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A COMBINED METHOD TO LOCATE AND IMPROVE DNA RECOVERY FROM FABRICS

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ABSTRACT

The purpose of this thesis project is to identify touch DNA on different clothing surfaces and improve DNA recovery and efficiency. This study undertook a multi-method approach to improve identify touch DNA on clothing and enhance recovery rates. The approach was initiated after Vacuum Metal Deposition (VMD) processing to visualize the location of DNA on the fabric's surface. The multi-method approach involved comparing wet-swab and M-Vac® techniques for DNA collection, evaluating silica-membrane-based (QIAmp™) and magnetic silica-based (DNA IQ™) DNA extraction methods, and gauging the efficacy of size exclusion chromatography in eliminating metal ions from VMD-processed DNA samples. Nine fabrics with duplicates, eighteen in total, were collected for processing. Dark colored fabrics were processed with silver and zinc, and the light-colored fabric samples were processed with gold and zinc. After collection, each sample fabric type had quadruplicates for statistical analysis. Results indicated that the multi-method approach did not apply a statistically significant effect on the concentration of DNA in a sample. The multi-method approach did apply a statistically significant effect on the number of allele peaks present in an electropherogram ($p<0.05$). QIAmp™, no size exclusion chromatography performed, and M-Vac® collection samples performed better than their respective counterparts in the general linear mixed effect model. A technique that employs the M-Vac® wet vacuum for gathering touch DNA samples, followed by DNA extraction utilizing the QIAmp™ kit, and bypasses size exclusion chromatography, can offer more detailed genetic profiles.

1. INTRODUCTION

The aim of this study is to enhance the efficiency of evidence processing in identifying touch DNA on clothing, thereby improving DNA recovery rates. Typically, forensic laboratories rarely process or do not process victim clothing for touch DNA as its location is not easily identifiable. Processing clothing without knowing the location of the touch DNA, so the whole item of clothing, would lead to possible dilution of the sample rendering it unusable. Currently, forensic laboratories encounter challenges in obtaining touch DNA from fabrics without some form of liquid staining, potentially compromising the integrity of DNA material through dilution. Utilizing liquid staining for touch DNA is a singular technique that is used to obtain the location of samples. This research endeavors to develop a comprehensive methodology to locate and enhance touch DNA recovery across various fabric types. This methodology, known as a multi-method approach, involves the integration of multiple techniques to optimize results.

Unlike current methodologies, which often rely on singular approaches, the multi-method approach utilized in this study combines various techniques to address three primary objectives. The approach compares the efficacy of wet-swab and Microbial wet-vacuum (M-Vac®) techniques for DNA collection, evaluates silica-membrane-based and magnetic silica-based DNA extraction methods, and assesses the effectiveness of size exclusion chromatography in removing metal ions from VMD-processed DNA samples.

This multi-method approach is initiated following the application of vacuum metal deposition (VMD) processing. It encompasses the careful selection of DNA collection techniques, extraction kits, and the application of size exclusion chromatography. Together, these components form a holistic approach to optimizing DNA recovery and efficiency in forensic investigations. Knowing the precise methods to blend from each category is vital for optimizing DNA extraction from fabrics treated with VMD.

The combined processing technique, VMD processing and a multi-method approach to obtain a DNA profile, can help identify and collect DNA evidence that could go unnoticed. When collecting touch DNA, most samples on surfaces are not easily identifiable. The common identifiers used in today's field are non-invasive detection systems, such as alternative light sources that emit wavelengths at a controlled range to visualize evidence through fluorescence (Van Oorschot, Ballantyne, & Mitchell, 2010). The use of vacuum metal deposition, VMD, on evidence can help identify the location of touch DNA and provide joint service where latent print evidence and DNA evidence coincide upon a singular item of evidence within cases. VMD represents a technique wherein metal is vaporized and deposited onto a surface, resulting in the formation of a discernable negative print (Fraser, Sturrock, Deacon, Bleay, & Bremner, 2011). The use of the M-Vac® wet vacuum system can help obtain more complete DNA profiles from evidence than the current DNA collection methods used today. Evidence using VMD for visualization can be processed in the latent evidence unit to search for prints and then any DNA present can be collected in a DNA laboratory, especially if quality prints are not obtained after VMD has occurred. Processing touch DNA evidence using a multi-method technique can

provide a streamlined protocol for evidence processing that can save time and resources. The combination of VMD processing and multi-method techniques (the collection methodology, specific extraction kit usage, and the utilization of size exclusion chromatography) may be the answer to some of the DNA missing in criminal cases that have been going undetected due to no protocol being established for touch DNA on fabrics.

The research design presented in this thesis is a multi-method approach that is used to determine the most efficient method for obtaining touch DNA on different fabrics. The main instrumentation used in this study is the vacuum metal deposition (VMD) machine and the M-Vac® wet vacuum. Silica-membrane based (QIAmp™) extraction kits, paramagnetic silica bead resin-based (DNA IQ™) extraction kits, and Micro Bio-spin® columns with Bio-Gel® P-30 are also used to improve touch DNA recovery from fabrics. Vacuum metal deposition (VMD) is a technique that is used to detect latent fingerprints on nonporous surfaces by the creation of negative prints (Fraser, Sturrock, Deacon, Bleay, & Bremner, 2011). The VMD uses a thin layer deposition of metal onto a substrate and applies thermal evaporation in a high vacuum environment to the metal to visualize latent prints (Fraser, Sturrock, Deacon, Bleay, & Bremner, 2011). The Microbial wet-vacuum instrument (M-Vac® Systems Inc.) was developed for microbial sampling of large surfaces, using a chemical-based buffer for collection and stabilization. Butterfield's buffer, the typical buffer used by the M-Vac® wet vacuum system, contains 34 grams of monobasic potassium phosphate, 175 mL of 1N sodium hydroxide, and 925 mL of demineralized water (Thermo Fisher Scientific, 2010). Eighteen total fabrics samples were processed by VMD using gold/zinc or silver/zinc mixtures, and the DNA present was

collected by both wet swabbing and the use of the M-Vac®. Half of the DNA samples collected were extracted using different silica-based extraction kits, silica-membrane based and silica bead resin-based kits, and amplified for capillary electrophoresis. Of the extracted DNA samples, 25 µL were subjected to size exclusion chromatography, using Micro Bio-Spin® columns, to remove metals that still may be present in the DNA extracted. Profiles were analyzed for completeness to determine the best method for obtaining touch DNA.

The literature review below provides a full and thorough justification for research regarding the use of VMD and the M-Vac® wet vacuum in obtaining touch DNA from evidence. The process of forensic DNA testing has changed and improved over the years; however, the collection methods have remained the same. The conventional methods, i.e., swabbing, cutting, and taping, have limited effectiveness on porous substrates (McLamb, Adams, & Kavlick, 2020). Mini tape is no longer used in the forensic science field due to discontinuation by manufacturer. Touch DNA has become a common occurrence in forensic labs for analysis (Van Oorschot, Ballantyne, & Mitchell, 2010). Touch DNA is recovered from skin cells that are transferred onto an object when an individual touches it, making it unable to be seen by the naked eye. The VMD provides analysts with the ability to visualize these prints and locate the DNA associated with the skin cells. Touch DNA samples are susceptible to degradation due to environmental factors and improper handling (Aloraer, Hassan, Albarzinji, & Goodwin, 2017). Using a chemical-based stabilization, such as the Butterfield buffer used by the M-Vac® wet vacuum, is advantageous when working with forensic touch DNA samples collected at crime scenes as it stabilizes the DNA collected and prevents deterioration. The utilization of vacuum metal

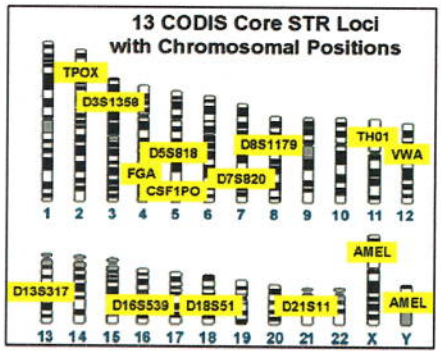
deposition and the M-Vac® wet vacuum could be an essential tool in the forensic science field, but further research needs to be conducted to advance the technique.

2. LITERATURE REVIEW

2.1 DNA Analysis in the Forensic Science Field

Throughout the years since its first use in 1987, DNA analysis has become a key piece of advancing criminal investigations. Initially, the analysis of DNA samples was limited to biological samples that contained nucleated cells (Lee & Ladd, 2001). Since the 1990's, mitochondrial DNA (mtDNA) has become desirable for forensic analysis when nucleated DNA is unavailable. In forensic biology, mtDNA is used mainly for its matrilineal inheritance, lack of recombination, and high copy numbers (Budowle, Allard, Wilson, & Chakraborty, 2003). Samples that DNA has been successfully extracted from and typed are as follows: blood and bloodstains, tissues, bones, semen and seminal stains, hair, saliva, teeth, urine, tissue, organs, fingernails, skin cells, and other biological fluids (Lee & Ladd, 2001). DNA is extracted from the biological sample, quantified, PCR amplified, and detected through capillary electrophoresis to obtain a DNA profile of the individual for comparative analysis. The 13 CODIS (Combined DNA Indexing System) loci, pictured in Figure 1, are employed by the United States Federal Bureau of Investigation to create a complete genetic profile regarding criminal investigations. DNA profiles from evidence collected at a scene have increased in presence at court due to its power of exclusion/inclusion. These techniques cannot prove that a person committed a crime, but they can prove if they were there at some point in time and left DNA evidence (Luftig & Richey, 2001). DNA analysis has revolutionized the forensic science field by determining an individual's presence at a crime scene by use of a minute sample.

Figure 1: *The 13 CODIS core STR loci*



Note. Loci were chosen by the FBI as a standardized set used for DNA profile generation and were entered into the CODIS database. Figure is taken from NIST STRBase.

2.1.1 Current DNA Collection Methods

Before DNA testing can be performed, the sample must be collected appropriately depending on the type of sample. Currently, the most common DNA collection method is the double swabbing technique. The technique utilizes the use of one wet swab and a dry swab. The first swab is moistened with deionized water and rubbed across the surface of the evidence item. A dry swab is then applied to collect possible skin cells that were left behind (Pang & Cheung, 2007). Another DNA collection method was the use of mini-tape lifters, that was first introduced into the forensic science field in the early 2000's, to recover residual cellular material from evidence (Hall & Fairley, 2004). Mini-tape lifting was preferred by forensic science laboratories due to it being quick, easy to apply, cost-effective, and it ensured invisible samples were collected and preserved correctly (Hess & Haas, 2017). Several forensic laboratories use a scraping method for porous items collected from a crime scene. The item of

interest is scraped with a sterile scalpel blade and collected for possible skin cells that are present (Williamson, 2012).

In a study, four different collection methods were compared to determine the most efficient method for recovering DNA from evidence (Hess & Haas, 2017). Researchers used dry swabbing, wet swabbing, mini-tape lifting, and scraping methodology to collect DNA samples from clothing. Eighteen clothing garments were analyzed with contact stains deposited in triplets in four places (three times for each donor), resulting in 207 samples. The STR results showed that DNA recovered by dry swabbing and wet swabbing had 52% and 58% of alleles detected, respectively. The mini-tape lifting and scraping methods had 67% and 73% of alleles detected, respectively (Hess & Haas, 2017). More complete DNA profiles can be obtained using the mini-tape lifting or scraping method, but they are recommended to not be performed at crime scenes due to the increased risk of contamination or the potential of DNA loss in an uncontrolled environment (Williamson, 2012).

2.1.2 Short Tandem Repeats in DNA Profiling

The current method used to obtain forensic DNA profiles is to examine short tandem repeats (STRs) using PCR amplification and capillary electrophoresis. STRs are microsatellites that are highly polymorphic (Panneerchelvam & Norazmi, 2003). STRs are small segments of the noncoding region of DNA that contain 2-7 base pairs in a sequence that repeats a variable number of times (Craig, Fowler, Burgoyne, Scott, & Harding, 1988). STR's constitute a cornerstone in forensic science by virtue of their pronounced polymorphic nature,

characterized by significant variations among individuals. This variability is instrumental in establishing unique genetic profiles for individuals. Moreover, the short repeat lengths inherent to STR's provide an ease for amplification, rendering them particularly advantageous for forensic analyses, notably in degraded DNA samples. The STRs used for DNA profiles in the forensic science field are mainly tetranucleotide repeats comprised of alleles of discrete size (Panneerchelvam & Norazmi, 2003). An advantage of using tetranucleotide STR loci in forensic DNA typing is that the small allele size range allows for several loci to be amplified simultaneously through multiplexing, increasing the discriminating power with observed heterozygosity (Edwards & Gibbs, 1994).

The STR sequencing project was formed to show all allelic diversity in specific regions of the human genome (Gettings, Ballard, Devesse, King, Parson, Phillips, & Vallone, 2017). Four laboratories (the National Institute of Standards, King's College London, University of North Texas Health Sciences Center, and University of Santiago de Compostela) placed all the available STRseq data into a GenBank record for the National Center for Biotechnology Information (NCBI). The records are sub-divided into common autosomal STRs, alternate autosomal STRs, Y-chromosomal STRs, and X-chromosomal STRs (Gettings, Ballard, Devesse, King, Parson, Phillips, & Vallone, 2017).

2.1.3 DNA Extraction

DNA analysis became increasingly common in the forensic science field, after its first use in a case in 1987, as an effective piece of evidence (Haddrill, 2021). Due to its commonality, the

methods for extracting DNA advanced significantly to achieve higher yields and improve overall efficiency. The Chelex®- 100 method is known to be used in the forensic science field due to its efficiency, low cost, and simple methodology. Unfortunately, the Chelex®- 100 method has shown that inhibition issues are present in blood samples due to heme components remaining in the extracted product (Idris & Goodwin, 2015). To remove inhibitors found in the extracted DNA, new solid phase methods have been developed and implemented in extraction kits. QIAmp™ and PrepFiler™ extraction kits have gained popularity in the forensic science field for the new solid-phase methods present (Idris & Goodwin, 2015). Although DNA extraction kits are successful in recovering higher DNA yields, the large number of transfer steps allows for an increased risk of contamination (Walsh, Metzger, & Higuchi, 1991).

DNA IQ™ is an extraction method that utilizes paramagnetic silica beads to separate DNA from inhibitors in a sample. In a study conducted in 2015, the DNA IQ™ and Chelex®-100 methods were compared to determine which will result in a more complete DNA profile from samples. The STR genotyping results showed the DNA IQ™ method effectively removed PCR inhibitors from the sample and obtained more complete DNA profiles (Hu, Liu, Yi, & Huang, 2015).

2.1.4 Multiplex PCR Amplification

The forensic science community has greatly benefitted from the use of the polymerase chain reaction (PCR) technique. PCR is an enzymatic process that uses a cell's normal mechanisms for replication and amplification to create hundreds of copies of DNA for analysis.

A specific region of the DNA, within the noncoding region, is selected for analysis, and primers are generated (twenty to thirty base pairs) on either side of the desired region. The primers and DNA sample are added to a test tube together, along with DNA polymerase to catalyze the synthesis of the new strands of DNA. The mixture is heated and cooled slowly to anneal the DNA strands and allow the primers to bind to the single strands. The mixture is heated once again to enact the DNA polymerase to start the production of copies of the strand, and the heating and cooling cycle is repeated until the replication of the DNA strands reaches an exponential rate. (Luftig & Richey, 2001). PCR amplification allows analysts to obtain a large amount of the DNA sequence they need to analyze that would usually be undetectable.

