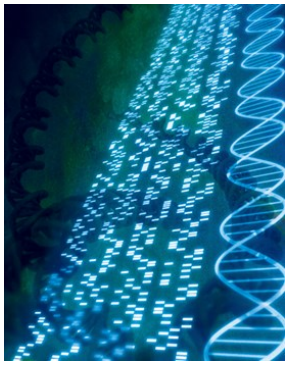
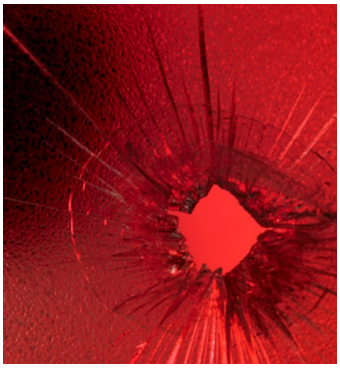
DUQUESNE UNIVERSITY

Forensic Science & Law Graduate Research Symposium

April 2-3, 2020
Online*

FORENSIC SCIENCE AND LAW PROGRAM
DUQUESNE UNIVERSITY

*Online due to Covid-19 Pandemic

Graduate Research Symposium Day 1

Thursday, April 2, 2020
Online
11:30am—4:00pm

Time	Title	Presenter
11:30am	LCMS Method Development and Optimization for the Identification and Quantification of Illicit Substances Introduced into Correctional Facilities	Erica Maney
12:00pm	Developing a Microsatellite Multiplex for the Individual Identification of African White-Bellied Pangolins	Amelia Bullard
12:30pm	Testing Kinship via Mitochondrial DNA on Colony vs. Non-colony Cats	Ashley Ruddy
1:00pm	*Break*	
1:15pm	Using Loop-Mediated Isothermal Amplification (LAMP) to Identify At-Risk Species in the Field	Brooke Driscoll
1:45pm	Longitudinal Study of The Effects Of Storage Conditions On DNA Recovery From Condoms	Claire Loretta
2:15pm	Recombinant Expression of Human Semenogelin Proteins and Creation of Novel Antibodies for the Detection of Human Semen	David Brown
2:45pm	*Break*	
3:00pm	Sex Determination of Ancient Human Skeletal Remains	Rhianna Beaver
3:30pm	DNA Contamination on Sharpie Markers Used to Outline Bodily Fluids in a Forensic Laboratory	Danielle Guckin

11:30am Erica Maney

LCMS Method Development and Optimization for the Identification and Quantification of Illicit Substances Introduced into Correctional Facilities

As illicit drug use continues to rise in America, there is a need to detect drugs being smuggled into correctional facilities. More individuals attempt to conceal illicit materials in articles of mail, believing that facilities will be unable to detect the presence of these substances. This project's focus was to develop LCMS methodologies to identify and quantify commonly trafficked drugs: methamphetamine, ketamine, heroin, cocaine, PCP, fentanyl, and methadone.
This project is in collaboration with ChemImage to improve their hyperspectral imaging system, the VeroVision™ Mail Screener. In addition to analyzing samples received from customer facilities, two sets of control samples were prepared using Methamphetamine HCl dissolved in Acetone. Samples were analyzed on the Agilent 6460 LC-QqQ-MS, with accurate results and relatively high efficiency rate of ~70%. Currently, 38% of confiscated mail samples tested have high concentrations of methamphetamine, a common and dangerous illicit substance responsible for overdoses all over the country.
Committee Members: Stephanie Wetzel, Logan Miller, Sean Fischer, Jeff Beckstead

12:00pm Amelia Bullard — **Developing a Microsatellite Multiplex for the Individual Identification of African White-Bellied Pangolins**

