 nm laser and a confocal spectrometer. Discriminant Analysis (DA) models were developed

to differentiate mixed samples from pure body fluids at different mass percent ratios (% m/m). Raman interferences were also analyzed using these models to detect the body fluid mixture stains in situ on textile substrates. Results showed that mixed samples can be discriminated against and that certain body fluids contribute more than others in the classification analysis.

Cognitive Bias Mitigation Techniques: Overcoming Barriers and Finding Solutions

Taylor Hopkins, Duquesne University, Sandra Sachs, Ph.D., Oakland Police Department Criminalistics Laboratory, Mandy Tinkey, M.S., Allegheny County Office of the Medical Examiner, Lyndsie Ferrara, Ph.D., Duquesne University

Abstract: One of the challenges in the modern forensic science world is acknowledging and mitigating bias. The 2009 National Academy of Sciences (NAS) report, Strengthening Forensic
Science in the United States: A Path Forward, recommendation number 5 called for more research on bias, its impact on professionals in the field, and solutions to help remedy its effects. 1 Much research on the topic has focused specifically on cognitive bias within the field of forensic science. Cognitive bias refers to variance in an individual's sound judgement or decision-making and is caused, in part, by emotional inputs and social influences. 2 While often carrying a negative connotation, such bias is a natural phenomenon that can unintentionally impact an examiner's means of collection, analysis, and/or final conclusions in relation to a case. Literature provides a variety of potential solutions such as the case manager or linear sequential unmasking models to reduce the risk of bias in the laboratory. 3, 4 While these and other mitigation techniques may appear simple in concept, implementation of such techniques requires effort and commitment to overcome barriers. This project investigated the specific barriers to implementing techniques faced by forensic laboratories across the United States. Those employed by forensic laboratories in the U.S. were contacted primarily via email and asked to voluntarily participate in an interview or complete a survey. Regardless of the mode of participation, participants were asked to share their role in their laboratory, knowledge of bias mitigation methods, what methods, if any, are being used at their laboratory of employment, and what barriers the laboratory faced to implement such changes, if applicable. Furthermore, they were asked to share what solutions they felt were needed to make implementation of such techniques easier. While a majority of participants answered affirmatively to the need for bias mitigation methods within the laboratory, barriers such as lack of lab-wide training, support, and resources make implementation difficult. These and other barriers provided by participants, along with proposed solutions, gave insight into possible organizational changes to implement when attempting to reduce the effects of cognitive bias. Overall, input from over 50 professionals ranging in position and experience, revealed relevant information that can help the field better understand current stances and future steps regarding the discussion of bias in forensic science.

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Updates from the Organization of Scientific Area Committees (OSAC) for Forensic Science: Standards Development, Implementation, and a Call for Participation

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Abstract: Published in 2009, the National Research Council (NRC) Report "Strengthening Forensic Science in the United States - A Path Forward" criticized the practice of forensic science in America for (among other things) its failure to have in place a network of nationally recognized, consensus-based standards with scientific merit. In answer to this call, NIST and the U.S. Department of Justice (USDOJ) responded in 2014 by creating the Organization of Scientific Area Committees (OSAC) for Forensic Science. With its primary mission to strengthen the nation's use of forensic science by facilitating the development of scientifically sound standards and guidelines and encouraging their use throughout the forensic science community, the members of OSAC have been working diligently for the past ten years.

In its current state, OSAC consists of seven Scientific Area Committees (SACs) encompassing 22 subcommittees with representation from more than two dozen forensic science disciplines. There are over 400 members and 300 affiliates from all over the world volunteering to contribute to OSAC's ongoing efforts. As of August 2023, more than 150 standards have been posted on the OSAC Registry and more than 130 forensic science service providers (FSSPs) have declared implementation of many of these standards in their laboratories.

Over the past ten years, OSAC has built an infrastructure that supports the review and revision of existing standards, the development of new standards, and the shepherding of documents through the standards development process. The primary goal of these activities being the population of the OSAC Registry with thoroughly vetted, high quality documents. As the number of standards on the OSAC Registry continues to increase, the focus of the program has recently shifted towards implementation.

This presentation will provide a brief review of the OSAC structure, discuss the progress that has been made over the last ten years, and provide a more in-depth overview of the ongoing implementation efforts. Additionally, information regarding the OSAC Research and Development needs program will be provided.
It is important to note that OSAC activities rely heavily on the voluntary efforts of practitioners from within and surrounding the forensic community. To this end, various opportunities for input, participation, and assistance will also be presented.

Improving CSI Response: An Early Roadmap for the Increased Quality and Effectiveness of Crime Scene Investigations

Joe Treviño, New York City Police Department, Michael Kessler, Denton Police Department

Abstract: The Crime Scene Investigation Subcommittee within the Organization of Scientific Area Committees for Forensic Science (OSAC) has highlighted the need for empirical research on adequate crime scene investigator (CSI) staffing levels via crime scene response. OSAC, policymakers, and fellow professionals cannot make appropriate recommendations to increase the quality and effectiveness of an investigation with staffing or crime scene response because of this data gap.

Other related professions benefit from full demographic studies and censuses that detail the tasks and services performed, which in turn make things like policymaking, budgeting, and improvements easier to do. Crime scene investigation is unique in that it exists inside and outside of the sphere or law enforcement. It is a mixture of civilian and sworn personnel, hybridized in some areas, and at all levels of government; and can be performed by part-time and full-time personnel, who may or may not be crime laboratory personnel who were cross-trained or are responsible for all the crime scene response of their employer. No matter the level or layer of employment, CSIs face the same tasks at a scene and only get one chance to do things correctly. That is compounded by the unique pressures they face from their employer or agency. That again is also compounded by work factors outside their control.

A study was designed to investigate what CSIs considered adequate response via a survey with questions that targeted: the factors affecting the quality and effectiveness of an investigation, what is feasible to handle alone without sacrificing quality and effectiveness, when extra staffing is needed, the barriers to hiring more personnel, and what the amount of extra staffing needed is within the focus established by the OSAC CSI Subcommittee. The survey was taken by both investigators and managers across many levels of government and

in different areas of the United States.

With few exceptions, there was consensus between investigator and manager responses throughout the study. The study established: which investigations are more resource intensive; which types of scenes are more resource intensive; which tasks increase the complexity and in turn, increase the need for improved response; how increased task loads affect investigations; where the stresses of an investigation affect the quality and effectiveness of an investigation; and early answers to what might be preventing increased staffing to alleviate inadequate crime scene response.

The data can be used for quick pitches for extra personnel to supervisors or command staff or for more meaningful conversations about staffing and CSI response throughout our discipline. Forensic science providers can use these numbers and address staffing or response according to their agency-specific needs, thereby increasing the quality and effectiveness of crime scene investigations at a customizable scale.

It is recommended that future research focus on more data and responses to these types of questions as well as address the lack of specificity of employment numbers. Full-scale studies on either of the two topics requires large amounts of time and resources, and one large study might be too unwieldy to administer. A pragmatic approach would be to collect a more robust data set on quality and effectiveness and then extrapolate on a complete employment survey of the discipline.

Tire failure – accidental or intentional

Pete Diaczuk, Ph.D., John Jay College of Criminal Justice

Abstract: This is a case study about a truck tire failure that was blamed on the truck operator. The tire failure caused the truck operator to lose control and crash the vehicle. Upon examination of the damage to the tire, the accident investigator at the scene accused the driver of the government vehicle of sabotaging the tire after the accident took place to blame the tire instead of admitting he made the mistake while driving the vehicle. The truck driver admitted he brushed against the curb, and the tire immediately blew out, but the driver was fired for trying to conceal his error by stabbing the tire sidewall with a knife. A close examination of the hole in the tire sidewall revealed evidence of the true cause of the failure. The Polarized Light Microscope is the workhorse for trace evidence analysis. Polarized Light Microscopy of the damaged reinforcing fibers in the sidewall confirmed whether the tire failed on its own or the driver sabotaged the tire by stabbing it.

Northeastern Association of Forensic Scientists

Proceedings of the 2023 Annual Meeting

Poster Session Abstracts

P1. Recovering Trace Biological Evidence from Archival Samples

Natalia Pedraza, Michael Marciano, Forensic and National Security Sciences Institute – Syracuse University

Many times, forensic evidence is highly limiting, therefore the ability to obtain intact cells or DNA from evidentiary items such as slides can be highly impactful to the investigations. Typically, this problem is associated with older samples. Although methods to remove coverslips and recover tissue have been done, this method does not translate well to forensics due to the sometimes-limited amount of target cells and the presence of mixed samples where only one cell type may be of interest. This project focuses on developing a method to remove coverslips from slides, the collection of the cells and the isolation of the DNA from forensic samples. Slides collected in forensic cold cases may be decades old with minimal amounts of target cells (potential perpetrator), making the cells more fragile. Thus, an approach must be taken to ensure the cells are collected efficiently and with care to avoid loss or further damage to the cells. This is an in-progress study.

This approach will seek to develop a method for coverslip removal from slides that were mounted using Eukitt or Cytoseal. This method will use humidification rather than any harsh chemicals such as xylene that may degrade or destroy cells or DNA. This method has shown to be effective at removing coverslips and yielding DNA profiles from the slides. This in progress study will continue attempting to isolate decreasing amounts of cells from slides as well as employ the use of the DEPArray to isolate cells when only few exist on the slide.