The PCR amplification allows for more than one region to be copied simultaneously when multiple primer sets are added to the reaction mixture. This amplification of multiple DNA regions concurrently is called multiplex PCR (Edwards & Gibbs, 1994). The first commercial kits used for typing multiple STRs in a single reaction became available in the late 1990s (Van Oorschot, Ballantyne, & Mitchell, 2010). For the multiplex PCR in these amplification kits to work properly, the primer sets need to be compatible. The primer sets should have similar annealing temperatures and avoid excessive regions of complementarity (Butler, Ruitberg, & Vallone, 2001). The primer sets for each locus are fluorescently tagged to differentiate the specific STR region that is being amplified. PCR amplification has become an essential tool in the creation of DNA profiles in the forensic science field.

2.1.5 Detection of PCR Products Through the Use of Capillary Electrophoresis

Once PCR amplification is completed, the STR loci must be individually separated to generate a DNA profile. The forensic science field uses capillary electrophoresis to achieve the separation of STR loci. The use of capillary electrophoresis for STR fragment separation in the forensic science field was first presented in the early 1990s. The capillary electrophoresis system sends the fluorescently labeled DNA fragments through the capillary tubes with a polymer (McCord, Jung, & Holleran, 1993). The capillary electrophoresis system is an efficient way to separate PCR products and create DNA profiles. When an electrical current is applied, the negatively charged DNA particles travel from the cathode to the anode end of the capillary. As the DNA moves, the smaller fragments travel through the polymer more quickly than the larger fragments. The PCR products are then separated individually when they reach the detection window of the capillary system. The fluorescent tags on the PCR products are then excited by a laser causing them to emit the corresponding light. The fluorescent lights are recorded by a computer, compared to the sizing standard, and separated by fluorescent dye color to generate the electropherogram (Butler & McCord, 2004).

2.2 Touch DNA and its Presence in Forensic Cases

Since 1997, touch DNA analysis has become an integral part of the forensic science community when other biological evidence might not be available and serves as a tool for investigators (Van Oorschot & Jones, 1997). When analyzing touch DNA, touch deposit composition must be considered. The outermost layer of the epidermis sheds skin cells when in

contact with an item; however, some researchers propose the DNA obtained may originate in several places. Fragmented cells, cell-free DNA, transferred exogenous nucleated cells, and endogenous nucleated cells are present on the epidermis layer and are all possible sources of touch DNA recovered from an evidence sample (Burrill, Daniel, & Frascione, 2019). Determination of the origin of DNA in touch deposits can help optimize the recovery of samples and the methodology used for analysis.

In 1997 it was determined that DNA profiles could be generated from touched objects, leading to investigators collecting DNA from a wider array of exhibits (Van Oorschot & Jones, 1997). The nature of the substrate touch DNA has been recovered from could have an influence on DNA extraction (Alkebti, 2018). Surfaces retain touch DNA samples differently and it is imperative that investigators consider the appropriate collection method for the different types of surfaces they encounter. Through the results presented in a study conducted by Alketbi & Goodwin, it is recommended that collectors use cotton swabs on non-porous surfaces, such as glass, and mini-lifting tapes to develop higher DNA quality on porous surfaces (Alketbi & Goodwin, 2019). In Table 1 (displayed below) a summary of DNA levels recovered from differing touched surfaces provides insight into how much influence the surface type has on the quantification levels of touch DNA recovered (Burrill, Daniel, & Frascione, 2019).

Table 1. *Summary of studies reporting amounts of DNA recovered from touched items*

Surface	Length of Contact	Nature of Contact	Total Quantity recovered (ng)	Author	Publication Year
Swabbing of hand	--	--	2-150	Van Oorschot and Jones	1997

Plastic knife handle, mug, glass	15 min	Holding	7-34	Van Oorschot and Jones	1997
Swabbing of hand	--	--	0.1-6.4	Bright and Petricevic	2004
New lower bed sheet	1 night	Sleeping	0-8	Petricevic et. al.	2006
Glass slides	5 sec	Pressure	0-2	Allen et. al.	2007
Paper	30 sec	Pressure	0-110	Sewell et al.	2008
Door frame	1 min	Grabbing	0->0.2	Raymond et. al.	2008
Cartridge casing	30 sec	Handling	0.3-0.7	Horsman-Hall et. al.	2009
Cotton	10-15 sec	Rubbing	6-12	Goray et. al.	2010
Plastic	10-15 sec	Rubbing	0.4-0.5	Goray et. al.	2010
Melamine-coated board	10 sec	Pressure	0-160	Kamphausen et. al.	2012
Glass	1 min	Holding	0-5	Daly et. al.	2012
Fabric	1 min	Holding	0-15	Daly et. al.	2012
Wood	1 min	Holding	0-169	Daly et. al.	2012
Infant's clothing	1 min	Rubbing	0.3-9	Goray et. al.	2012
Plastic block	1 min	Rubbing	0-2.5	Goray et. al.	2012
Plastic syringe	10 sec	Holding	0-80	Poetsch et. al.	2013
Glass slides	Brief	Fingerprint pressure	0-17.6	Thomasma and Foran	2013
Knife handle	1 min X 4	Simulated regular use	~1-10	Meakin et. al.	2015
Glass slides	15 sec	Fingerprint pressure	0-1.5	Oleiwi et. al.	2015
Glass	10 sec	Pressure	0-5	Goray et. al.	2016
Knife handle	Brief	Grip/stabbing	0-4.8	Samie et. al.	2016
Non-porous cables	Brief	Fingerprint pressure	0-3	Lim et. al.	2016
Plastic tubes	10 sec	Holding	0.04-3.8	Fonnelop et. al.	2017
Car steering wheel	2-60 min	Holding	0.21-134	Kirgiz and Calloway	2017
Plastic cable ties	Brief	Used to bind objects	0-39.8	Steensma et. al.	2017
Polycarbonate board	Brief	Fingerprint pressure	0-3.5	Tobias et. al.	2017

Note. The table was updated and adapted from table published by Meakin and Jamieson (2013).

2.3 Latent Print and DNA Collection Considerations

When an individual comes into contact with an item, by hand placing or grabbing, residues are found to be present on the item. These residues can leave a latent mark (Horvath, 2022). The composition of latent prints can be affected by many factors such as sweat secretion, diet, environmental factors, and chemical properties of the substrate (Horvath, 2022). Touch DNA can be recovered from the nucleus of skin cells that are left behind when a person touches an item (Hess & Haas, 2017). However, when collecting touch DNA, most

samples on surfaces are not easily identifiable (Van Oorschot, Ballantyne, & Mitchell, 2010). Skin cells are microscopic residues that are left behind through contact and cannot be seen by the naked eye. Latent prints may contain biological materials that can be pertinent to the development of the forensic analysis of evidence.

DNA and latent prints evidence is known to overlap in cases; however, it is hard to know which takes precedence over the other at times. Some crime labs have created procedures for these types of evidence items. Most crime labs require the latent evidence unit to process evidence first and ensure they wear gloves. The latent print examiner is encouraged to collect and preserve the touch DNA that can be obtained from the latent prints (Amick, Bivins, Cathcart, Hammer, & Pippin, 2015). An example case item reviewed is the processing of a latex glove found at a crime scene. The examiner swabs the area in between the fingers of the glove to obtain touch DNA that is present, while still preserving prints that are present of the fingers on the glove. The acknowledgment and development of collection procedures for touch DNA found in latent prints could be essential to the production of a case (Amick, Bivins, Cathcart, Hammer, & Pippin, 2015).

2.3.1 Vacuum Metal Deposition

Vacuum metal deposition, VMD, is a technique used to detect latent fingerprints on nonporous surfaces (Fraser, Sturrock, Deacon, Bleay, & Bremner, 2011). The VMD uses a thin layer deposition of metal onto a substrate and applies thermal evaporation in a high vacuum environment to the metal to visualize latent prints (Horvath, 2022). In a study regarding the

Visualization of fingermarks and grab impressions on fabrics, researchers observed the VMD's ability to produce impressions on different types of fabrics. Researchers used fifteen donors, who had not washed their hands thirty minutes prior to the experiment, to leave fingermarks on cotton, nylon, polyester, and polycotton fabrics. The VMD used a mixture of gold and zinc to create visible impressions on each fabric sample. Out of the fabrics present in the study, the shiny nonporous textiles showed greater ridge detail in the prints than the other fabrics. The VMD can be a useful tool to visualize fingermarks present on clothing obtained from a crime scene (Fraser, Sturrock, Deacon, Bleay, & Bremner, 2011). However, an article over fingerprint development states that prints obtained using the VMD method will fade, sometimes within a few hours (HOSDB, 2005). A more recent study explored the effects alternative metal deposition mixtures had on the detail of the developed prints. Eight metal processes were placed and tested on each fabric type; gold/zinc, silver, aluminum/zinc, silver/zinc, sterling silver, sterling silver/zinc, copper, and copper/zinc. The metal mixtures, aluminum/zinc, and copper/zinc, produced latent prints with the greatest detail (Horvath, 2022).

Regardless, the VMD's success in producing quality ridge details is not a necessary component when looking for DNA. This process can show finger-like markings which can be targeted for DNA. When using the VMD to locate touch DNA, one must take the risk of metal inhibition of processes that utilize DNA, such as PCR, into account. Transition metals can be extracted with the DNA in a sample, and they can become a PCR inhibitor by interfering with the fluorescent dyes. In a study conducted by Lilliana Moreno and Bruce McCord, researchers observed the effects copper had on the DNA extracted and the electrophoresis process. Copper

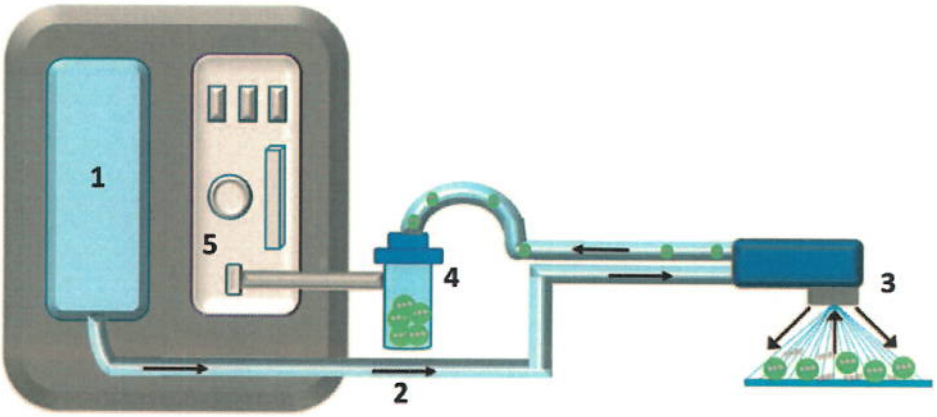
chloride was added to the control DNA in a serial dilution form and allowed to sit for an hour before the samples were sent for amplification. The samples were then run through capillary electrophoresis using the manufacturer's recommendations. The control DNA sample with a 0.001 M concentration of copper chloride exhibited no DNA present in the results. As a cleanup step, samples were incubated in formamide for 1-4 hours before re-attempting capillary electrophoresis. The results show the metal ions present causing complete polymerase inhibition, but if a cleanup step is presented before amplification, then the expected DNA product can be obtained. Metal ions present in a biological sample can affect the conformation and charge of the DNA and can ultimately lead to the loss of a DNA profile for a key piece of evidence in the forensic science field (Moreno & McCord, 2017).

2.3.2 M-Vac® Wet Vacuum DNA Collection System

The process of forensic DNA testing has changed and improved over the years; however, the collection methods have remained relatively the same. Swabbing and cutting collection methods have been used since the beginning of DNA analysis in the forensic science field. The conventional methods, swabbing, cutting, and taping, are known to have limited effectiveness on porous substrates due to DNA's ability to diffuse and be unavailable for surface sampling. The M-Vac® wet-vacuum-based collection method can be used as a possible alternative for DNA collection from porous surfaces (McLamb, Adams, & Kavlick, 2020). The Microbial wet-vacuum instrument (M-Vac® Systems Inc.) was developed for microbial sampling of large surfaces, using a chemical-based buffer for collection and stabilization. Since 2011, the M-Vac® wet vacuum has been applied in the forensic science field for human identification purposes:

sampling of saliva from human skin, bloodstains on different surfaces, dried saliva on porous and non-porous surfaces, and shed cells on clothes and touch DNA (Hedman, Agren, & Ansell, 2015). Figure 2 (below) shows the M-Vac® mechanism and how the sterile buffer is applied and vacuumed into a collection bottle when obtaining a sample (Vickar, Bache, Daniel, & Frascione, 2018).

Figure 2: *Schematic of the M-Vac® system*



Note. The solution buffer (1) is pressurized and distributed through tubing (2) to the sampling head. The buffer solution is sprayed (3) onto the sample surface while simultaneously vacuumed into an adjacent tube. The DNA sample collected in the solution is then transferred into the collection bottle (4) due to the vacuum pressure (5). Figure is taken from Vickar, Bache, Daniel, & Frascione, 2018

Touch DNA samples are susceptible to degradation due to environmental factors and improper handling. In a study conducted in 2017 by Aloraer, Hassan, Albarzinji, and Goodwin, the researchers determined whether detergent-based wetting agents could increase the DNA collected from touch samples. Touch DNA samples were taken from glass, plastic, and metal bottles after applying thirty seconds of contact using medium pressure. The DNA was collected

using a double swab technique. The buffer used to obtain the samples was made in-house using 1% n-lauroylsarcosine, 10 mM Tris-HCL, 0.1 mM EDTA, and 50 mM NaCl. Five batches of samples were collected from each object and extracted using the PureGene® Extraction kit. The results showed that the DNA concentration recovered with a buffer was significantly higher than compared to samples collected with water. The DNA profiles of samples collected with water showed higher deterioration than those using the cell lysis solution. Using a chemical-based stabilization is advantageous when working with forensic touch DNA samples collected at crime scenes (Aloraer, Hassan, Albarzinji, & Goodwin, 2017).

In a recent experiment conducted by McLamb, Adams, and Kavlick, researchers obtained twenty-two substrates, varying in porosity. The substrates had twelve diluted blood drops placed onto their surface using a dropper (1:100 Butterfield's buffer dilution for each drop). The samples were then allowed to dry overnight. Each sample was collected by two methods, the wet swab using deionized water and the wet vacuum using a buffer. The collected samples were quantified using the Quantifiler® Human Plus DNA Quantification kit. The results showed that the wet vacuum method yielded twelve times more DNA than the wet swab method on eighteen samples. No samples recovered yielded more DNA by the wet swab method than the M-Vac® wet vacuum. The M-Vac® wet vacuum may be able to serve as an alternative DNA collection method on porous items collected at a crime scene (McLamb, Adams, & Kavlick, 2020).

The DNA concentrations yielded by the M-Vac® method may be dependent on the type of sample being collected. Researchers compared the traditional swabbing DNA collection

technique to the use of the M-Vac® wet vacuum system (Vickar, Bache, Daniel, & Frascione, 2018). Researchers used a diluted saliva solution to represent a realistic concentration of touch DNA and sprayed the solution onto tiles and bricks. Half of the surface (for both tile and brick) was swabbed using the double swab technique, and the other half was collected using the M-Vac® and poured through a PES membrane filter, connected to the M-Vac® vacuum pump. The bricks were also assessed for touch DNA from twelve volunteers, the collection was carried out in the same manner as the previous samples. After extraction and quantification, the results showed that the M-Vac® collected a significantly higher concentration of DNA on the bricks than double swabbing. However, the tile results showed that the M-Vac® collected less DNA than the double swabbing, likely due to the surface being nonporous. The touch DNA obtained from the bricks showed no distinct trend in whether the double swabbing or the M-Vac® had higher collection rates. The data shows that the M-Vac® could be a useful tool in the forensic science field, however, more research needs to be done on differing surfaces to accurately determine the amount of DNA it can collect (Vickar, Bache, Daniel, & Frascione, 2018).