Pangolins are the world's most trafficked mammal due to poaching and their use in traditional Chinese medicine. Because of their elusive nocturnal behavior, and a lack of previous research, not much is known about them. In this study, we attempt to come up with an individual identification technique for pangolins to assist with the conservation of their species. Pangolins have not been fully genotyped and the discrepancies between several species have long been ignored. By developing a multiplex to individually identify African white- bellied pangolins, we can begin down the long road of separating species. By taking blood samples, conducting PCR with the extracted DNA, and capillary electrophoresis, we can then coalesce the most efficient primers into a multiplex to provide a technique capable of differentiating between individuals. This is pertinent to the wildlife forensic community by providing knowledge that will assist with the conservation of the worlds most trafficked mammal.
Committee Members: Jan Janecka, Lisa Ludvico, Kenneth Kaemmerer

12:30pm Ashley Ruddy — **Testing Kinship via Mitochondrial DNA on Colony vs. Non-colony Cats**

Feral cat (*Felis catus*) populations have drawn public attention for several reasons, including increasing population size and decreasing native fauna populations. Several communities have implemented the Trap-Neuter-Return (TNR) method, developed in Rome. This study examined whether cats that live in a certain social environment (colony cats) are more likely to be related than cats of the general population (non-colony cats). The degree of relatedness was determined separately for each population.
Frankie's Friends Clinic, headed by Dr. Becky Morrow, provided the samples for this research. The sample size was n=40 colony cat and n=40 non-colony cat ear tips. The extraction method employed was Qiagen QIAamp DNA Mini Tissue Kit, followed by quantitation using the NanoDrop Lite. Primer sequences, Lf15926 and Hf3 were used to amplify the HV1 region of mtDNA, followed by sequencing with the ABI Big Dye Kit, 3130 Genetic Analyzer and Chromas Software.
Committee Members: Lisa Ludvico, Jan Janecka, Becky Morrow

1:00pm BREAK

1:15pm Brooke Driscoll — **Using Loop-Mediated Isothermal Amplification (LAMP) to Identify At-Risk Species in the Field**

Species identification through DNA analysis is relevant to wildlife forensics but can be challenging in resource-limited areas. A potential solution to this issue is loop-mediated isothermal amplification (LAMP), an alternative to polymerase chain reaction (PCR). LAMP requires a thirty-minute reaction time and offers the potential for in-tube visualization. The main question to be answered is: can a field- accessible kit be developed for species identification using the LAMP method? Through this research, a method is developed using the Lucigen© LavaLAMP™ kit, a fluorescent dye, and snow leopard scat. Optimization of this method has shown that calcein and manganese (II) chloride provide a fluorescent indication of amplification with the use of a UV light source. The LAMP reaction has shown success with amplifying scat samples at isothermal temperatures, requiring only a heat block. The complete method has demonstrated speed, ease of use, and minimal instrumentation, making it ideal for field use.
Committee Members: Jan Janecka, Lisa Ludvico, Nickolas Walker

1:45pm Claire Loretta — **Longitudinal Study of the Effects of Storage Conditions on DNA Recovery from Condoms**

With increased awareness of DNA value within criminal investigations, the number of sexual assaults involving condoms has also increased. Although the consequences of DNA degradation are well-established, the influence of condoms on this process is undetermined. By understanding DNA degradation within condoms, standardized processing and preservation procedures can be implemented. To establish a preliminary understanding of condoms' effects on DNA recovery, DNA quantity and quality were measured across three variables: time, temperature, and condom brand. Human semen was aliquoted into condoms from three different brands (Trojan, Durex, Sustain), followed by sample storage under three temperatures (25°C, 4°C, -20°C) for a total storage duration of one year with sample analysis at four intervals: t_0 = 0 weeks, t_1 = 16 weeks, t_2 = 32 weeks, and t_3 = 52 weeks. This line of inquiry can aid in sexual assault investigations as well as supplement the development of a sexual lubricant database.
Committee Members: Pamela Marshall, Lisa Ludvico, Stephanie Wetzel, Benjamin Cooley

2:15pm David Brown — **Recombinant Expression of Human Semenogelin Proteins and Creation of Novel Antibodies for the Detection of Human Semen**