P2. The Comparative Evaluation of EESClt Likelihood Ratios for Single Cells Across Two Models

Liliana Berrios, Graduate Program of Forensic Science, Rutgers University; Madison M. Mulcahy, Department of Chemistry, Rutgers University; Nidhi Sheth, Center for Computational and Integrative Biology, Rutgers University; Desmond S. Lun, Department of Computer Science, Rutgers University; Catherine Grgicak, Department of Chemistry, Rutgers University

Interpreting electropherograms can be an arduous task as bulk mixture pipelines produce electropherograms containing information from any number of, potentially partial, contributors, rendering Weights of Evidence (WoE) that approach zero as the mixture becomes more complex. Single cell treatments offer a way forward, by isolating, amplifying, and analyzing each cell, individually. This creates a set of electropherograms (EPGs) for each cell isolated, which are then grouped by similarity into clusters, where it is reasonable to assume a single common donor. By supposing the group of single cell EPGs (scEPGs) are replicates of one another, the logarithm of the likelihood ratio – i.e., WoE – for the cluster can be determined by comparing the probabilities of the cluster given a Person of Interest (PoI) contributed divided by the probability given a random person contributed. Though a variety of clustering approaches exist, we accomplish this by the model-based clustering application *mclust* in R. Once clustered, the group of scEPGs are evaluated and the LR for each cluster was calculated with EESCIt™, which stands for **Evidentiary Evaluation of Single Cells.** With EESIt™ being able to rapidly and reproducibly assess any number of scEPGs in any number of clusters in seconds, we perform a large-scale analysis on the implementation of two models to the EESCIt™ system: that of the normal and log-normal distributions to describe peak heights.

Specifically, 1,210 single cells were processed through a validated single cell pipeline to produce 1,210 scEPGs. The scEPGs were tested in EESCIt against the true contributor, s_{true}, and a false contributor, s_{false}, to produce logLRs with both models. As a result, there were 1,210•4 = 4,840 outcomes that were explored. When testing a sample against its true contributor, a positive logLR value is expected. Similarly, when testing a scEPG against a false contributor, a negative logLR value is expected.

Adhering to SWGDAM's guidelines for the validation of probabilistic genotyping systems, we tested the sensitivity of each model by calculating the proportion of scEPGs for which the logLR$(scEPG, s_{true}) > 0$, and tested the specificity by calculating the proportion of scEPGs for the logLR$(scEPG, s_{false}) < 0$. Preliminary

results show that the normal peak model resulted in a logLR($scEPG, s_{true}$) > 0 of 89.4% and a logLR($scEPG, s_{false}$) < 0 of 94.3%. The log normal model resulted in a logLR($scEPG, s_{true}$) > 0 of 88.4% and a logLR(EPG, s_{false}) < 0 of 92.4%. Notably, the reported sensitivities and specificities include the results when the scEPG carried minute levels of information or contained much allele drop-out. Therefore, in this study we go further and will report the robustness of these models by evaluating the logLR for each state, s_{true} or s_{false}, across total peak intensity. Additionally, Type I and Type II errors will be explored by genotype. The results for both models will be compared in the aggregate to determine which one to implement for single-cell applications.

P3. The Sound of Gunfire

Andrew J. Winter Ed.S., Middlesex County Prosecutor's Office; Peter J. Diaczuk, Ph.D., John Jay College of Criminal Justice; Ashley Buchman, Centenary University

Often the sound of gunfire is confused with other sounds like fireworks, machinery, etc. This confusion impacts the false reporting of alleged gunfire incidents to law enforcement. The purpose of this research is to document the sound of gunfire utilizing a sound meter and comparing it to that of non-firearms. The data collected will be documented and analyzed as part of this research. A total of six firearms, one blank gun (Hollywood gun), and four varieties of fireworks were used. The firearms and fireworks were discharged from two distances: approximately 50 feet and 200 feet from the recording devices. The sound of each discharge was recorded using an MP3 player application and a sound meter. This experiment was conducted in wooded terrain in a residential area commonly seen at crime scenes.

P4. Assessing PreCR Repair Mix for Effectiveness in the Repair, Interpretation, and Deconvolution of Y-STR DNA Mixtures

Alexandrina Durkee, University of New Haven

Advances in the technology and methodology of forensic DNA analysis has allowed for DNA mixtures and low level DNA to make up an increasing amount of evidence samples. Thus, the repairing and deconvolution of damaged mixture samples is the logical next step in furthering the applicability and accuracy of this field. This research focused on determining if there is a way to recover and repair sufficient information to help deconvolute and identify donor profiles in Y-STR mixtures, as well as if the repair process influenced the resulting peak height ratios. This research utilized the Y-Filer+ PCR amplification kit (Thermofisher Scientific) on UV damaged DNA and PreCR® (New England Biolabs) repaired DNA, from saliva samples in a 1:1 two-person mixture.

The peak height ratios of non-repaired mixtures versus repaired mixtures showed a slight decrease in haplotype loci peak height ratios and a larger decrease in diplotype-like loci peak height ratios, thus demonstrating the PreCR® repair mix did in fact influence the peak height ratios. Analysis on diplotype-like locus DYS385 demonstrated PreCR's® potential ability to be able to deconvolute 1:1 Y-STR mixtures by decreasing the peak height ratio of the diplotype-like loci. However, the unpaired T-Test performed for the statistical analysis of this data determined the data obtained was not statistically significant. If PreCR® mix is found to have an effect on allelic peak height, especially within mixtures, it would be an indication that PreCR® repair mix either shouldn't be used in forensic crime labs or should be used very carefully, due to the weight allelic heights and peak height ratios have on mixture analysis. This research highlights the potential benefits and drawbacks to repairing evidentiary DNA samples and the need for additional research before evidentiary items are attempted to be repaired and deconvoluted in forensic laboratories.

P5. Separation of Spores from Complex Matrices for Single Cell Characterization

Julia Dirre, Syracuse University

Biological threat agents have historically been used for bioterrorism and warfare, emphasizing the significance of the identification of the agents for investigative purposes. The escalating risks of these agents being used have remained a continuous concern. Numerous microorganisms, like bacteria, can be used as potential agents for these attacks. For instance, *Bacillus anthracis,* a bacterium that is found in soil, causes the infectious disease anthrax. This bacterium can generate spores that can persist in soil for extended periods and pose a severe threat upon entering the human body. The current identification methods are time-consuming and often insufficient, particularly when dealing with complex matrices. These methods are used to isolate the target cells from the matrix before doing genomic sequencing.

In this study, *Bacillus subtilis* was examined and analyzed as a substitute for *Bacillus anthracis* due to their similar morphologies as members of the same genus. Both species are sporulating, rod-shaped, and aerobic but *B. subtilis* is nonpathogenic, making it safer to handle. Additionally, *B. subtilis,* like *B. anthracis*, is found in soil and is a gram-positive bacterium. *B. subtilis's* distinct size and shape facilitate easier identification, thereby speeding up the identification process.

The study investigates the application of the DEPArray™ system to separate various cells within a mixture, specifically focusing on separating *Bacillus subtilis* from complex matrices like soil. The DEPArray™ system enables the automated isolation of cells from complex matrices and the collection of individual cells through fluorescence-based methods. By employing antibodies labeled with fluorophores, the DEPArray™ system can differentiate between various organisms based on their fluorescence pattern. This is achieved through the antibody-antigen binding exclusively to target species. Additionally, the DEPArray™ system provides high-resolution images of the cells as they transverse through the device. These images of the cells aid in the elimination of false positives or cell clusters, ensuring that only the target cells are collected. Moreover, the images enable the identification of the correct target cells based on their fluorescence intensity and morphology. The target cells can then be used for sequencing.

This study employs single-cell or "trace" amplification and sequencing techniques to identify and characterize isolated cells. The sequencing process quickly generates accurate genome sequences for downstream identification purposes and facilitates comparative studies. For sequencing, the study utilizes the minION flow cell with Nanopore technology. The minION is beneficial due to its ability to give near real time data, enabling rapid and actionable results.

P6. Multi-ancestry Phenome-wide Association Study of SNPs Used to Predict Externally Visible Characteristics in a Forensic Setting

Brendan Newton, University of Toronto, Mississauga; Kairavi Parikh, Ellie Wan, University of Toronto, Mississauga; Cristina Abbatangelo, Mary Anne Panoyan, Esteban Parra, Department of Anthropology, University of Toronto, Mississauga; Frank Wendt, Forensic Science Program & Department of Anthropology, University of Toronto, Mississauga; Michela Marcellino, Department of Epidemiology, Dalla Lana School of Public Health

Background: HIrisPlex-S and SNIPPER forensic DNA phenotyping tools permit prediction of eye, hair, and skin colour. These phenotyping tools accurately predict extreme colour classifications (e.g., blue versus brown eyes) but struggle to predict intermediate phenotypes (e.g., green eyes) with the same accuracy. Pigmentation genetics varies greatly across populations, but little has been done to evaluate the pleiotropic effects (the influence of a single variant on the phenotypic expression of multiple traits) of these single nucleotide polymorphism (SNP)-trait associations across different ancestries. To this extent, we performed 368,458 SNP-trait associations with SNPs present in HIrisPlex-S and/or SNIPPER panels to detect potential off-target effects

of pigmentation prediction panels.