2.4 Significance of Combined Method to Locate and Improve DNA Recovery from Fabrics

The combined utilization method of the VMD and the M-Vac® wet vacuum system could be efficient in the DNA downstream processing on fabric-based evidence. The first step to collecting touch DNA is determining the location to target as touch DNA on surfaces is not readily identifiable (Van Oorschot, Ballantyne, & Mitchell, 2010). Touch DNA samples do not contain large amounts of biological material and must be carefully collected to prevent the risk of degradation or contamination (Aloraer, Hassan, Albarzinji, & Goodwin, 2017). Currently,

analysts employ swabbing techniques to collect touch DNA from clothing from crime scenes if the location is known. In a study conducted in 2010 by researcher Van Oorschot, results showed that swabs of touch DNA samples from clothing did not produce high-quality results as expected. The swabs contained PCR inhibitor factors and produced difficult-to-interpret DNA profiles (Van Oorschot, Ballantyne, & Mitchell, 2010). The chemical-based buffer utilized by the M-Vac® wet vacuum system allows for the stabilization of the touch DNA collected from a sample. The stabilization of the DNA sample can allow for the creation of a more complete DNA profile. The M-Vac® obtains a higher concentration of DNA on clothing because it is pulling the wearer and the depositor, which is not ideal. The combined method to locate and improve touch DNA recovery on fabrics could be an essential tool in the forensic science field, especially in regards to assault/homicide cases. Further research needs to be conducted to further advance the multi-method DNA recovery technique.

This study tested a multi-method approach to enhance the efficiency of evidence processing by identifying touch DNA on clothing, thus improving DNA recovery rates. The research conducted in this study is important because forensic laboratories face challenges in obtaining touch DNA from fabrics without liquid staining, which can compromise DNA integrity. Developing a comprehensive methodology is crucial for overcoming these challenges and improving DNA recovery rates. The multi-method approach integrates multiple techniques to optimize touch DNA recovery. The approach is implemented following the application of VMD processing to visualize the location of the touch DNA on the fabrics. The multi-method approach includes comparing wet-swab and M-Vac® techniques for DNA collection, evaluating

silica-membrane-based and magnetic silica-based DNA extraction methods, and assessing the effectiveness of size exclusion chromatography in removing metal ions from VMD-processed DNA samples. Unlike current practices that often rely on a singular approach, the multi-method approach combines various techniques to address multiple objectives simultaneously. It offers a more comprehensive and effective approach to location and DNA recovery from fabrics. The data will provide insights into the efficacy of different DNA collection and extraction methods, as well as the effectiveness of size exclusion chromatography in enhancing DNA recovery. Specifically, it will reveal which combination of methods yields the highest DNA recovery rates from VMD-treated fabrics, thus informing future forensic practices.

3. MATERIALS AND METHODS

3.1 Preparation of Samples Using VMD and Collection

Nine fabrics with duplicates, eighteen in total, were collected and cut into hand sized squares for processing. Seven of the nine fabrics were from worn garments to simulate clothing obtained from victims of a crime, and the two remaining clothing items were purchased new from a store. One individual was used to deposit a DNA sample to create a uniform "suspect" profile to determine the different collection method's efficiency in retrieving DNA from possible mixed samples. The donor was previously determined to have a medium shedder status using the methodology highlighted in the 2021 article *Determination of shedder status: A comparison of two methods involving cell counting in fingerprints and the DNA analysis of handheld tubes.* The donor refrained from handwashing for one hour prior to the experiment, and each fabric sample was pressed for five seconds to obtain a palm print. The palm prints were pressed over the course of a month at the same time each day. Once the fabric samples were pressed to produce a palm print containing touch DNA, VMD was performed based on the fabric color. VMD was performed over a month based on the availability of the latent print unit at the Oklahoma State Bureau of Investigation. Dark colored fabrics were processed with 0.002g of silver and 1.0g of zinc, and the light-colored fabric samples were processed with 0.002g of gold and 1.0g of zinc. The pressure of the VMD chamber was set to 3×10^{-4} mbar to vaporize both metals until the print could be visualized, approximately one hour. Each fabric sample was then placed into a labeled manila folder for identification during collection. A description of the

fabrics used within this study and the metal they were processed with is displayed below in Table 2. Fabrics made of various materials and colors were used in this study. The metal used for VMD processing was chosen based on the fabric color.

Table 2. *Selection of fabric descriptions subjected to VMD analysis*

Item	Material	Color	VMD Metal
1- Fabric square (6.5"x 7.5")	100% polyester (dri-fit) (worn)	Orange	Gold
2- Fabric square (6.5"x 7.5")	95% nylon/5% spandex (lace) (worn)	Coral	Gold
3- Fabric square (6.5"x 7.5")	100% cotton (ribbed) (worn)	Beige	Gold
4- Fabric square (6.5"x 7.5")	65% cotton/18% rayon/16% polyester/1% spandex (Jean) (worn)	Black	Silver
5- Fabric square (6.5"x 7.5")	100% polyester (brushed) (worn)	Black/white plaid	Silver
6- Fabric square (6.5"x 7.5")	98% cotton/2% spandex (smooth) (worn)	Coral	Gold
7- Fabric square (6.5"x 7.5")	80% silk/16% nylon/ 4% spandex (medium weave) (worn)	Blue	Silver
8- Fabric square (6.5"x 7.5")	100% polyester (upholstery) (new)	Blue/brown	Silver
9- Fabric square (6.5"x 7.5")	100% polyester (fleece) (new)	White/multi	Gold

For touch DNA collection, each fabric sample was photographed with scale and then marked on butcher paper into two sections. The first half, the left side of the fabric, was processed by the wet swabbing technique for DNA collection. The swabs were placed back into their packaging, labeled, and placed into a manila folder. The right half of each sample fabric was collected using the M-Vac® wet vacuum method. The DNA filtrate of the M-Vac® wet vacuum was poured onto the polyether sulfone (PES) filter paper twice, using a 2" diameter

funnel to concentrate the filtrate to the center of the filter. The filters were left to completely dry, and then placed into clear coin envelopes. The coin envelopes were labeled and then placed in a manila folder. Both collection folders, the swabs and filters, sat at room temperature until DNA extraction could be conducted.

3.2 DNA Extraction

Two extraction methods were utilized in this study. The two swabs for each sample were cut in half and the filters were cut into quarter pieces to create replicates for each fabric type. Two copies of each DNA sample were extracted using QIAmp™ mini kit, a silica--membrane--based extraction method. The samples were extracted according to the *QIAmp™ mini kit Quick-Start Protocol* (Qiagen, 2018). Thirty-six swab samples and thirty-six M-Vac® wet vacuum samples were placed in separate 1.5 mL microcentrifuge tubes, respectively. 180 µL of buffer ATL and 20 µL of proteinase K were added to each sample tube. The samples were then vortexed and placed to incubate at 56°C for one hour. Next, 200 µL of buffer AL was added and then vortexed for 15 seconds. The samples were then incubated at 70°C for 10 minutes. After incubation, 200 µL of ethanol was added and vortexed for 15 seconds. The samples were then pipetted into a QIAmp™ Mini spin column and centrifuged at 6000 x g (8000 rpm) for 1 minute. The flow through was discarded and the spin column was placed in a new 2 mL collection tube. 500 µL of buffer AW1 was added to each spin column and centrifuged at 6000 x g (8000 rpm) for 1 minute. The flow through was discarded and the spin column was placed in a new 2 mL collection tube. 500 µL of buffer AW2 was added to each spin column and centrifuged at full speed for 3 minutes. The flow through was discarded and the spin column was placed in a new

1.5 mL collection tube. The next step was to place 50 µL of buffer AE into the spin columns and incubate at room temperature for 1 minute. The samples were then centrifuged at 6000 x g (8000 rpm) for 1 minute to elute the DNA. 25 µL DNA were placed into new 2 mL microcentrifuge tubes for size exclusion chromatography. The DNA samples were placed in the -20°C freezer for storage until quantification.

The other half of DNA sample replicates were extracted using the DNA IQ™ kit, a paramagnetic silica bead resin extraction method. The samples were extracted according to the *DNA IQ™ System- Database protocol* (Promega, 2016). Thirty-six swab samples and thirty-six M-Vac® wet vacuum samples were placed separately in ClickFit® Microtubes. 250 µL of lysis buffer, with 1 M DTT present, was added to the sample and incubated at 70°C for 30 minutes. The samples were then centrifuged for 2 minutes at maximum speed, and the spin basket was removed. The stock resin was vortexed for 10 seconds to thoroughly mix the resin. 7 µL of resin was added to the samples, and vortexed for 3 seconds at high speed. The samples were then incubated at room temperature for 5 minutes, vortexed for 3 seconds once every minute during the incubation period. The tubes were then vortexed for 2 seconds at maximum speed, and then placed into the magnetic stand. The solution was discarded without disturbing the resin pellet. 100 µL of lysis buffer was added to the tubes and vortexed for 2 seconds at maximum speed. The tubes were returned to the magnetic stand and the lysis buffer was discarded. 100 µL of 1X wash buffer was added to the tubes and vortexed for 2 seconds at maximum speed. The tubes were then returned to the magnetic stand and the wash buffer was discarded. The washing steps was repeated two more times. The sample tube was placed on

the magnetic stand with the lids open to allow for the resin to air-dry for 5 minutes. 50 µL of elution buffer was added to the tubes and vortexed for 2 seconds at maximum speed. The tubes were then incubated at 65°C for 5 minutes. The tubes were removed from the heating block, vortexed for 2 seconds at maximum speed, and immediately placed in the magnetic stand. The solution was removed and placed into new 2 mL microcentrifuge tubes. 25 µL DNA were placed into new 2 mL microcentrifuge tubes for size exclusion chromatography. The DNA samples were placed in the -20°C freezer for storage until quantification.

3.3 DNA Quantification

25 µL of DNA samples that were extracted were quantified using the Quantifiler® DNA Quantification Kit on a Bio-rad® FX96 Real-Time System C1000 Touch Thermal Cycler. The samples were quantified according to the protocol found in the *Quantifiler® Kits User's Manual* (Applied Biosystems, 2006). To prepare the reagents, the primer mix was thawed and vortexed for 5 seconds. The Quantifiler® PCR mixture was swirled, not vortexed, before beginning sample preparation. In a 2 mL microcentrifuge tube, 10.5 µL of primer mix and 12.5 µL of Quantifiler® PCR reaction mix (for each sample) was added and vortexed for 5 seconds. The master mix tube was then centrifuged briefly. Next, 23 µL of the master mix was pipetted into each reaction well of the plate. 2 µL of sample, standard, or control was added to the appropriate wells. The reaction plate was then sealed using a MicroAmp® Optical Adhesive Film and centrifuged at 3700 rpm briefly to remove any air bubbles that were present at the bottom of the wells. The reaction plate was then loaded onto the Bio-rad® FX96 Real-Time System C1000 Touch Thermal Cycler, and the standard and sample names were entered into the plate set-up program in the

software. The plated samples underwent a 10-minute hold at 95°C to activate the Taq polymerase that was present. The samples then underwent 40 PCR cycles, each consisting of 15 seconds at 95°C and then followed by 1 minute at 60°C. Once the system had gathered all data, showing the negative control samples did not show any detectable DNA and the standard curve was acceptable, the concentration of each sample was used to target the appropriate amount of DNA for the amplification process.

3.4 Size Exclusion Chromatography

The remaining 25 µL of extracted DNA samples underwent size exclusion chromatography to remove any metal ions that might be present that could inhibit PCR. The samples were pipetted into a Micro Bio-spin® column with Bio-Gel® P-30 and centrifuged at 8000 rpm for 5 minutes (or until all the sample was spun down). The columns were removed, and the samples were then placed into 2 mL microcentrifuge tubes. The DNA samples were then placed into a -20°C freezer until quantification. The size exclusion chromatography DNA samples were quantified using the same Quantifiler® kit as the samples from the previous section.

3.5 DNA Amplification

Following quantification, all samples were amplified using the PowerPlex® Fusion amplification kit on the Applied Biosystems® GeneAmp® PCR System 9700. The samples were amplified according to the protocol found in the *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* (Applied Biosystems, 2020). The

master mix was prepared consisting of 5 µL of PowerPlex® Fusion 5X master mix and 5 µL of PowerPlex® Fusion 5X primer pair mix per sample. 10 µL of the prepared master mix was added to each reaction plate well. 15 µL of DNA was added to the corresponding wells, and then vortexed for 10 seconds. The samples were then pipetted into the reaction well plate. The positive amplification control used the 2800M control DNA dilution, and the negative amplification control used distilled water in place of the 15 µL of DNA. The plate was briefly centrifuged to remove any air bubbles from the bottom of the wells. The reaction plate was amplified using a GeneAmp® PCR System 9700 thermal cycler according to the protocol outlined in the *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* (Applied Biosystems, 2020). PCR cycling was preceded by a 1-minute initial activation hold at 96°C to activate the Taq polymerase. Next, the samples underwent 30 cycles at 94°C for 10 seconds, 59°C for 1 minute, and 72°C for 30 seconds. The final step was an extension step at 60°C for 10 minutes before cycler returns to a 4°C hold until the samples were removed from the instrument. The samples were stored in a -20°C freezer until capillary electrophoresis could be performed.

3.6 Genetic Analysis

The amplified DNA samples were analyzed using capillary electrophoresis on an Applied Biosystems® 3500 Series Genetic Analyzer. The capillary electrophoresis of samples was conducted according to the protocol outlined in the *PowerPlex® Fusion System for Use on the Applied Biosystems® Genetic Analyzers Technical Manual* (Applied Biosystems, 2020). A master mix was prepared by combining 9 µL of formamide and 1 µL of internal lane standard (WEN)

per sample. The mix was then vortexed for 15 seconds. 10 µL of the master mix was pipetted into each sample well of the plate and 9.75 µL of master mix in the allelic ladder wells of the plate. 1 µL of amplified sample (or 1.25 µL of allelic ladder) was then added to each corresponding well. The plate was then covered by the appropriate 96 well septa and centrifuged briefly at 3700 rpm to remove any air bubbles. The plate was loaded into the genetic analyzer, and the appropriate run parameters were selected (15 seconds for injection time and 15kV run voltage).

3.7 Data Analysis

In this study the independent variables encompassed various factors that were controlled by the researchers. These included the type of collection method employed, the specific extraction kit utilized, the application or omission of size exclusion chromatography, and the metal type in the VMD process. The dependent variables were the responses that were measured and analyzed based on the changes of the independent variables. In this context, the dependent variables consisted of the DNA concentration obtained and the number of allele peaks observed in an electropherogram.

The electropherograms resulting from capillary electrophoresis were analyzed using GeneMapper® ID-X Software v1.4 from Life Technologies™. The negative controls and reagent blanks were examined to ensure that peaks were not above the analytical thresholds designated for each dye channel. The profiles of the electropherograms were analyzed for completeness by determining if any allele drop-out had occurred or if any artifacts were

present. Each sample received a score of 0 to 44 for the number of alleles present on the electropherogram, not accounting for the sex determining loci. The concentration of the DNA samples was compared to determine the efficiency of each collection method. The statistical analysis was performed using R (R Core Team, 2024) and lme4 (Bates, Maechler, Bolker, & Walker, 2015) to obtain a general linear mixed effect model. The metal type, collection method, size exclusion chromatography, and extraction kit used were entered as fixed effects into the model. Fixed effects represent the independent variable, average trends that should persist across experiments (Winter, 2013). The sample ID and whole plot were entered as random effects into the model. Random effects represent all things that might affect the dependent variable that are not within the experimental design (Winter, 2013). P-values were obtained for each fixed effect through the general linear mixed effect models.

