EVALUATION OF COLLECTION METHODS FOR EXTRACTION OF TRACE AMOUNTS OF DNA FROM CLOTH SUBSTRATES

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EVALUATION OF COLLECTION METHODS FOR EXTRACTION OF TRACE AMOUNTS OF DNA FROM CLOTH SUBSTRATES

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Abstract: DNA analysis has become the golden standard in many crime laboratories around the world. As technology advances, new possibilities arise in using evidence left at crime scenes, touch DNA being one of these possibilities. Touch DNA, available in trace amounts, is often available on surfaces handled at a crime scene. Due to the limited amount of DNA in these samples, recovery efficiency is crucial if the samples are to be used as evidence in an investigation. Fabric was chosen as a substrate for its high prevalence in everyday life. A common technique of recovery from these materials is cutting extraction, however several additional methods are available. A total of 5 methods (cutting, tape lifting, and 3 swab types) were evaluated for their efficiency in recovery of DNA from these fabric substrates. Known amounts of DNA were spotted onto marked fabric during the first portion of the study in order to estimate the percentage of DNA recovered. In the second portion, volunteers were asked to wear garments to put this information into real world perspective. While cutting was the best option for most of the substrates in the laboratory portion, no one method showed greater efficiency among all the garments in the real world portion. The majority of samples from the garments were sufficient for STR typing, according to the limit of detection given by the PowerPlex multi-locus STR kit.

TABLE OF CONTENTS

Chapter Page

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

ABBREVIATIONS

ALS	alternate light source
BCI	Bureau of Criminal Investigation
DNA	deoxyribonucleic acid
dNTPs	deoxynucleoside triphosphates
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
IRB	Institutional Review Board
LCM	laser capture micro dissection
uL	microliter
mL	milliliter
ng	nanogram
PCR	polymerase chain reaction
pg	picogram
TPD Lab	Tulsa Police Department Forensic Laboratory
UCR	Uniform Crime Report
UV	ultraviolet
xg	G-force

CHAPTER I

INTRODUCTION

In July 2009, police discovered the bodies of Alan Grna and his mother, Julianna Grna, violently beaten to death in their own home. After a week, law enforcement located a suspect, Johnnie Cook, but did not have enough evidence to charge him with the double homicide. Investigators contacted the Ohio Bureau of Criminal Investigation (BCI) for help. As investigators walked through the crime scene, they searched for an object the perpetrator touched during the commission of the attack or afterwards when he tried to clean up. During their search, the investigators noted evidence in the bathroom that indicated he may have washed. Eyes fell to a roll of toilet paper possibly used by the assailant to dry his hands. Investigators collected and submitted the roll to the crime lab. Technicians later extracted and analyzed touch DNA left on the roll from the suspect's skin cells sloughing from his hands. Two of the three profiles found belonged to Alan and Julianna Grna; the third matched the DNA of Johnnie Cook.[1] As a result, the suspect was charged, later convicted of aggravated murder, and sentenced to life in prison without the possibility of parole. The family and friends of the victims could begin to heal knowing the man who bludgeoned their loved ones was being held accountable for his crimes.[2]

According to the FBI's Uniform Crime Reporting (UCR) Program, an estimated 1,163,146 violent crimes were committed in 2013 across the country. Approximately 48.1% of these

crimes were cleared by arrest or by exception, leaving approximately 51.9% of violent offenders on the streets.[3] While forensic science has made significant technological advances in recent decades, technology changes and researchers continue to find new ways to connect suspects to their crimes. Convictions, such as that of Johnnie Cook, show the importance of these advancements.

Deoxyribonucleic acid, commonly known as DNA, has become the "golden standard" for the identification of perpetrators at crime scenes. This molecule contains the instructions necessary to create every type of cell in a person's body. With the exception of identical twins, approximately 0.1% of DNA varies among people. This 0.1% is the main focus of forensic DNA investigations.[4] Due to DNA's abundance in the body, multiple fluids can be used as a source for DNA. Good sources of DNA include blood, saliva, and semen, often visible to the naked eye. Presumptive tests available commercially narrow the possibilities for the type of fluid available. For example, a stain believed to be blood is tested using phenolphthalein. A negative result confirms the stain as not blood, whereas a positive result suggests the stain as probably blood. A second test, a confirmatory test, is necessary in order to declare a stain as blood.[5]

A less commonly known source of DNA is touch DNA, defined as a sample containing less than 200pg of genomic DNA.[6] According to Locard's Exchange Principle, contact of objects results in an exchange between those objects.[5] This principle applies when a person touches an object, possibly leaving fingerprints and touch DNA behind. Depositing touch DNA occurs when cells slough off the surface of the skin. At this time, no established presumptive tests for touch DNA exist, making locating the evidence difficult. Additionally, due to the small amount of sample available, prevention of contamination and degradation of the DNA is critical.[7,8]

DNA analysis begins with the collection of the evidence that may harbor DNA. Studies show that as much as 86% of DNA deposited on a surface may remain uncollected. This statistic varies

depending upon the surface from which the DNA is collected and the method used to collect (swabbing, taping, cutting).[9] While lab technicians and crime scene investigators cannot control the surface upon which the DNA is deposited, options exist for the most appropriate method for sample collection. For example, the cutting method, involving cutting a sample from the substrate itself, is realistic for clothing or bedding, but not realistic for doorknobs and countertops.

Fabrics are everywhere: from clothing and bedding to carpet and upholstery. Fabrics come in all colors, sizes, and textures imaginable. Dictionary.com defines fabric as "a cloth made by weaving, knitting, or felting fibers".[10] Because individual fibers are woven together, countless minute spaces are created, as seen in Figure 1. These spaces often trap molecules, including DNA, within the fabrics. The uneven surface of the fabric also creates an abrasive surface as skin brushes across the fibers. These features make fabric an excellent source of touch DNA; however collecting the DNA from between the fibers may be difficult. Investigating collection methods helps to determine the most efficient method. Common methods include cutting, taping, and swabbing.

Figure 1- Microscopic View of Fabric Fibers[11]

Fabric may contain minute spaces in which DNA becomes trapped.

The purpose of this experimental study was to test the hypothesis that touch DNA adheres to glass more efficiently than to other substrates used for the collection of biological material from fabric. Testing compared 5 collection methods (cutting, taping, Dacron swabbing, cotton

swabbing, and glass fiber swabbing). A total of 6 fabric types were tested, including cotton, denim, polyester, silk, spandex, and wool. The specific goals of this study included:

(a) To develop controlled laboratory testing procedures to compare 5 collection methods,

(b) To conduct real-world testing of clothing worn by volunteers, and

(c) To statistically determine efficiency of a glass fiber swab in comparison to traditional swabbing methods.

CHAPTER II

REVIEW OF LITERATURE

Deoxyribonucleic acid

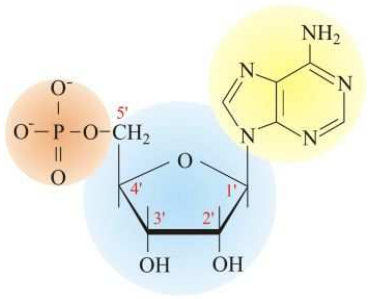
An individual's DNA houses the blueprints for every cell in the body. With about 99.9% of DNA consistent from one person to the next, forensic scientists concern themselves with the remaining 0.1%. As the likelihood of one person sharing the exact same DNA with another is approximately 1 in 594 trillion, this biological material often connects a certain person to a crime scene. Concerns relating to DNA analysis include prevention of unauthorized use and reliability in the results.[4] In light of these concerns, scientists are constantly revising old techniques and establishing new ones.

Properties of DNA

Nucleotides make up a segment of double-stranded DNA. Each nucleotide contains a phosphate group, a sugar (deoxyribose), and one of four nitrogen bases (adenine, cytosine, thymine, or guanine). The basic structure of a nucleotide is shown in Figure 2. Adenine and cytosine carry a positive or neutral charge, thymine has a negative or neutral charge, and guanine carries any of the three charges. While the nitrogen base may vary in charge, the overall DNA molecule

possesses a net negative charge. In a solution containing a high concentration of salt, the phosphate groups of the nucleotide backbone can associate with a positively charged cation in the salt molecule and, if the cation is of the appropriate type, it can form an ionic "bridge" between the phosphate and negatively charged silica molecules in glass and thus cause the DNA to bind to the silica.[12]

Figure 2- Structure of a Nucleotide Base[13]



A nucleotide base contains a phosphate group (red), deoxyribose (blue), and 1 of 4 nitrogen bases (yellow). Adenine is pictured.

Touch DNA

Touch DNA has become an area of interest in recent years. When a person touches an object, cells slough off the skin and leave genomic DNA behind that may be of sufficient quantity and quality to produce a DNA profile. In a cross-section of human skin, the first few layers are keratinized and lack nuclei. Not until the third layer, the stratum granulosum, does a nucleus, containing the DNA of interest, remain in the cell.[14] Daly et al speculate two possibilities to explain how sloughed cells leave DNA: (1) sloughing of the external cells leaves nucleated cells exposed and vulnerable to sloughing themselves, and (2) hands are used as a carrier of DNA from other parts of the body such as the mouth and eyes.[15] Several difficulties arise when considering

the use of touch DNA: quantities deposited, lack of visibility precluding easy detection, lack of a presumptive test to localize DNA in the touched item, and interpretation of the result.[7,8]

Touch DNA, also referred to as low template DNA (LTDNA), is defined as a sample containing less than 200pg of template.[6] With such a small amount of DNA available, great care must be taken during collection and analysis to prevent loss and contamination. Collecting touch DNA from cloth is further complicated by the challenge of knowing where to find it. The material is neither visible on the substrate from which it is collected nor does a presumptive test exist to make it visible, except in cases in which obvious touch evidence is visible (i.e. fingerprint or palmprint). Haines et al performed a study to determine whether or not SYBR Green, an intercalating dye used to visualize DNA during electrophoresis, would be an effective presumptive test for visualizing DNA. DNA could be visualized but it was uncertain as to whether human or bacterial DNA was being detected.[16]

Following collection and analysis of biological material, interpretation of STR results may be difficult as well. Factors that must be taken into consideration include when the sample was transferred to the substrate and whether secondary transfer is a possibility. Secondary transfer refers to someone "picking up" DNA on their hands through contact and then depositing that DNA onto another substrate, perhaps along with their own DNA. Consider a case of sexual assault in which touch DNA is collected from the victim's jeans; the suspect being her ex-boyfriend. For the evidence to be admissible, the prosecution must prove the DNA was deposited during the crime rather than during a previous encounter. Secondary transfer in this case could be the transfer of the ex-boyfriend's DNA to the perpetrator who then deposits the DNA onto the jeans. Lowe et al performed a study to investigate the occurrence of secondary transfer.[17] Each subject was classified as a good shedder or a poor shedder. A good shedder was defined by the deposition of a full DNA profile 15 minutes after hand washing. A poor shedder left only partial profiles 15 minutes after hand washing. Experiments were conducted by assessing the amount of

DNA from the good shedder deposited on an object versus the poor shedder. Variables within the experiments included the time period since the subjects last washed their hands (wash interval) and the length of time between contact of the individuals and deposition of DNA (contact interval). Results of the studies performed by Lowe et al show that as the wash interval increases, the level of touch DNA deposited increases. As the contact interval increases, the likelihood of detecting a mixture of DNA from both parties increases.[17]

Collection of Samples

Given the low recoveries of touch DNA possible, each step in the STR typing process from sample collection to data interpretation becomes crucial. Individual steps must be optimized if the process as a whole is to be of greatest use. A variety of collection techniques are available to analysts for collecting touch DNA. Cutting, taping, and swabbing are a few of the commonly used techniques and they form the focus of this study.

Cutting is a method in which the lab technician removes a section of the fabric from the piece of evidence and performs DNA extraction directly from the substrate. This method decreases the loss and contamination of DNA by reducing the number of steps and containers used during extraction. A possible disadvantage to this particular technique arises in the difficulty of locating touch DNA on a piece of fabric. In a study conducted by Petricevic et al, DNA was extracted from sheets taken from volunteers' beds after one night of use.[18] Full STR profiles were produced from the individual and a mixed profile could also be detected from the individual and a guest also sleeping in the bed.

Taping is a common method when collecting biological material from porous surfaces, such as fabrics. In a study by Verdon et al, Scotch tape, Scenesafe FAST tape, and cotton swabs were compared for their effectiveness in collecting trace DNA.[19] Taping was deemed more effective than swabbing and Scenesafe FAST tape was more effective than Scotch tape, due to the

increased adhesion of the Scenesafe FAST tape, as well as the greater ability to extract DNA. The number of times a tape lift is applied to the area was also taken into account. The authors determined that taping 16 to 32 times resulted in significantly more DNA than a single application; however, once up to 64 tapings, the yield decreased. This decrease is a result of the collection of fibers on the tape with the DNA as well as some loss of DNA back to the substrate.[7]

Swabs have several advantages over other methods. Swabs are not only inexpensive, but also simple to use and able to collect a number of biological materials. The swabs can vary based upon the swab material, the dimensions of the tip, and the density of the tip.[19] Verdon et al conducted a separate study comparing the efficiency of various swabs for trace DNA collection.[19] Of the types examined, including cotton, nylon, polyester, rayon, and foam, cotton tips were deemed most efficient. The action of dragging the swab across the surface containing DNA causes the mechanical trapping of the DNA within the fibers of the swab. In a study by Wilkins[20], swabs composed of glass fiber filter were explored as a device trace DNA collection due to DNA's high affinity for glass in a high salt environment. Thus the extension of the knowledge that DNA binds to glass would be that a glass fiber swab, rather than trapping DNA in the swab matrix, would actually bind the DNA to the matrix like a magnet.