Methods: The UK Biobank (UKB) is a population-based cohort of >500,000 participants with genotype and deep phenotypes related to diet, anthropometrics, mental health, body imaging, etc. Participants are genetically classified into six ancestry groups: African (N ≤ 6,636), admixed American (N ≤ 980), Central/South Asian (N ≤ 8,876), East Asian (N ≤ 2,709), European (N ≤ 420,531), and Middle Eastern (N ≤ 1,599). There are 55 SNPs in the SNIPPER and HIrisPlex-S panels. UKB samples have information for 52 of these SNPs: 14 SNIPPER-exclusive SNPs, 32 HIrisPlex-S-exclusive SNPs, and 9 SNPs found in both panels. SNP-phenotype association was performed with linear or logistic mixed models in SAIGE including a kinship matrix as a random effect. Age, sex, age-by-sex, age^2, age^2-by-sex, and the first 10 within-ancestry principal components were used as fixed-effect covariates. Enrichment of trait domains was performed with hypergeometric tests. Nominal p-values were adjusted using false discovery rate (FDR) multiple testing correction to account for possible linkage disequilibrium between SNPs, panels, and UKB phenotypes. A more stringent Bonferroni correction also was applied to determine the robustness of enrichment findings.

Results: Despite individual SNPs associating with dermatological traits across all six populations, many SNPs also had genome-wide association ($p<5x10^{-8}$) with other domains, including psychiatry, cognition, and body structure. HIrisPlex-S and SNIPPER SNPs were unsurprisingly enriched for dermatological traits (12.05-fold to 39.76-fold in the HIrisPlex-S panel and 13.0-fold to 39.76-fold in the SNIPPER panel). Additionally, full panels were enriched for psychiatric (1.42-fold to 2.53-fold in HIrisPlex-S, 1.51-fold to 2.40-fold in SNIPPER) and hematological (1.05-fold to 2.16-fold in HIrisPlex-S, 1.33-fold to 3.11-fold in SNIPPER) associations across all populations. Cognitive (1.86-fold to 3.79-fold in HIrisPlex-S, 1.70-fold to 3.24-fold in SNIPPER) and ophthalmological (1.06-fold to 1.48-fold in HIrisPlex-S, 1.08-fold to 1.98-fold in SNIPPER) traits were enriched for all but European and admixed American populations respectively across both panels. Body structure associations were enriched across all populations using HIrisPlex-S SNPs (2.33-fold in AFR, 2.76-fold in EUR, 3.08-fold in MID) but were scarcely enriched among SNIPPER SNPs (2.33-fold to 3.08-fold, p-values>0.05).
Conclusion: We performed a large-scale multi-ancestry phenome-wide investigation of SNPs used to predict externally visible characteristics in the UKB and uncovered thousands of potentially informative off-target effects. Though not individually predictive of EVCs, further investigation into their additive pleiotropic effects will inform potential biases in commonly implemented pigmentation predictive panels.

P7. Unraveling Clues from Cigarette Butts: Analyzing DNA Extracted from Gasoline-Soaked Filters
Faith Ruggiero, Bay Path University

Perpetrators attempting to start a fire may throw a lit cigarette into gasoline, examples of which include cigarette butts being found in automobile tanks or puddles of gasoline. These cigarette butts do not normally result in a fire but rather are extinguished in the liquid, and as a result gasoline-soaked cigarette butts are found at crime scenes. These cigarette butts have the potential to contain valuable DNA evidence that can assist investigators in identifying the perpetrators. Gasoline is a volatile, flammable mixture of liquid hydrocarbons derived from petroleum, and as a result is a powerful solvent. This has led crime scene responders to question whether usable DNA profiles can be generated after prolonged exposure to gasoline. A study was performed to determine whether gasoline affects the structural integrity of DNA and thus the quality of the DNA profiles obtained from gasoline-soaked cigarette butts. The study examined whether usable DNA can be collected from smoked cigarette butts soaked in gasoline for 2 hours, 1 day, 7 days, and 28 days. DNA was extracted from twelve soaked butts using an EZ1 Biorobot, quantified with the Quantifiler Trio kit, and amplified using the Globalfiler STR Kit. The DNA profiles were generated with a 3500 Genetic Analyzer. DNA quantification indicated slight degradation in only one of the three 1-day samples, while the rest of the samples showed no

significant degradation. All twelve samples were amplified and produced complete DNA profiles. This work demonstrates that usable DNA can be extracted from cigarette butts exposed to gasoline and supports crime scene responders in collecting these possibly valuable items of evidence.

P8. Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC/MS/MS

Emily Eng, Kevin Crowshaw, Stephanie Reichardt, United Chemical Technologies

Xylazine is a veterinary sedative that has been emerging as a popular adulterant in the illicit drug market. It is often seen with powders and tablets containing fentanyl. This combination of drugs is commonly referred to as "Tranq". Although xylazine is not an opioid, its use with fentanyl is having a major impact on the opioid epidemic for a number of reasons. It can induce a state of unconsciousness, worsen addiction, potentially increase the risk of fatal overdose, and its effects are not counteracted by naloxone. According to the DEA, in 2022, xylazine was detected in 48 out of 50 states. Of all of the fentanyl positive samples tested by the DEA, 23% of powder samples and 7% of tablet samples also contained xylazine. The trends of xylazine usage parallel to fentanyl, indicating that it is likely to persist. This poster details an effective method for the simultaneous analysis of fentanyl, fentanyl analogs, xylazine, and other common adulterants by SPE and LC-MS/MS.

UCT's Clean Screen® DAU cartridges were utilized for extraction. Samples were prepared at low, medium, and high concentrations of 5, 25, and 80 ng/mL from a stock solution standard mix. Urine samples were prepared by adding 1 mL of sample, 0.5 mL of methanol (MeOH), and 2.5 mL of phosphate buffer (pH 6.0, 0.1 M). Blood samples were prepared by diluting 0.5 mL of sample in 3 mL of phosphate buffer, followed by mixing and centrifugation. DAU cartridges were conditioned with MeOH and equilibrated with water and phosphate buffer. The samples were loaded onto the cartridge and washed with 100 mM HCl followed by MeOH. The target analytes were eluted with 2% ammonium hydroxide in MeOH. After evaporating, the extracts were reconstituted in 5% MeOH.

Samples were analyzed on SelectraCore® DA UHPLC column (100 mm x 2.1 mm, 2.7 μm) using a Shimadzu Nexera LC-30AD with MS-8050. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B was MeOH.

Analytes were successfully extracted from urine and blood with high recoveries and low matrix effects at low, medium, and high concentrations of 5, 25, and 80 ng/mL (n=3). Extraction recoveries of analytes from urine ranged from 88-119%. Aside from acepromazine, which was not the focus of this panel, relative standard deviations (RSDs) were ≤ 20% and matrix effects were within ± 25%. Extraction recoveries from blood ranged between 80-113%, RSDs were ≤ 20%, and matrix effects were within ± 25%.

Para-, meta-, and ortho-fluorofentanyl were effectively separated on the LC-MS/MS method utilizing the SelectraCore® DA UHPLC column with a convenient 12-minute total run time. Fentanyl, para-fluorofentanyl, xylazine, and the other eight additional drugs included in the panel were successfully extracted from urine and blood using the Clean Screen® DAU cartridges with excellent recoveries, matrix effects, and RSDs. These results indicate that this optimized method is highly efficient and can be readily implemented in high-throughput laboratories and that xylazine and other adulterants can easily be easily incorporated into existing fentanyl panels.

P9. Investigation into the Ex Vivo Aging of Bloodstains Post Deposition Using Steady-State Fluorescence Spectroscopy

Alexis Weber, Igor K. Lednev Ph.D., University at Albany, SUNY/SupreMEtric LLC

Blood is one of the most common body fluids discovered at crime scenes involving violent actions. It is

one of the most important types of forensic evidence since it allows for the identification of the individual providing that there is a match with a known DNA profile. Determining the time since deposition (TSD) could further assist investigators by establishing when the crime occurred or if a bloodstain present is actually related to the investigated event. Additionally, if crime scenes contain multiple sets of bloodstains, the TSD determined for individual bloodstains should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed.
To develop a forensically sound method for determining the TSD of a bloodstain, it is necessary to understand the underlying biochemical mechanisms occurring during aging. As biochemical processes occurring in blood are necessary for the continued survival of living organisms, they are important subjects of human biology and biomedicine and are well understood. However, during a violent criminal event where bleeding occurs, the blood will be deposited onto a surface. And the biochemistry of bloodstain aging *ex vivo* is primarily of interest to forensic scientists as it has not yet been thoroughly researched.

This preliminary study utilizes steady-state fluorescence spectroscopy to probe and compare the changes in fluorescence properties of peripheral and menstrual blood up to 24-hours post deposition. Peripheral and menstrual blood exhibited similar kinetic changes over time, assigned to the presence of the fluorophores: tryptophan, nicotinamide adenine dinucleotide (NADH), and flavins in both biological fluids. The biochemical mechanism of blood aging *ex vivo* will be discussed.

P10. Commercializing a Universal Method for Trace Body Fluid Identification for Forensic Purposes
Alexis Weber, SupreMEtric LLC; Igor K. Lednev Ph.D., University at Albany, SUNY

The ability to identify body fluid traces at crime scenes, while preserving any DNA present, is critically important in forensic science. Currently in forensic science laboratories, the identification can be difficult and many of the current techniques are specific to one body fluid. Additionally, typical biochemical methods are destructive – preventing any further analysis. When there is a problem within the scientific field, research laboratories are the main group to solve this problem. After conducting research in the laboratory, the next step in the process is to commercialize the research. Commercialization is bringing a product to market and selling it for financial gain. Within the Lendev Laboratory, in order to develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling.
All six forensically relevant body fluids (blood, semen, saliva, sweat, urine, and vaginal fluid) were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids. The development of this product has occurred over several years to prepare it for sale, with the culmination of this being the creation of the start-up company SupreMEtric LLC. SupreMEtric's mission is to streamline the forensic analysis of biological stains by creating a universal nondestructive method for the identification of all main body fluids. This presentation covers the process from research to commercialization process of this technology.