4. RESULTS

4.1 DNA Concentration

The complete quantification data for all samples and replicates are represented in Table 3 below. QIAmp™ exhibited a DNA presence in 14 of the 36 swab samples and 28 of the 36 filter samples. In contrast, DNA IQ™ demonstrated DNA detection in 27 of the 36 swab samples and 23 of the 36 filter samples. In the context of size exclusion chromatography, QIAmp™ revealed DNA presence in 8 of the 36 swab samples and 18 of the 36 filter samples, whereas DNA IQ™ size exclusion chromatography identified the presence of DNA in 20 of the 36 swab samples and 17 of the 36 filter samples. Fourteen samples did not have concentration results to report from the DNA IQ™ size exclusion chromatography samples, labelled as "fail" in the table below (Table 3). In the "fail" samples the reaction failed during qPCR and no data was collected. The size exclusion chromatography column could have contributed to the reaction failure. The "fail" samples were not applied to any statistical analysis performed.

Table 3. *DNA sample concentrations*

	QIAMP		DNAIQ		QIAMP Size Exclusion		DNAIQ Size Exclusion	
sample	Swab	Filter	Swab	Filter	Swab	Filter	Swab	Filter
1.1	0.007006	0.005853	0.001464	0	0	0.006194	0.000971	0.001636
1.2	0	0.00229	0.00302	0.000198	0	0	fail	fail
2.1	0.003451	0	0	0.001098	0	0.035575	0	0.079022
2.2	0.003142	0.007868	0	0.000623	0	0.001287	0	0.014838
3.1	0	0.005323	0.005062	0.002015	0.000571	0.011387	0	0
3.2	0	0.013092	0	0.003104	0	0	11.4978	0
4.1	0.019111	0.017945	0.000915	0	0	0.002313	2.536923	0
4.2	0.003617	0.015195	0	0.000391	0	0.001464	fail	fail
5.1	0	0.002428	0.000163	0	0	0.004997	0.039397	0.038069
5.2	0	0	0.058117	7.54E-05	0	0.00012	0	9.04E-05
6.1	0.00143	0.008946	0.005992	0	0	0	0.021151	0.00089

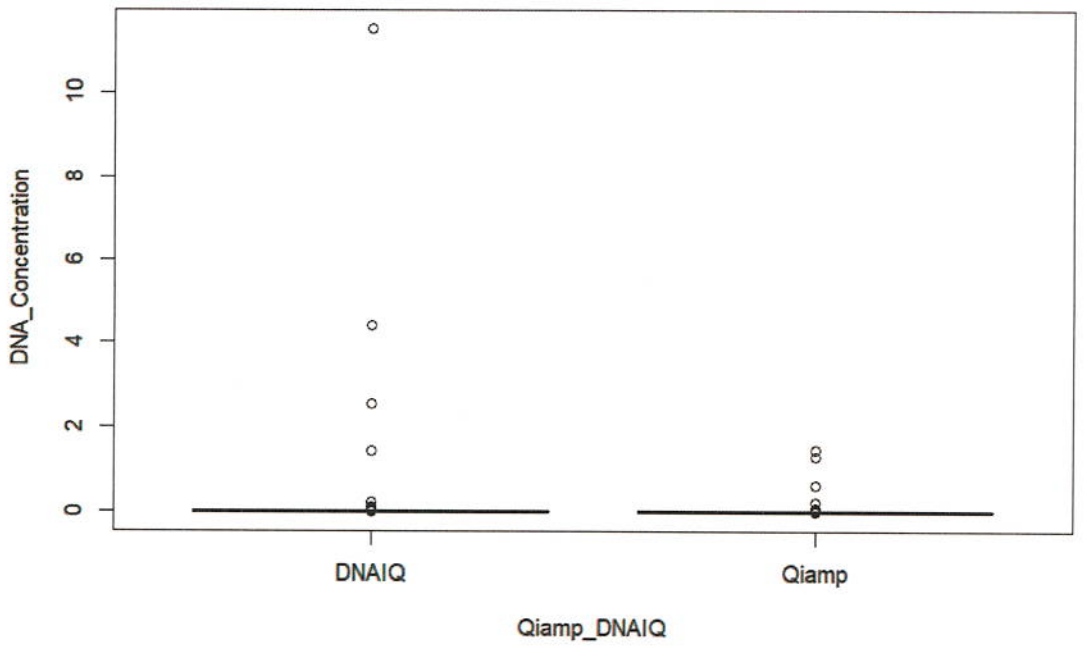
6.2	0	0.003872	0.000143	0.110389	0	0.000214	fail	fail
7.1	0.006858	0	0	0.000208	0.00352	0	0.000444	0.001226
7.2	0.004467	0.004178	6.23E-05	0.005794	0	0	0.004248	0.000861
8.1	0	0.001567	0	0.000362	0.003331	0.003977	0	9.61E-05
8.2	0	0.011857	0.010187	0	0	0	0.000216	0
9.1	0	0.005567	0.000442	0	0.632715	0	fail	fail
9.2	0.007304	0.001504	0.009782	0.006404	0.004658	0	0.001645	0.001353
10.1	1.466744	0.00306	0.003403	0	0	0.001956	0.000118	0
10.2	0	0	0.001015	0.000992	0	0	0	7.88E-05
11.1	0	0.003803	0.000149	0.032015	0	0.00335	0	0.001805
11.2	0	0	0.216104	0.002098	0	0	fail	fail
12.1	0	0.021325	0.001115	0	0	0.03966	0.001701	0.009198
12.2	0.004587	0.022318	0.006479	0	0	0.011796	0.001876	0.018056
13.1	0	0.005405	8.95E-05	0.000563	0	0	0	0
13.2	0	0.01787	0.000997	0.000903	0	0.003445	0.006329	0
14.1	0	0.044946	0	4.80E-05	0	0	0.000274	0.001018
14.2	0	0.087862	1.432871	4.416219	0	0.001606	4.34E-05	0.019993
15.1	0.00478	0.004545	0.002091	0	0.003136	0.003364	0.093789	0
15.2	0	0.002927	0.118067	0	0.206698	0	0.001124	0
16.1	0.000463	0.007262	0.008996	4.90E-05	1.29029	0.003521	fail	fail
16.2	0	0.006404	0.000207	0.015057	0	0	fail	fail
17.1	0	0	0	0	0	0	0.00206	0
17.2	0	0	0	0	0	0	0.003771	0.000472
18.1	0	0.003063	0.000216	0.002842	0	0	0.001344	0
18.2	0.002026	0	0.00253	0.000406	0	0	0.000148	0.002946
Average	0.042638	0.009396	0.052491	0.127829	0.059581	0.003784	0.490185	0.006609
Std. dev (+/-)	0.240746	0.015958	0.236782	0.725111	0.23453	0.008714	2.130875	0.016059

4.1.1 Model for Extraction Kits

The generalized linear mixed effect model yielded estimates of each fixed effect, including their corresponding standard errors, t-values, and p-values. The estimate for the categorical effect "QIAmp™" was reported to be -0.483. This suggests that QIAmp™ sample concentrations were, on average, lower than DNA IQ™ sample concentrations by 0.483 ng/µL. The standard error associated with this fixed effect was calculated to be +/- 0.535. The resulting t-value was -0.903, with a corresponding p-value of 0.366. In the figure below (Figure 3) sample

results are displayed in a box plot. The fixed effect, the extraction kit, is displayed as the x-axis. The response vector, DNA concentration, is displayed as the y-axis.

Figure 3: DNA Concentration (QIAmp™ V. DNA IQ™)



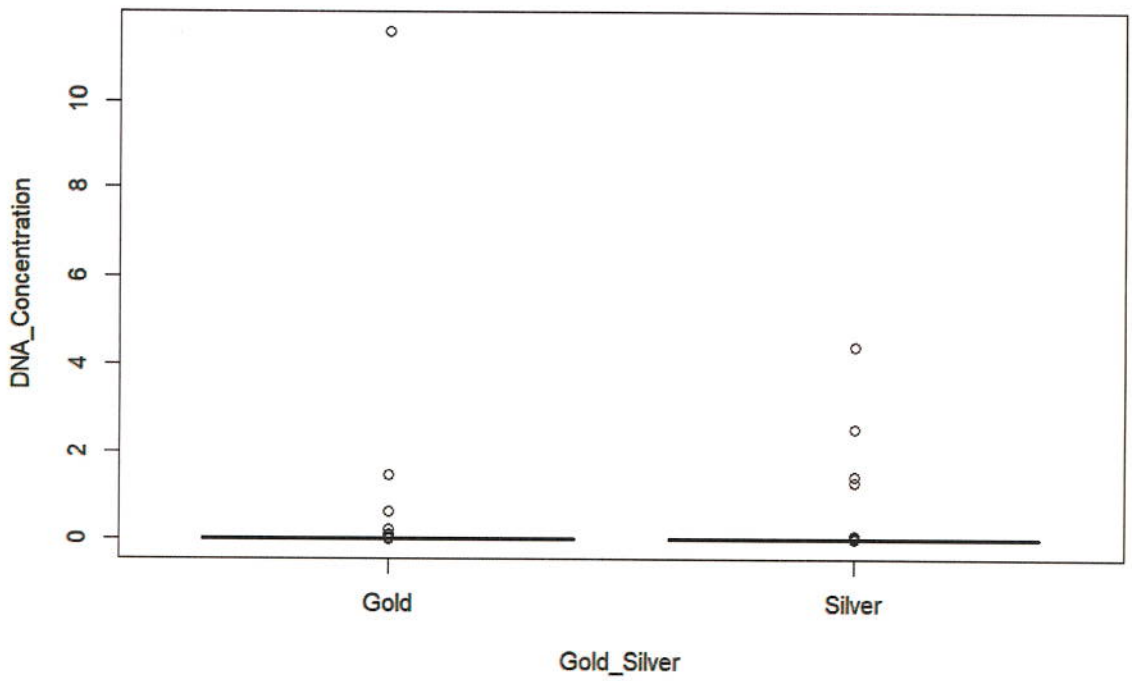
Note. n=72 for DNA IQ™ and n=72 for QIAmp™

4.1.2 Model for Metal Type

Silver-treated sample concentrations were, on average, lower than gold-treated sample concentrations by 0.415 ng/µL. The standard error associated with this fixed effect was calculated to be +/- 0.683. The resulting t-value was -0.607, with a corresponding p-value of 0.544. In the figure below (Figure 4) sample results are displayed in a box plot. The fixed effect,

the metal type, is displayed as the x-axis. The response vector, DNA concentration, is displayed as the y-axis.

Figure 4: *DNA Concentration (Gold V. Silver)*



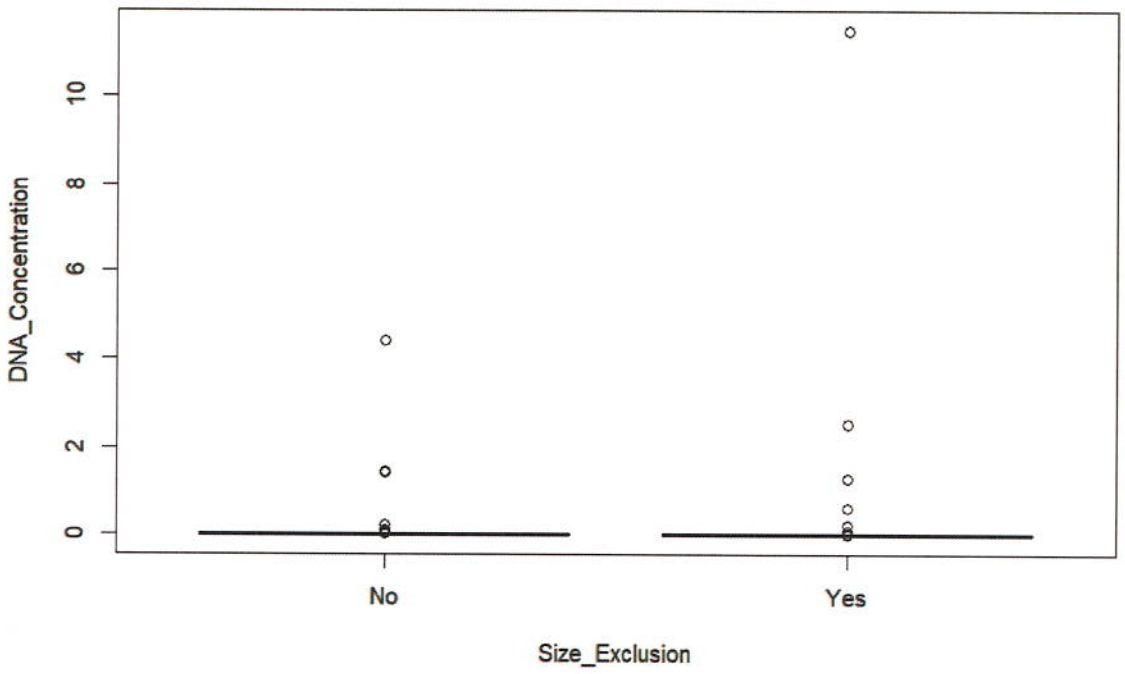
Note. n=160 for Gold and n=128 for Silver

4.1.3 Model for Size Exclusion Chromatography

Size exclusion chromatography sample concentrations were higher than non-size exclusion chromatography sample concentrations by 0.022 ng/µL, on average. The standard error associated with this fixed effect was calculated to be +/- 0.564. The resulting t-value was 0.038, with a corresponding p-value of 0.970. In the figure below (Figure 5) the fixed effect, if

size exclusion chromatography was performed, is displayed as the x-axis. The response vector, DNA concentration, is displayed as the y-axis.

Figure 5: *DNA Concentration (Size Exclusion Chromatography Yes V. No)*

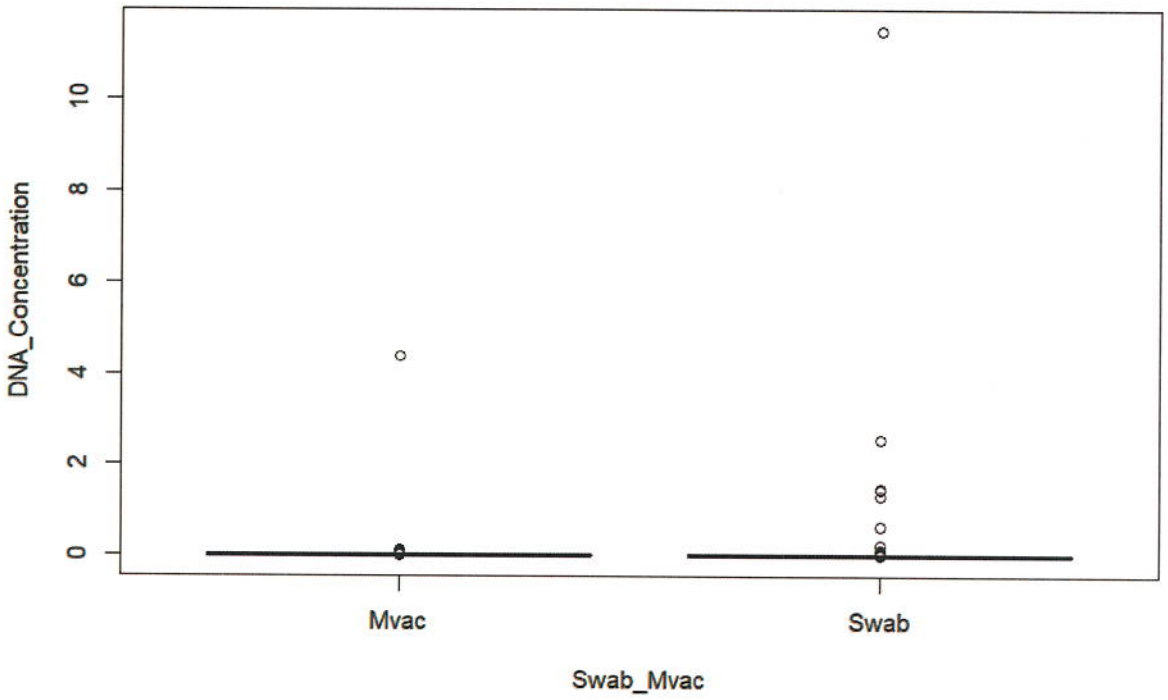


Note. n=144 for no size exclusion chromatography and n=130 for size exclusion chromatography

4.1.4 Model for Collection Type

Swab collected sample concentrations were higher than M-Vac® collected sample concentrations by 0.687 ng/µL, on average. The standard error associated with this fixed effect was calculated to be +/- 0.643. The resulting t-value was 1.069, with a corresponding p-value of 0.285. In the figure below (Figure 6) the fixed effect, the collection method, is displayed as the x-axis. The response vector, DNA concentration, is displayed as the y-axis.

