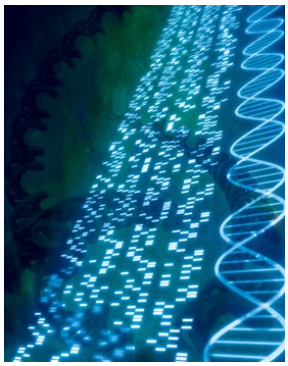
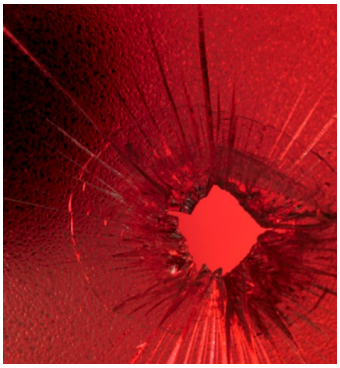
DUQUESNE UNIVERSITY

Forensic Science & Law Graduate Research Symposium

April 8-9, 2021
Hy-Flex*
Join Zoom Meeting

FORENSIC SCIENCE AND LAW PROGRAM
DUQUESNE UNIVERSITY

*Hy-Flex due to Covid-19 Pandemic

Graduate Research Symposium Day 1

Thursday, April 8, 2021
9:00am—4:00pm

Time	Title	Presenter
9:00am	Improving the Efficiency of Mitochondrial DNA Extraction from an Ancient Skeletal Sample by Comparing Hi-Flow® and Organic Extraction Protocols	Lissa Patterson
9:30am	Optimization of Solid Phase Extraction for Organic Gunshot Residue Analysis via LC-QqQ-MS	Jackson Dimalanta
10:00am	The Effects of Fingerprint Development Techniques on Bullet Casing Identification	Andrew Nickischer
10:30am	A Landscape Study on Massively Parallel Sequencing Technology for Forensic Science Applications	Erin Estus
11:00pm	*Break*	
11:30am	Extraction, Detection, and Quantification of Illicit Substances Used in Drug-Facilitated Sexual Assaults from Gummy Bear Matrices	Alysha Donlan
12:00pm	Detection and Quantification of Ketamine in Alcoholic Beverages Using Paper Spray Coupled with Tandem Mass Spectrometry	Dylan Arrigo
12:30pm	Developing a Method to Stop the Degradation of Δ9-THC in Oral Fluid	Colette Miranda
1:00pm	Contamination of Crime Investigation and Lab Analysis Tools – A Review	Rachel Jacobs
1:30pm	*Break*	
2:00pm	Extraction and Genotyping of Human DNA in a Still Body Aqueous Environment	Samantha Border
2:30pm	Use of PCT to Enhance Extraction of DNA from Strangulation Devices	Christina Scott
3:00pm	Osteological, Biochemical and DNA Analysis of Ancient Human Remains from Lithuania	Chelsea Timmerman
3:30pm	A Landscape Study: Familial-DNA Searching in The Criminal Justice System	Samantha Minoski

9:00am Lissa Patterson — **Improving the Efficiency of Mitochondrial DNA Extraction from an Ancient Skeletal Sample by Comparing Hi-Flow® and Organic Extraction Protocols**

This presentation aims to provide insight on an extraction method that produced the highest quality and highest molecular weight mitochondrial DNA (mtDNA) from a challenged skeletal sample found at the Flevaeis Plot Archaeological Site in Rhodes, Greece. A comparison between an Organic PCI extraction method and a Hi-Flow ® silica-column extraction method was performed. This research also attempted to generate a complete sequence of the hypervariable 1 region (HV1) of the sample's mtDNA.
MtDNA analysis is an efficient method for comparative identification of related individuals due to its polymorphic nature and maternal inheritance. The skeletal sample consisted of ancient DNA (aDNA), DNA that has aged and degraded over time. Skeletal material is useful in the analysis of aDNA due to its mineralized tissue that can resist degradation and extreme environments.
This research could not determine a difference between Hi-Flow® silica-column extraction method and the Organic PCI extraction.