P11. Capabilities and Limitations of Particle Correlated Raman Spectroscopy (PCRS) for the Analysis of Forensic Soil Minerals
Jasmine Kaur, Joshusa Christensen, Ella Galvan, Marisia Fikiet Ph.D., Virginia Maxwell Ph.D., Brooke Kammrath Ph.D., University of New Haven; Ethan Groves, Skip Palenik, Chris Palenik Ph.D., Microtrace; Peter De Forest D. Crim., Forensic Consultants

This study evaluated the capabilities and limitations of Particle Correlated Raman Spectroscopy (PCRS)

for the analysis of soil samples. Given the long-stated criticisms of forensic soil analysis (e.g., subjective and time consuming), there is a need for an automated system which can provide an efficient and statistically comparable approach to the interrogation of soil samples. PCRS, an integrated technique that combines image analysis with Raman spectroscopy, has the ability to provide morphological and chemical information from a mixture of discrete particles. To develop PCRS for inclusion in a forensic soil workflow, the limitations and advantages of the method need to be evaluated, which has been the goal of the research that will be presented.
For the evaluation of PCRS as a tool to analyze soil minerals, single-blind PCRS was completed on four unknown, four-component mixtures of comminuted minerals and an additional ten soil samples. Following the dispersion of particles on a Low-e slide, 90-180 μm fraction of minerals was analyzed using image analysis and Raman spectroscopy using two lasers excitations (532 nm and 785 nm). The resulting Raman spectra were identified via spectral library searching of the RRUFF[2] mineral database. The results of the PCRS method were then compared to those obtained using traditional methods for mineral identification, including polarized light microscopy and scanning electron microscopy equipped with energy dispersive X-ray spectroscopy. Similarities and distinctions of the results between these approaches have been evaluated to explore the utility of the present PCRS method for use in forensic soil casework.

P12. DNA degradation at Room Temperature for Saliva Stains Collected with Two Different Swab-Moistening Solutions

Kuanwei Lu, Mechthild Prinz Ph.D., John Jay College of Criminal Justice

Trace DNA evidence has assisted police departments in forensic investigation of crime scenes since the 1990s. With the importance of trace DNA increasing, each process needs to be optimized to avoid DNA loss. Factors that could impact DNA quantity and quality during transport or storge are environmental temperature, swabbing materials, packaging, and any possible contaminations. The purpose of this study was to compare the stability of DNA from saliva stain swabs stored at room temperature for swabs moistened with deionized water and Sample Keeper. Sample Keeper (Microread Genetics, Beijing) is a proprietary buffer marketed as a DNA stabilizing agent. We swabbed 120 saliva stains from five different donors with either deionized water and Sample Keeper at defined time intervals from day 1 to 180. Swabs were extracted with Qiagen Investigator Chemistry on a Qiacube robot and analyzed with a Quantifiler Trio quantification kit (Thermo Fisher Scientific). The small and large autosomal targets were used to calculate a degradation index (DI). The results showed that the average DI was higher in the samples that were moistened with water. From day 1 to day 30 the values for both moistening solutions are close to 1 indicating intact DNA. Starting with day 60 the samples swabbed with water showed increasing DI's, while the DI's for Sample Keeper stayed close to 1, this difference is significant at $p < 0.05$ for a paired t-test. The water swabs' DI values did increase but indicate only moderate degradation unlikely to affect STR typing results. This research should be repeated for touch DNA collection. This sample type is more prone to DNA degradation and Sample Keeper may be able to preserve this sample type even better.

P13. Detection of Δ^9-THC and Other Cannabinoids in Cannabis Plant Materials with Potential Interfering Pesticides by Ambient Ionization Mass Spectrometry

Megan I. Chambers Ph.D., Walter B. Wilson Ph.D., National Institute of Standards and Technology

In recent years, ambient ionization mass spectrometry has become more prevalent in forensic science laboratories due to its targeted and non-targeted capabilities, as well as the ability to rapidly screen for trace level contaminants. One of the areas that leverages these advantages is seized drug analysis, which includes the detection of cannabinoids in materials derived from *Cannabis sativa*. To accurately identify these compounds, it is important to understand the potential interferences from contaminants and analytes likely to exist within the

matrices of interest. Therefore, the focus of this study was to investigate a subset of pesticides to determine possible interferences with the mass spectral analysis and identification of cannabinoids (e.g., Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), other neutral and acid cannabinoids) using a common ambient ionization mass spectrometric technique referred to as direct analysis in real time – high-resolution mass spectrometry (DART-HRMS).

A DART ion source coupled to an ion trap mass spectrometer was used in positive-ion mode at 350 °C to analyze an 11-cannabinoid mixture and 20 pesticides from different classes (e.g., organophosphorus, organonitrogen, and carbamate/uron compounds). These pesticides were selected for this study because they yield protonated high-resolution masses within 1 millimass unit (mmu) of one or more cannabinoids. After demonstrating that the protonated mass $[M+H]^+$ of each cannabinoid and pesticide was detected in their respective standards, the pesticides were spiked into aliquots of the 11-cannabinoid mixture and onto small amounts of homogenized hemp plant material. DART-HRMS analysis of these samples revealed the following: (1) all the cannabinoids and pesticides included in this study were detected in the mixtures and spiked plant samples; (2) there were no interferences with the peak at nominal m/z 315 consistent with the protonated mass of THC and CBD in any of the samples; (3) although none of the protonated pesticides interfered with the cannabinoids, the isotope peak of an organonitrogen pesticide was not fully resolved from the cannabinol (CBN) peak; (4) a few pesticides were not fully resolved from other pesticide peaks; and (5) a few lower intensity peaks in the plant matrix prevented some cannabinoid peaks from being baseline resolved. Additional pesticides and contaminants will be investigated to continue identifying potential interferences in the detection of major and minor cannabinoids. These results will aid forensic laboratories that utilize ambient ionization mass spectrometry-based methods (i.e., DART-HRMS) in their seized drug analysis workflows by providing them with information regarding the possible interference of pesticides and other contaminants in *C. sativa* plant material.

P14. Improving the Δ^9-THC, THCA, Moisture Measurements in Forensic Laboratories through NIST CannaQAP

Megan I. Chambers Ph.D., Walter B. Wilson Ph.D., Andrea Yarberry Ph.D., National Institute of Standards and Technology

Cannabis (marijuana and hemp) and its psychoactive constituent, delta-9-tetrahydrocannabinol (Δ^9-THC), have been classified as Schedule I controlled substances since the 1970s. In the past, seized *Cannabis* samples have been tested by forensic laboratories through macro- and microscopic evaluation, colorimetric tests for the presence of Δ^9-THC, and confirmatory chemical testing via gas chromatography – mass spectrometry (GC-MS). In 2018, the Farm Bill defined hemp as *Cannabis* containing less than or equal to 0.3% decarboxylated Δ^9-THC content and removed hemp from the controlled substances list. Federal, state, and local crime laboratories are now required throughout the United States to implement quantitative analytical methods to distinguish *Cannabis* seizures as marijuana or hemp despite little to no experience in or accreditation to perform quantitative drug analysis.

To help the forensic community, the National Institute of Standards and Technology established a *Cannabis* Laboratory Quality Assurance Program (CannaQAP) to help ensure the quality of routine analysis in forensic laboratories through a series of interlaboratory comparison studies. To date, CannaQAP has completed three sets of comparisons with over 200 participants focusing on the determination of cannabinoids Δ^9-THC, THCA (the acidic precursor of Δ^9-THC), total Δ^9-THC, and moisture in *Cannabis* plants and/or oils. These studies are designed to allow forensic laboratories to demonstrate the accuracy and precision of their analytical methods and ability of their forensic scientists. Results from CannaQAP participants are evaluated with respect to the consensus of submitted results as well as to NIST results, and all studies are summarized in publicly available NIST Reports. This presentation will reveal the overall performance of forensic and cannabis testing

laboratories in Exercise 2 and 3 of CannaQAP. Data will be presented comparing the participants results with NIST results obtained by liquid chromatography with either a photodiode array detector and/or tandem mass spectrometry. Additionally, moisture measurement comparison's will be provided to demonstrate inaccuracy across the cannabis industry.

P.15 Utilizing Blood Molar Metabolites to Determine Recent Cannabis Use

Trevor Koppy, David Calixte, Rhoda Nankabirwa, University of Massachusetts, Boston; Xueling Zou, New England College of Optometry; Holly Kailher, University of Rhode Island; Allen Mello, Wisconsin State Laboratory of Hygiene; Denise A. Valenti, IMMAD LLC; United States of America

The legalization of cannabis in many states has resulted in an increase in the number of crashes on the road and work place incidences. Δ9-Tetrahydrocannibinol (THC) and the metabolites, 11-hydroxy- Δ9-Tetrahydrocannibinol (11-Hydroxy-THC) and 11-nor-9-carboxy- Δ9-Tetrahydrocannibinol (11-nor-9-carboxy-THC) are important in determining potential impairment. Whole blood samples of THC cannot be used to accurately determine time since cannabis use. It has been shown that two molar metabolite ratios can be used to identify recent cannabis smoking more accurately: [Δ 9-THC] to [11-nor-9-carboxy-THC] and [Δ 9-THC] + [11-hydroxy-THC] to [11-nor-9-carboxy-THC] (Kosnett et al., 2023). We utilized these ratios as another method to confirm the time since cannabis smoking of casual users, chronic recreational users, and chronic medicinal users.