Figure 6: *DNA Concentration (Swab V. M-Vac®)*



Note. n=137 for M-Vac® and n=137 for Swab

4.2 Genetic Profile Quality

All sample genetic profiles were compared to the donor profile and the number of matching allele peaks present were recorded into a data table. QIAmp™ exhibited allele peaks within their electropherogram in 12 of the 36 swab samples and 19 of the 36 filter samples. In contrast, DNA IQ™ demonstrated allele peaks in 5 of the 36 swab samples and 14 of the 36 filter samples. In the context of size exclusion chromatography, QIAmp™ revealed allele peaks within their electropherogram in 15 of the 36 swab samples and 17 of the 36 filter samples, whereas DNA IQ™ size exclusion chromatography identified allele peaks in 11 of the 36 swab

samples and 10 of the 36 filter samples. The complete genetic profile data for all samples and replicates are represented in Table 4 below.

Table 4. *Number of allele peaks present in a sample's electropherogram*

	QIAMP		DNAIQ		QIAMP Size Exclusion		DNAIQ Size Exclusion	
sample	Swab	Filter	Swab	Filter	Swab	Filter	Swab	Filter
1.1	15	0	12	0	2	0	0	0
1.2	13	0	2	1	0	0	0	0
2.1	0	0	0	0	1	0	0	0
2.2	0	0	0	1	0	0	0	0
3.1	0	0	0	0	1	2	0	0
3.2	0	0	0	3	1	0	0	0
4.1	0	0	0	0	1	1	0	0
4.2	0	1	0	1	0	0	0	0
5.1	1	0	0	0	1	0	0	0
5.2	1	0	0	0	2	0	1	0
6.1	0	0	0	0	1	0	1	2
6.2	2	8	0	0	0	1	0	0
7.1	0	8	2	14	0	3	0	0
7.2	3	1	0	15	0	7	0	0
8.1	0	2	0	0	0	3	0	0
8.2	4	2	0	1	0	1	1	1
9.1	0	3	0	1	0	0	1	2
9.2	1	0	0	38	1	3	1	1
10.1	0	5	0	0	4	1	0	0
10.2	1	1	2	0	0	1	3	1
11.1	0	0	0	0	0	4	0	0
11.2	0	0	0	0	0	2	1	1
12.1	0	1	0	3	0	7	0	1
12.2	0	20	0	0	1	12	1	0
13.1	2	1	0	0	0	0	0	0
13.2	0	8	0	0	0	0	0	1
14.1	0	1	0	1	0	0	0	1
14.2	0	2	0	0	1	0	1	0
15.1	0	3	0	0	0	1	0	0
15.2	0	0	0	0	1	0	2	1
16.1	0	1	0	0	0	4	0	0
16.2	2	0	0	0	0	0	2	0
17.1	0	1	1	0	0	1	0	0
17.2	1	0	0	2	1	0	0	0
18.1	0	0	0	1	1	0	0	0
18.2	0	2	0	1	0	0	0	0
Average	1.27778	1.97222	0.52778	2.30556	0.55556	1.5	0.41667	0.33333
St. dev (+/-)	3.23703	3.79683	2.02054	6.87919	0.86148	2.55495	0.72169	0.57735

A model was created to obtain the p-values for each fixed effect and the R^2 value for the complete genetic profile mixed effect model. The results, presented in Table 5, indicate that the utilization of size exclusion chromatography, the extraction kit, and the type of collection method have a statistically significant impact on the number of allele peaks obtained in a genetic profile ($p< 0.05$). The complete model had a marginal R^2 of 0.186, assessing the proportion of variance explained by the fixed effects included in the model.

Table 5. *Results for genetic profile mixed effect model*

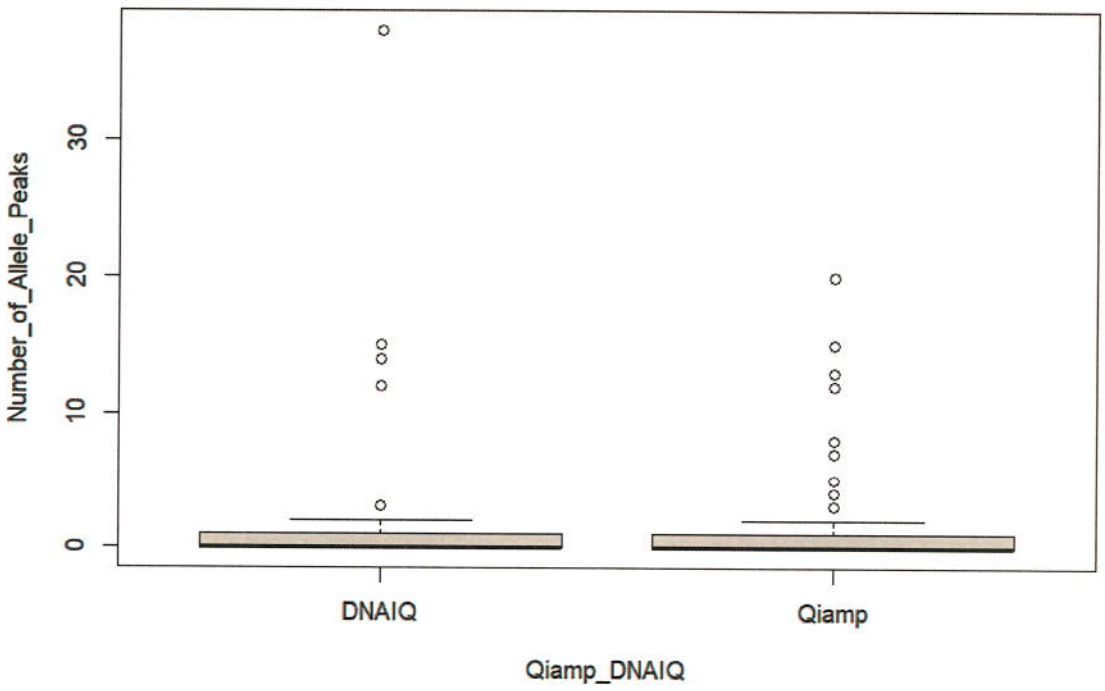
Number of Allele Peaks			
Predictors	*Incidence Rate Ratios*	*CI*	*P*
(Intercept)	1.09	0.55-2.15	0.812
Gold Silver [Silver]	0.69	0.30-1.55	0.367
Swab Mvac [Swab]	0.47	0.28-0.78	0.003
Qiamp DNAIQ [Qiamp]	1.92	1.19-3.10	0.007
Size Exclusion [Yes]	0.59	0.36-0.96	0.035
Random Effects			
σ^2	1.56		
$\tau_{00\ Sample\ ID}$	0.00		
$\tau_{00\ Whole\ Plot}$	0.50		
$N_{Whole\ Plot}$	18		
$N_{Sample\ ID}$	36		
Observations	288		
Marginal R^2/ Conditional R^2	0.186/NA		

4.2.1 Model for Extraction Kits

The generalized linear mixed effect model yielded estimates of each fixed effect, including their corresponding standard errors, z-values, and p-values. The estimate for the categorical effect "QIAmp™" was reported to be 0.654. This suggests that QIAmp™ samples, on average, had more peaks present than DNA IQ™ samples by 0.654. The standard error

associated with this fixed effect was calculated to be +/- 0.244. The resulting z-value was 2.675, with a corresponding p-value of 0.00748. In the figure below (Figure 7) sample results are displayed in a box plot. The fixed effect, the extraction kit, is displayed as the x-axis. The response vector, number of allele peaks present, is displayed as the y-axis. The distributions in the box plot are difficult to discern statistical difference, suggesting the difference may be significant but not meaningful in a practical sense.

Figure 7: Number of Allele Peaks Present (QIAmp™ V. DNA IQ™)

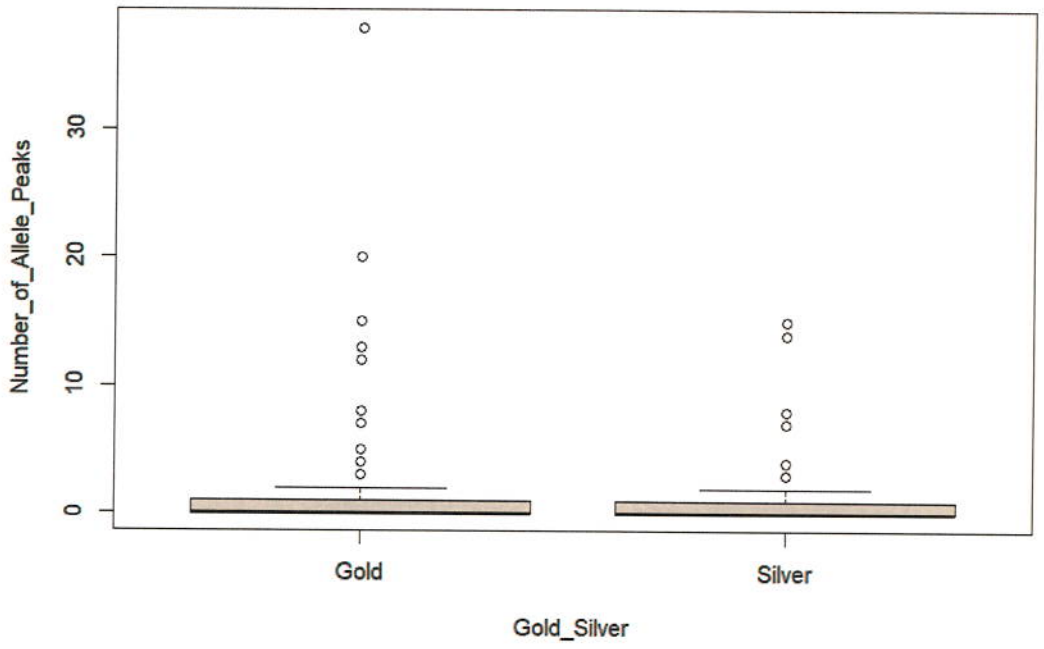


Note. n=72 for DNA IQ™ and n=72 for QIAmp™

4.2.2 Model for Metal Type

Silver-treated samples, on average, had less peaks present than gold-treated samples by 0.376. The standard error associated with this fixed effect was calculated to be +/- 0.416. The resulting z-value was -0.903, with a corresponding p-value of 0.367. In the figure below (Figure 8) sample results are displayed in a box plot. The fixed effect, the metal type, is displayed as the x-axis. The response vector, number of allele peaks present, is displayed as the y-axis.

Figure 8: Number of Allele Peaks Present (Gold V. Silver)

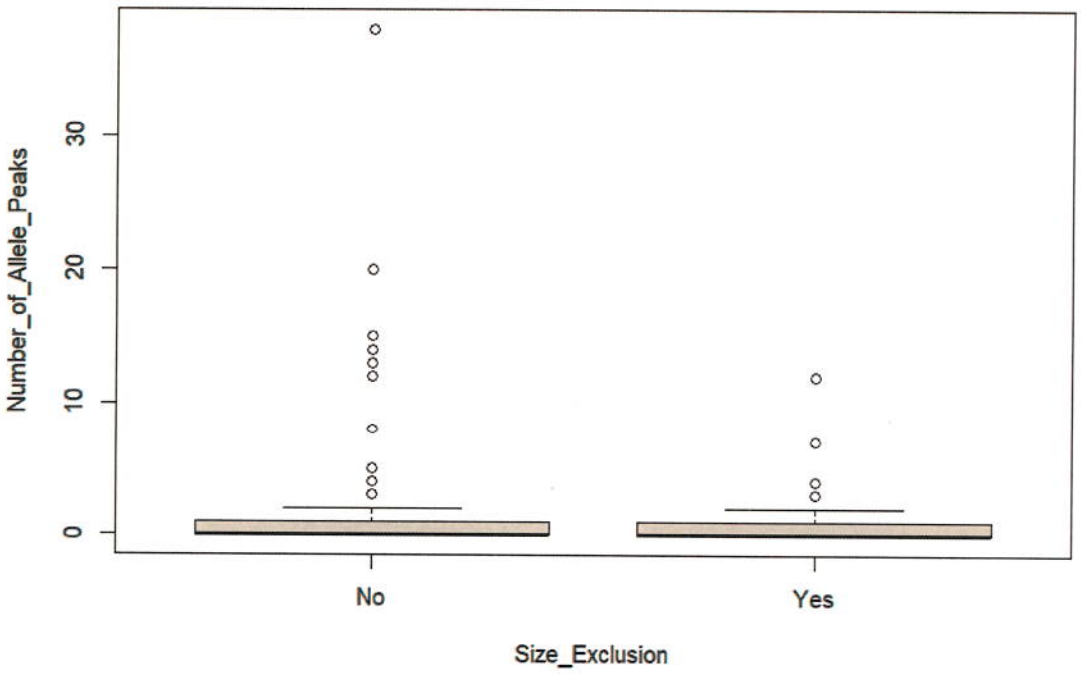


Note. n=160 for Gold and n=128 for Silver

4.2.3 Model for Size Exclusion Chromatography

Size exclusion chromatography samples had less peaks present than non-size exclusion chromatography samples by 0.528. The standard error associated with this fixed effect was calculated to be +/- 0.250. The resulting z-value was -2.109, with a corresponding p-value of 0.03493. In the figure below (Figure 9) the fixed effect, if size exclusion chromatography was performed, is displayed as the x-axis. The response vector, number of allele peaks present, is displayed as the y-axis.