The protein component of human semen largely consists of a group of proteins called the semenogelins. After ejaculation, they undergo protein-protein interactions to form the coagulation of seminal fluid for protection of spermatozoa prior to fertilization. Because of their abundance, the semenogelin proteins are an effective marker to detect human semen at crime scenes, even after substantial degradation. The goal of this study is to create an optimized method for producing novel antibodies for the semenogelin proteins that can be used to detect seminal fluid. Segments of the semenogelin 1 gene were amplified, cloned into three mammalian expression vectors, and transfected into human cells for expression. Samples were taken across five days from spent media and cell lysate, separated on SDS- PAGE, and detected with immunoblots. After this expression is fully optimized, antibodies can be created against the purified recombinant semenogelins and tested for their ability to detect traces of semen.
Committee Members: Michael Seaman, Pamela Marshall, Benjamin Cooley

2:45pm BREAK

3:00pm Rhianna Beaver — **Sex Determination of Ancient Human Skeletal Remains**

The forensic science community has made several advancements since the discovery of Ancient DNA (aDNA) extraction in 1984. Because the decomposition of soft tissue occurs almost immediately, bone material has been found to be a suitable substrate for the extraction of DNA. The bones in this study belong to several individuals whose skeletal remains were found in the Flevaeis Plot in Rhodes, Greece; these bones have been estimated to be approximately 1700 years old. For this project, they were extracted using a Qiagen, Quick-Start Protocol with the addition of Pressure Cycling Technology (PCT). The AMEL locus (AMELX and AMELY) was amplified using Amelogenin markers in order to determine the sex of the bone samples. One fragment at 106 base pairs (bp) showed the presence of only X chromosomes (XX; indicating female). Two lengths, at 106 and 112 bp, showed both an X and a Y chromosome (XY; indicating male).
Committee Members: Lisa Ludvico, Pamela Marshall, Sara Bitner, Elisabeth Wisbon

3:30pm Danielle Guckin — **DNA Contamination on Sharpie Markers Used to Outline Bodily Fluids in a Forensic Laboratory**

This research examines the secondary transfer of DNA between items of evidence and Sharpie markers. Forensic biologists outline bodily fluids with markers to indicate where to swab or cut evidence, and this marker is used multiple times on different pieces of evidence. A preliminary study proved DNA can be extracted and quantified from the tips of Sharpie markers. Afterwards, mock samples were created using donated clothing, blood and semen from six different donors, and reference samples to better simulate casework. The Sharpie markers were used to outline the bodily fluids as a two-step DNA transfer event. As a result, DNA contamination was present on certain samples via fragment analysis data, indicating a potential source of contamination in crime laboratories. Identifying contributing factors of DNA contamination in a crime laboratory is imperative so that measures can be taken to prevent the contamination.
Committee Members: Lisa Ludvico, Pamela Marshall, Sara Bitner, Elisabeth Wisbon

Graduate Research Symposium Day 2

Friday, April 3, 2020
Online
8:00am—12:00pm

Time	Title	Presenter
8:00am	Probing the Intrinsic Conformation of Anionic Uranyl Complexes using Quantum Chemical Calculations and IRMPD Spectroscopy	Scott Rissler
8:30am	Degradation Studies of Derivatized Spice Compounds using Paper Spray Ionization with Mass Spectrometry	Susan Kline
9:00am	Exploring Derivatization Methods to Improve the Detection and Quantification of Surrogates for Illicit Monoamine Compounds	Hannah Zimmerman
9:30am	Analysis of Cannabinoids in Vitreous Fluid	Haley Berkland
10:00am	Methamphetamine Confirmation Analysis after Controlled Vick's VapoInhaler Injection into Oral Fluid	Julia Canello
10:30am	The Effects of Storage Conditions and Time on Extracted Ignitable Liquids Using GC-MS	Sierra Strnisa
11:00am	The Effects of the Evidence Preservation System on the Storage of DNA Samples	Devin Doyle
11:30am	The Disconnect Between Forensic Science and the Lawyers and Judges Who Represent It	Hannah Reidenbaugh

8:00am Scott Rissler — **Probing the Intrinsic Conformation of Anionic Uranyl Complexes using Quantum Chemical Calculations and IRMPD Spectroscopy**