Users were classified into three different groups using a standardized, validated survey of cannabis use, the three categories were casual users, chronic recreational users, and chronic medicinal users. The research was undertaken as part of an IRB approved protocol funded by the National Institute of Justice. The protocol uses an opportunistic dosing strategy of having volunteers using their own legal product in their own home. Whole blood samples were taken at baseline and after a second visit after consuming a volunteer preferred cannabis product. The whole blood Δ9-Tetrahydrocannibinol and its metabolites 11-hydroxy- Δ9-Tetrahydrocannibinol and 11-nor-9-carboxy- Δ9-Tetrahydrocannibinol were measured by liquid chromatography with tandem mass spectrometry for both baseline and post-cannabis use samples. The blood metabolites were then examined using two different sets of ratios:

1) [Δ9-Tetrahydrocannibinol] to [11-nor-9-carboxy- Δ9-Tetrahydrocannibinol]
2) [Δ9-Tetrahydrocannibinol] + [11-hydroxy- Δ9-Tetrahydrocannibinol] to [11-nor-9- carboxy- Δ9-Tetrahydrocannibinol]

The ratios were evaluated in relationship to Δ9-Tetrahydrocannibinol alone as an indicator of recent cannabis smoking. The reported time since prior use ranged from 20-40 min.

The average Δ9-Tetrahydrocannibinol concentrations at baseline and after dosing for the three categories of users, casual, chronic recreational, and chronic medicinal was compared against the molar metabolite ratios at baseline and after dosing. Data collection is ongoing however, it is expected that there will be ten users in each group for a total of 30 users. Trends have shown a better relationship to time since cannabis use using the molar metabolite ratios compared to Δ9-Tetrahydrocannibinol concentration alone.

Based off of the publication of others, the molar metabolite ratios should provide a more precise estimation of time since dosing compared to Δ9-Tetrahydrocannibinol concentration alone in casual, chronic recreational, and chronic medicinal users alike. In order to fully validate this generalization more work is needed on a larger population scale to confirm.

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P16. Comparing Nanoparticle Type in Nanoparticle-Enhanced Spectroscopy Methods for Detecting Gunshot Residue

Kristopher O'Brien, University of New Haven

The spectroscopy method of Laser-Induced Breakdown Spectroscopy (LIBS) was enhanced with metal nanoparticles (NPs), namely gold (Au) and silver (Ag), in order to increase the sensitivity of the method in the detection of characteristic elements in gunshot residue (GSR). GSR analysis is a staple in the examination of forensic cases where a firearm is discharged, as the presence of GSR on the hands of a suspect shows that they have recently discharged a firearm. Current practice in GSR analysis is to use a Scanning Electron Microscope/Energy Dispersive X-Ray Spectroscopy (SEM/EDS) to detect characteristic spherical GSR particles containing lead (Pb), barium (Ba), and antimony (Sb), which are the characteristic elements of GSR. However, SEM/EDS requires the scanning of a large area for trace amounts of GSR particles, which can prove to be time-consuming and difficult. The use of metal NPs allows for enhancement of the detection limit and better spectral intensity when analyzing GSR particles via spectroscopy methods. Three types of samples were analyzed, both with and without the enhancement of NPs, and analyzed using Nanoparticle-Enhanced Laser-Induced Breakdown Spectroscopy (NELIBS): three salts solution containing barium nitrate ($Ba(NO_3)_2$), antimony sulfide (Sb_2S_3), and lead oxide (PbO), respectively, for the establishment of peak wavelengths identifying each characteristic element, burnt gunpowder samples for concentrated samples made in a controlled setting, and finally real-life GSR samples collected from the hands of a shooter following the discharge of a firearm. Experimentation was conducted in order to determine which type of metal NP reports the best enhancement of spectral intensities when detecting the characteristic elements of GSR in a sample. Following data collection and analysis, none of the three salt solutions provided a clear trend on the optimal NP type. However, the burnt gunpowder samples reported an almost doubling of intensities when using Au NP-enhancement over no NP-enhancement, as well as almost doubling of intensities over Ag NP-enhancement. GSR samples did not report a clear trend about optimal NP type when considering intensities, but Au NP-enhanced samples reported signals for every characteristic peak, while some characteristic peaks failed to provide signal in the Ag NP-enhanced samples. Considering all of the samples, Au NPs are the optimal NP type in the enhancement of peak intensities in NELIBS when analyzing GSR samples.

P17. Determining Fluid Dynamic Properties of the Interaction of Blood on Surfaces of Different Roughness Values at Varying Angles of Impact

Autumn Reynolds, Dr. Paul V. Quinn Sr., Cedar Crest College

Bloodstain patterns are used within crime scene reconstruction to illustrate past events that occurred at the scene. Impact spatter is one type of bloodstain pattern created when a source of liquid blood receives a force resulting in the dispersion of smaller stains onto nearby surfaces at various angles. These angles can be combined to locate the area of origin of the spatter; however, discrepancies exist between current models and the data gathered from impact spatter patterns at crime scenes. One such discrepancy could be due to the interaction of the blood with various types of surfaces since current models only exist for smooth, non-porous surfaces. Most surfaces found at crime scenes are not smooth and non-porous but rather have variations of roughness and absorption characteristics. The lack of applicable models for the wide variety of rough surfaces at a crime scene may possibly result in forensic reports with inconclusive or weakly supported conclusions. This research project creates an empirical model to account for a variation of roughness in various surfaces to increase the accuracy of conclusions made when analyzing impact spatter at a crime scene. The roughness of various surfaces was measured and the flow of blood on those surfaces was observed at varying angles. These empirical results are used to derive a roughness factor which is an adjustment applied to previous models for smooth surfaces by accounting for roughness. This will allow for a more accurate description of the interaction between the blood

and the surface on which it flows, and therefore, more accurate conclusions when analyzing impact spatter.

P.18 Evaluating the Potential of Differentiating Somatic Origin of Hair Using Microscopy and Amino Acid Analysis

Alyssa Campbell, Ted Schwartz, Alyssa Marsico Ph.D., University of New Haven; Dan Rothenberg, Westchester County Forensic Laboratory

Forensic hair examination is a common procedure utilized during forensic investigations, as hair is a commonly found trace evidence item. Due to the nature of hair, this type of evidence is easily left behind during the commission of a crime and is easily transferred between individuals or objects. When hairs are analyzed, somatic origin analysis is often overlooked, thereby bypassing crucial contextual information. In the event that somatic origin analysis is conducted, conclusions may be unreliable due to the overlapping characteristics seen across various body locations. Thus, somatic origin analysis cannot rely on microscopy or DNA alone.

Proteins, on the other hand, are much more robust and provide a possible solution to somatic origin determination. Non-synonymous amino acid changes in the protein sequence cause single nucleotide polymorphism profiles that can differentiate individuals. Proteomic techniques have been heavily researched for the use of genetically variant peptides (GVPs) as a differentiating factor between individuals. Amino acid analysis is another alternative approach that may compensate for the weaknesses of the proteomics approach. Recent studies have demonstrated the use of amino acid quantities in differentiating between individuals based on general class characteristics such as age group, geographical origin, and sex. Macri et al. successfully showed that different amino acid ratios of two individuals with morphologically similar hairs were able to differentiate between them. A study conducted by Yaroshuk demonstrated the possibility of amino acid ratios being used as an exculpatory method.

The central dogma of molecular biology describes the flow of genetic information from DNA to RNA to amino acid sequences that make up proteins. This pathway is crucial for life since each piece of the puzzle directly affects the next step. Each three nucleotides in DNA, a codon, is eventually transcribed into RNA and then translated into a protein. Therefore, any change or mutation in one of the three nucleotides may result in a change in the protein and thus protein expression. Based on this pathway, any changes within the DNA will result in the inclusion of different amino acids that make up a protein which could explain the differences we see between somatic origin of hairs.

This research combines the methodology of current microscopic hair analysis with gas chromatography-mass spectrometry (GC-MS) to obtain amino acid quantities. Somatic origins analyzed include the following: axillary, chest, facial (beard, eyebrow, mustache), limb (arm, leg), pubic and scalp hairs. Microscopy results confirm that buckling, a characteristic often thought to indicate a pubic hair, is present in beard, chest, and scalp hairs in addition to pubic hairs. Inconsistent diameters were also observed in hairs from almost all somatic origins examined. These trends support the claim that no one microscopic characteristic alone can distinguish one somatic origin from the others. With the use of GC-MS, we will determine if amino acid compositions may contain unique significant differences that can be used to differentiate among somatic origin of hairs. This will be determined by calculating the amino acid ratios for each somatic origin and a multivariate statistics analysis will be utilized.

P.19 Using Machine Learning Models to Predict Drop-in and Drop-out of Single Sourced STR Profiles Using the PROVEDit Dataset

Ellie Wan, Forensic Science Program, University of Toronto, Mississauga; Mary Anne Panoyan, Frank Wendt, Forensic Science Program and Department of Anthropology, University of Toronto, Mississauga and Division of Biostatistics, Dalla Lana School of Public Health, Toronto

Background: Short tandem repeat (STR) genotyping is crucial for forensic identification. Crime scene and reference DNA profiles are compared to generate match statistics and evaluate the weight of evidence. DNA from forensic samples is often degraded or present in low quantity, which may produce increased stochastic sampling and elevated genotyping error rates. Allele drop-in and allele drop-out are two types of stochastic errors that can occur during the forensic workflow. These could alter the recorded genotypes and severely affect all downstream source attribution of the profile. While some studies have been performed to estimate the allele dropout rate, further research is needed to understand when and how these events occur and what approaches may mitigate their effects. With the recent availability of large public data sets, we created machine learning models to predict allele drop-in and drop-out rates, as well as identify important thresholds, parameters, and workflow conditions to minimize error rates.