Figure 9: *Number of Allele Peaks Present (Size Exclusion Chromatography Yes V. No)*

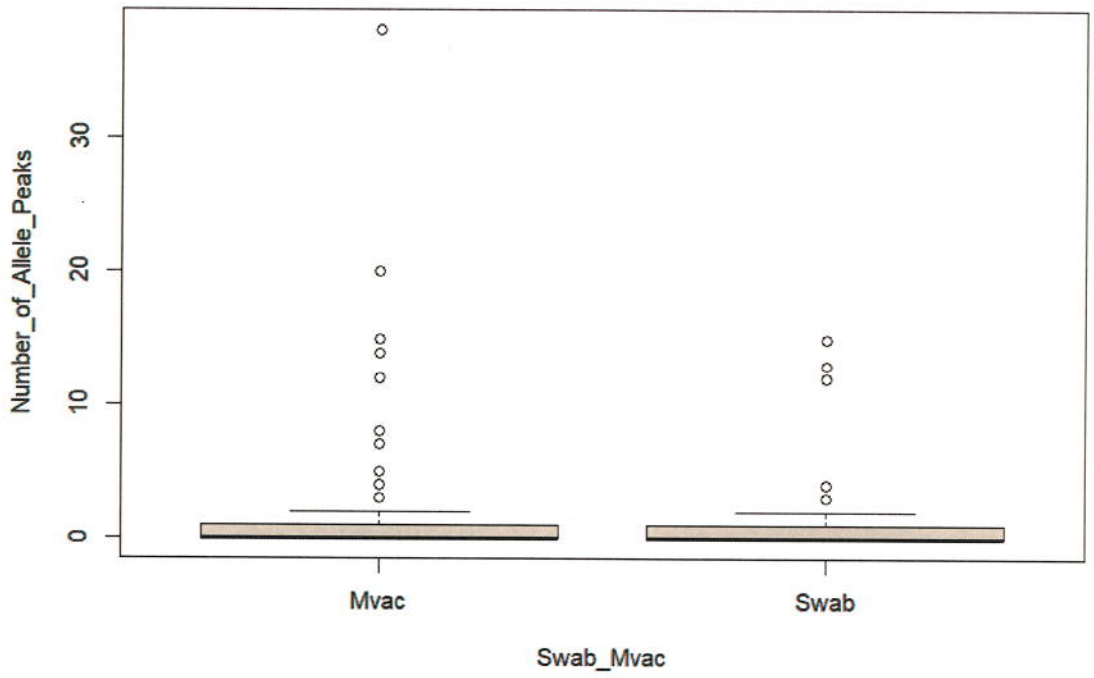


Note. n=144 for no size exclusion chromatography and n=144 for size exclusion chromatography

4.2.4 Mixed Effect Model for Collection Type

Swab collected samples had less peaks present than M-Vac® collected samples by 0.755 alleles on average. The standard error associated with this fixed effect was calculated to be +/- 0.256. The resulting z-value was -2.945, with a corresponding p-value of 0.00323. In the figure below (Figure 10), the fixed effect, the collection method, is displayed as the x-axis. The response vector, number of allele peaks present, is displayed as the y-axis. The distributions displayed in the box plot are difficult to discern statistical difference, suggesting the difference may be significant but not meaningful in a practical sense.

Figure 10: *Number of Allele Peaks Present (Swab V. M-Vac®)*



Note. n=144 for M-Vac® and n=144 for Swab

5. DISCUSSION

This study undertook a multi-method approach to improve the efficiency of evidence processing by identifying touch DNA on clothing and enhancing recovery rates. The multi-method approach employed various techniques to optimize touch DNA recovery. The approach was initiated after VMD processing to visualize the location of DNA on the fabric's surface. The multi-method approach involved comparing wet-swab and M-Vac® techniques for DNA collection, evaluating silica-membrane-based and magnetic silica-based DNA extraction methods, and gauging the efficacy of size exclusion chromatography in eliminating metal ions from VMD-processed DNA samples. The data generated provided insights into the combination of methods that yields the highest DNA recovery rates from VMD-treated fabrics, thus providing a guideline for future practices.

5.1 DNA Concentration

Following the collection of concentration data from each sample, the study examined the connections between the fixed and random effects to assess the effectiveness of the multi-method approach in recovering touch DNA from fabrics. The efficiency of each independent variable, including metal type, collection method, the utilization of size exclusion chromatography, and the type of extraction kit, was assessed through statistical analysis employing a general linear mixed effect model with a gamma distribution.

When examining individual fixed effects, it was found that gold-treated samples outperformed silver-treated samples by 0.41505 ng/µL on average. Among collection methods, double swabbing yielded higher DNA concentrations compared to samples collected using the M-Vac® wet vacuum method by 0.68715 ng/µL. Samples that were subjected to size exclusion chromatography exhibited higher overall DNA concentrations, on average, compared to those not undergoing this process by 0.02154 ng/µL. However, it is worth noting that these effects had minimal impact on the DNA concentration obtained. Additionally, DNA IQ™ extracted samples performed better than their counterpart, QIAmp™ extraction samples by 0.48307 ng/µL. None of the observed average estimates among the methods demonstrated significance or substantive relevance to the concentration of DNA recovered. In the examination to the statistical significance pertaining to each fixed effect, the metal type exhibited a p-value of 0.544, denoting insignificance in its impact on the concentration of DNA yielded. Similarly, the collection type manifested no statistical significance (p=0.285) concerning the concentration of touch DNA retrieved. The statistical analysis revealed that the extraction kit utilized yielded a p-value of 0.366, suggesting insignificance in its influence on the concentration of obtained DNA. Finally, the utilization of size exclusion chromatography exhibited no statistically significant association (p=0.970) with the concentration of recovered DNA from fabric samples. The p-values from the mixed effect model suggest that different independent variables (i.e. the metal type used, collection method, type of extraction kit used, and the utilization of size exclusion chromatography) do not significantly influence the concentration of touch DNA retrieved from fabrics.

5.2 Genetic Profile Quality

The quality of a genetic profile obtained from each sample was completed by receiving a score of 0 to 44 for the number of alleles present on the electropherogram, not accounting for the sex determining loci. The maximum number of allele peaks obtained for a sample was 38 and the minimum was no allele peaks obtained. The relationships between the study's fixed effects and random effects were used to evaluate the efficiency of the multi-method approach. As stated previously, the efficiency of each independent variable was evaluated by statistical analysis using a general linear mixed effect model with a negative binomial distribution.

Individually, gold-treated samples yielded more allele peaks in an electropherogram, on average, compared to silver-treated samples by 0.37597 peaks. Samples collected using the M-Vac® wet vacuum method outperformed samples collected using the double swabbing method by 0.75465 peaks. Samples that were not subjected to size exclusion chromatography exhibited more complete overall genetic profiles compared to those that underwent this process by 0.52774 peaks on average. Finally, QIAmp™ extracted samples performed better than their counterpart, DNA IQ™ extraction samples by 0.65385 peaks. None of the observed average estimates among the methods demonstrated substantive relevance and had a minimal impact on the quality of genetic profiles obtained from samples. In the analysis of the statistical significance associated with each fixed effect, it was found that the metal type yielded a p-value of 0.36657, indicating a lack of significance in its effect of the quality of a genetic profile. However, the collection type showed a statistically significant impact (p=0.00323) on the number of allele peaks obtained in a genetic profile. Additionally, the choice of extraction kit

resulted in a p-value of 0.00748, suggesting that it significantly influences the completeness of a genetic profile. Lastly, the use of size exclusion chromatography demonstrated statistically significant correlation (p=0.03493) with the number of allele peaks obtained in a genetic profile recovered from fabric samples.

As depicted in Table 5, employing a multi-method approach that includes M-Vac® wet vacuum as the collection method, the QIAmp™ mini kit for extraction, and does not undergo size exclusion chromatography as an additional cleaning step yields a statistically significant impact on the quantity of allele peaks obtained in a genetic profile. The utilization of size exclusion chromatography, the extraction kit, and the chosen collection method exhibit a correlation with the number of allele peaks discerned on an electropherogram. However, it is noteworthy that none of these factors, nor their combination, substantially predicts the variability in the overall quantity of allele peaks. These factors (utilization of size exclusion chromatography, the extraction kit, and the chosen collection method) merely explain around 18% to 20% of the total variation.

5.3 Limitations

Several limitations were encountered during the execution of this study, primarily stemming from equipment availability and the visibility of Vacuum Metal Deposition (VMD) prints. The University of Central Oklahoma lacked ownership of a VMD instrument, necessitating the involvement of the Oklahoma State Bureau of Investigation for processing fabric samples when accessible. However, this arrangement revealed a second constraint: VMD

prints on fabrics appeared visible only within a narrow time frame post-processing. Upon return to researchers, the prints ceased to be fully discernible, leaving behind a residual film where the metal had been deposited but failing to yield identifiable palm prints. Investigations concerning the duration of print visibility through VMD remains sparse in existing literature. An article authored by the Home Office Scientific Development Branch in 2005 asserts that VMD prints have been observed to retain visibility for a duration of a few hours. Notably, metal particles persist on the fabric surface over time but the quality of the print does not. Although this phenomenon did not have a significant influence on the current study, given that the visualization of ridge detail was not required, the residual presence of metal ions on the fabric surface offered valuable indications concerning the potential location of touch DNA.

6. CONCLUSION

The goal of this study was to address the streamlining of evidence processing for the identification of touch DNA on clothing, with a focus on improving DNA recovery and efficiency. The multi-method approach had three main objectives: compare the wet-swab and Microbial wet-vacuum (M-Vac®) techniques for collection of touch DNA, compare silica-membrane-based and magnetic silica-based DNA extraction methods, and assess the effectiveness of size exclusion chromatography in the removal of metal ions from samples. With regards to the collection method, there was not a significant impact on the concentration of DNA obtained when comparing the double swabbing method and the M-Vac® collection method. However, the M-Vac® collection method produced genetic profiles with more allele peaks present compared to the double swabbing collection method. In the case of the extraction kits comparison, there was no statistically significant effect found between the extraction kits in this study to the concentration of touch DNA recovered. Although, the silica-membrane-based extraction method (QIAmp™) produced genetic profiles with more allele peaks present than its counterpart, the magnetic silica-based extraction method (DNA IQ™). Regarding the efficacy of size exclusion chromatography, the samples that were subjected to the size exclusion chromatography process and the samples that bypassed this process showed no significant differences in regards to the concentration of DNA. Nevertheless, samples that were not subjected to size exclusion chromatography yielded genetic profiles exhibiting a higher count of allele peaks in comparison to the samples that underwent the size exclusion chromatography process.

With these results in consideration, the techniques within the multi-method approach (M-Vac® collection method, QIAmp™ mini kit extraction, and bypassing size exclusion chromatography) employed in this study significantly impacts touch DNA genetic profiles but does not affect the concentration obtained during collection. The utilization of size exclusion chromatography, the type of extraction kit used, and the collection method can affect the quality of a genetic profile obtained from a sample. However, it is worthy to note that the difference is minute and based on the limited dataset presented in this study. When considering these findings in a casework context, the number of allele peaks detected in a genetic profile holds greater importance than the concentration of DNA acquired. Utilizing a technique involving the M-Vac® wet vacuum for touch DNA sample collection, followed by DNA extraction using the QIAmp™ kit, without the need for size exclusion chromatography, can yield more comprehensive genetic profiles. This method could be employed by analysts in cases where there is overlap with the latent prints unit or when they encounter difficulties in locating potential touch DNA on fabric evidence. The application of vacuum metal deposition and the M-Vac® wet vacuum holds promise as a tool in the field of forensic science. Future research should be conducted outside the scope of this study. One possibility is replicating this study with test subjects of differing shedder statuses. Repetition with more subjects may show a more noteworthy difference in the collection methods for touch DNA on fabrics. Another option is replicating this study but simulating a crime or some form of physical exercise before depositing the prints onto the fabric. In actual casework, suspects would likely be sweatier and grab or push more firmly, allowing for the possibility of more skin cells to be deposited in the

print. Simulating some form of physical exercise before depositing the print may lead to higher DNA concentration yields and more complete DNA profiles. Additionally, a study on the maximum time for VMD to stay visible on fabrics should be conducted. There is limited literature that discusses how long VMD metal remains visible on fabrics and it would be worthwhile to have a time range for processing fabrics. Currently, analysts that perform VMD should consider marking around the area that showed print-like patterns.

References

Alketbi, S.K. (2018). The affecting factors of touch DNA. *Journal of Forensic Science Research,* 9(3), 1-4. http://dx.doi.org/10.4172/2157-7145.1000424

Alketbi, S.K., Goodwin, W. (2019). The effect of surface type, collection, and extraction methods on touch DNA. *Forensic Science International: Genetics,* 7(1), 704-706. https://doi.org/10.1016/j.fsigss.2019.10.145

Aloraer, D., Hassan, N. H., Albarzinji, B., Goodwin, W. (2017). Improving recovery and stability of touch DNA. *Forensic Science International: Genetics Supplement Series*, 6, e390-e392. https://doi.org/10.1016/j.fsigss.2017.09.166.

Amick, J., Bivins, D., Cathcart, K., Hammer, L., & Pippin, T. (2015). Integrating DNA collection into the latent print section. Journal of Forensic Identification, 65(4), 630-637. http://alaskapolicyforum.org/wp-content/uploads/Integrating-DNA-Collection-into-the-Latent-Print-Section.pdf

Applied Biosystems. (2020). PowerPlex® Fusion system for use on the Applied Biosystems® genetic analyzers technical manual. *Applied Biosystems.* PowerPlex Fusion System TMD039.pdf

Applied Biosystems. (2006). Quantifiler® kits-- Quantifiler® human DNA quantification kit and Quantifiler® Y human male DNA quantification kit—User's manual. *Applied Biosystems.* https://projects.nfstc.org/workshops/resources/articles/AB%20Quantifiler%20Kits%20User's%20Manual.pdf

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Budowle, B., Allard, M.W., Wilson, M.R., & Chakraborty, R. (2003). Forensics and mitochondrial DNA: Applications, debates, and foundations. *Annu. Rev. Genomics Hum. Genet.*, 4, 119-141. https://doi.org/10.1146/annurev.genom.4.070802.110352

Burrill, J., Daniel, B., & Frascione, N. (2019). A review of trace "touch DNA" deposits: Variability factors and an exploration of cellular composition. *Forensic Science International: Genetics*, 39, 8-18. https://doi.org/10.1016/j.fsigen.2018.11.019

Butler, J., & McCord, B. (2004). Capillary electrophoresis in DNA analysis: Introduction to CE and ABI 310. *NEAFS CE-DNA Workshop.* https://strbase.nist.gov/pub_pres/NEAFS_CEintro.pdf

Butler, J.M., Ruitberg, C.M., & Vallone, P.M. (2001). Capillary electrophoresis as a tool for optimization of multiplex PCR reactions. *Fresenius' Journal of Analytical Chemistry*, 369, 200-205.

Craig, J., Fowler, S., Burgoyne, L., Scott, A., & Harding, H. (1988). Repetitive deoxyribonucleic acid (DNA) and human genome variation- A concise review relevant to forensic biology. Journal of Forensic Sciences, 1111-1127.

Edwards, M., & Gibbs, R. (1994). Multiplex PCR: Advantages, development, and applications. Genome Research, S65-S75.

Fraser, J., Sturrock, K., Deacon, P., Bleay, S., & Bremner, D. H. (2011). Visualization of fingermarks and grab impressions on fabrics, part 1: gold/zinc vacuum metal deposition. Forensic Science International, 208, 74-78. https://doi.org/10.1016/j.forsciint.2010.11.003

Gettings, K. B., Ballard, D., Devesse, L., King, J. L., Parson W., Phillips, C., Vallone, P. (2017). STRSeq: a catalog of sequence diversity at human identification short tandem repeat loci. *Forensic Sci. Int. Genet.*, 31, 111-117. https://doi.org/10.1016/j.fsigen.2017.08.017

Hall, D., Fairley, M. (2004). A single approach to the recovery of DNA and firearm discharge residue evidence. *Science & Justice: Journal of the Forensic Science Society*, 44(1), 15-19. https://doi.org/10.1016/s1355-0306(04)71680-9

Haddrill, P.R. (2021). Developments in forensic DNA analysis. *Emerg Top Life Sci*, 5(3), 381-393. https://doi.org/10.1042/ETLS20200304

Hedman, J., Ågren, J., Ansell, R. (2015). Crime scene DNA sampling by wet-vacuum applying M-Vac®. *Forensic Science International: Genetics Supplement Series*, 5, e89-e90. https://doi.org/10.1016/j.fsigss.2015.09.036.