Extraction

Once a sample has been collected, DNA extraction begins. Three common types of DNA extraction methodology are available:(1) extraction with detergent and protease followed by removal of contaminants with organic solvents. (2) Extraction of DNA with chaotropic salts followed by binding to silica during removal of contaminants through washing. DNA is eluted from the silica with water or dilute buffer and DNA thus recovered is ready for amplification and analysis. (3) Laser capture micro dissection (LCM), in which individual cells can be captured

from a microscopic slide using a laser beam and then subjected to DNA extraction by one of the variations of the methods above.

The type and amount of biological material suspected in evidence determine the most appropriate method of extraction. Each method involves the same basic steps: disruption of the cell, lysis of membranes, and removal of contaminants. Cell disruption, most commonly performed by digestive enzymes and detergent such as proteinase K and SDS, may also be done by a boiling or alkali treatment. Lysis, or the breaking open of membranes, is carried out by a lysis buffer containing five components: (1) detergents, used to break down membranes and proteins, (2) buffer, for maintenance of pH, (3) high concentration of salt, used to dissociate histones from the DNA, (4) reducing agents, to prevent oxidation from damaging DNA, and (5) chelating agents, used to capture divalent cations which serve as cofactors promoting the hydrolysis of DNA.

Extraction with organic solvent removes contaminants and further strips chromatin proteins from the DNA. DNA, once liberated and clean, can be recovered through any number of final steps, including ethanol precipitation. Another common extraction method used widely today involve dissolution of cellular structures with the aid of a concentrated chaotropic salt, such as guanidium isothiocyanate. DNA is then captured on the surface of silica-coated magnetic beads. This binding allows the washing away of proteins without the risk of losing the DNA. The phosphodiester backbone of DNA is dehydrated, and the phosphate residue binds to the silica. Once immobilized, a mixture of dilute buffer with a 70% (v/v) mixture of alcohols is used to wash away chaotrophic agents and contaminating cellular material. Aqueous low-salt solutions rehydrate the DNA backbone, resulting in the elution of DNA from the silica, ready for amplification and further analysis.[21]

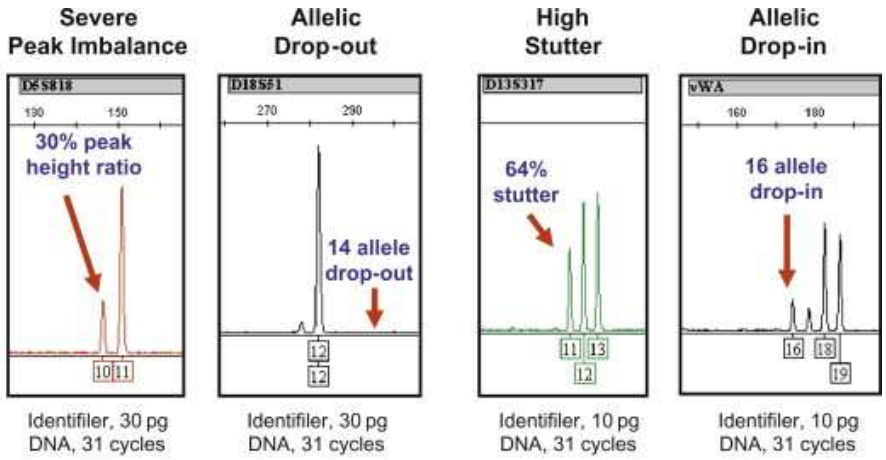
Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique used in many forensic laboratories to analyze DNA. Real-time PCR is an adaptation of the basic PCR process and was developed in the early 1990s. During real time PCR, the accumulation of PCR product is quantified through various fluorescent means which means real time PCR (i.e. qPCR) is quantitative.

Since the goal of this study was to evaluate different collection methods for the recovery of trace DNA from fabric, we chose real-time PCR to quantify the amount of DNA collected from different fabrics with each recovery method. Samples were collected using 5 methods described earlier (i.e. cutting, taping, Dacron swabbing, cotton swabbing, and glass fiber swabbing). Ultimately, qPCR was used to estimate the amount of DNA recovered with each recovery method.

Stochastic effects when dealing with a limited quantity of DNA may affect the interpretation of analysis. The four common problems are allelic drop-out, allelic drop-in, increased stutter, and peak imbalance.[4,8,22] Drop-out occurs following a failure of an allele to amplify. Drop-in shows what is described by Butler as "sporadic contamination". [8] All PCR results show background noise with peaks typically 5-10% the height of an allele. Stutter refers to instances in which this background noise presents peaks greater than typical. Peak imbalance occurs when one allele is preferentially amplified over the other in a heterozygous pair.[4,8,22] Examples of these phenomena are shown in Figure 3.[8] Care must be taken to assess for these problems prior to comparing to suspect samples as bias is a major concern.[23] In order to combat stochastic effects, a consensus profile is generated in which an allele is only documented if it appears in at least two replications.[4]

Figure 3- Stochastic Effects that may occur during Analysis of Low Template DNA[8]



Stochastic effects may result in misinterpretation of peak data.

CHAPTER III

MATERIALS AND METHODOLOGY

Touch DNA (or trace DNA) is generally characterized as that containing less than 200pg of DNA.[6] Given such a small amount of DNA template available for amplification and analysis, it is critically important to initially recover as much DNA from evidence as possible. An effective combination of optimal sample collection method(s) and use of STR typing kits with increased sensitivity could make the production of DNA profiles from trace evidence more routine. Common methods used for collection of DNA from fabric include cutting and direct extraction, Dacron swabbing, cotton swabbing, and tape-lifting. A newly developed glass fiber swab[20] was also included in the experimental plan. Each of these methods were adopted by the OSU-CHS DNA laboratory either from the literature or were developed in house. These procedures were used as a starting point and were optimized for use in extracting touch DNA from fabrics.

The methodology presented here has been reviewed and approved by the Institutional Review Board (IRB) of OSU-CHS.

Materials

Fabrics selected for this study included white cotton, blue denim, polyester, silk, wool, and spandex, chosen for their high prevalence in everyday life. While fabrics used for clothing, blankets, and other items are commonly made up of a combination of different types of fabrics, the samples of each of the substrates for this study will be as close to 100% composition as

possible to eliminate variability in blends. Fabrics were purchased at JoAnne Fabrics and the fabric composition reported.

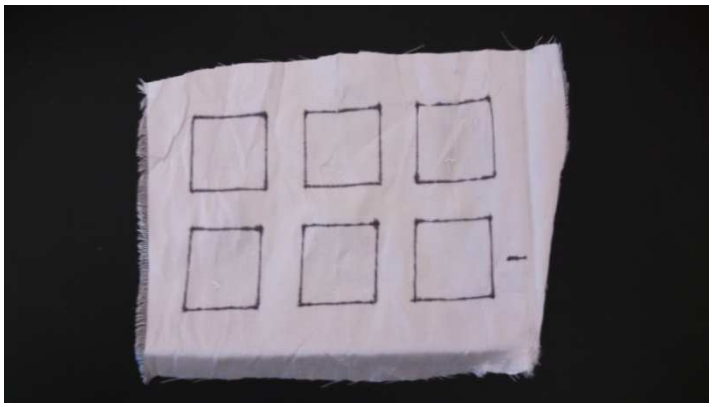
Instruments necessary for this study include a UV Stratalinker (Stratagene, San Diego, CA) and an ABI 7500 RealTime PCR instrument (Applied Biosystems, Foster City, CA) with associated software. Kits sold for use in forensic laboratories included the Applied Biosystems' Quantifiler Human DNA Quantification Kit (Applied Biosystems Inc, Foster City, CA) and the DNA IQ extraction kit (Promega Corp, Madison, WI). Collection materials include Scotch tape, Dacron swabs (Puritan, Guilford, ME), cotton swabs (Puritan, Guilford, ME), and swabs manually manufactured using Whatman glass fiber paper (VWR Scientific, Radnor, PA). A complete list of the materials and instruments used in this study are listed in Appendix A.

Fabric Preparation

Prior to using the fabric, they were all hand washed in warm tap water for 2 minutes. The fabrics were then wrung out and allowed to air dry, without the use of fans, over 24 hours on a drying rack at room temperature. Once dry, the fabrics were folded and stored in separate Ziploc bags until their use in the laboratory. Gloves were worn at each step of this cleaning process to prevent contamination of the fabrics while handling.

The fabrics were marked using an ink marker to locate where on the fabric DNA was to be spotted. A fine-tipped Sharpie was used to draw 6 squares, measuring 2cm across, on each of the fabrics. A space measuring 1cm was left between each square, as shown in Figure 4. These measurements were chosen as 2uL of DNA does not appear to wick beyond these squares when spiked onto the fabric. Of these 6 squares, 5 were spiked with DNA with the last square left as a negative control and marked as such.

Figure 4- Setup of Fabric Swatches for Spiking DNA



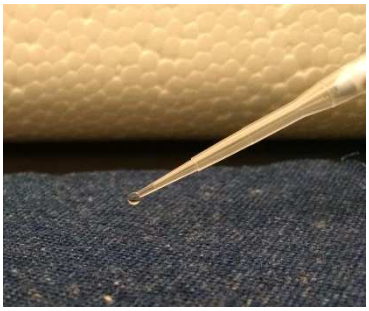
A total of 6 squares are drawn on the fabric to locate spiked DNA with 1 left as a negative control.

Once the fabrics were marked, they were placed under an ultraviolet (UV) light in order to eliminate any residual amplifiable DNA left on the fabric. The instrument used for this purpose was a UV Stratalinker 1800. The energy setting was programmed at 3000, representing the number of microjoules/cm^2 x 100. This number translates to a total of 300,000 microjoules/cm^2 to crosslink any DNA on the fabric preventing it from being a suitable template for PCR amplification. The pieces of fabric were then carefully transferred to a clean workbench for the process of spiking DNA onto the materials.

DNA was spotted directly onto the fabric squares. Spiking a known amount of DNA onto each square provides a starting point with which to compare the amount of DNA that is collected with the various collection methods. A 5ng/uL stock of DNA in TE^{-4} (10mM Tris-Cl, pH 8.0, 0.1mM EDTA) was prepared using the concentrated standard DNA (200ng/ul) provided with the Quantifiler kit also used for quantification. Each fabric square was then spiked with 2uL of diluted DNA for a total of 10ng of naked DNA deposited on the fabric. A reliable way to ensure no DNA was left in the pipette tip was to depress the plunger on the pipette to form a bead of DNA on the tip then lightly touch the bead to the fabric, allowing it to be wicked into the fabric.

This technique is shown in Figure 5. DNA was allowed to soak into the fabric and dry overnight as it might in a real world situation. The fabric swatches were allowed to dry on the lab bench without fans to reduce the risk of contamination.

Figure 5- Spiking DNA onto Fabric Swatches



Slowly depressing the plunger on the pipette forms a bead which can easily be deposited on the fabric.

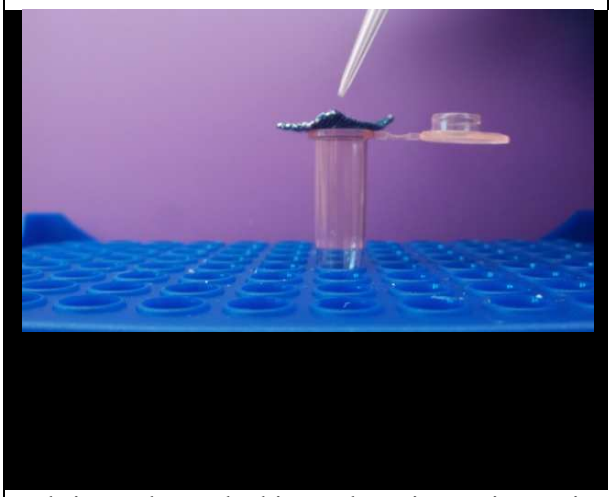
Extraction Methods

There are several extractions available to researchers. Two basic extraction types explored for this study include organic extraction via a 9:0.96:0.04 mixture of phenol-chloroform-isoamyl alcohol and, extraction via chaotropic salts using silica-coated magnetic beads as a binding agent. A comparison of these extraction methods preceded the comparison of the collection methods. Cuttings of cloth were subjected to extraction using the organic extraction method and the DNA IQ method employing a chaotropic salt extraction followed by binding of DNA to magnetic beads. Both extraction methods yielded similar recovery of DNA. However, the DNA IQ methodology reduced the time necessary for extraction and uses nonhazardous reagents, and so this extraction method was chosen for the remainder of the study. As the collection methods use different materials (polyester, cotton, glass fiber, tape), there were slight variations to the basic DNA IQ methodology developed and each modification is discussed in the remainder of this section. The procedures as discussed serve as a starting point and were optimized for this study.