Methods: The PROVEDit (Project Research Openness for Validation with Empirical Data) dataset contains >25,000 multiplex STR profiles ranging from one to five contributors. We used 5,836 untreated single source samples and 14,648 single source samples subjected to treatments that simulate DNA degradation. Some variables had incomplete observations (e.g., quality index was missing in 4050 single source profiles). Five imputation methods were used to fill in these gaps (K-nearest-neighbor, mean, multiple imputation, regression, random forest). Imputed variables were evaluated with root mean square error, and results from the K-nearest-neighbor method were used. Categorical data was one-hot-encoded to minimize bias within the models. Inverse rank normalization was applied so the data better fit a normal distribution. Principal component analysis, t-distributed stochastic neighbor embedding and uniform manifold approximation and projection (UMAP) were used to determine patterns and clusters within the data. Supervised machine learning models were trained to predict delta true allele count (the difference between observed allele count and true allele count). Multiple models were constructed to increase the robustness and confidence in the predictions. The sensitivity and specificity of the models was assessed using the area under the receiver operating curve.

Results: Among untreated samples, template mass (Pearson=0.80) and capillary electrophoresis (CE) instrument (Pearson=0.42) were correlated with ΔTAC. Among treated samples, template mass (Pearson=0.79), dilution ratio (Pearson=0.42), CE instrument (Pearson=0.39), and PCR cycle number (Pearson=0.39) were correlated with ΔTAC. For feature engineering, UMAP resulted in the most distinct clustering patterns. Six clusters were formed for the untreated data and five clusters were formed for the treated data. For both datasets, PCR cycle number, multiplex kit and CE instrument clustered together the most, representing similar, yet unsurprising, inherent structures in the original high dimensional data.

Conclusion: Machine learning models can help prioritize key features in the forensic DNA profiling workflow with large and independent effects on allele drop in and drop out. Preliminary results showcase significant correlations between multiplex instrument, PCR cycle number, and CE instrument with ΔTAC. Our work highlights the importance of the selection of multiplex and CE instruments for STR genotyping. Future efforts will evaluate how these PCR-related features of the forensic DNA workflow interact with environmental insults to contribute to ΔTAC.

P.20 Comparison of Nile Red and Diamond Dye Fluorescence on Non-Porous Surfaces

Casey Rech, Cedar Crest College

Nile Red, a lipophilic fluorescent stain, was compared with Diamond Nucleic Acid Dye in its ability to yield fluorescence on touched non-porous surfaces. Diamond Nucleic Acid dye which binds to single and double-stranded DNA and RNA is commonly used in forensic applications to detect touch DNA on a variety

of substrates. Both dyes are commercially available with Nile Red lower in cost. In this study, subjects touched with one finger various substrates including glass, metal, and tile after systematically rubbing their own hair, face, and neck at 15, 30, 45, and 60 seconds respectively. Touched areas were treated with each dye respectively in pentaplicate immediately after touch. Resultant fluorescence was photographed with a camera phone with an orange filter at an optimal distance away from the surface of the substrate. Photographic parameters were optimized through validation. RGB values were measured on fluorescent areas on each photograph in Adobe Photoshop. Non-parametric pair-wise testing (Mann-Whitney test) for results obtained with both dyes showed that there were no significant differences in fluorescence on touched surfaces regardless of substrates or duration of touch. Thus, Nile Red can be a lower cost alternative to Diamond Nucleic Acid dye for the detection of touched areas on non-porous substrates.

P.21 What's Left Behind: Understanding the Potential for DNA to be Recovered from Latent Blood at an Arson Scene

Teresa DiStefano, University of New Haven

Perpetrators of violent crimes may resort to setting fire to a crime scene to eliminate evidence or hamper the investigative process. First responders and crime scene investigators would likely overlook the potential for traditional forensic techniques to locate blood and obtain DNA because of the high temperatures at fire scenes. This research explores the possibility that blood and DNA can potentially be recovered from some common flooring materials despite being exposed to elevated temperatures normally detrimental to these thermally-labile compounds, specifically because of seepage into crevices and porous areas of thosesubstrates. The literature regarding the recovery of blood DNA after a residential house fire is limited but suggests a potential for recovery.

To investigate whether blood that seeped into common household floor materials can withstand typical household fire temperatures and still yield positive blood reagent reactions and sufficient DNA for amplification, three types of flooring were tested: hardwood, tile, and carpet. As part of the experiment, wooden floor samples were joined together using typical joints, while tile samples were prepared using two tiles connected by a grout line. A consistent volume of human blood was then pipetted onto the seams of each sample to encourage seepage into these areas. Once dried, the samples were placed in an oven from 100 to 200 degrees for 3 minutes. Once removed from heat and returned to ambient temperatures, Kastle-Meyer presumptive testing and ABAcard HemaTrace confirmatory testing was done to each sample to test for the presence of blood. The samples were then extracted using Qiagen QIAamp DNA Investigator Kit, and quantified using ThermoFisher Scientific Applied Biosystems Quantifiler Trio DNA Quantification Kit.

It is hypothesized that the data from the samples discussed in this presentation will support further research to develop protocols for detecting blood and recovering DNA from fire scenes.

P.22 Recovery of Nuclear DNA from Skeletal Remains Decomposed in Freshwater

Zoe Sikon, Ashley Morgan Ph.D., University of New Haven

DNA testing of skeletal remains may be difficult under normal circumstances, but degradation due to environmental conditions can make the process even more difficult. In cases when ante-mortem dental records are not available, DNA analysis may be the only pathway for identification of an individual. This research compared freshwater and dry decomposition environments and their impact on DNA recovery in *Sus scrofa* femora over a 3-month period. Bone cuttings were collected at 3-week intervals from waterlogged and dry bone samples. Samples from both decomposition environments were extracted using the recommended protocol from the PrepFiler™ BTA extraction kit by Applied Biosystems™ and an updated method of the PrepFiler™ BTA protocol. DNA quantitation was performed using custom primer sets specific to *Sus scrofa*, using the

QuantStudio™ 5 Real-Time PCR System by Applied Biosystems™ and PowerUp™ SYBR™ Green Master Mix. DNA quantitation results showed that dry decomposition samples extracted using the updated protocol had a higher average quantity of DNA recovered than those extracted with the PrepFiler™ BTA protocol. A decrease in DNA recovery was observed at week three for both decomposition conditions with both extraction methods. By weeks six and nine, DNA was recovered only from the dry decomposed samples using the updated extraction method. Wet decomposed samples and samples extracted with the PrepFiler™ BTA protocol yielded no DNA. The data suggests the updated protocol improves DNA recovery from dry decomposed skeletal elements and may be easily implemented in a forensic laboratory for analysis of decomposed remains. This research improves our understanding of how the quantity of DNA changes over time in specific decomposition environments and may provide a framework for analysts to further investigate and develop time frames for sample collection and analyses.

P.23 Assessing the Impact Dynamics of Less Lethal Bean Bag Ammunition

Brooke Fontaine, Peter Diaczuk Ph.D., John Jay College

Less lethal ammunition is used to control situations without deadly force. Bean bag ammunition has become a popular type of less lethal ammunition with law enforcement, especially during the 2020 protests. However, bean bag ammunition has been reported to be the cause of life threatening injuries, some of which lead to long lasting effects. This study investigates some of the potential issues of bean bag ammunition in an effort to determine whether they are an effective option for law enforcement. In order to make this determination, both regular 12 gauge shotgun ammunition and the less lethal ammunition were compared. Both types of ammunition were weighed while completely intact, then disassembled to weigh the individual components to determine if there is any significant variability in weight. After weighing each ammunition type, both intact and their individual components, it was determined that there was no significant variability in weight between the different rounds. The bean bag ammunition used in this study only contained lead shot, unlike some of the other brands which contain other material. The next step was to fire both ammunition types at ballistic gelatin, a skin simulant (chamois cloth), and a cotton t-shirt to analyze accuracy, precision, changes in velocity, impact force, and damage. An adjustable gun mount was used to stabilize the shotgun during testing. The bean bag ammunition was fired at multiple distances including, 4, 21 and 30 ft. The less lethal bean bag ammunition did not penetrate the ballistic gelatin, however some rounds ripped through the chamois cloth. Using a chronograph, a potential variance in velocity was observed. A regular 12-gauge shotgun round was fired at a distance of 30 ft at the ballistic gelatin for comparison. The round passed through the ballistic gelatin entirely. Further testing is ongoing to determine whether there is a definite issue with inconsistent velocity and to come to a conclusion as to whether or not less lethal bean bag ammunition is an effective option for law enforcement officials.

P.24 The Development of a Device to Propel a Single Drop of Liquid

Alyssa Ricca, Ralph Ristenbatt, Pennsylvania State University

Despite the growing development in forensic science methods and practices, currently, there is no easy-to-use, inexpensive, reliable device to project single liquid drops of varying mass at speeds faster than a drop's terminal velocity. Although there has been substantial previous research investigating various aspects of radial ("impact") spatter and single drop impacts on substrates, this project focuses on the propulsion of individual drops. It involves the design and construction of a device to propel a single liquid drop onto a target substrate. The device has been designed to enable alteration of drop mass and velocity. A high-speed camera is used to calculate the size of the propelled drop and its velocity at impact. This device can have a significant impact in

the field of criminalistics by providing data and information on high-speed drop-substrate interactions and the morphology of resultant drop deposits, particularly in the evaluation of non-contact blood traces, including single drop deposits and radial spatter patterns. High-speed video also permits analysis of drop morphology in flight, which can be affected by drop oscillations and drag.