Committee Members: ***Lisa Ludvico Ph.D.****; Pamela Marshall Ph.D.; Angie Ambers Ph.D.*

9:30am Jackson Dimalanta — **Optimization of Solid Phase Extraction for Organic Gunshot Residue Analysis via LC-QqQ-MS**

Traditionally, GSR has been analyzed using Scanning Electron Microscopy with Energy Dispersive X-Ray analysis (SEM-EDX), which as provided a benchmark for GSR analysis within the legal system for decades.[1] SEM-EDX analysis has become more obsolete as manufacturers are producing more lead-free ammunition. This has paved the way for new alternative methods to analyze OGSR. OGSR is the residues of the nitrogen compounds used as the propellant in firearms, which does not contain lead, barium, and antimony. OGSR consists of 7 organic chemical compounds; diphenylamine (DPA), 2-nitrodiphenylamine (2-NO_2-DPA), 4-nitrodiphenylamine (4-NO_2-DPA), N-nitrosodiphenylamine (N-NO-DPA), ethylcentralite (EC), akardite II (AKII), and methyl centralite (MC).[3] The goal of this study is to determine if OGSR can be captured by SPE and analyzed by LC-QqQ-MS to establish if a firearm has been discharged. Preliminary data has demonstrated that the LC-MS method used was efficient and the stock solution was able to be analyzed prior to SPE extraction.

Committee Members: ***Stephanie Wetzel, Ph.D.****; Lyndsie Ferrara, Ph.D.; Allison Laneve, M.S.*

10:00am Andrew Nickischer — **The Effects of Fingerprint Development Techniques on Bullet Casing Identification**

Fingerprint development is a procedure that is routinely performed on bullet casings in crime labs. This study was undertaken to identify the effect of fingerprinting techniques on bullet casing identification. Fired cartridges with casing materials of brass, steel, nickel-plated brass and aluminum were exposed to the most optimized casing fingerprint development techniques: cyanoacrylate fuming, gun bluing solution, and Basic Yellow 40 fluorescent dye stain. Casings were exposed to each process individually as well as in a sequence as listed above. Casing marks such as Firing pin impressions, ejection port marks, chamber marks, and extractor marks were analyzed using a comparison microscope. Qualitative results between developed and control casings of this study show that the gun blue solution is quite destructive to small casing marks (particularly steel and brass casings). All other fingerprinting procedures resulted in no change of the casing marks, allowing typical identification.

Committee Members: ***Lyndsie Ferrara, Ph.D.****; Allison Laneve, M.S.; Melissa Meredith, M.S.*

10:30am Erin Estus — **A Landscape Study on Massively Parallel Sequencing Technology for Forensic Science Applications**

A landscape study defines stakeholders and their products to enable end users to make more informed purchasing decisions. This report provides a landscape view of currently available Massively Parallel Sequencing (MPS) technology. MPS is a massively parallel process which enables the sequencing of an entire genome in a single day. It allows analysts to sequence by size and base pair. This permits the distinction between two alleles of the same length at the same locus. MPS allows forensic analysts to achieve an increased level of accuracy for DNA analysis over current gold standard sequencing methods. It is pertinent that forensic scientists can quickly and reliably keep up with the evolution of forensic DNA analysis. This report will allow forensic decision makers to gain a better understanding of MPS technology and what they need to consider before they chose to implement the technology in their crime laboratory.

Committee Members: ***Lyndsie Ferrara, Ph.D.****; Pamela Marshall, Ph.D.; Lisa Ludvico, Ph.D.; Evan Penrod, M.S.*

11:30am Alysha Donlan — **Extraction, Detection, and Quantification of Illicit Substances Used in Drug-Facilitated Sexual Assaults from Gummy Bear Matrices**

There is currently a need in forensics for a standardized set of methods for the analysis of various drug-laced foods. Current methods typically involve costly, time-consuming, and laborious pretreatments before the preferred mass spectrometry techniques can be performed. Labs have a limited amount of funding, and with caseloads continuously increasing, it will be useful to explore faster, and less expensive methods for the analysis of these adulterated food products. The goal of this study was to determine whether three drugs commonly encountered during investigations of drug-facilitated sexual assaults, specifically alprazolam, 3,4-methylenedioxymethamphetamine (MDMA), and γ-hydroxybutyric acid (GHB), could be extracted, detected, and quantified from gummy bear matrices. Spiked gummy bears were prepared, and a liquid-liquid extraction with low temperature partitioning (LLE-LTP) was used to separate the drugs from the complex sample matrix. Following extraction, paper spray ionization mass spectrometry (PSI-MS) experiments were used for both detection and quantification purposes.