Cutting Extraction

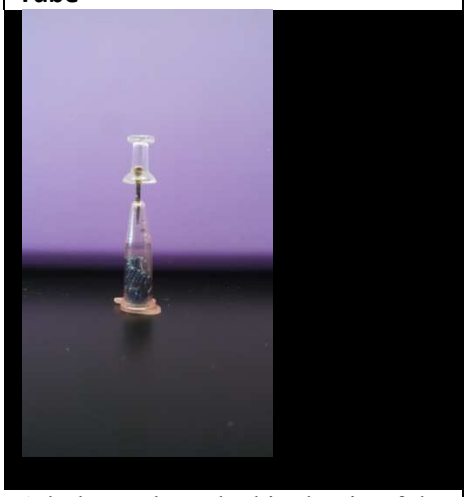
Cutting extraction via magnetic beads requires lysis/DTT solution. Each milliliter of lysis/DTT solution contains 900µL of chaotropic lysis solution (supplied with the DNA IQ kit) and 100µL of 1M DTT. Extraction of DNA from a cutting involved inserting the fabric cutting into the microfuge tube. The square was first placed over the opening of a labeled 0.65mL tube and a 1000uL pipette tip was used to push the square into the tube, as in Figure 6. Lysis/DTT (200µL) was added; the sample then vortexed and incubated at 70°C for 15 minutes. Turning the sample tubes upside down on the lab bench and flicking the solution away from the tip of the tube preceded poking a hole in the bottom so the extract and not the matrix could be recovered in a microfuge tube during centrifugation, as shown in Figure 7. These 0.65mL microfuge tubes were then placed into 1.5mL tubes and centrifuged for 3 minutes at 6000xg. To the recovered extract (containing any DNA) was added 7µL of magnetic beads, included in Promega's DNA IQ System kit (Madison, WI). Extracts were incubated with beads for 5 minutes with intermittent vortexing. At this point, the DNA is bound to the beads which can be immobilized using a magnetic tube stand and lysis/DTT (and any contaminants) can be aspirated out of the tube without losing DNA. The samples were washed with 50µL lysis/DTT, followed by further washing with 50µL of a wash buffer composed of TE^{-4} containing 35% (v/v) ethanol and 35% (v/v) isopropanol twice, with aspiration of the solution each time. These aspiration steps were performed with the samples on the magnetic stand to reduce the loss of DNA. The samples were allowed to air dry for 5 minutes before being eluted with 50µL of TE^{-4} buffer (65°C) and incubating at 65°C for 5 minutes. The DNA, no longer bound to the magnetic beads, was pipetted out of the sample tube and placed into a clean tube and the sample was quantified using real-time PCR and the Quantifiler kit.

Figure 6- Pushing Cut Swatch into Tube



Fabric can be pushed into tube using a pipette tip

Figure 7- Poking a Hole in Small Tube



A hole can be poked in the tip of the tube using a pushpin

Dacron and Cotton Swab Extraction

To prepare for Dacron or cotton swab extraction, a hole was punctured in the tip of a 0.65mL microfuge tube for each sample and these tubes were placed into 1.5mL tubes. A sterile swab was moistened with 75µL of TE^{-4} buffer and each fabric square was swabbed using a scrubbing motion; these swabs were placed into individual 0.65mL tubes, cutting off the excess swab applicator stick. Lysis/DTT solution from the DNA IQ extraction kit (150µL) was added to each sample, which was then centrifuged at 6000xg for 3 minutes and the flow-through containing the DNA was collected. Magnetic beads from the DNA IQ extraction kit (7µL) were then added to each sample and the solution was incubated at room temperature for 5 minutes, with occasional mixing. The samples were placed on the magnetic stand in order to aspirate the liquid, the DNA being bound to the beads. The sample was washed with an additional 150µL of lysis/DTT and then with 200uL of wash buffer, included with the DNA IQ kit as described above. The liquid was aspirated after each wash since the DNA was bound to the silica coated magnetic beads,

which were immobilized on the side of the microfuge tube magnetically. DNA was eluted from the silica twice independently with 25uL aliquots of TE^{-4}, which were then pooled.

Glass Fiber Swab Extraction

Glass fiber swabs were manually prepared as described in Wilkins[20]. A piece of glass fiber filter paper, measuring 3.4 x 1-cm, was attached, using superglue, to the plastic applicator supplied with the Dacron swabs typically used in the Human ID laboratory (Puritan, obtained from Fitzco Corp in Spring Park, MN) at the opposite end of the Dacron swab head. The glass fiber filter was glued to the plastic applicator using superglue and then the filter was manually wound around the applicator stick and the free end was secured with superglue. A finished glass fiber swab is shown in Figure 8. Any contaminating DNA in the glass fiber matrix was inactivated through exposure to UV irradiation (Stratalinker 1800, Stratagene, San Diego, CA), set at 300,000 microjoules/cm^2.

Figure 8- Finished Glass Fiber Swab

Glass fiber paper is wrapped around the opposite end of a Dacron swab and secured with superglue.

DNA was collected from the fabrics by wetting the swab with 75µL lysis/DTT and using a rolling motion vertically and horizontally across the substrate until the entire area was swabbed. The swab head was placed in a 0.65mL tube with a hole punched in the bottom, which in turn was placed in a 1.5mL tube. The excess applicator stick was cut and discarded and the apparatus was centrifuged for 3 minutes at 6000xg. The liquid collected in the 1.5mL tube was discarded. A wash step was performed twice, with 200µL of wash buffer, provided with the DNA IQ extraction kit. Wash solutions were removed from the swab head (with bound DNA) by centrifugation at 6000xg for 1.5 minutes at room temperature and washes were discarded. After washing, the 0.65mL tube with the swab head was placed in a new 1.5mL tube and DNA was eluted from the swab twice consequtively with 25uL of TE^{-4} buffer. Ultimately, DNA collected from the fabric existed in a 50uL total volume.

Tape-Lift Extraction

Tape-lifting uses Scotch tape to capture DNA-containing materials from the fabric. Scotch tape was used rather than SceneSafe FAST due to the availability of Scotch tape in the laboratory. A piece of tape, approximately 0.5-1 inch long, was removed from the roll and firmly pressed onto the area of fabric containing the DNA. The portion of tape on the roll that has been exposed, displayed in Figure 9, was not used in order to reduce possible contamination. The tape was pulled from the fabric and this taping motion was repeated 25 times before being placed in a 1.5mL tube. Lysis/DTT (300µL) was added to the sample, which was incubated at 70°C for 15-30 minutes. The lysis/DTT, containing any DNA recovered, was then transferred to a new 1.5mL tube. Magnetic beads (7µL) were added; the sample was vortexed and incubated at room temperature for 5 minutes. Following this incubation period, the samples were processed with washing and elution of DNA in the typical manner when the DNA IQ extraction system was used.

Figure 9- Portion of Tape to be Discarded[24]

The portion of tape indicated in red should not be using to collect DNA as it may be contaminated.

DNA Quantitation

The Quantifiler Human DNA Quantification Kit, supplied by Applied Biosystems (Foster City, CA) was used to quantify DNA recovered from the different fabrics using the different methodologies. The qPCR reaction mix contains Taq DNA polymerase, deoxynucleotide triphosphates, divalent cations, and the primers that direct the amplification of the different products used for quantitation. A master mix of reaction mix and primer mix was created to simplify the process of setting up the sample plate, as well as to reduce the risk of contamination. Each well of a 96-well optically clear plate contained 9uLof a master mix composed of 5µL of reaction mix and 4µL of primer mix. Enough reagents were added for a few extra reactions to allow for pipetting error. Once the master mix was created and mixed, 9µL of reaction mix was added to each well of the 96 well reaction plate, followed by 1uL of DNA, either as a quantification standard or as an unknown. Positive and negative reagent controls were included on each plate. To complete the reactions, 1µL of DNA was added to each well, TE^{-4} was used as the negative reagent control. For this study, DNA was quantified from each extraction in triplicate. Once all the reagents and DNA extract or quantitation standard had been added to each

well, optical adhesive film was used to cover the plate to prevent evaporation. Plates were briefly centrifuged to remove any air bubbles and force the reagents to the bottom of the wells.

Real time PCR was performed using an ABI 7500 real time instrument (Applied Biosystems, Foster City, CA) using 7500 System SDS Software supplied with the instrument, following instructions provided by the manufacturer. Included in the Quantifiler kit is a synthetic DNA template (the IPC) that is present in every reaction at a constant concentration. This synthetic template is also amplified during the PCR reaction and serves to reveal PCR inhibitors if they are present. If the IPC quantifies normally, an analyst can conclude that the DNA extract being quantified does not contain PCR inhibitors.

Data Analysis

Real time PCR is able to estimate the concentration of human DNA in an extract by comparing the accumulation of fluorescence in the unknown with that detected in a sample of known concentration.[25] Thus a standard curve is prepared for use in estimating DNA quantity in unknowns. The analysis software supplied with the instrument is able to determine when the accumulation of fluorescence in each reaction reaches a logarithmic phase known as the cycle threshold (or CT). So the CT values for unknowns is compared with that of known samples, composing the standard curve to arrive at an estimate of the concentration of DNA in an unknown. The dynamic range of qPCR is about 25pg at the low end and extends up to 20ng at the upper end. The concentrations used for the standard curve can be seen in Table 1.

Table 1- Dilutions used to generate standard curves [26]		
Standard	Concentration (ng/µL)	Solution Amounts
1	50.000	50µL [200ng/µL stock] + 150µL TE^{-4} buffer
2	16.700	50µL [Std 1] + 100µL TE^{-4} buffer
3	5.560	50µL [Std 2] + 100µL TE^{-4} buffer
4	1.850	50µL [Std 3] + 100µL TE^{-4} buffer
5	0.620	50µL [Std 4] + 100µL TE^{-4} buffer
6	0.210	50µL [Std 5] + 100µL TE^{-4} buffer
7	0.068	50µL [Std 6] + 100µL TE^{-4} buffer
8	0.023	50µL [Std 7] + 100µL TE^{-4} buffer
A standard curve is produced in which Ct values are compared in order to calculate the amount of DNA present in unknown samples.		

Application of Data

Once data had been gathered to estimate the amount of DNA collected using each method, the results were applied to real-world situations. In order to conduct this portion of the study, volunteers, selected from those available at OSU-CHS, were asked to wear articles of clothing for 45-60 minutes performing various activities based upon the type of clothing. Clothing articles were rinsed in warm tap water in Tulsa, Oklahoma and air-dried before being placed into butcher paper folded in a druggist fold. Gloves worn at each step decreased the possibility of contamination. Common clothing types were chosen for use in this study, including t-shirts, ball caps, and gloves. Each of the items chosen were as close to 100% cotton as possible. In addition to these items, leather gloves were also tested due to their high prevalence as evidence received by crime labs, such as Tulsa Police Department. The volunteer receiving the t-shirts was asked to perform their normal 45 minute workout while wearing one of the shirts. The volunteer with the ball caps wore each hat for 60 minutes while going about normal daily routine. The volunteers with gloves (cotton or leather) wore one glove on their dominant hand for 60 minutes while going about a normal day. Details of instructions given to volunteers are available in Appendix F. After wearing the garments, the volunteers folded the clothing in the butcher paper and returned

the articles to the researcher. Information about the volunteers was limited to gender and age. They were also free of any skin conditions, such as psoriasis and eczema that may alter the rate of cell shedding.

After the volunteers submitted the clothing, samples were collected from prominent areas on the articles. DNA was extracted from the fabrics with each of the optimized methods and the results compared to determine the ideal procedure for collecting and analyzing touch DNA from fabrics. Leftover materials were destroyed upon completion of the research. Volunteer data will be kept in a secure location, accessible only to the researcher, for 7 years before destruction.

Processing Clothing for Wearer

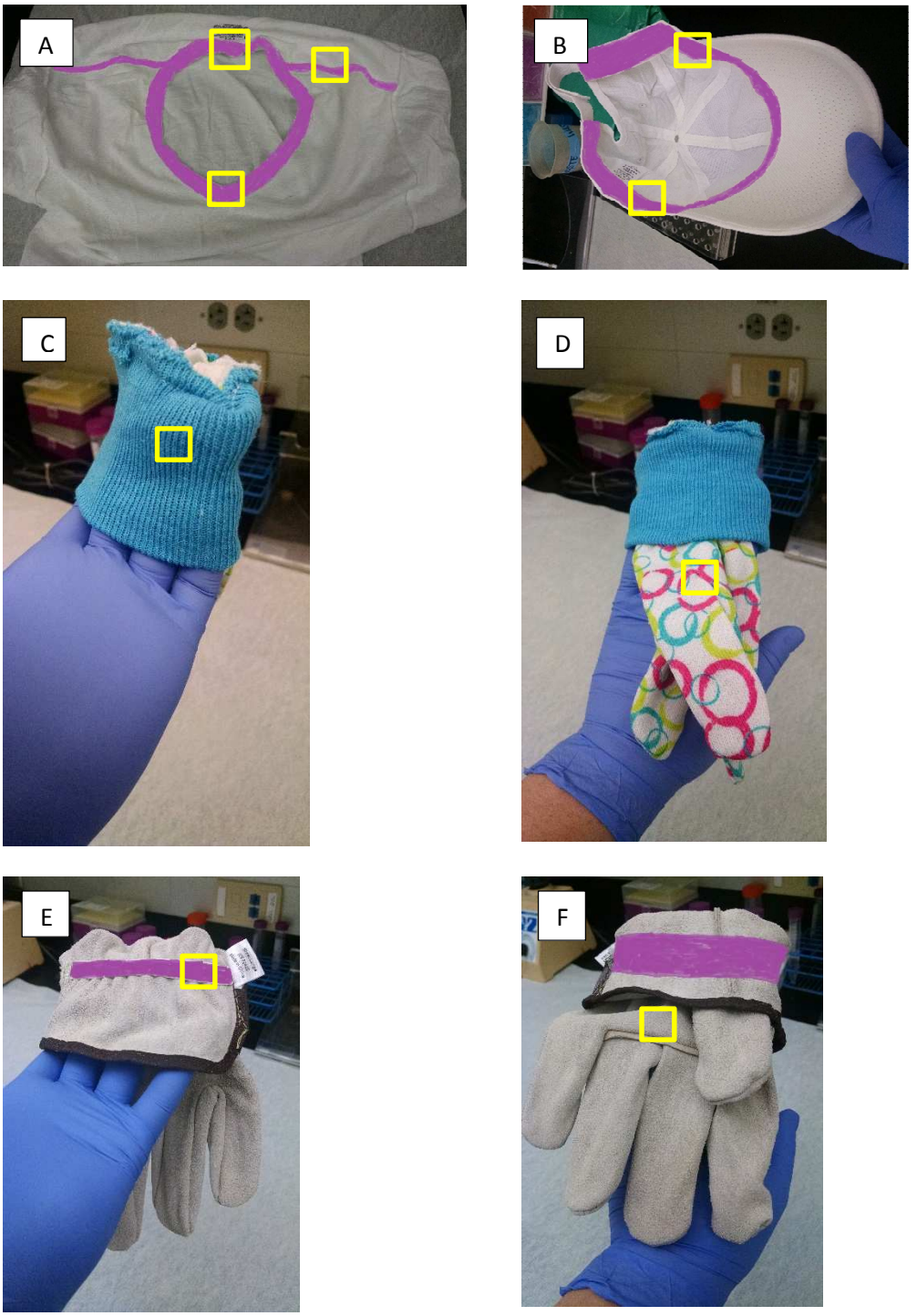
A validated sample collection protocol promotes consistency among analysts of a laboratory. While specific procedures may differ between crime labs, the overall process is similar. In an interview with Byron Smith at the Tulsa Police Department's Forensic Laboratory (March 2015), a detailed demonstration of collection was provided. The protocol is divided into four procedures: (1) note identifiers, (2) address stains, (3) identify biologicals, and (4) recover touch DNA. Each piece of clothing is unique in specific actions, however these steps apply to all clothing types. A complete outline of the protocol, including tips, is available in Appendix G.