P.25 Differentiation of Hair from Individuals with Similar Hair Color Using Amino Acid Ratios Obtained from GC-MS Analysis

Emma Brownlie, Alyssa Marsico Ph.D., University of New Haven

Hair trace evidence is currently analyzed using microscopic hair comparison (MHC) and DNA analysis. Both nuclear and mitochondrial analysis can be done but only nuclear DNA analysis can individualize samples. Traditional MHC is subjective, and DNA cannot be performed on inadequate samples (1-4). Recently, single nucleotide polymorphisms which result in non-synonymous amino acid changes in the hairs protein sequences, are being explored as a potential alternative method for forensic hair analysis. Genetically variant peptides have been used in proteomic analysis for non-related individuals, but amino acids may provide a less complicated method of analysis (4-6). This method has been used for differentiating between plants, bee propolis and humans (7,8). In humans, amino acids have been shown to differentiate individuals based on their demographic and geographic characteristics (9-11). A study by Yaroshuk evaluated the discriminating power of amino acid ratios in mainly dark-haired individuals (12). Expanding on this study, this research will include alternative hair colors to further evaluate the use of amino acid ratios to differentiate between individuals. This continued research aims to develop a method that can be used to supplement traditional MHC when DNA analysis cannot be conducted.

Thus far, 11 hair samples, from consenting female participants, were obtained and randomized. Four individuals self-reported having brown/dark brown hair, one reported as red, five as blonde/dark blonde and one as a blonde and gray mix. The hairs were first visualized and photographed via microscopy. Adobe Photoshop was used on the images to generate RGB values which were used to create 4 sample groups based on color similarity. The first group with the lowest RGB values had 4 samples, the second group had 3 samples and the remaining two groups each had 2 samples.

A mixed 11 amino acid standard derivative in ethyl acetate, and 11 hair samples mixed with an internal standard of L-norvaline, run in triplicate, were analyzed using gas chromatography mass spectrometry (GCMS) (12). Identification of amino acids was confirmed by comparing the sample retention times to standards and the mass spectra library. In early sample analysis, the following amino acid derivatives were observed L-valine, L-leucine, L-proline, L-serine, L-threonine, and L-phenylalanine with variation in their peak intensities. Quantitation relative to the internal standard will be completed to construct amino acid ratios to further compare the differences in peak values. If preliminary comparison of mean and standard error between the ratios determines that individuals can be differentiated from one another, then statistical analysis will be done using ANOVA. Additionally, with success, male samples may be collected and analyzed following the same protocol for additional comparison. Yaroshuk's previous use of this method allowed for the differentiation between all 10 participants using 45 amino acid comparisons obtained through GCMS data and quantitation calculations (12). Given early GCMS results and Yaroshuk's research using the same method, it is suggested that there will be similar differentiability in other hair colors.

P.26 Evaluation of Cannabidiol (CBD) and delta-9-tetrahydrocannabinol (THC) in Connecticut based CBD oils using High Performance Liquid Chromatography-Mass Spectrometry

Lydia Clifford, Marisia Fikiet Ph.D., University of New Haven

Cannabidiol (CBD) is a non-scheduled cannabinoid from the Cannabis plant that is marketed across the nation for its therapeutic effects of treating anxiety, chronic pain, and insomnia[1]. A variety of products have been created including oils, lotions, creams, and dietary supplements to help treat consumers with the targeted symptoms[2]. CBD is similar in structure but has different effects to delta-9-tetrahydrocannabinol (THC), the main psychoactive agent in the Cannabis plant[3]. THC is often used by many people to experience the psychoactive effects it produces such as euphoria, a sense of relaxation, laughter, and an increased appetite[4]. Marijuana is listed as a Schedule 1 Controlled Substance in which THC is believed to be the main ingredient producing the psychoactive effect. Since the Farm Bill was passed in 2018, hemp was removed from the definition of marijuana in the Controlled Substances Act allowing products to be created with no more than 0.3% THC on a dry weight basis[5]. Due to the rapid expansion of the CBD market, not enough quality control of these products is occurring, resulting in users ingesting more or less CBD than desired. In some instances, users could possibly ingest scheduled compounds like THC unknowingly if the oil was labeled as broad spectrum (not containing THC)[6]. The long-term effects of CBD usage are not fully known but it has been found to interact with other medications like blood thinners[1]. This study tested 20 locally (Connecticut) produced CBD oil samples from 20 different suppliers and compared the label values to the experimentally determined values of CBD and delta-9-tetrahydrocannabinol (THC). The samples consist of broad spectrum, full spectrum, and flavored tincture oils to be applied to the tongue in the mouth of the user. The samples were chosen so there was a variety of CBD concentrations ranging from 10 mg/mL to 70 mg/mL. Using the High-Performance Liquid Chromatography coupled with tandem mass spectrometry, a method was designed to extract CBD and THC and quantify them. The method consists of a 5-point calibration curve along with high and low controls. Matched deuterated internal standards were used for the quantification of both CBD and THC. The method consists of a binary gradient with starting conditions of 30:70 (A:B,) using water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B. The acquisition of results is currently still ongoing, and any conclusions drawn will be useful in assessing if users are consuming the correct dosages of the product, and how well reported values match experimental ones.

P.27 Development of a Rapid Precipitation Test to Identify Xylazine via Microscopy and Raman Spectroscopy

Grace Patzer, Lindsey A. Welch Ph.D., Department of Chemical, Physical & Forensic Sciences, Cedar Crest College

Xylazine, an adulterant in illicit drugs, is a growing threat to the health and safety of drug-using individuals across the United States. Frequently referred to as "tranq" from a substance-user standpoint, xylazine is a veterinary drug that can cause detrimental central nervous system depression. In the event of overdose, naxalone cannot reverse the effects of xylazine. This leads to concern that the overdose medication will become less effective as the spread of xylazine grows, and death rates will spike along with it. The purpose of this study was to develop a method of identification for xylazine in a solution to aid in rapid laboratory testing of drug samples. The combination of a known xylazine sample with a previously established amine test using nickel (II) chloride, carbon disulfide, and concentrated ammonium hydroxide solution proved to be a reliable and reproduceable test with a distinct result. The formation of precipitate indicates the presence of a secondary amine in the solution. Complications arise with this test when primary and tertiary amines as well as amides may also produce positive tests. Lidocaine hydrochloride is an amide that frequently produces a false positive and was chosen to further examination. Microcrystalline microscopy was employed to further characterize the drugs. Precipitates from xylazine, lidocaine, and other various drugs were examined and distinguishing characteristics of the crystals were identified with both microscopy and Raman spectroscopy. While multiple drugs produce similar precipitates, analysis of their crystals with Raman spectroscopy is an effective tool to confirm the substance that produced the positive test. It was found that drugs that produce crystals in the nickel (II) chloride

carbon disulfide test tend to have distinct crystalline shapes, and therefore can be identified through this analysis. This application is fast, simple, and has the potential to aid in the analysis of potentially harmful drug mixtures.

P.28 Limitations of Chemiluminescent Detection of Cannabinoids in Edibles and Urine Samples
Cristina E. Lemus, Lindsey A. Welch Ph.D., Department of Chemical, Physical & Forensic Sciences, Cedar Crest College

Chemiluminescence, characterized by its high sensitivity, offers a robust means of detecting pharmaceuticals and controlled substances such as cannabinoids and their metabolites. In forensic science applications, chemiluminescence can provide a rapid and accurate presumptive test for the presence and concentration of Δ^9-tetrahydrocannbinol (Δ^9-THC) in urine samples and aqueous solutions. However, current literature has raised concerns that individuals could tamper with urine samples using adulterants such as bleach, vinegar, or food dyes to produce false negative results. This research aims to address these challenges by evaluating the limitations of a chemiluminescent detection method for the presumptive detection of natural cannabinoids. This is achieved by utilizing a tube luminometer to detect natural cannabinoids and their metabolites in aqueous solutions, both in the presence and absence of common adulterants. Cerium (IV) sulfate and rhodamine B were used in the chemiluminescent detection of Δ^9-THC and tartrazine in water. The Δ^9-THC exhibited a linear correlation of 0.0636-3.18 μM and tartrazine of 7.71-0.964 μM across the compounds. When spiked with vinegar the chemiluminescence of Δ^9-THC diminished while spiking with bleach and tartrazine inhibited chemiluminescence. Additional work to be presented will show the impact of these adulterants on the detection of other cannabinoids. This research holds the potential to equip forensic toxicologists with a cost-effective and rapid method for detecting cannabinoids. These results indicate that chemiluminescent detection of cannabinoids may be deterred in the presence of adulterants or ingredients in edible products. The continuous pursuit of optimized chemiluminescent detection methods for cannabis edibles and biological samples, improvements in matrix compatibility, and the establishment of standardized protocols will undoubtedly contribute to the accuracy and reliability of forensic analyses in this evolving and complex field.