Committee Members: ***Michael Van Stipdonk, Ph.D.****; Lyndsie Ferrara, Ph.D.; Timothy Evans, Ph.D.*

12:00pm Dylan Arrigo — **Detection and Quantification of Ketamine in Alcoholic Beverages Using Paper Spray Coupled with Tandem Mass Spectrometry**

Drug facilitated sexual assault (DSFA) cases have become more common in club scenes and isolated settings. It is achieved by spiking common sedatives in a victim's beverage. Usually, a toxicological analysis would be performed for the presence of the drug or testing the drink. Current methods are extraneous and time extensive, and an alternative method of Paper Spray-Tandem Mass Spectrometry (PSI-MS/MS) can be a solution to these methods, with little to no sample preparation, and a run time that only takes minutes. The drinks analyzed were a vodka soda and vodka cranberry spiked with ketamine. For quantitation, a calibration curve was created in the two matrices. The limit of detection (LOD) and limit of quantitation (LOQ) was determined as well. This method can be held to the same standard as ones used in labs today and can also be extended to an infield instrument that can be used on the crime scene.

Committee Members: ***Michael Van Stipdonk, Ph.D.****; Stephanie Wetzel, Ph.D.; Frederick Fochtman, Ph.D.*

12:30pm Colette Miranda — **Developing a Method to Stop the Degradation of Δ9-THC in Oral Fluid**

With more states legalizing medicinal and recreational marijuana every year, it is important to be able to identify how much is in a person's system. Δ9-Tetrahydrocannabinol (THC) can be detected in oral fluid for up to 22 hours. Oral fluid provides a narrower window to identify the accused was under the influence at the time of the crime. This project utilized Solid Phase Extraction and Gas Chromatography- Mass Spectrometry to extract and analyze the samples. This project studied what type of material was best to store the spiked samples in: unsilanized glass, silanized glass, and plastic. The samples were prepared by using donor oral fluid and a Δ9-THC drug standard to make a concentration of 375 ng/mL in the oral fluid. An internal standard (Δ9-THC-d3) was used to account for potential errors in the extraction process. It was hypothesized that the silanized glassware will yield the least amount of degradation.

Committee Members: ***Stephanie Wetzel, Ph.D.****; Pamela Marshall, Ph.D.; Frederick Fochtman, Ph.D.*

1:00pm Rachel Jacobs — **Contamination of Crime Investigation and Lab Analysis Tools – A Review**

Contamination can make something impure or corrupt by contact and has potential to be detrimental to the forensic science analysis process. Contamination has the largest potential to be introduced to evidence during collection and analysis. This can be observed through secondary transfer and evidence exchange whether that's unintentional DNA profiles being present, or evidence being stored together. Evidence collection and analysis tools can be a prime vector for contamination during investigations, as can be seen through previously conducted research. Contamination results in the loss of integrity for evidence and it is impossible to return evidence to any previous condition after the introduction of contaminants. Evidence that was corrupted will not produce routine results, and cannot be uncontaminated, which can have a large impact in the courtroom. This study will analyze articles from scientific journals to form a deeper understanding of contamination and how it can affect forensic analysis.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Benjamin Cooley, M.S.*

2:00pm Samantha Border — **Extraction and Genotyping of Human DNA in a Still Body Aqueous Environment**

This research attempts to provide a novel forensic technique for improving the outcome of missing person cases associated with bodies of water. Using DNA processing methods directed at isolating and analyzing environmental DNA (eDNA), introduces forensic applications which reduces the time required for locating victims and improving the efficiency of the searching process. This study focuses on migration, degradation, and genotyping of human DNA from an aqueous environment. Trials were conducted during spring and summer. Human epidermal tissue, secured in a small container, was placed into a pond. 15 mL collections were made every other day for thirty-days, every 3ft from the tissue out and including 12ft. Samples were filtered, extracted, quantified, and select samples were amplified and genotyped. STR loci of the known and unknown were compared. This study's results support the use of eDNA in missing person cases.