Use of uranium has increased as it has become useful in industry and in the nuclear fuel cycle. In order to develop methods for identifying and alleviating uranium from the environment it is necessary to understand its intrinsic behavior. Having a reliable computational model aids in streamlining the process when trying to provide support for experimental findings.
Anionic uranyl complexes were modeled using the Gaussian software package. The theoretical models that were assessed included B3LYP, PBE0, and M06-L functionals. Thermodynamic and frequency data were collected. Experimental data for the model complexes, $UO_2(FA)_3$, $UO_2(AC)_3$, and $UO_3(NO_3)_2$ were collected at FELIX.
Results show that it is possible to compute multiple conformations for all species, but with the addition of comparing IR spectra it is possible to identify species specific signatures. The results also indicate that B3LYP may serve as the most reliable functional in terms of the IR spectra comparisons.
Committee Members: Michael Van Stipdonk, Lyndsie Ferrara, Benjamin Bythell

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8:30am Susan Kline — **Degradation Studies of Derivatized Spice Compounds using Paper Spray Ionization with Mass Spectrometry**

Homemade explosives (HMEs) are an increasing threat due to the wide accessibility of the starting materials and ease of synthesis. One category of ingredients are spice compounds such as cinnamaldehyde, cuminaldehyde, and vanillin. These compounds are not monitored like other HME ingredients, and detection methods are not as well developed compared to other categories. Previous research in our lab determined that paper spray ionization coupled with mass spectrometry was a viable option for detection. Solutions of each of the three previously mentioned spice compounds were made, and two different studies were of focus in this research. The first study was to determine how long the samples could be detected on a surface with exposure to the surroundings. The second study was to determine the lifetime of prepared samples for analysis. Storage samples were made with varying conditions including derivatized vs. underivatized, room temperature vs. refrigerated, and sealed vs. open.
Committee Members: Michael Van Stipdonk, Lyndsie Ferrara, Joseph Bennett

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9:00am Hannah Zimmerman — **Exploring Derivatization Methods to Improve the Detection and Quantification of Surrogates for Illicit Monoamine Compounds**

America is facing an epidemic where monoamine drugs like amphetamine and methamphetamine are abused and paraphernalia is often found at crime scenes. These monoamines often require derivatization for proper analysis. Derivatizing agents improve detection, separation, ionization, and quantification through various spectrometry and ionization methods. This study was conducted to determine if aldehyde based derivatizing agents allowed for improvement of the stated factors using several experimental methods. Experiments were first conducted on illicit monoamine surrogates like aniline and phenethylamine, and then on stock samples of illicit monoamine drugs. Derivatizing agents previously used were not robust one step derivatizations suited for several different instrumentation methods. Derivatization was deemed successful with paper spray ionization, electrospray ionization, and gas chromatography mass spectrometry methods. The next step was testing derivatized mixtures of monoamines with the determined instrumentation methods, to determine if the individual compounds could be differentiated.
Committee Members: Michael Van Stipdonk, Lyndsie Ferrara, Frederick Fochtman

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9:30am Haley Berkland — **Analysis of Cannabinoids in Vitreous Fluid**

Cannabis is currently the most widely abused illicit drug in the world.[1] As medical and recreational uses of this substance become increasingly legal, there is a need for reliable analytical methods that can detect and analyze cannabinoids in death cases where there is a question relating to the cause and manner of death when typical matrices are not available. Vitreous humor is a gelatinous fluid located in the eyeball and is regularly drawn during autopsy and used as a matrix to test for drugs and alcohol by a postmortem toxicologist. In this study, a method was developed and validated to identify and quantitate tetrahydrocannabinol, 11-hydoxy-Δ9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, and cannabidiol in bovine vitreous humor. Separation and quantitation were conducted using LC-MS/MS instrumentation. Samples were screened using enzyme-linked immunosorbent assay and quantitatively confirmed using the developed LC-MS/MS cannabinoid assay. Limits of quantitation are typically 0.5 ng/mL.
Committee Members: Stephanie Wetzel, Frederick Fochtman, Christopher Divito