P.29 Comparison of DNA Extraction Methods for Bone Samples
Jenna Mercer, Ashley Morgan Ph.D., University of New Haven

This research was designed to compare magnetic particle extraction and silica gel-based extraction efficiencies. *Sus scrofa* trotters (feet) were separated into two treatment conditions, unburned (fresh) and burned. Fresh samples were prepared for extraction within a few days of purchase. Burned samples were prepared in an open burn with wood fuel and burned for approximately 38 minutes. Samples were extracted with both the QIAamp DNA Investigator Kit and the Prepfiler BTA kit. QIAamp DNA Investigator Kit for bones and teeth uses a silica-based membrane that binds DNA, allowing for the cleaning and subsequent elution of concentrated DNA. The Prepfiler BTA kit uses magnetic particles to bind DNA instead of a membrane. One benefit of using magnetic particles in the extraction process is the decrease in tube transfers, allowing for less DNA to potentially be lost during tube transfer in each washing step. The minimization of tube transfers was hypothesized to be especially beneficial when extracting DNA from burned remains, as low quantities of DNA were expected due to denaturation and degradation from heat during burning. In the forensic biology field, this comparison of kits may provide guidance for optimizing extraction methods for both fresh and burned bones. Additionally, this work may provide data on how these kits potentially differ in performance, including which one is more successful in DNA recovery from challenging bone samples. DNA analysis is critical in the field of forensic science due to its individualizing potential. However, DNA extraction can be difficult with bone samples, as there are elements of bones that make it difficult to access and clean the DNA while still yielding a sufficient

amount for analysis. When dealing with challenging samples such as skeletal remains, optimizing DNA extraction methods could greatly impact DNA identification efforts.

P.30 Differentiation of Biological Sex by Color Coding (ULW) Latent Fingerprint Images

Riley Eagleson, Josep De Alcaraz-Fossoul Ph.D., University of New Haven

Latent fingerprint analysis can be a relevant part of crime investigations in which a timeline is vital to help to reconstruct when and how an event occurred. It has been reported that fingerprints degrade over time in predictable ways under certain conditions; but less is known about how factors such as biological sex of the donor and the substrate where the impressions are deposited on may affect this process. Some of the uncertainty surrounding the natural aging process may be eliminated by studying the visual patterns of ridge degradation regarding different variables. For this research, 756 images of latent fingerprints were obtained from fourteen donors (seven male and seven female) to investigate whether the biological sex of a donor had a discernable effect on fingerprint degradation over time. Three replicates per donor were deposited on glass microscope slides and on conventional plastic petri dishes to also determine the influence of the type of substrate. The prints were developed using titanium dioxide (TiO2) powder and aged in the dark for three months. Random fingerprints were enhanced and photographed at nine time periods, the first being on the day of deposition, and then on days 3, 6, 16, 26, 36, 46, 60 and 90. The color, resolution, and dimensions of these images were standardized using a scale and imaging software. This allowed the images to be properly processed by the Federal Bureau of Investigation's (FBI) Universal Latent Workstation (ULW). ULW software provided a series of metrics, including Blue-Green (BG) data and color- coded maps overlaid on the images, which assessed the visual clarity of ridges. The BG values represented the relative percentage area of a fingerprint that exhibits "good ridge flow" and "clear level-3 detail". The data was then input into a spreadsheet and statistical analysis performed. The analysis of the BG values revealed a "divergence" of aging trends between males and females after day 16 on the glass substrate, with the female donors experiencing a faster rate of degradation after that point. However, this may be due to error, as ridge patterns were not properly detected by ULW in cases where ridges were clearly visible. The BG morphometric did not discover any sex effect on aging patterns on plastic substrate. The data obtained through this research can help to increase the confidence with which information is deduced from latent fingerprints in a criminal investigation. For example, sex of a donor would not need to be considered in an investigation where fingerprints have been enhanced with TiO2 and examined with ULW if the analysis occurs within 90 days after deposition.

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Educators' Forum Abstracts

Project Based Learning in the Introductory Forensic Science Classroom

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Abstract: The "Staten Island Tech Nutshells" are a cumulative, content-rich, project-based learning experience for a college-level introductory forensic science course. The project, carried out over the last several years and originally published in the Journal of Forensic Science Education, was inspired by the Nutshell Studies of Unexplained Death created by Frances Glessner Lee in the 1930s. Students were required to create their own miniature death scenes at a scale of one inch to one foot. They conducted research over extended periods of time, designed their scene using CAD or Revvit, wrote supporting documents (witness statements, autopsy report, forensic testing report) based on their case scenario, and constructed three-dimensional work products in the school's makerspace. This project emphasized the essential "4 C's" of 21st century global skills: communication, collaboration, critical thinking, and creativity; and culminated in a Science and Technology Showcase at the end of the school year. Caveats included time limitations due to short class periods and the use of a shared makerspace. The students gained in-depth knowledge of crime scene analysis and death investigation and were highly engaged throughout the process.

Promoting College and STEM Education through the Forensic Science Initiative (FSI)

David Fisher, M.S. Barbara Elder Weller, Ed.D. Jacqueline, L. Cusack, Ed.D., Kevin Belfield, Ph.D., Forensic Science Program, New Jersey Institute of Technology

Abstract: Launched in 2022, the Forensic Science Initiative (FSI) is a collaborative effort between New Jersey public school districts, the New Jersey Institute of Technology (NJIT) College of Science & Liberal Arts, and NJIT's Center for Pre-College Programs. FSI addresses the critical need to increase diversity in STEM fields by offering underrepresented high school students in New Jersey a unique pathway to higher education through the engaging lens of forensic science. Supported by a $1.4M grant from the US Department of Higher Education's Governor's Emergency Education Relief (GEER) fund, FSI emerged as a response to the "Opportunity Meets Innovation" challenge in the state. Spearheaded by NJIT, the initiative introduces students to the world of forensic science via an intensive summer experience encompassing classroom instruction, hands-on laboratory work, field research, tutoring, and college preparation. Students then dive into a college-level course, "FRSC 201 - Introduction to Forensic Science," during the Fall semester. This foundation lays the groundwork for their Spring capstone research project, guided by experienced faculty mentors. By utilizing forensic science as a covert mechanism, FSI empowers students to develop the skills and readiness required for collegiate level STEM studies. Impressively, the program's inaugural year saw a remarkable 100% college acceptance rate among its participants, with 62% choosing to further their education at NJIT. The relatively small number of underrepresented students in STEM programs is a well recognized issue, partially attributed to the scarcity of highly qualified STEM educators in K-12 schools across the nation. In tandem with the student-focused curriculum, FSI also extended professional development opportunities to high school forensic science teachers. This development encompassed lesson plan creation, gap lesson design, and the establishment of a successful dual enrollment course titled "Introduction to Forensic Science." FSI aims to ensure a sustainable educational model that students can pursue within their respective high schools. The projected enrollment for Fall 2023 anticipates a cohort of 240 high school seniors, poised to benefit from FSI's holistic approach to STEM education. Through the convergence of collaborative partnerships, targeted student engagement, and faculty mentorship, the Forensic Science Initiative at NJIT continues to forge a transformative pathway.

Creating a More Resilient Future Forensic Scientist - Making the Argument for Better Communication Between FEPAC, ASCLD, and Other Stakeholders

Pamela Marshall, Ph.D., Forensic Science and Law Program, Duquesne University

Abstract: The COVID pandemic impacted all of us in different ways. In academia, student mental health moved to the forefront of education. Educators in FEPAC-accredited programs struggled to find ways to deliver content and hands-on laboratory experiences in a remote format, internships that dissolved at most laboratory agencies, and other challenges, all while supporting our student's well-being. These students are now graduating and preparing for the workforce; many of them are prepared academically but may not be ready to face the workplace and the day-to-day trauma that is encountered in a crime lab.

Building off of the author's workshop at the 2023 International Symposium on Human Identification, best practices in forensic education and crime lab training of new hires will be discussed to help develop a more resilient workforce. The presentation will also allow current educators to come together, network, and discuss challenges at their own institutions. Finally, a critical focus will be on improved communication between key criminal justice stakeholders, such as FEPAC, the accrediting body of university forensic education programs, ASCLD, an organization of crime laboratory directors and forensic science managers, and the FBI, which creates standards and decision-making for Quality Assurance guidelines (hiring standards), making the argument for enhancing communication amongst these stakeholders. It is without a doubt that if these stakeholders came together, we could better align our missions, with the objective being a higher-quality graduate who is ready to tackle the forensic landscape.

Keywords: *Forensic Education, FEPAC, Forensic Workforce*

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Student Forum Abstracts

"Empowerment in STEM: A Discussion about Supporting Women Scientists"

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STEM NOW (Nourishing Opportunities for Women) is a support group for women in STEM at the University at Albany, SUNY. Our mission is to facilitate a safe environment where people of all backgrounds and genders can work together to promote equality in STEM through professional development, networking, and advocacy. Our very first event was a meet and greet for current members and prospective members to network and bond. This meeting was a great opportunity for women in STEM and their allies to make connections and discuss their experiences in their respective fields. Since then, STEM NOW has hosted bring-your-own-lunch events every other Thursday, where graduate students are able to connect with other women in STEM. STEM NOW also co-hosted a panel discussion, where four women in STEM with diverse backgrounds answered questions from the audience and spoke on challenges and adversities they have faced throughout their careers. This panel discussion was not only for graduate students but invited postdoctoral and undergraduate researchers as well. The goal of our past and future events is to create comradery among all scientists. This organization has written a letter of support for an NSF grant for the University to work with STEM NOW in career development workshops (networking, resume/CV writing, 3-minute thesis advisement, and career chats with established women in STEM careers). Together we are looking to emphasize the voices of scientists of all backgrounds. The success of this group has been supported by a faculty organization Women in Science and Health (WISH).

There is an overwhelming need to create a safe space for education and continued support for women who are beginning or advancing their careers. This talk will emphasize the challenges and rewards of being a woman in STEM and how to balance a challenging career while struggling with adversity. These adversities include the intersectionality of race, ethnicity, sexuality, etc. We will also have an interactive discussion on important topics, including advancement of women in education and the workplace through collaboration and comradery, the need for adaptability and flexibility, and how you can be an ally

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