Hess, S. & Haas, C. (2017). Recovery of trace DNA on clothing: comparison of mini-tape lifting and three other forensic evidence collection techniques. *Journal of Forensic Sciences*, 62, 187-191. https://doi.org/10.1111/1556-4029.13246

Home Office Scientific Development Branch (HOSDB). (2005). Fingerprint development and imaging newsletter. *Home Office*, 47(05).

www.assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/895950/Fingerprint_Update_October_2005_47-05.pdf

Horvath, A. (2022). Visualization of latent fingerprints on fabrics using vacuum metal deposition. Journal of Forensic Identification, 72(3), 339-370. https://libproxy.uco.edu/login?url=https://www.proquest.com/scholarly-journals/visualization-latent-fingerprints-on-fabrics/docview/2697726327/se-2

Hu, Q., Liu, Y., Yi, S., & Huang, D. (2015). A comparison of four methods for PCR inhibitor removal. *Forensic Science International: Genetics,* 16, 94-97. https://doi.org/10.1016/j.fsigen.2014.12.001

Idris, B., Goodwin, W. (2015). Comparison of chelex®-100 with two solid phase DNA extraction techniques. *Forensic Science International: Genetics Supplement Series,* 5, e274-e275. https://doi.org/10.1016/j.fsigss.2015.09.109

Johannessen, H., Gill, P., Roseth, A., & Fonnelop, A.E. (2021). Determination of shedder status: A comparison of two methods involving cell counting in fingerprints and the DNA analysis of handheld tubes. *Forensic Science International: Genetics,* 53. doi: 10.1016/j.fsigen.2021.102541. Epub 2021 May 28. PMID: 34090062.

Lee, H.C. & Ladd, C. (2001). Preservation and collection of biological evidence. *Croatian Medical Journal,* 42(3), 225-228. https://abacusdiagnostics.com/Collection_of_Evidence.pdf

Luftig, M. A., & Richey, S. (2001). DNA and forensic science. *New England Law Review*, 35(3), 609-614.

McCord, B.R., Jung, J.M., & Holleran, E.A. (1993). High resolution capillary electrophoresis of forensic DNA using a non-gel sieving buffer. *Journal of Liquid Chromatography*, 9, 1963-1981. https://doi.org/10.1080/10826079308019908

McLamb, J.M., Adams, L.D., & Kavlick, M.F. (2020). Comparison of the M-Vac® wet vacuum-based collection method to a wet swabbing method for DNA recovery on diluted bloodstained substrates. *Journal of Forensic Sciences*, 65(6), 1828-1834. https://doi.org/10.1111/1556-4029.14508

Meakin, G., Jamieson, A. (2013). DNA transfer: Review and implications for casework. *Forensic Science International: Genetics*, 7(4), 434-443. https://doi.org/10.1016/j.fsigen.2013.03.013

Moreno, L. I. & McCord B. R. (2017). Understanding metal inhibition: The effect of copper (Cu^{2+}) on DNA containing samples. *Forensic Chemistry*, 4, 89-95. https://doi.org/10.1016/j.forc.2017.03.005

NIST. (2015). FBI CODIS core STR loci. https://strbase.nist.gov/fbicore.htm

Pang, B.C.M., Cheung, B.K.K. (2007). Double swab technique for collecting touched evidence. *Legal Medicine*, 9, 181-184. https://doi.org/10.1016/j.legalmed.2006.12.003

Panneerchelvam, S., Norazmi, M.N. (2003). Forensic DNA profiling and database. *The Malaysian Journal of Medical Sciences*, 10(2), 20-26. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3561883/

Promega. (2016). DNA IQ™ system- Database protocol. *Promega.* https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/101/dna-iq-system-database-protocol.pdf

Qiagen. (2018). QIAamp® DNA mini kit: Quick-start protocol. *Qiagen.* HB-2530-001_1113198_Pcard_QIAamp_DNA_Mini_0418_WW (1).pdf

R Core Team. (2024). R: A language and environment for statistical computing. *R Foundation for Statistical Computing,* Vienna, Austria. <https://www.R-project.org/>.

Thermo Fisher Scientific. (2010). Phosphate buffer (butterfield's buffer). *Thermo Fisher Scientific.* https://assets.thermofisher.com/TFS-Assets/LSG/manuals/IFU112526.pdf

Van Oorschot, R. A., Ballantyne, K. N., & Mitchell, J. R. (2010). Forensic trace DNA: a review. *Investigative Genetics,* 1, 1-17. https://doi.org/10.1186/2041-2223-1-14

Vann Oorschot, R.A., & Jones, M.K. (1997). DNA fingerprints from fingerprints. *Nature,* 387, 767. https://doi.org/10.1038/42838

Vickar, T., Bache, K., Daniel, B., & Frascione, N. (2018). The use of the M-Vac® system as a method for DNA recovery. *Science & Justice,* 58, 282-286. https://doi.org/10.1016/j.scijus.2018.01.003

Walsh, P.S., Metzger, A., & Higuchi, R. (1991). Chelex® 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques,* 10(4), 506-513. https://www.future-science.com/doi/pdf/10.2144/000114018

Williamson, A.L. (2012). Touch DNA: Forensic collection and application to investigations. *J Assoc Crime Scene Reconstr.*, 18(1), 1-5. https://www.acsr.org/wp-content/uploads/2012/01/Williamson.pdf

Winter, B. (2013). A very basic tutorial for performing linear mixed effects analyses. *University of California*. http://www.bodowinter/tutorial/bw_LME_tutorial2.pdf

Appendix A: R Markdown of a Combined Method to Locate and Improve DNA Recovery from Fabrics

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2024-03-28

Load Libraries

```
library(tidyverse)

library(lme4)

library(DHARMa)

library(dplyr)
```

Load Data

```
getwd()
```

```
## [1] "C:/Users/maken/OneDrive/Desktop/Thesis/R_analyses"
```

```
setwd("C:/Users/maken/OneDrive/Desktop/Thesis/R_Analyses")
dat <- read_csv("Thesis data table CSV.csv") |>
mutate(Sample_ID = factor(Sample_ID),
         Whole_Plot = str_extract(Sample_ID, "[0-9]*(?=.)"),
         DNA_Concentration2 = DNA_Concentration + 0.00000000001)
```

.

Summary of Data

```
summary(dat)
```

```
##  Sample_Number      Sample_ID   Fabric_Type         Hand_Side
##  Min.   :  1.00   1.1    :  8   Length:288         Length:288
##  1st Qu.: 72.75   1.2    :  8   Class :character   Class :character
##  Median :144.50   2.1    :  8   Mode  :character   Mode  :character
##  Mean   :144.50   2.2    :  8
##  3rd Qu.:216.25   3.1    :  8
##  Max.   :288.00   3.2    :  8
##                   (Other):240
##  Gold_Silver          Swab_Mvac         Qiamp_DNAIQ         Size_Excl
usion
##  Length:288         Length:288         Length:288         Length:28
```

```
8
##  Class :character   Class :character   Class :character   Class :ch
aracter
##  Mode  :character   Mode  :character   Mode  :character   Mode  :ch
aracter
##
##
##
##
##  DNA_Concentration   Number_of_Allele_Peaks  Whole_Plot
##  Min.   : 0.000000   Min.   : 0.000         Length:288
##  1st Qu.: 0.000000   1st Qu.: 0.000         Class :character
##  Median : 0.000216   Median : 0.000         Mode  :character
##  Mean   : 0.091434   Mean   : 1.111
##  3rd Qu.: 0.003795   3rd Qu.: 1.000
##  Max.   :11.497805   Max.   :38.000
##  NA's   :14
##  DNA_Concentration2
##  Min.   : 0.000000
##  1st Qu.: 0.000000
##  Median : 0.000216
##  Mean   : 0.091434
##  3rd Qu.: 0.003795
##  Max.   :11.497805
##  NA's   :14
```

Linear Mixed Effect Models

Linear Mixed effect models are used for data that is collected and summarized in groups. These models simultaneously represent fixed and random effects of a data set. Fixed effects represent average trends that should persist across experiments. Random effects represent the extent these trends vary across levels of a grouping element. For both dependent variables, a general linear mixed effect model was used.

DNA Concentration Linear Mixed Effect Models

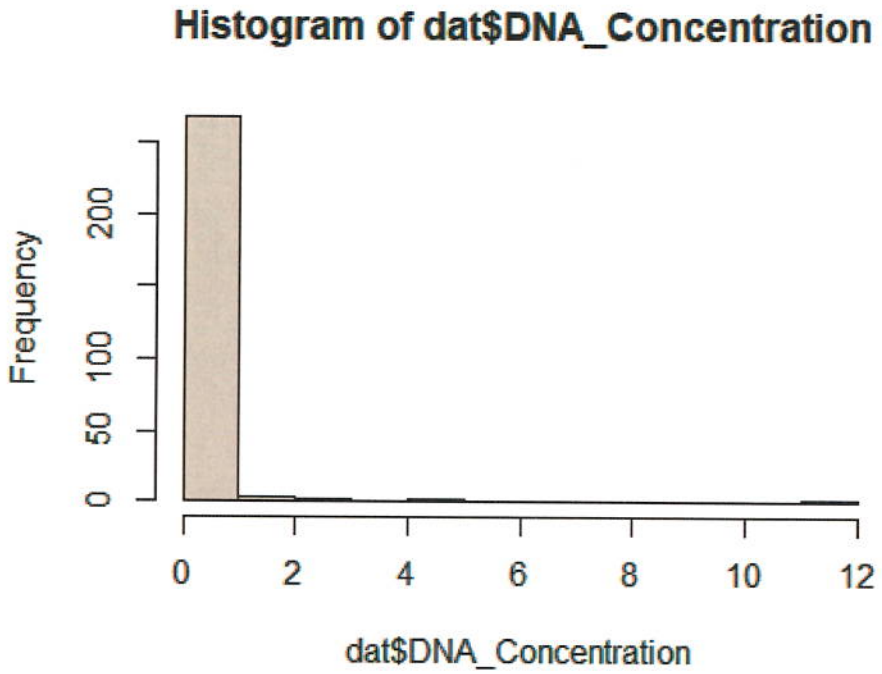
The metal type, collection method, size exclusion chromatography, and extraction kit used were entered as fixed effects into the models. The sample ID and whole plot error terms were entered as random effects into the model. A gamma distribution was applied.

```
dna_mod <- glmer(DNA_Concentration2 ~ Gold_Silver + (1|Whole_Plot)
                 + Swab_Mvac + (1|Sample_ID) + Qiamp_DNAIQ + Size_Excl
usion, data = dat, family = Gamma(link = "log"))

summary(dna_mod)
```

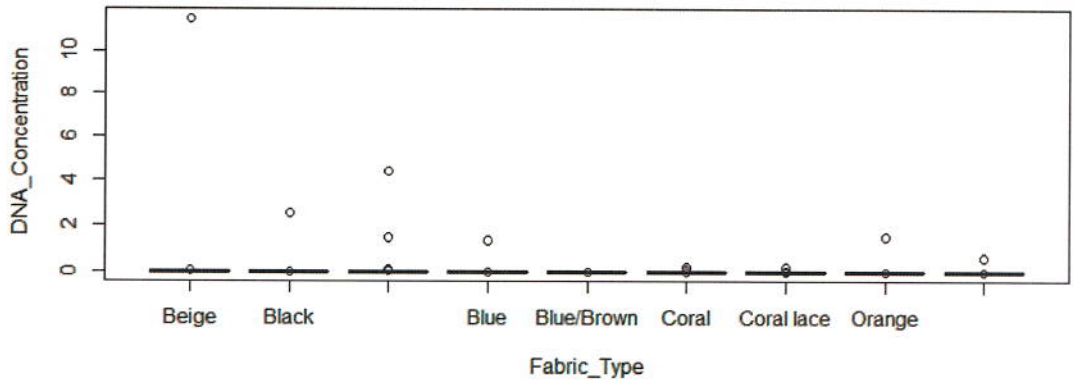
```
## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Gamma  ( log )
## Formula: DNA_Concentration2 ~ Gold_Silver + (1 | Whole_Plot) + Swab
_Mvac +
##     (1 | Sample_ID) + Qiamp_DNAIQ + Size_Exclusion
##    Data: dat
##
##      AIC      BIC   logLik deviance df.resid
##  -5562.8  -5533.9   2789.4  -5578.8      266
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.5091 -0.5091 -0.4970 -0.0474  4.1479
##
## Random effects:
##  Groups     Name        Variance Std.Dev.
##  Sample_ID  (Intercept) 13.5770  3.6847
##  Whole_Plot (Intercept)  0.7119  0.8438
##  Residual                3.8583  1.9643
## Number of obs: 274, groups:  Sample_ID, 36; Whole_Plot, 18
##
## Fixed effects:
##                   Estimate Std. Error t value Pr(>|z|)
## (Intercept)       -4.82907    0.66742  -7.235 4.64e-13 ***
## Gold_SilverSilver -0.41505    0.68324  -0.607    0.544
## Swab_MvacSwab      0.68715    0.64278   1.069    0.285
## Qiamp_DNAIQQiamp  -0.48307    0.53480  -0.903    0.366
## Size_ExclusionYes  0.02154    0.56412   0.038    0.970
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##
## Correlation of Fixed Effects:
##             (Intr) Gld_SS Swb_MS Q_DNAI
## Gld_SlvrSlv -0.448
## Swab_MvcSwb -0.458 -0.011
## Qmp_DNAIQQm -0.529 -0.018  0.201
## Sz_ExclsnYs -0.360  0.014 -0.100  0.101
```

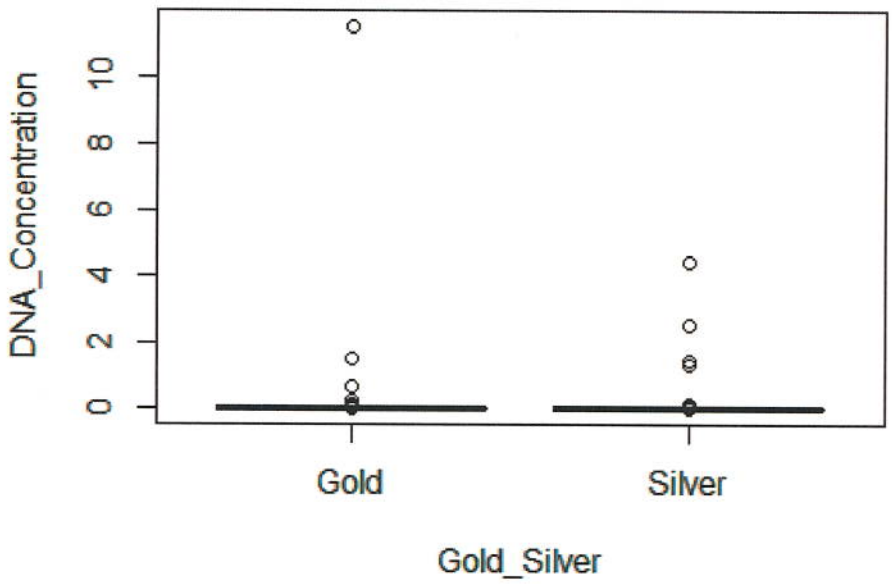
```
hist(dat$DNA_Concentration)
```



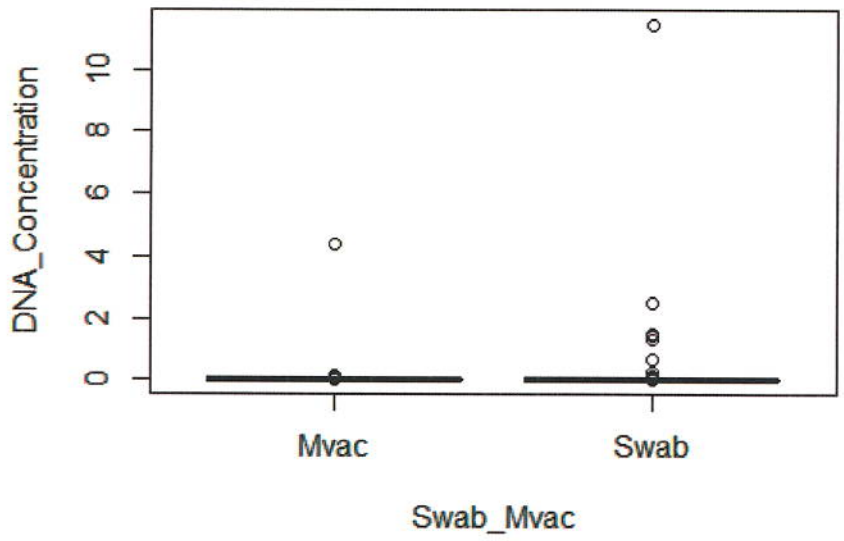
```
boxplot(DNA_Concentration ~ Fabric_Type, data = dat)
```