Per the Tulsa Police Forensic Lab (TPD Lab) identifiers are distinguishing characteristics unique to the clothing examined. The analyst describes the item, including the brand, size, and logos. Functionality of zippers, buttons, and snaps is noted, as well as any stains or tears in the article. Any items in the pockets are recorded. Visible stains are localized and described in a general way (i.e. "reddish brown stain", "crusty yellow stain", etc). Each stain is swabbed separately. The swabs are packaged in the swab wrapper then sealed in a labeled envelope. Pockets should also be checked for possible blood stains. Biologicals are identified via an alternative light source (ALS). The ALS is used to locate any stains on the garment, which are circled for ease of

swabbing. One swab is used to collect from all areas positive for ALS. This swab is labeled as a combined swab for ALS and is packaged as previously described.

Touch DNA was collected as a single swab obtained anywhere on the garment believed to be a point of contact between the article and the wearer. The areas chosen for each item in this study is shown in Figure 10. Using one swab for the entire article increases the chances of a full profile. As the amount of DNA available is limited, dividing this amount between several swabs is undesirable. Buttons and zipper pulls are also areas that may collect DNA, which is collected with the same swab. An additional swab may be used for touch DNA for any articles that have pockets.

Figure 10- Swabbed Areas of Clothing

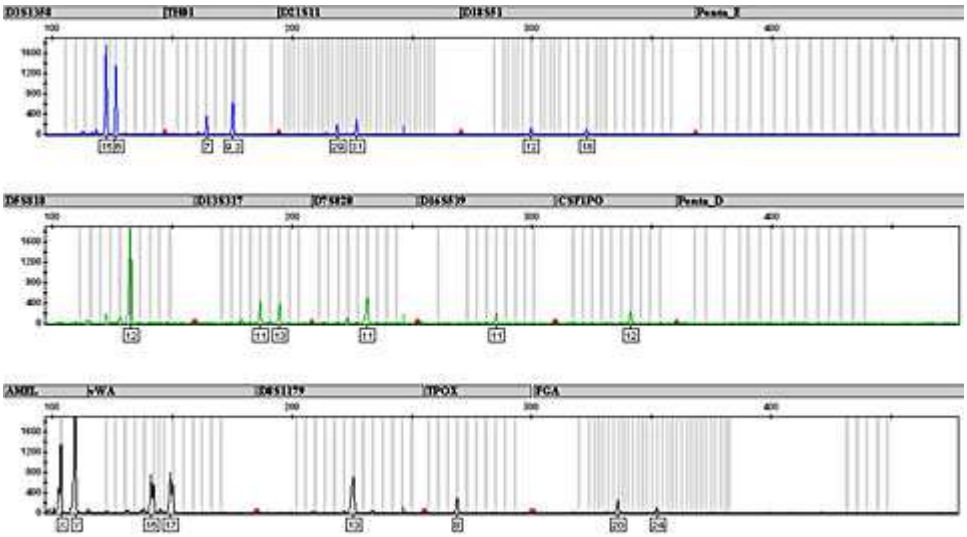


Photos showing the areas from which DNA is collected. The yellow boxes indicate where cuttings were taken. (A) Tee is swabbed inside collar and seam across top of shoulders. Shirt was flipped inside out for picture only, not for collection. (B) Sweat band of hat was swabbed. (C D) Cotton gloves were swabbed completely around blue portion of wristband. (E F) Leather gloves were swabbed completely around the wristband where shaded.

Comparing DNA Profiles

Once the DNA is collected, extraction is performed to release the cells from the swab and isolate the DNA from those cells. Many labs, such as the TPD Lab, have automated the DNA IQ extraction process, as previously described as silica-based extraction. This not only reduces the time necessary for extraction, but also decreases the risk of contamination. Following extraction, the samples are amplified using the PowerPlex Fusion system (Promega Corp, Madison, WI). In this PCR-based assay, the DNA is amplified at 24 loci simultaneously. These loci include 13 CODIS loci, 12 European standard loci, and Amelogenin.[27] The complete list of loci is listed in Appendix H. Once PCR is complete, the amplified product is subjected to electrophoresis on a 3130xl genetic analyzer (Applied Biosystems Inc, Foster City, CA) in order to separate the fragments by size. Applied Biosystems produces capillary electrophoresis instruments, 3130 and 3130xl Genetic Analyzers. In capillary electrophoresis, the PCR products are separated by size in a capillary tube filled with dimethyl polyacrylamide which serves as the sieving agent. As electrophoresis is run, smaller fragments travel through the matrix more quickly than larger fragments, resulting in the fragments being sorted by size. Toward the end of the capillary, a laser excites a fluorescent dye attached to the primer and a detector records the results. A DNA standard is run through the matrix simultaneously in order to accurately estimate the size of the fragment. To interpret the results, the instrument is programmed to recognize a specific locus based on the time in which the fragment moves through the matrix and the color of fluorescence recorded. The level of fluorescence is used to determine the alleles present in that locus.[28] This information is used to produce a profile, such as the one in Figure 11.

Figure 11- DNA Profile Example [29]



Producing a profile simplifies the information used to interpret results.

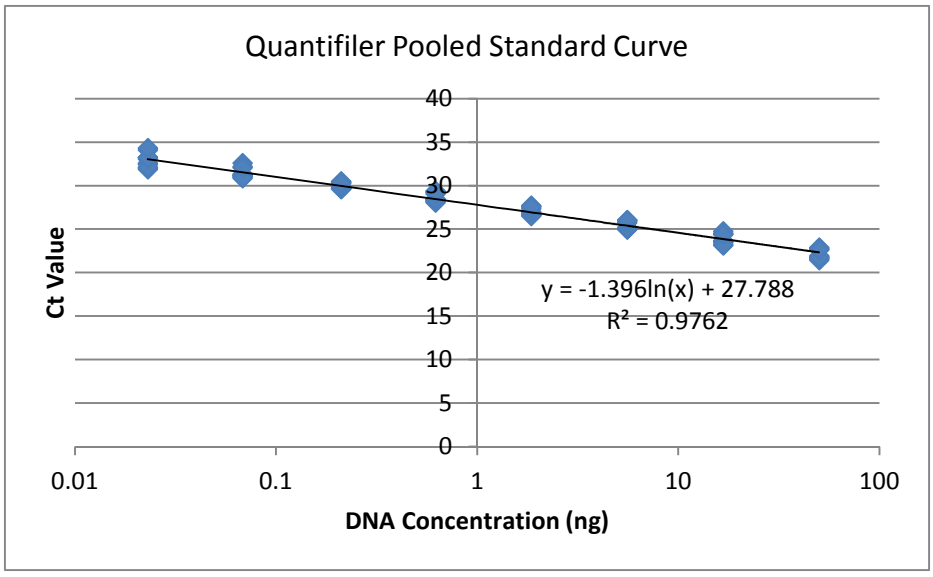
CHAPTER IV

FINDINGS

Standard Curve

The standard curve used for this study involved combining 10 individual, complete curves into an average curve, as described in Table 1. The resultant CT values were plotted on the y-axis with the known concentrations plotted on the x-axis of the graph. A linear best-fit line was plotted and an equation generated. This equation was used throughout the study to convert the CT values to nanograms of DNA. The graph and equation are shown in Figure 12.

Figure 12- Standard Curve



A standard curve was compiled to which CT values of unknown samples were compared. Nanograms are shown on the x-axis with CT values on the y-axis.

Preliminary Results

Upon completion of the first portion of the study, preliminary results were evaluated to assist in determining how to move forward with the second portion of the study. In the real-world application, a decision needed to be made as to which buffer to use with each swab. The preliminary results were evaluated by recording the number of samples for each method/buffer combination that gave a positive result. These results are shown in Table 2. Based on these numbers, TE^{-4} was used to moisten the cotton swabs and lysis/DTT was used for the Dacron and glass fiber swabs. The overall percentages were used to make this decision, as well as the spread of the numbers. In the case of the Dacron swab, the percentage of results being positive were about equal so lysis/DTT was chosen based on the recovery across the substrates. In looking at the glass swab, TE^{-4} had a higher percentage, but the majority of these positives came from spandex. Lysis/DTT resulted in a better overall spread of recovery. While the percentages are listed in the table for consistency, no determinations were made from the preliminary results with regards to cutting or taping methods. Taping with 10 lifts does appear to yield higher results, however, in the application of this method, analysts will not limit their collection to 10 tape lifts, rather they will collect from the entire area.

Table 2- Percentages of Results Giving Positive Value

	Cutting	Cotton-TE	Cotton-Lysis	Dacron-TE	Dacron-Lysis	Glass-TE	Glass-Lysis	Taping (1)	Taping (10)	Taping (25)	Total
Cotton	15	1	1	0	0	1	1	1	0	2	22 (16%)
Denim	15	1	0	0	3	0	3	2	5	2	31 (23%)
Poly	11	3	0	1	3	0	4	0	1	0	23 (17%)
Silk	15	2	0	0	0	0	2	0	0	0	19 (14%)
Spandex	15	11	12	3	6	5	9	2	9	6	78 (58%)
Wool	11	15	13	15	7	0	12	2	11	9	95 (70%)
Total	82 (91%)	33 (37%)	26 (29%)	19 (21%)	19 (21%)	6 (10%)	31 (34%)	7 (8%)	26 (29%)	19 (21%)	268

Preliminary results were calculated by recording the number of positive results within each method and substrate type.

The complete table of raw data is available in Appendix I. To simplify the results, each swabbing method will be referred based on the type of swab and buffer used to wet the swab (i.e. cotton-TE, glass-lysis). When describing the tape lifting methods, taping1, taping10, or taping25 will be used based upon the number of lifts from the substrate. A two-way ANOVA was performed on the data, which allows for a comparison of the means using two different factors. In this case, this test is comparing the method of collection and the substrate type. If the means showed a significant difference with a p-value of ≤ 0.05, a post hoc comparison was run to test hypotheses based on the sample data. In each of the comparisons, the null hypothesis is that there is no significant different between the methods or substrates tested, the alternative hypothesis being that there is a significant difference.

For the remaining of the document, methods will be referred to using abbreviations. Swabbing methods are classified by the swab type and the buffer used (i.e. Dacron-Lysis is a Dacron swab moistened with Lysis/DTT). Taping is classified by the number of lifts from the same area (i.e. Taping10 is 10 lifts from a single area).

Method of Collection Results

In looking at the results for the cotton substrate, there was a significant difference among the method of collection used. Using the post hoc comparison, cutting extraction produced significantly higher results than the remaining methods. This would probably be expected inasmuch as forensic analysts have been using cuttings as a source of DNA for years. The goal currently is to simplify and streamline recovery methods that are less laborious. Among the rest of the methods, there was no significant difference.

Table 3- Statistical Comparison of Methods on Cotton Substrate

Fabric	Method	Mean Recovery		SE Recovery
Cotton	Cutting	1.82467	a	0.18840
Cotton	Cotton-TE	0.01000	c	0.01000
Cotton	Cotton-lysis	0.00733	c	0.00733
Cotton	Dacron-TE	0.00000	c	0.00000
Cotton	Dacron-lysis	0.00000	c	0.00000
Cotton	Glass-TE	0.02700	bc	0.02700
Cotton	Glass-lysis	0.00133	c	0.00133
Cotton	Taping1	0.01667	c	0.01667
Cotton	Taping10	0.00000	c	0.00000
Cotton	Taping25	0.01667	c	0.01162

Shown is the mean recovery for each collection method on cotton fabric. $P<0.0001$
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at $P=0.05$.

Cuttings of denim showed a significant difference at a $p<0.0001$. As seen in cotton, cutting extraction yielded significantly more DNA in comparison to other extractions. Taping1 and taping10 are grouped with the remaining methods resulting in significantly less DNA than cutting and taping1 or taping25.

Table 4- Statistical Comparison of Methods on Denim Substrate

Fabric	Method	Mean Recovery		SE Recovery
Denim	Cutting	2.83600	a	0.14559
Denim	Cotton-TE	0.00375	c	0.00375
Denim	Cotton-lysis	0.00000	c	0.00000
Denim	Dacron-TE	0.00000	c	0.00000
Denim	Dacron-lysis	0.05200	c	0.03046
Denim	Glass-TE	0.00000	c	0.00000
Denim	Glass-lysis	0.05067	c	0.03272
Denim	Taping1	0.90067	b	0.89001
Denim	Taping10	0.52933	bc	0.47501
Denim	Taping25	0.02133	c	0.01467

Shown is the mean recovery for each collection method on denim fabric. P<0.0001
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

Examining polyester as a substrate, there was no significant difference between collection methods using a p-value of 0.05. No significance was found until a p-value of 0.2640. Given this result, no post hoc comparison was conducted.