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10:00am Julia Canello — **Methamphetamine Confirmation Analysis after Controlled Vick's VapoInhaler Injection into Oral Fluid**

Falsely screening positive for illicit drug use has consistently been an issue in the forensic community for years, leading to an increased need for confirmatory methods. Specifically, immunoassays have been known to screen positive for Methamphetamine use after Vick's Vapoinhaler (and other nasal decongestants) intake, resulting in a false-positive for the illegal substance d-methamphetamine. Vick's VapoInhaler contains both Methamphetamine isomers, dextro-Methamphetamine (d-MAMP) and levo-Methamphetamine (l-MAMP). In this study, a methamphetamine ELISA kit was used to detect d-MAMP-positive results in samples injected with Vick's VapoInhaler. Different concentrations of Vick's (0-500ng) were injected into separate samples and then screened for methamphetamine. Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) was subsequently used to separate the enantiomers and quantitate specific concentrations of d-MAMP. Separating and quantitating Methamphetamine isomers in these Vick's products can eventually lead to more specific immunoassays which will produce less false-positives.
Committee Members: Stephanie Wetzel, Lyndsie Ferrara, Frederick Fochtman, Mandy Tinkey

10:30am Sierra Strnisa — **The Effects of Storage Conditions and Time on Extracted Ignitable Liquids Using GC-MS**

In arson investigation, the presence of ignitable liquid is important in determining intent. In some cases, extracts of fire debris recovered from the scene must be stored for later analysis; though little is known about how to properly store extracted samples while upholding analytical integrity over time. Three petroleum distillates were selected as samples and extracted onto activated charcoal strips using a passive headspace method. Samples were transferred to a nylon fire debris bag or PTFE-lined glass vial. Once contained, samples were stored at room temperature or refrigerated for 3, 6, and 9 months then analyzed using gas chromatography mass spectrometry. Analysis was performed through compound identification according to ASTM protocol by utilizing the NIST Mass Spectral database. Comparisons were made between all variables noting any loss of compounds. This research indicates a limited difference in compound retention over time between nylon fire debris bag and PTFE-lined glass vial.
Committee Members: Stephanie Wetzel, Lyndsie Ferrara, Frederick Fochtman, Mandy Tinkey

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11:00am Devin Doyle — **The Effects of the Evidence Preservation System on the Storage of DNA Samples**

Storage of collected samples is a concern for all disciplines of forensic science. Without proper storage, samples could become unusable due to degradation, cross contamination, etc. This research focuses on the storage of samples in the Evidence Preservation System (EPS) by Forensic Solutions, Inc., which is a controlled environment able to be programmed to control temperature and humidity while also preventing UV radiation exposure and bacterial growth on samples. The purpose of the research was to perform comparative studies between storage conditions of -20°C, 4°C, Room Temperature, and the EPS Unit storage environments by examining their effects on the quantity and quality of degraded DNA samples as well as the drying weight of samples. Due to the importance of the storage of forensic samples across all disciplines, the EPS unit could a very useful resource that could change and potentially improve the way various forensic samples are stored.
Committee Members: Pamela Marshall, Lisa Ludvico, Mark Dale

4:00pm Hannah Reidenbaugh — **The Disconnect Between Forensic Science and The Lawyers and Judges Who Represent It**

The goal of this research is to demonstrate a knowledge gap in the forensic science education of lawyers. In law schools across the country, there is minimal curriculum requirements focused on educating law students in forensic science. Of the top 50 law schools in the U.S., 20% offer a forensic science elective. After the rigor of law school, there is no requirement set to educate lawyers in forensic science. In fact, based on survey responses, 51% of criminal lawyers do not take part in forensic science continuing education programs. Though educators as well as professionals have voiced the need for scientific education, there has been little progress made. Criminal lawyers and judges should be adequately educated in forensic science in order to uphold the integrity of the justice system. Forensic Science education for lawyers should be offered in law schools as a possible solution for this disconnect.
Committee Members: Lyndsie Ferrara, Pamela Marshall, Bobbi Jo Wagner

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