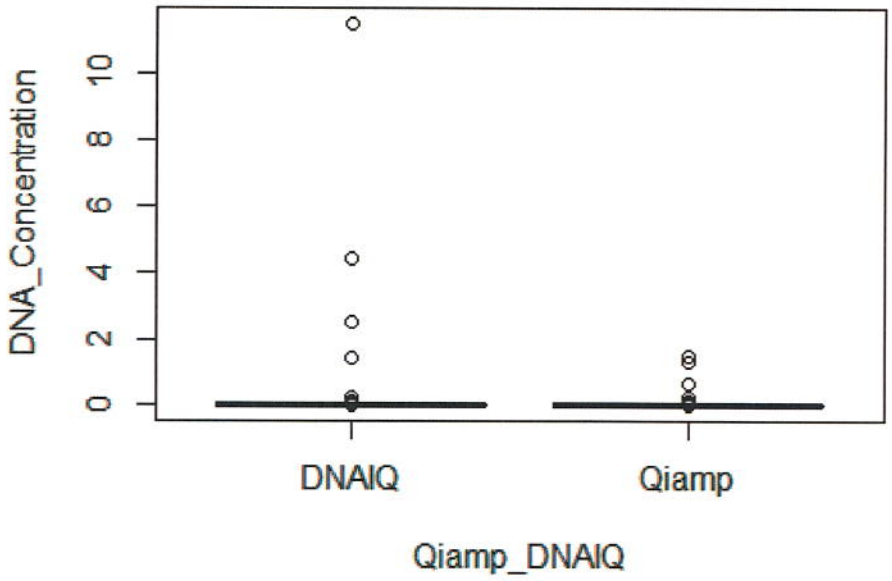

```
boxplot(DNA_Concentration ~ Gold_Silver, data = dat)
```



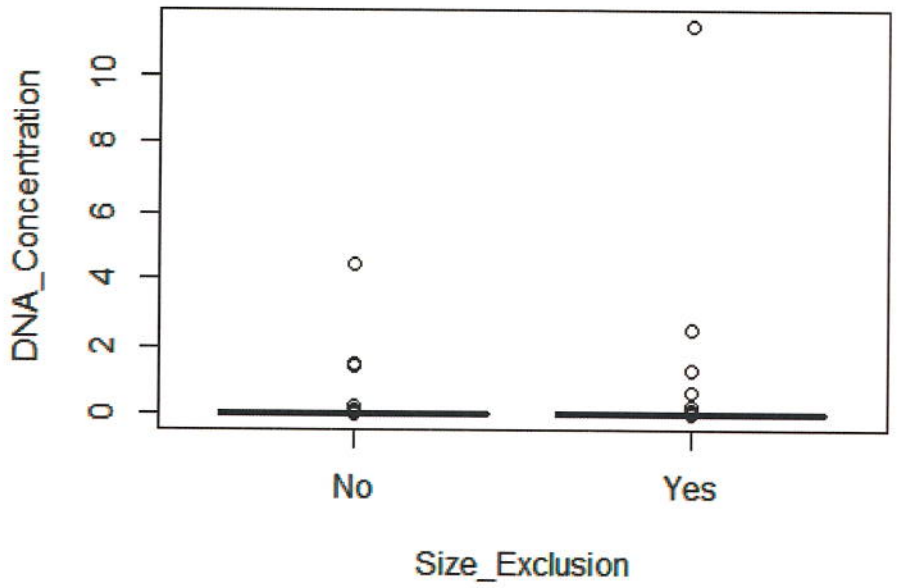
```
boxplot(DNA_Concentration ~ Swab_Mvac, data = dat)
```




```
boxplot(DNA_Concentration ~ Qiamp_DNAIQ, data = dat)
```



```
boxplot(DNA_Concentration ~ Size_Exclusion, data = dat)
```



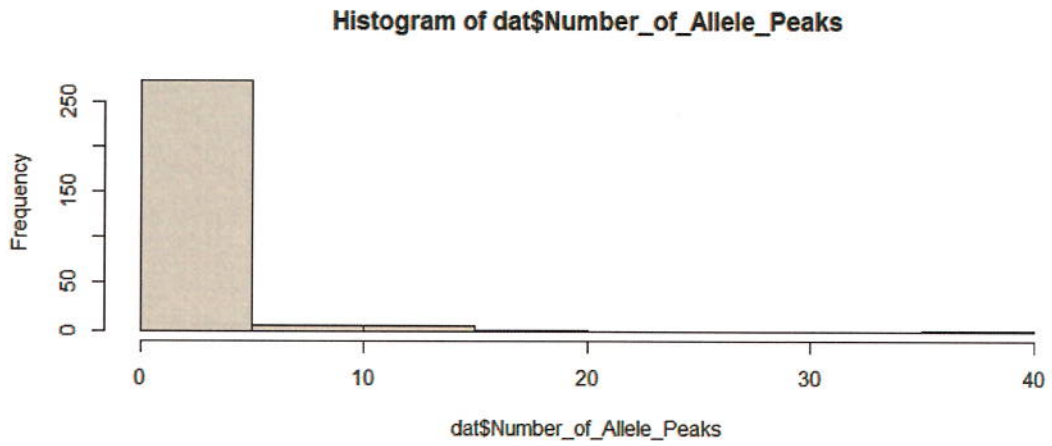
Genetic Profile Quality Linear Mixed Effect Models

The metal type, collection method, size exclusion chromatography, and extraction kit used were entered as fixed effects into the model. The sample ID and whole plot error terms were entered as random effects into the model. a negative binomial distribution was applied.

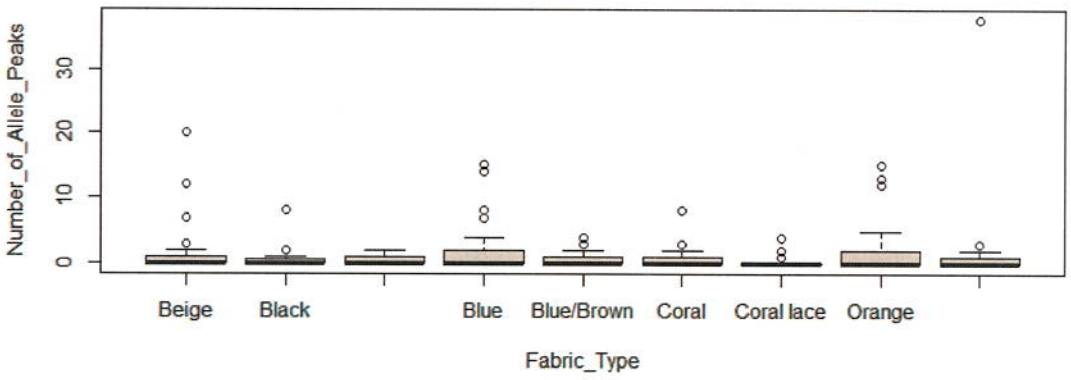
```
allele_mod <- glmer.nb(Number_of_Allele_Peaks ~ Gold_Silver + (1|Whole
_Plot)
                       + Swab_Mvac + (1|Sample_ID) + Qiamp_DNAIQ + Siz
e_Exclusion, data = dat)

## boundary (singular) fit: see help('isSingular')

 summary(allele_mod)

## Generalized linear mixed model fit by maximum likelihood (Laplace
##   Approximation) [glmerMod]
##  Family: Negative Binomial(0.409)  ( log )
## Formula: Number_of_Allele_Peaks ~ Gold_Silver + (1 | Whole_Plot) +
```

```
Swab_Mvac +
##     (1 | Sample_ID) + Qiamp_DNAIQ + Size_Exclusion
##    Data: dat
##
##      AIC      BIC   logLik deviance df.resid
##    725.4    754.7   -354.7    709.4      280
##
## Scaled residuals:
##     Min      1Q  Median      3Q     Max
## -0.6177 -0.5073 -0.4290  0.1565  7.6013
##
## Random effects:
##  Groups     Name        Variance  Std.Dev.
##  Sample_ID  (Intercept) 5.795e-11 7.613e-06
##  Whole_Plot (Intercept) 4.957e-01 7.040e-01
## Number of obs: 288, groups:  Sample_ID, 36; Whole_Plot, 18
##
## Fixed effects:
##                    Estimate Std. Error z value Pr(>|z|)
## (Intercept)         0.08279    0.34746   0.238  0.81167
## Gold_SilverSilver -0.37597    0.41639  -0.903  0.36657
## Swab_MvacSwab     -0.75465    0.25628  -2.945  0.00323 **
## Qiamp_DNAIQQiamp   0.65385    0.24445   2.675  0.00748 **
## Size_ExclusionYes -0.52774    0.25021  -2.109  0.03493 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Correlation of Fixed Effects:
##             (Intr) Gld_SS Swb_MS Q_DNAI
## Gld_SlvrSlv -0.533
## Swab_MvcSwb -0.293  0.009
## Qmp_DNAIQQm -0.368 -0.027 -0.007
## Sz_ExclsnYs -0.314  0.034 -0.085 -0.058
## optimizer (Nelder_Mead) convergence code: 0 (OK)
## boundary (singular) fit: see help('isSingular')
```

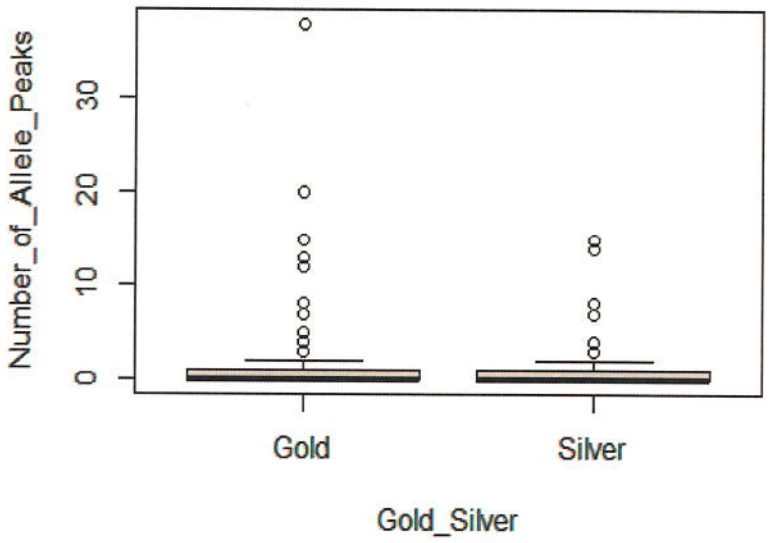
```
hist(dat$Number_of_Allele_Peaks)
```



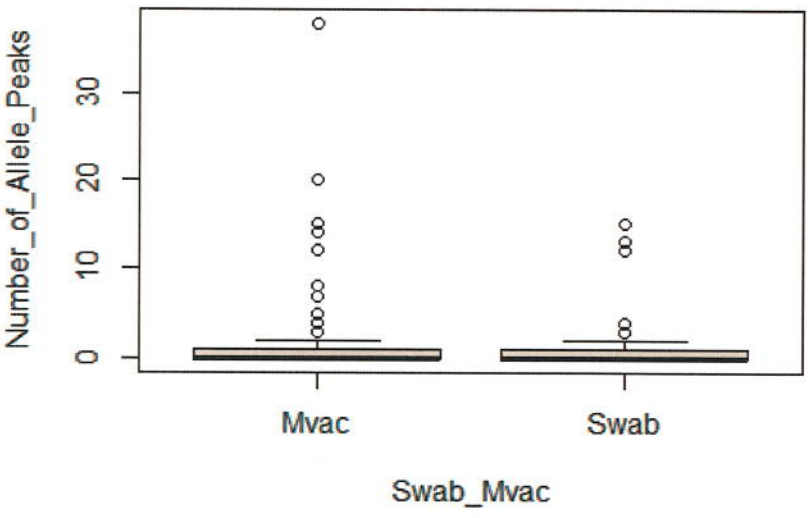

```
boxplot(Number_of_Allele_Peaks ~ Fabric_Type, data = dat)
```



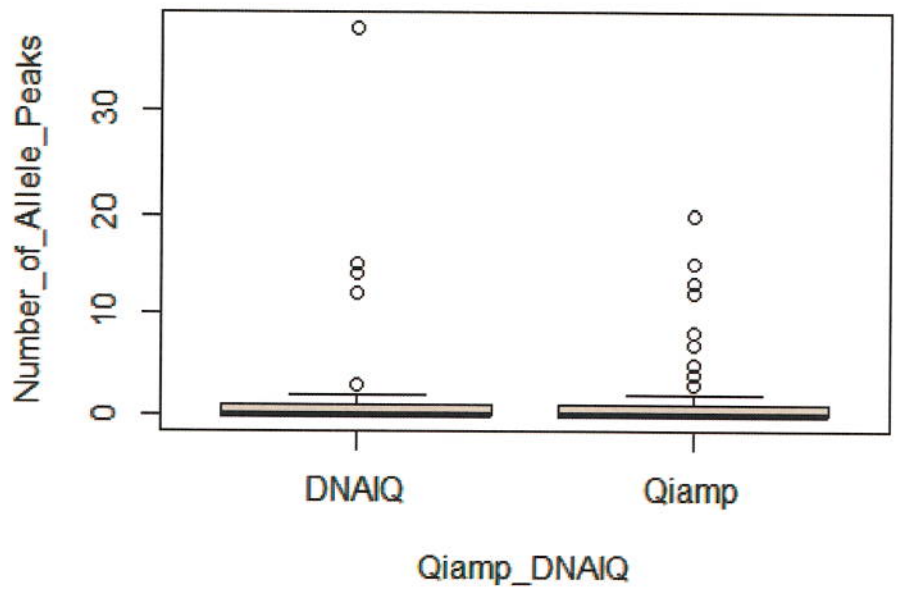
```
boxplot(Number_of_Allele_Peaks ~ Gold_Silver, data = dat)
```




```
boxplot(Number_of_Allele_Peaks ~ Swab_Mvac, data = dat)
```



```
boxplot(Number_of_Allele_Peaks ~ Qiamp_DNAIQ, data = dat)
```




```
boxplot(Number_of_Allele_Peaks ~ Size_Exclusion, data = dat)
```