Committee Members: ***Lisa Ludvico, Ph.D.****; Pamela Marshall, Ph.D.; Shannon Mahoney, M.S.*

2:30pm Christina Scott **Use of PCT to Enhance Extraction of DNA from Strangulation Devices**

Strangulation frequently occurs during violent assaults where the attacker may suppress their victims using clothing, pantyhose, or rope. This contact leads to the transfer of small quantities of DNA from the victim and/or perpetrator to the device. Often, these low quantities of DNA are not high enough to yield a full profile. Pressure Cycling Technology (PCT) is used to increase the amount of DNA that is extracted from different objects through alternating cycles of hydrostatic pressure. A human blood sample was selected based on its heterozygosity and then diluted to quantities of DNA meeting the definition of low copy. Dilutions were then placed on two different substrates, nylon pantyhose and cotton rope, and extracted with and without PCT. PCT allowed for more DNA to be obtained when compared with the standard cell lysis methods for extracting DNA, leading to a fuller profile that can be used to identify a suspect.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Nathan Lawrence, Ph.D.*

3:00pm Chelsea Timmerman **Osteological, Biochemical and DNA Analysis of Ancient Human Remains from Lithuania**

An archaeological site in Lithuania was excavated in 2012, containing the remains of nine individuals, estimated to be from 200-400AD. An assemblage of bones was sent to Duquesne University where osteological, biochemical and DNA analysis were performed for identification of the remains. Osteological analysis using morphological features and archaeological reports determined identifying factors of the individuals, including sex, age, and trauma. Biochemical analysis of the outer sanded layer using gas chromatography-mass spectrometry was performed to determine components present due to decomposition or environmental factors that act as contaminants. DNA analysis was performed using the hypervariable region I of mitochondrial DNA due to its high copy number per cell, since ancient DNA is often highly degraded. Hi-Flow® extraction, quantification and amplification using Armed Forces DNA Identification Lab mini primer set, and sequencing was performed to compare sequences to known data set sequences from regions around Lithuania to infer possible maternal lineages.

Committee Members: ***Lisa Ludvico, Ph.D.;*** *Pamela Marshall, Ph.D.; Stephanie Wetzel, Ph.D.; Anne Burrows, Ph.D.*

3:30pm Samantha Minoski **A Landscape Study: Familial-DNA Searching in The Criminal Justice System**

Familial DNA searching (FDS) is an emerging investigative tool in the forensic community. It can detect and statistically rank potential candidates in CODIS that may be close biological relatives to an unknown DNA profile. Many states will either not use FDS as an option or will only use it under very specific circumstances, such as all investigative leads having been exhausted. States that do use FDS have their own methods and policies regarding it. Though FDS has its positive uses, it carries current ethical and constitutional concerns as well.
Data was collected using an anonymous survey through Qualtrics sent to participants in the following roles in the criminal justice system: Crime lab director, DNA crime lab practitioner, crime scene investigator, criminal prosecutor, or criminal defense attorney. These diverse roles were chosen to obtain the different points of view between the science and legal perspective regarding FDS.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Phillip Palmer, Ph.D.*

Graduate Research Symposium Day 2

Friday, April 9, 2021
8:30am—10:30am

Time	Title	Presenter
8:30am	An Analysis of Extraction Efficiencies of Various Swabs on Sperm Recovery	Lindsey Campany
9:00am	Investigating the Probative Value of Touch DNA Evidence - A Landscape Study	Annaliese Black
9:30am	A Comparison of DNA Quantity and Quality in Sexual Assault Evidence Kits Using Varying Conditions of Storage Types and Storage Durations	Dana Voris
10:00am	Quantification of Toxins in Commercial Dietary Supplements Using Stir-Bar Sorptive Extraction, GC-MS, and Isotope Dilution Mass Spectrometry	Ashley Dillard