Table 5- Statistical Comparison of Methods on Polyester Substrate

Fabric	Method	Mean Recovery		SE Recovery
Poly	Cutting	0.26800	a	0.06967
Poly	Cotton-TE	0.06000	a	0.03225
Poly	Cotton-lysis	0.00000	a	0.00000
Poly	Dacron-TE	0.00400	a	0.00400
Poly	Dacron-lysis	0.12867	a	0.07778
Poly	Glass-TE	0.00000	a	0.00000
Poly	Glass-lysis	0.07467	a	0.03897
Poly	Taping1	0.00000	a	0.00000
Poly	Taping10	0.02267	a	0.02267
Poly	Taping25	0.00000	a	0.00000

Shown is the mean recovery for each collection method on polyester fabric. P=0.2640
Post hoc comparison was not performed.

It is interesting that even cuttings of polyester were not different than the other collection methods. Perhaps something in the chemical structure of polyester fiber traps or binds DNA in a

way not easily liberated. As seen in cotton, silk showed a significant difference in the amount of DNA extracted via the cutting method. Among the other methods, there was no significant difference.

Table 6- Statistical Comparison of Methods on Silk Substrate

Fabric	Method	Mean Recovery		SE Recovery
Silk	Cutting	2.46533	a	0.23652
Silk	Cotton-TE	0.02000	b	0.01447
Silk	Cotton-lysis	0.00000	b	0.00000
Silk	Dacron-TE	0.00000	b	0.00000
Silk	Dacron-lysis	0.00000	b	0.00000
Silk	Glass-TE	0.00000	b	0.00000
Silk	Glass-lysis	0.03333	b	0.02435
Silk	Taping1	0.00000	b	0.00000
Silk	Taping10	0.00000	b	0.00000
Silk	Taping25	0.00000	b	0.00000

Shown is the mean recovery for each collection method on silk fabric. $P<0.0001$
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at $P=0.05$.

With respect to spandex, collection methods showed a significant difference at $p<0.0001$. Cutting extraction recovered more DNA than the remaining methods. Cotton-lysis, cotton-TE, dacron-TE, glass-lysis, glass-TE, and taping10 recovered more DNA than taping1 or taping25.

Table 7- Statistical Comparison of Methods on Spandex Substrate

Fabric	Method	Mean Recovery		SE Recovery
Spandex	Cutting	3.25267	a	0.06903
Spandex	Cotton-TE	0.83800	bc	0.26825
Spandex	Cotton-lysis	0.52000	bcd	0.10739
Spandex	Dacron-TE	0.17400	bcd	0.13681
Spandex	Dacron-lysis	0.09800	cd	0.09730
Spandex	Glass-TE	0.10800	bcd	0.00000
Spandex	Glass-lysis	0.54800	bcd	0.40253
Spandex	Taping1	0.01933	d	0.04097
Spandex	Taping10	0.91333	b	0.35085
Spandex	Taping25	0.10600	cd	0.05776

Shown is the mean recovery for each collection method on spandex fabric. P<0.0001
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

When using wool as a substrate, a significant difference was found among the collection methods. Cotton-TE and glass-lysis recovered significantly more DNA than the rest of the methods, with glass-lysis and taping10 showing no difference. Cotton-lysis, glass-TE, and taping1 recovered significantly less DNA than any other methods.

Table 8- Statistical Comparison of Methods on Wool Substrate

Fabric	Method	Mean Recovery		SE Recovery
Wool	Cutting	0.30000	de	0.06903
Wool	Cotton-TE	2.29667	a	0.26825
Wool	Cotton-lysis	0.40133	de	0.10739
Wool	Dacron-TE	0.79467	cde	0.13681
Wool	Dacron-lysis	0.21133	de	0.09730
Wool	Glass-TE	0.00000	e	0.00000
Wool	Glass-lysis	1.90200	ab	0.40253
Wool	Taping1	0.04933	e	0.04097
Wool	Taping10	1.32933	bc	0.35085
Wool	Taping25	0.18200	de	0.05776

Shown is the mean recovery for each collection method on wool fabric. P<0.0001
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

Substrate Type Results

When examining the cutting method, spandex and denim gave a significantly higher yield of DNA. Polyester and wool had the least recovery of DNA using this method.

Table 9- Statistical Comparison of Substrates using Cutting Method

Method	Fabric	Mean Recovery		SE Recovery
Cutting	Cotton	1.82467	c	0.18840
Cutting	Denim	2.83600	ab	0.14559
Cutting	Poly	0.26800	d	0.06967
Cutting	Silk	2.46533	bc	0.23652
Cutting	Spandex	3.25267	ab	0.42072
Cutting	Wool	0.30000	d	0.06903

Shown is the mean recovery for each fabric using the cutting method. $P<0.0001$
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at $P=0.05$.

Using cotton-TE, wool provided significantly greater extraction than other substrates. Cotton, denim, polyester, and silk yielded significantly less DNA than spandex and wool. Cotton-lysis did not provide significantly different extraction between fabrics.

Table 10- Statistical Comparison of Substrates using Cotton Swabbing Method

Method	Fabric	Mean Recovery		SE Recovery
Cotton-TE	Cotton	0.01000	c	0.01000
Cotton-TE	Denim	0.00375	c	0.00375
Cotton-TE	Poly	0.06000	bc	0.03225
Cotton-TE	Silk	0.02000	c	0.01447
Cotton-TE	Spandex	0.83800	bc	0.19597
Cotton-TE	Wool	2.29667	a	0.26825

Method	Fabric	Mean Recovery		SE Recovery
Cotton-lysis	Cotton	0.00733	a	0.00733
Cotton-lysis	Denim	0.00000	a	0.00000
Cotton-lysis	Poly	0.00000	a	0.00000
Cotton-lysis	Silk	0.00000	a	0.00000
Cotton-lysis	Spandex	0.52000	a	0.12844
Cotton-lysis	Wool	0.40133	a	0.10739

Shown is the mean recovery for each fabric using the cotton swabbing method.
P<0.0001 for cotton-TE. P=0.6061 for cotton-lysis.
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

Dacron-TE and dacron-lysis did not result in a significant difference among substrates. Because of this reason, post hoc comparisons were not performed.

Table 11- Statistical Comparison of Substrates using Dacron Swabbing Method

Method	Fabric	Mean Recovery		SE Recovery
Dacron-TE	Cotton	0.00000	a	0.00000
Dacron-TE	Denim	0.00000	a	0.00000
Dacron-TE	Poly	0.00400	a	0.00400
Dacron-TE	Silk	0.00000	a	0.00000
Dacron-TE	Spandex	0.17400	a	0.12083
Dacron-TE	Wool	0.79467	a	0.13681

Method	Fabric	Mean Recovery		SE Recovery
Dacron-lysis	Cotton	0.00000	a	0.00000
Dacron-lysis	Denim	0.05200	a	0.03046
Dacron-lysis	Poly	0.12867	a	0.07778
Dacron-lysis	Silk	0.00000	a	0.00000
Dacron-lysis	Spandex	0.09800	a	0.03518
Dacron-lysis	Wool	0.21133	a	0.09730

Shown is the mean recovery for each fabric using the dacron swabbing method.
P=0.2781 for dacron-TE. P=0.9947 for dacron-lysis.
Post hoc comparisons were not performed.

Glass-TE did not provide a significant difference between fabrics. In glass-lysis extraction, wool allowed for greater DNA to be collected in comparison to other substrates. Post hoc comparison was run on glass-lysis only.

Table 12- Statistical Comparison of Substrates using Glass Fiber Swabbing Method

Method	Fabric	Mean Recovery		SE Recovery
Glass-TE	Cotton	0.02700	a	0.02700
Glass-TE	Denim	0.00000	a	0.00000
Glass-TE	Poly	0.00000	a	0.00000
Glass-TE	Silk	0.00000	a	0.00000
Glass-TE	Spandex	0.10800	a	0.03915
Glass-TE	Wool	0.00000	a	0.00000

Method	Fabric	Mean Recovery		SE Recovery
Glass-lysis	Cotton	0.00133	b	0.00133
Glass-lysis	Denim	0.05067	b	0.03272
Glass-lysis	Poly	0.07467	b	0.03897
Glass-lysis	Silk	0.03333	b	0.02435
Glass-lysis	Spandex	0.54800	b	0.18742
Glass-lysis	Wool	1.90200	a	0.40253

Shown is the mean recovery for each fabric using the glass fiber swabbing method.
P=0.9999 for glass-TE. P<0.0001 for glass-lysis.
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

In collection by tape lift, no significant difference was noted using 1 lift or 25 lifts. There was a significant difference when lifting 10 times. In this method, spandex and wool resulted in greater recovery of DNA. Significantly less DNA was recovered from cotton, denim, polyester, and silk.

Table 13- Statistical Comparison of Substrates using Taping Method

Method	Fabric	Mean Recovery		SE Recovery
Taping1	Cotton	0.01667	a	0.01667
Taping1	Denim	0.90067	a	0.89001
Taping1	Poly	0.00000	a	0.00000
Taping1	Silk	0.00000	a	0.00000
Taping1	Spandex	0.01933	a	0.01325
Taping1	Wool	0.04933	a	0.04097

Method	Fabric	Mean Recovery		SE Recovery
Taping10	Cotton	0.00000	c	0.00000
Taping10	Denim	0.52933	bc	0.47501
Taping10	Poly	0.02267	c	0.02267
Taping10	Silk	0.00000	c	0.00000
Taping10	Spandex	0.91333	ab	0.48146
Taping10	Wool	1.32933	a	0.35085

Method	Fabric	Mean Recovery		SE Recovery
Taping25	Cotton	0.01667	a	0.01162
Taping25	Denim	0.02133	a	0.01467
Taping25	Poly	0.00000	a	0.00000
Taping25	Silk	0.00000	a	0.00000
Taping25	Spandex	0.10600	a	0.04210
Taping25	Wool	0.18200	a	0.05776

Shown is the mean recovery for each fabric using the taping method.
P=0.1496 for taping1. P=0.0017 for taping10. P=0.9967 for taping25.
The letters correspond to grouping based upon post hoc comparisons. Those means with the same letter are not statistically different at P=0.05.

Real-World Study Results

In the real-world portion of the study, the small sample size does not allow for ANOVA testing, such as that used in the laboratory section. Due to this limitation, these values were compared using means only, as shown in Table 14. Multiple samples were taken from each item for the cutting method. As for the swabbing and taping methods, one combined sample was taken from each article. The first trend noted involves the negative controls. Even with a thorough washing in warm water (without the use of detergents), several of these samples came back with positive

results. This presence of DNA can be attributed to the handling of these articles before their purchase from the store. This shows how well DNA can be trapped within the fibers of the fabrics and how important context is when interpreting these results.

The second noteworthy comparison is the quantity of DNA collected from the hat using the cutting method. Since the sweatband of the hat is so thick, three extractions were performed: (1) entire thickness, (2) fabric covering only, and (3) padding only. This was necessary because the first sample was packed so tightly into tube, the lysis-DTT used during incubation may not have been able to penetrate the entire sample; in addition, the DNA may have become trapped during centrifugation. As a result, more touch DNA was extracted from the padding than from either the fabric or the entire thickness.

In looking across the collection methods, one type does not show overall efficiency over the others. Cutting, dacron-lysis, and taping do appear to collect more touch DNA than does cotton-TE or glass-lysis, but which of these collected the most for any particular article varies.

Table 14- Results of Real-World Study

	Cutting	Cotton-TE	Dacron-lysis	Glass-lysis	Taping
Hat neg	0	0	0	0.05	0
Fabric	0				
Padding	0.14				
Hat pos	0.4	0.56	0.65	0.74	1.57
Fabric	0.34				
Padding	2.52				
	Cutting	Cotton-TE	Dacron-lysis	Glass-lysis	Taping
Tee neg	0.04	0.15	0	0.06	0
Tee pos		0.05	1.12	0.14	0.32
Back neck	0.4				
Shoulder	0.44				
V-neck	0.36				
	Cutting	Cotton-TE	Dacron-lysis	Glass-lysis	Taping
C. Glove neg	0.05	0.03	0	0	0.11
C. Glove pos	0	0.17	1.15	0.48	0.09
Palm	0				
	Cutting	Cotton-TE	Dacron-lysis	Glass-lysis	Taping
L. Glove neg	0.15	0	0.1	0	0
L. Glove pos	0.55	0.12	0.11	0	0.55
Palm	0.32				

Shown are the means of the real-world results. Multiple cuttings were taken from each article of clothing. A combined sample was taken for each swab or tape lift.

Application of Results

After looking at the results of this study, one must next ask what these numbers mean for their use in a crime lab. The ultimate goal of DNA typing is the generation of a profile that can be compared to a reference sample from a suspect or victim. The PowerPlex kit, manufactured by Promega, is a multi-locus STR typing kit often used in crime labs. According to the specifications of this kit, a full profile can be generated with as little as 100pg (or 0.1ng) of DNA.[27] Using this information, the majority of samples, both in the laboratory portion and the real-world portion, giving positive results should produce a partial, if not full, profile.

CHAPTER V

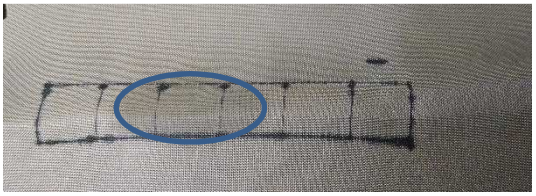
DISCUSSION

Advantages and Disadvantages of Each Method

Recovery of DNA extracted via each collection method is clearly the top priority, an examination of the processes themselves is also necessary to gain a complete picture of the study. The ease of use of the procedure is important when applying it for use in the crime lab. Each collection method will be discussed, including advantages and disadvantages that arose for this study in particular.

Before even beginning the extraction process for any of the methods, the methodology had to be altered. The original plan called for each sample square to measure $1cm^2$. This original measurement was based on the cutting extraction protocol. During the first setup of the study, the analyst observed that the 2µL of DNA spiked onto the fabric was wicking past the outline of the square drawn, as shown in Figure 13. Due to this problem, the amount of DNA truly within the square, and therefore subjected to extraction was not known. In order to combat this, all squares after this point measured $4cm^2$ to ensure a known starting amount of 10ng within each square.

Figure 13- Wicking of DNA



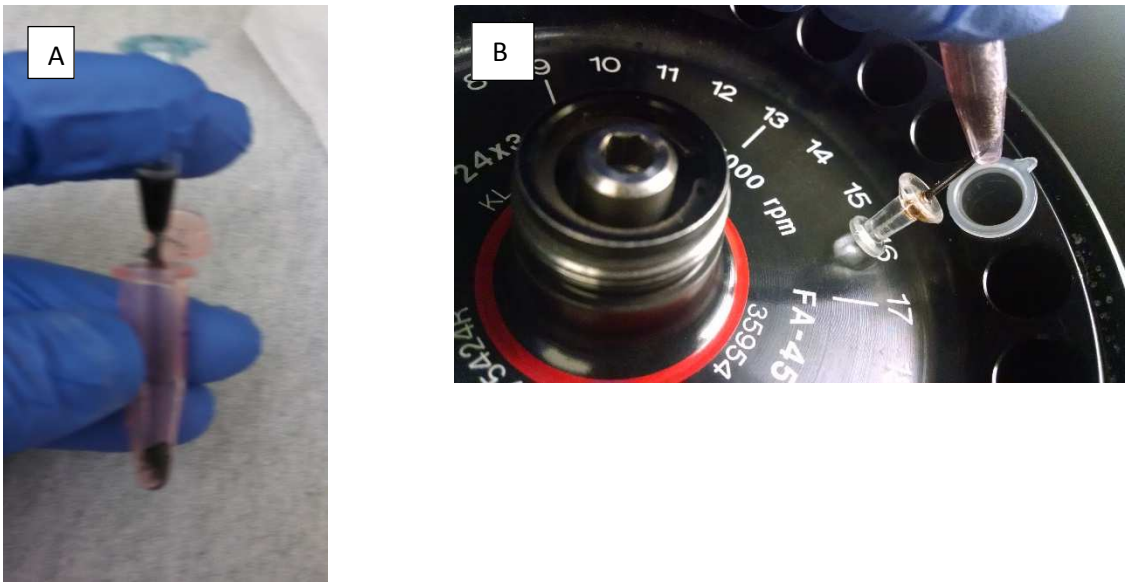
When 1cm^2 were used, the DNA spiked onto the fabric was observed wicking past the outline of the square, as shown by the blue outline. 4cm^2 squares were used to correct this problem.

Cutting Extraction

Examining the first portion of the study, the cutting method showed significantly greater collection when comparing the amount of DNA collected, as shown in Table 2 in the "Preliminary Results" section. In a controlled laboratory setting, cutting extraction is generally the most efficient method for recovering DNA. This does not, however, easily transfer to a real-world setting. Without a presumptive test to aid in locating DNA on the object, choosing an area to cut for extraction is purely a guessing game, and, with backlogs growing in crime labs, an easy, reliable recovery method is priority 1.

Another challenge was encountered when using the cutting method on wool. During the centrifugation step, the wool itself was pulled through the hole in the small tube. In order to combat this problem, the hole poked in the bottom of the tube was offset to allow the DNA through but keeping the wool in the tube. For this to work, the tube must be placed in the centrifuge with the hole toward the center. The problem and solution are depicted in Figure 14.

Figure 14- Cutting Extraction Problem with Wool



(A) Wool came through the hole in the bottom of the tube during centrifugation in cutting extraction. (B) Protocol was adjusted by moving the hole in the tube.

A difficulty that arose during the second portion of the study is the amount of material in the tube. In the laboratory portion, each fabric had one layer. When transferring this method into a real-world setting, the thickness of the cutting, sometimes 3 times thicker, made fitting the samples into the tube difficult. Since the protocol called for a larger tube into which the DNA would wash, the size of the tube for the cutting could not be changed. The cutting being packed into the tube may result in a loss of DNA as the buffer may not entirely penetrate the sample during incubation. The DNA may also become trapped within the folds of the fabric during centrifugation and be unable to wash into the larger tube.

Cotton and Dacron Swab Extraction

Of all the methods examined in this study, the cotton and Dacron swabs were simplest to use in collection and extraction. The swabs were prepackaged and sterile, greatly reducing the risk of contamination of the swabs. The extraction protocol allowed for simple isolation and purification

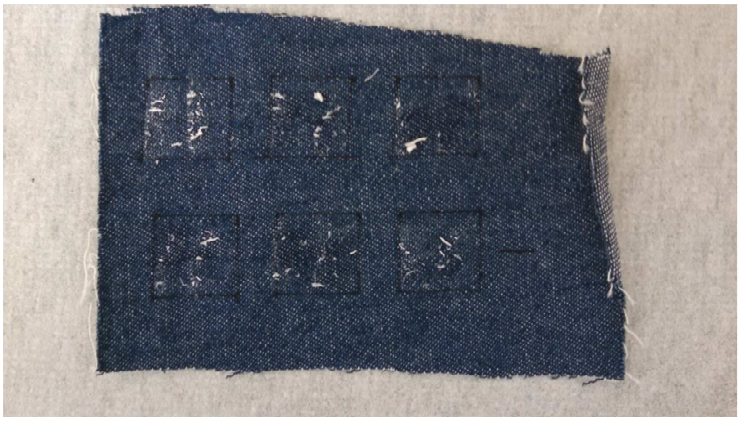
of the DNA. The cotton and Dacron extraction methods did not account for any specific problems within this study.

Glass Fiber Swab Extraction

As the glass fiber swab is still in its production stage, a few issues are unique to this swab type that need to be addressed. In creating the swabs themselves, consistency may pose a problem. The amount of glass fiber paper, and therefore the thickness of the swab, is equal across all swabs, but the amount of superglue used to secure the ends is not regulated at this stage. This can become a problem during swabbing and extraction because the buffer used to wet the swab is not absorbed where the glue is present. Another possible complication with the glass swab is the fact the superglue is composed of the same acrylate compound used for fuming fingerprints, which is known to compromise DNA recovery. Future studies need to find an alternative to superglue to secure the swab head.

During the swabbing step, another problem arose regarding the glass fiber swab. As the area of fabric was swabbed, the glass fiber paper was breaking apart on the rough surface of the fabric, as seen in Figure 15. This phenomenon was most noticeable when swabbing the denim, however the problem was present across all fabric types to some degree. This causes a major loss in the recoverable touch DNA. In order to combat this loss, the swab was used to pick up as many of the pieces as possible before continuing with extraction. This solution may still result in a loss of DNA if the pieces are small enough to be washed away during centrifugation.

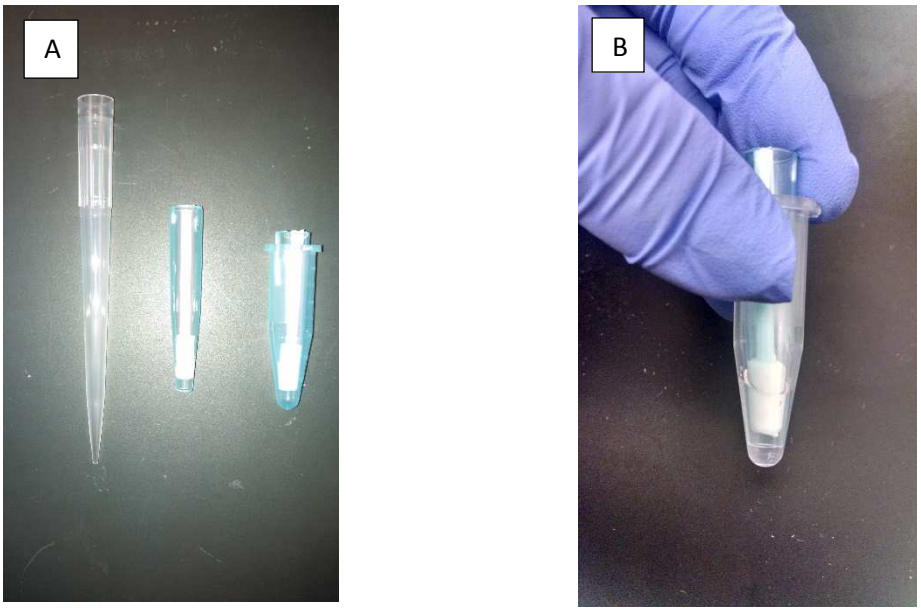
Figure 15- Breaking Apart of Glass Fiber Swab



The glass fiber swab was observed breaking apart as the surface of the fabric was swabbed.

The original protocol for glass fiber swab extraction called for an apparatus using a cut 1000µL pipette tip to house the swab during extraction. This apparatus did not provide optimal washing away of contaminants. Post centrifugation, a small volume of the previous solution wound up staying in the end of the pipette tip. The original apparatus and the problem is depicted in Figure 16. As a solution to this problem, the swab was placed in a 0.65mL tube with a hole punched in the bottom, rather than the cut pipette tip, then placed in the 1.5mL tube. This substitution allowed for the solution to completely drain away from the swab during centrifugation.

Figure 16- Glass Fiber Swab Apparatus and Centrifuging Problem



(A) The original apparatus for the glass fiber swab is shown. (B) The solution was not pulled away from the swab during centrifuging.

Using this modified protocol, the efficiency in time greatly increased for glass fiber swab extraction. Since the swab acted as the silica beads did in other extractions, pipetting the wash buffer containing the contaminants was not necessary. Centrifuging the samples to draw out the contaminants is much more efficient than pipetting the liquid and allows for better separation of the DNA, trapped on the swab or beads, and the wash buffer containing contaminants.

Taping Extraction

When examining the taping method, not as many problems came up as with other methods, however the method was not flawless. Because each tape lift is not separately packaged before use, contamination is going to need to be addressed. Contamination is greatly reduced by discarding the exposed portion, as previously shown in Figure 9.

During the extraction process, the samples are incubated and the solution, containing the DNA, is pipetted out of the tube with the tape and into a fresh tube. Another loss of DNA may be

accounted for in this transfer step. Because the DNA is pipetted rather than centrifuged into a new tube, solution containing DNA will always remain in the first tube. The samples cannot be centrifuged as in other methods due to the tubes used. Placing the tape in a smaller tube, allowing for the larger tube to be used as a collection tube, results in the tape becoming bunched and sticking to itself. This results in less contact area for the buffer and can result in a loss of DNA during this step.

Fabric Substrates

In order to fully understand how effectively fabric traps touch DNA and other materials, one must also look at the composition of the types of fabric in question, as well as their general properties. The fabrics tested in the Laboratory portion of this study included cotton, denim, polyester, silk, spandex, and wool. For ease of understanding I will discuss these fabrics as grouped into natural fibers and manufactured fibers.

Natural Fibers

Cotton and Denim

As denim is typically made from cotton fibers, these processes will be discussed together. Cotton comes from the cotton plant, grown mainly in the southern United States. During harvesting, the seed cotton is removed from the boll of the plant and transported to the gin. At the gin, the lint and the seed are separated and the lint is baled. These bales are classified based on the strength, length, and color of the fibers.[30]

In the production of the yarn, there are 4 major steps: blending, cleaning and carding, drawing, and spinning. In the first step, the lint from several bales are blended together to create a uniform product. From here, the lint is sent through cleaning machines and carding machines. The cleaning process removes most of the dirt and contaminants from the material. In the carding

machine, the fibers pass through metal teeth in order to remove any remaining dirt and to align the fibers into a parallel orientation. The fibers are then drawn through a funnel, known as a trumpet, to produce a single strand. These strands are spun together to produce the thread used to make fabric. Different types of spinning machines determine how tightly the strands are wound and ultimately the strength and thickness of the thread. This leads to the difference in texture and durability between cotton and denim fabric.[30]

Silk

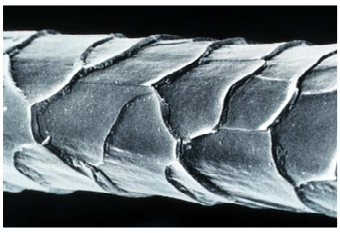
Silk is often used in clothing and bedding, among other uses. It is made from the cocoons of silkworms bred in captivity. The silkworms are raised on a mulberry plant and, after about a month, the worms encase themselves in a cocoon. The cocoons are collected and boiled to release the worm from inside and cooled to loosen the fibers so the thread can be unwound. Since a single strand of silk is too thin to be used, several strands are combined to make thread. The thread can be dyed; the unique triangular shape of the thread causes a change in color as the fabric moves. The thread is then used to weave fabric on a bamboo loom. Different types of silk can be produced based on the weaving style used.[31]

Wool

Wool is the number 1 animal fiber used in the United States. The fiber commonly comes from sheep, but can also be from goats, camels, or rabbits. As an animal hair, the structure of wool plays an important part in using it to make yarn. There are 3 layers to a hair: the outer cuticle, the cortex, and the inner medulla. The protective cuticle layer, as shown in Figure 17 consists of scale-like cells that, in wool, catch on adjacent hairs causing them to stick together. This property makes the production of yarn much easier. The arrangement of the cells of the cortex gives the natural crimp of the wool. Air spaces within the medulla provide the insulative property of wool. The production of wool fabric involves 7 major steps: (1) shearing, (2) grading and sorting, (3)

cleaning and scouring, (4) carding, (5) spinning, (6) weaving, and (7) finishing. The animal is sheared (shaved) to collect the wool before grading, in which the fleece is separated based on quality. In the cleaning step, alkaline baths are used to remove dirt and contaminants. The fleece is run through rollers to remove excess water and an oil treatment increases the material's manageability. Carding involves passing the fibers through metal teeth in order to straighten and blend the fibers. This process also removes any dirt remaining after the cleaning and scouring step. The fibers are then spun together to create yarn. In the weaving step, there are 2 general types of weaves: plain weave and twill. Twill is created using a tighter and produces a more durable fabric with a smoother surface. The finishing process is made up of 3 steps. In the fulling step, the fabric is immersed in water to interlock the fibers. The crabbing step permanently sets this interlocking. The decating step prevents the fabric from shrinking.[32]

Figure 17- Cuticle Layer of Wool[33]



The scale-like cells of the cuticle cause the wool fibers to stick together.