8:30am Lindsey Campany **An Analysis of Extraction Efficiencies of Various Swabs on Sperm Recovery**

With advancements in DNA technology and methodology, more complete DNA profiles can be obtained. Extraction procedures and their reagents have been optimized for maximal DNA yield, but the cotton swab used and provided for sexual assault examinations has not advanced. Despite research suggesting the cotton swab's absorbent nature and its inclination to retain cellular material, the field of forensic nursing has not yet implemented another swab. This study aims to address if the cotton swab is an efficient enough collection device for continued use in sexual assault examination kits by comparing the cotton swab to both the nylon flocked and cytobrush swabs. Seminal fluid was used to create mock samples and these mock samples were extracted to compare elution efficiencies between swab types. Supporting previous research, the results show that the nylon and cytobrush swabs have significant differences in male DNA concentration when compared to the cotton swab.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Elisabeth Wisbon, M.S.*

9:00am Annaliese Black **Investigating the Probative Value of Touch DNA Evidence - A Landscape Study**

Touch DNA evidence is received by forensic laboratories on a daily basis and the procedure for discerning each item's probative value varies between labs. Touch DNA is obtained via sloughed off skin cells on an object's surface, therefore brief interactions with objects or clothing often do not result in ample DNA. This study will consolidate data from crime laboratories to identify similar trends and distinguish differences in their procedures as they pertain to touch DNA evidence processing. Through the use of Qualtrics survey software and virtual interviews, members of crime laboratories will be consulted about their independent definitions of touch DNA, the frequency of its submission as evidence and the probative value that evidence holds. The results of this study will allow a comprehensive understanding of commonly practiced crime lab policies pertaining to touch DNA evidence and provide information regarding which objects hold the most probative value.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Lyndsie Ferrara, Ph.D.; Elisabeth Wisbon, M.S.*

9:30am Dana Voris **A Comparison of DNA Quantity and Quality in Sexual Assault Evidence Kits Using Varying Conditions of Storage Types and Storage Durations**

DNA evidence is the most important physical evidence in a sexual assault case. Poor storage can cause DNA evidence to degrade, affecting its quantity and quality for analysis. The Gentueri Sexual Assault Collection Pouch is a new sexual assault kit that contains a desiccation component to keep moisture out of samples and minimize DNA degradation. This research tested if the Gentueri Sexual Assault Collection Pouch preserved DNA samples better than the Pennsylvania State Police Sexual Assault Kit. Mock sexual assault samples using a 1:200 dilution of semen were created with both kits. The kits were put into storage for periods of zero, 60, and 90 days. Storage conditions of -20°C, 4°C, and 25°C were used. Once out of storage, testing was done on the samples and results were collected, which revealed how their DNA quantity and quality was affected by the storage conditions and lengths of time.

Committee Members: ***Pamela Marshall, Ph.D.****; Lisa Ludvico, Ph.D.; Elisabeth Wisbon, M.S.*

10:00am Ashley Dillard **Quantification of Toxins in Commercial Dietary Supplements Using Stir-Bar Sorptive Extraction, GC-MS, and Isotope Dilution Mass Spectrometry**

Toxins are chemicals that have adverse effects on human health. Dietary supplements (DS) consisting of botanical materials have been found to contain toxins. In this study, methods were developed to quantify toxins in 12 different dietary supplements using GC-MS/MS and GC-MS. These toxins included glyphosate and persistent organic pollutants (POPs), such as polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs). Stir-bar sorptive extraction was utilized to extract the toxins. Glyphosate was extracted after derivatization using N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) with 1% trimethylsilyl chloride (TMCS). Quantification was accomplished using isotope dilution mass spectrometry (IDMS). By adding an isotopically enriched form of these toxins into the samples, the isotope ratio could be measured. Preliminary studies have shown that utilizing IDMS increases the accuracy and precision especially at lower measured concentrations. The development and application of this method is important to further identify and quantify the presence of pollutants in DS.

Committee Members: ***H.M. Skip Kingston, Ph.D.****; Stephanie Wetzel, Ph.D.; Mandy Tinkey, M.S.*