Manufactured Fibers

Polyester

Polyester is a manmade fiber created by linking esters within the monomers. Ethylene is used as the main monomer in this product. There are multiple types of polyester, based on the manufacturing process. The 2 main types of for polyester fabric are filament fiber and staple fiber. Filament fibers are longer fibers that produce a smooth fabric. There are 3 main steps

followed in this process: (1) polymerization, (2) melt spinning, and (3) drawing. In the polymerization step, ethylene glycol and an acid are added in a vacuum at high heat. The product is cast as a ribbon, which hardens and is broken into chips to improve consistency. In the second step, these chips are dried and heated before being forced through spinnerets. These fibers cool when they hit the air. Chemicals may be added at this point in order to increase the flame retardant or antistatic properties. In the final step of the manufacturing of polyester thread, the fibers are stretched to about 5 times its original length. The fibers are made thinner and stronger as this process forces the fibers to align in a parallel orientation. The thread produced is used to make fabrics seen in common products.[34]

Staple fibers are shorter fibers that are more easily blended with other fibers, such as cotton or rayon. The process to make this type is similar to that to make filament fibers, with a few differences. The (1) polymerization and (2) melt spinning steps are equivalent except for an increase in the number of spinnerets through which the melted chips are forced. In the (3) drawing step, the fibers are only stretched to 3 or 4 times its original weight. Additional steps include (4) crimping, (5) setting, and (6) cutting. When crimping, the fibers are folded like an accordion with an average of 9-15 crimps per inch. This process helps to hold the fibers together after manufacturing. In the setting step, the fibers are heated to dry and set the crimp. The fibers are then cut into shorter lengths depending on the material with which they will be blended.[34]

Spandex

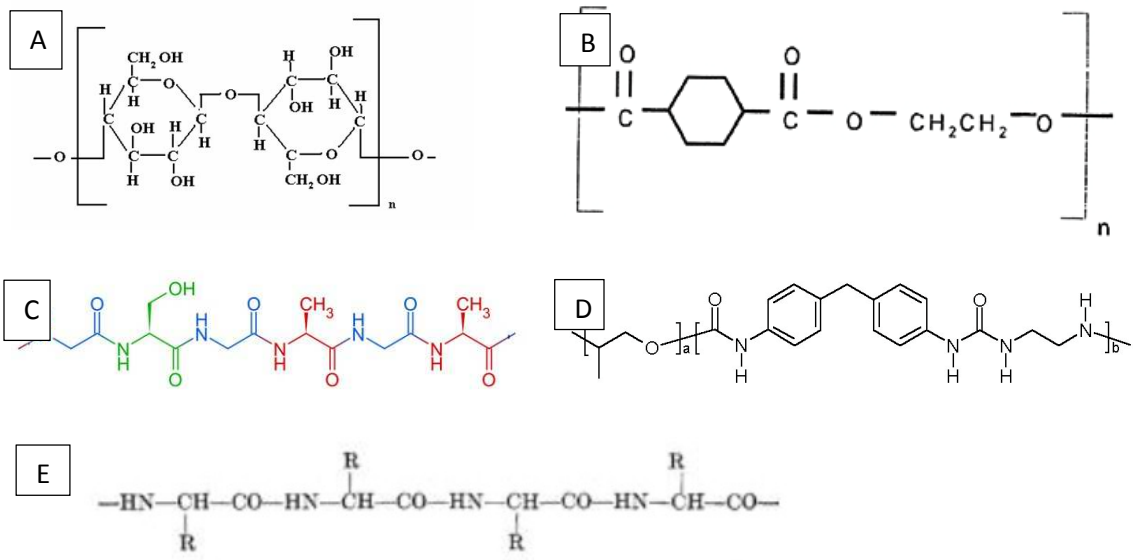
Spandex is another type of manmade fiber. 2 prepolymers are mixed in order to form spandex fibers: a flexible macroglycol and a stiff diisocyanate. The macroglycol is a long chain polymer with an alcohol (-OH) group on each end and the diisocyanate is a short polymer with an isocyanate (-NCO) group on each end. Spandex is produced in 4 different ways: melt extrusion, reaction spinning, solution dry spinning, or solution wet spinning. As over 90% of the world's

spandex is produced by solution dry spinning, this process will be explained. Solution dry spinning involves 5 steps: (1) prepolymer reaction, (2) chain extension reaction, (3) drawing, (4) twisting, and (5) finishing. During the prepolymer reaction, a 1:2 mixture of glycol:diisocyanate is created with the help of a catalyst. In the second step, this product is mixed with an equal amount of diamine and diluted to produce a solution. The solution is then drawn through a spinneret to form strands. These strands are solidified by being heated with nitrogen and solvent gas. In the twisting step, the fibers are twisted together to form the desired thickness. The natural stickiness of the fibers cause them to adhere. In the final step, the threads are coated in a finishing agent, such as magnesium stearate, to keep the threads from sticking to one another.[35]

Chemical Attraction of DNA

The chemical structure of the fibers also affects the amount of recoverable DNA. DNA has a greater affinity to some fabrics in comparison to others. A look at these structures, shown in Figure 18, gives an idea of the affinity. In structures with multiple hydroxyl (-OH) or amine (-NH) groups, tend to form strong hydrogen bonds with the backbone of DNA. This may result in less DNA recovered by swabs or tape lifts from fabrics such as cotton, denim, silk, and spandex. In contrast, structures containing carbonyl (-C=O) groups form dipole-dipole bonds, which are weaker than hydrogen bonds and allow for greater recovery of DNA. This can be seen in polyester and wool.[36] Polyester did not, however, show great recovery of DNA as the structure would suggest. Perhaps this occurrence is the result of chemical treatment to the fabric in order to increase longevity.

Figure 18- Chemical Structures of Fibers



Shown are the chemical structures of (A) cotton and denim[37], (B) polyester[38], (C) silk[39], (D) spandex[40], and (E) wool[41].

Negative Controls

Negative controls are run with each point to better pinpoint any contamination that may occur during the process. Controls monitoring the extraction process are run by swabbing an area of the fabric deliberately not spiked with DNA and performing the extraction process on this swab alongside the rest of the samples. A reagent negative control is run during amplification by loading the well with the reagents necessary for PCR and adding TE^{-4} buffer in place of DNA into the well. All reagent negatives run for this study came back negative, as expected. Several extraction negatives, however, came back with positive results. In the laboratory portion, 7.95% of negative controls gave a positive result and 20.6% of the real-world negative controls came back positive. In the entire study, only 7 controls gave a positive result in more than one of the replicates of the same sample. These controls are considered as true positives and may be attributed back to the fabric substrates. Because the fabrics came from a fabric store with

countless people handling them, DNA from these people is expected on the substrates. In the case of the clothing used in the real-world portion, the articles were, again, accessible to many individuals at the store and because they were hand washed and unable to be subjected to the UV light, DNA may have still been trapped within the fibers of the fabric.

Limitations

Several factors limit the scope of this study. Due to the number of influences on the various portions of a scenario, as with any real-world application, not everything can be accounted for in a case. Factors influencing the scope can be characterized as related to the individual, the clothing, or the procedure. Each of these factors alone can create variability and when brought together, the number of combinations is overwhelming.

Relating to Individual

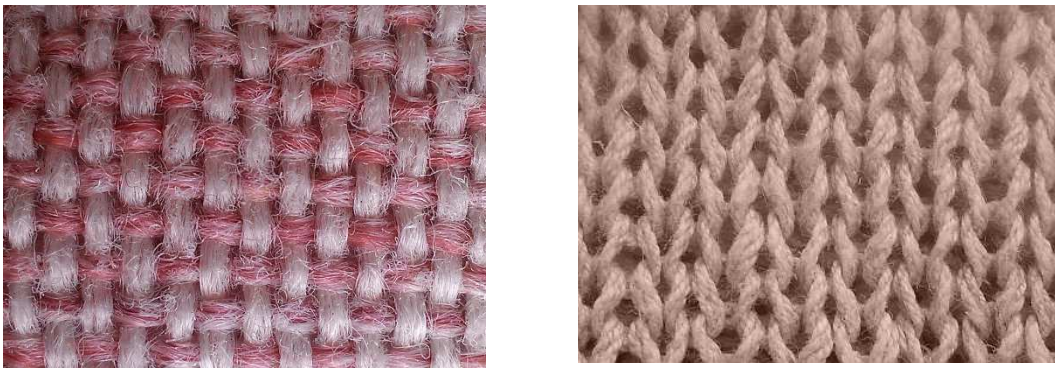
Much variation exists because of the individuals involved. Anything from age and gender to build and health can change how much a person's skin cells shed. Those individuals who suffer from skin diseases or general dry skin tend to slough off more cells than those with apparently healthy skin. Additionally, various individuals perspire more than others, increasing the amount of DNA available on the fabric. A person's habits may increase the amount of DNA transferred. If the person often touches their face, eyes, or mouth, more DNA is present on their hands to be transferred upon contact with an object. Individuals constantly washing their hands are likely to wash away skin cells and less likely to transfer DNA to an object such as fabric. Several studies, such as that conducted by Lowe et al, have made a differentiation between "good shedders" and "poor shedders" based upon the ability to obtain a full or partial profile from objects touched by the individuals.[17]

Relating to Clothing

The clothing to be analyzed also limits the ability to extract touch DNA. While several fabrics were examined within this study, much of the clothing on the market do not consist of 100% composition of a single fabric. The mixtures are used to create clothing that is more durable or softer also create variety that can be difficult to use in a controlled study. For example, clothing advertised as spandex often actually contains less than 10% spandex. The major contributor to the fabric is typically cotton or nylon. Additionally, loose-fitting clothing rather than tight is believed to have less contact with the skin and, therefore, has less opportunity to gather skin cells.

Also believed to be a factor is the pattern in which the threads are joined. Two common methods in creating fabric are weaving and knitting. These patterns, displayed in Figure 19, result in different sized spaces. How tightly the threads are woven or knitted may determine how easily DNA is caught and released from the fabric.

Figure 19-Woven and Knitted Fabric



Threads can be combined into fabrics by many methods such as (A) weaving[42] or (B) knitting[43].

When a piece of clothing is submitted as evidence, one can guess the article has been washed at least once. As a result, residue of the laundry detergent or softener used may be present on the

clothing. While the effects of these residues on touch DNA is beyond the scope of this study, research has been conducted regarding the use of commercially available laundry detergents during the extraction and isolation process of DNA.[44,45]

Variability is added to garments during the manufacturing process by treating and/or dyeing the fabrics. Much of the clothing on the market today has been dyed in some way to increase the aesthetic appeal. These dyes may interfere with the recovery of DNA, particularly during the PCR process. In addition, some fabrics require chemical treatment during the production of the threads or fabrics. For example, when a silkworm produces a cocoon, a substance known as sericin coats the fibers in order to hold the fibers together. In the production of silk thread, this substance must be removed by degumming using different chemicals such as a soap-soda ash combination or citric acid.[46] Further research opportunities may include the exploration of the effects of these dyes and chemicals on the recovery of DNA.

Relating to Procedure

Believed to be the most limiting factor in this study involves procedure and the lack of presumptive testing. When analyzing DNA from a piece of clothing stained with blood, a test such as a phenolphthalein assay can be used to locate the DNA.[47] For semen, acid phosphate can be used[48] and amylase can be analyzed for saliva.[49] Touch DNA, however, does not have a presumptive test with which to locate the sample. Common sense may suggest where touch DNA is likely to be present, such as the collar or under the arms, but it remains just that, an estimate. Sensitivity also plays a role in locating touch DNA as failed attempts to collect may destroy what little sample is available.

A small sample size is listed as a limitation of the real-world portion of this study. The purpose of the second portion was to give an idea of how the methodology could be applied to a crime lab. With only 4 clothing types and 1 subject for each type, these results are simply introductory.

Many other factors must be taken into consideration, such as those previously discussed in this section.

Conclusions

Touch DNA is an area in Forensics that needs much more research conducted in order to fully take advantage of its presence at crime scenes. In the comparison of collection methods, taping and cutting resulted in the best recovery of DNA. Across substrates, wool was, by far, the best surface from which to collect touch DNA; spandex was also noteworthy in recovery. Somewhat surprisingly, polyester and, to a lesser extent, wool were rather stingy in releasing their DNA using cutting as the recovery method. This study opens up future opportunities in research such as looking at other common fabrics and blends, the effects of laundry detergents on DNA collection, and a greater sample size in real-world sample.

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APPENDICES

APPENDIX A: MATERIALS AND INSTRUMENTS

Instruments

UV Stratalinker 1800 (Stratagene, San Diego, CA)

Centrifuge

Heat block (one at 65°C, one at 70°C)

ABI 7500 RealTime PCR system (

Kits

Quantifiler Human DNA Quantification Kit (Applied Biosystems Inc, Foster City, CA)

- Quantifiler PCR Reaction Mix
- Quantifiler Human Primer Mix
- Quantifiler Human DNA Standard (200ng/uL)

DNA IQ System Kit (Promega Corp, Madison, WI)

- Resin
- Lysis buffer
- 2X Wash buffer
- Elution buffer

Chemicals

TE^{-4} buffer (10mM Tris-Cl, pH 8.0, 0.1mM EDTA)

DTT

Collection Materials

Scotch tape

Dacron swabs (Puritan, Guilford, ME)

Cotton swabs (Puritan, Guilford, ME)

Whatman Glass Fiber Sheets (VWR Scientific, Radnor, PA)

Other Materials

Push pins

APPENDIX B: CUTTING EXTRACTION

Each mL lysis/DTT buffer contains:

- 900uL lysis
- 100uL DTT

1. Take a cutting (1cm x 1cm) containing the stain from the fabric.
2. Place the cutting in 1mL tube with 200uL lysis/DTT and vortex the sample.
3. Incubate on a heat block at 70°C for 15min.
4. Ensuring the tube is tightly closed, flick the sample away from the tip of the tube. Using a clean pushpin, punch hole in the tip of the tube containing the cutting. Place this tube in a 1.5mL tube and centrifuge for 3min at 6000xg.
5. Add 7uL silica-coated beads, vortex, and let the sample incubate at room temperature for 5min.
6. Vortex the sample and place on the magnetic stand. Allow the solution clear of beads.
7. Aspirate off lysis/DTT.
8. Wash the sample with 50uL lysis/DTT, place on magnetic stand, and aspirate.
9. Wash the sample with 50uL wash buffer, place on magnetic stand, and aspirate. Repeat step 9.
10. Allow the sample to air dry for 5min.
11. Add 50uL hot TE^{-4} buffer (65°C), vortex and incubate on a heat block at 65°C for 5min.
12. With the tube on the magnetic stand, transfer the TE^{-4} containing isolated DNA in a clean, labeled tube.

APPENDIX C: DACRON/COTTON SWAB EXTRACTION

Each mL lysis/DTT buffer contains:

- 900uL lysis
- 100uL DTT

1. Using a clean pushpin, poke a hole in the tip of a 0.5mL tube and place this tube in a 1.5mL tube.
2. Wet the swab with 75uL TE^{-4} buffer and swab the fabric at the location of the stain with a rolling motion.
3. Add 150uL lysis/DTT to the sample. Centrifuge for 3min at 6000xg. Discard the 0.5mL tube.
4. Add 7uL silica-coated beads and incubate at room temperature for 5min, vortexing occasionally.
5. Place the tube on the magnetic stand, let the solution clear of beads, and aspirate the liquid.
6. Wash with 150uL lysis/DTT. Place the sample on the magnetic stand, allow the solution to clear of beads, and aspirate the liquid.
7. Wash with 200uL wash buffer. Place the sample on the magnetic stand, allow the solution to clear of beads, and aspirate the liquid. Repeat step 7 for a total of 3 washes.
8. Elute the sample with 25uL TE^{-4} buffer at room temperature. While the tube is on the magnetic stand, transfer the solution containing the DNA to a new, clean tube. Repeat step 8.

APPENDIX D: GLASS FIBER SWAB EXTRACTION

Each mL lysis/DTT contains:

- 900uL lysis buffer
- 100uL DTT

1. Use 75uL warm lysis/DTT (65°C) to wet the swab. Swab the fabric using a rolling motion.
2. Place swab in a 0.65mL tube. Place this tube in labeled 1.5mL tube.
3. Centrifuge for 3min at 6000xg. Discard liquid in 1.5mL tube. Repeat if necessary.
4. Wash with 200uL wash buffer. Centrifuge for 90sec at 6000xg. Discard wash buffer in 1.5mL tube. Repeat centrifuging if necessary.
5. Repeat step 4.
6. Place tube containing swab in a new, labeled 1.5mL tube. Centrifuge for 30sec at 6000xg.
7. Place tube containing swab in a new, labeled 1.5mL tube.
8. Elute with 25uL TE^{-4} buffer. Centrifuge for 90sec. Repeat step 8.
9. Discard pipette tip and swab. DNA is contained in solution.

APPENDIX E: TAPE LIFT EXTRACTION

Each mL lysis/DTT contains:

- 900uL lysis buffer
- 100uL DTT

1. Discard exposed tape from roll (see Figure 9).
2. Take small piece of tape (1/2-1in long) and firmly press over area of interest on fabric.
3. Rip tape off the fabric and place in a 1.5mL tube.
4. Add 300uL lysis/DTT, vortex, and incubate at 70°C for 15-30min.
5. Pipette lysis/DTT into new, labeled 1.5mL tube. Discard old tube with tape.
6. Add 7uL silica-coated beads, vortex, and incubate at room temperature for 5min.
7. Vortex the sample and place on the magnetic stand. Let the solution clear of beads and aspirate lysis/DTT.
8. Wash the sample with 50uL lysis/DTT, place on magnetic stand, and aspirate lysis/DTT.
9. Wash the sample with 50uL wash buffer, place on magnetic stand, and aspirate. Repeat step 9.
10. Allow sample to air dry for 5min.
11. Add 50ul warm TE^{-4} (65°C), vortex, and incubate at 65°C for 5min.
12. Transfer TE containing DNA to a clean, labeled tube.

APPENDIX F: PARTICIPANT CONSENT FORM

OKLAHOMA STATE UNIVERSITY

Center of Health Sciences

PARTICIPANT INFORMATION AND CONSENT FORM

Title of Project**: Application of a Glass Fiber Swab as a Collection Device for Touch DNA on Fabrics**

Investigator(s): **Kaitlyn S Burgei, BS Department of Forensic Science 937-903-8629**

"You" refers to the participant.

"I" refers to the researcher.

You are being asked to participate in this research study based on your interest in the study and your willingness to complete the instructions provided in the timeframe specified.

What you should know about participating in a research study:

Participation in research is a voluntary choice, and this consent form will provide you with information about the risks, benefits or alternatives to participation in the study.

- Someone will explain this research study to you.
- You may volunteer to be in a research study.
- Whether or not you take part is up to you.
- You can choose not to take part in the research study.
- You can agree to take part now and later change your mind.
- Whatever you decide it will not be held against you.
- Feel free to ask all the questions you want before you decide.

Who can you talk to?

Although this consent form provides detailed information about this study, the researcher is available to answer any questions you may have about this study and/or participation in it. If you

have questions, concerns, or complaints, or think the research has hurt you, talk to the researcher at kaitlyn.burgei@okstate.edu or 937-903-8629

This research has been reviewed and approved by the Oklahoma State University Center for Health Sciences Institutional Review Board (IRB). You may contact the chairperson of this committee, Richard Wansley, PhD, at 918-561-8325 for any of the following:

- Your questions, concerns, or complaints are not being answered by the researcher..
- You cannot reach the researcher..
- You want to talk to someone other than the researcher..
- You have questions about your rights as a research participant.
- You want to get information or provide input about this research or your experience in this research study.

Why am I doing this research?

The purpose of the research is to determine the most efficient method in collecting touch DNA from clothing, using a variety of swabs and additional methods.

How long will the research last?

I expect that you will be in this research study for a maximum of 2 weeks after the distribution of materials.

How many people will be studied?

I expect about 4 people to be enrolled into this study.

What happens if you say yes, you want to be in this research?

You will be asked to wear an article of clothing (provided) for 45-60 minutes while completing an everyday task. The task will depend upon which article of clothing you receive.

The volunteer assigned t-shirts will be asked to wear one shirt while performing a 45 minute workout. Once you have completed the workout, if the shirt is wet, hang it to dry without fans, ensuring it does not come into contact with other clothing. Once dry, neatly fold the shirt inside the butcher paper in the same way it was received. With 2 pieces of masking tape (provided), secure the outer flaps of the package. Using a No.2 pencil, write the date of the workout on the outside of the paper and return the package to the paper bag supplied. Repeat this procedure for each of the 5 shirts on separate days. Once completed, return the bag with the shirts to the investigator.

The volunteer assigned ball caps will be asked to wear 1 ball cap for 1 hour while going about normal activities. After this time period, place the cap back into the paper bag, secure the flap using 2 pieces of masking tape (provided), and write the date on the outside of the paper bag using a No.2 pencil. Return the package back to the large bag supplied. Repeat this procedure for each of the 5 ball caps on separate days. Once completed, return the supplies back to the investigator.

The volunteers assigned gloves, either cotton or leather, will be asked to wear one glove on the dominant hand only for 1 hour while typing. After this time period, return the worn glove only to the butcher paper in the same way it was received. Secure the free edges of the paper with 2 pieces of masking tape (provided). Fold the unworn glove in a separate piece of butcher paper and tape the edges as previously described. Using a No.2 pencil, write the date worn and "worn" or "unworn" on the outside of the butcher paper. Return the packages to the paper bag supplied. Repeat this procedure for each pair of gloves on separate days. Once completed, return the bag with the gloves to the investigator.

What happens if you say no, you do not want to be in this research?

You may decide not to take part in the research and it will not be held against you. A refusal to participate in this research study will involve no penalty or loss of benefits to which you are otherwise entitled. There will be no effect on student status as a result of participation or refusal to participate.

What happens if you say yes, but you change your mind later?

You can agree to take part in the research now and stop at any time. It will not be held against you. Discontinuing participation will not result in penalty or loss of benefits to which you are otherwise entitled.

If you decide to leave the research, contact the researcher and return the supplies to the researcher.

If you stop participating in the research study, you will be asked whether the researcher can continue to collect data from the clothing items provided.

Is there a risk to being in this study?
There are no risks associated with this project which are expected to be greater than those ordinarily encountered in daily life.

Will it cost you anything to be in this study?
Participating in this study will not result in any cost to you.

What are your responsibilities?

Follow the instructions provided to you by the researcher. Notify the researcher if you develop any skin rashes during the course of the study. Compromised skin may lead to altered results in the study.

Will being in this study help you in any way?

There are no benefits to you from your taking part in this research. Participation in this study will not have any compensation, monetarily or academically.

What happens to the information we collect?

The information I collect will remain in the sole possession of the researcher. Information gathered from the DNA collected from the articles of clothing will be quantitative only. No other data, such as genetic information, will be collected. The articles of clothing will be destroyed at the end of the study, at maximum 6 months. Any written results will not include identifying information. Research data will be kept on a password protected computer. Data will be destroyed 7 years after the completion of the study.

Can you be removed from the research without your OK?

The researcher can remove you from the research study without your approval. This will result if you develop a skin rash during the course of the study.

When will it be destroyed?

The clothing containing the DNA of the participants will be destroyed following the completion of the study, at maximum 6 months after collection. The data collected during the analysis will be destroyed 7 years after the completion of the study.

Your signature below documents your consent to take part in this research and to the use and disclosure of your protected health information. You will receive a signed copy of this complete form.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

APPENDIX G: CRIME LAB PROTOCOL*

1. Note identifiers
    a. Brand, Size, Logos, Stains, Tears
    b. Are all parts of the clothing functional? (zippers, buttons, snaps)
    c. Is there anything in the pockets?
2. Are there any reddish brown stains?
    a. If yes, swab each stain with separate swab
    b. Check pockets for reddish brown stains
3. Examination with ALS
    a. Circle any stains located with ALS
    b. Use one swab to collect from all areas positive for ALS
4. Recovery of Touch DNA
    a. Use one swab to collect from any areas believed to be in direct contact with wearer (collar, seams, waistbands)
    b. Use additional swab to collect from inside pockets

Tips:

- Use separate bench paper and gloves for each article of clothing.
- Swab lengthwise on the fabric (with the grain).
- Avoid swabbing under the arms. Deodorant may inhibit analysis of DNA.
- Elastic is a great area to swab for touch DNA.
- If any hair or fibers are observed, leave them with the item.
- When swabbing bandannas or ligatures, keep the item knotted. Use one swab for the ends and a second swab for the loop. If the suspect brought the rope with him and the victim has long sleeves, suspect DNA may be recoverable from a previous use.
- When analyzing, only take ½ swab, leaving the rest for defense in the future if necessary. Storing DNA on a swab is more stable than storing DNA in solution.

*Interview notes from March 2015 as cited in section entitled “Processing Clothing for Wearer”

APPENDIX H: LOCI USED FOR HUMAN IDENTIFICATION [27]

13 CODIS Loci:

CSF1PO*
FGA*
TH01*
TPOX*
vWA*
D3S1358*
D5S818*
D7S820*
D8S1179*
D13S317*
D16S539*
D18S51*
D21S11*

12 European Standard Loci:

TH01
vWA
FGA
D21S11
D3S1358
D8S1179
D18S51
D10S1248*
D22S1045*
D2S441*
D1S1656*
D12S391*

Additional Loci:

Amelogenin*
DYS391*
Penta D*
Penta E*
D2S1338*
D19S433*

*24 loci used in a typical profile. There are some repeats between the CODIS and European Standard set.

APPENDIX I: LABORATORY RESULTS

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Cotton neg	0	0	0	0	0	0	0	0	0	0
	0	0	0	0.18	0	0	0	0	0	0
	0	0	0	0	0		0	0	0	0
Cotton 1	1.62	0	0	0	0	0	0	0	0	0
	0.29	0	0	0	0	0	0.02	0	0	0
	1.37	0	0	0	0		0	0	0	0
Cotton 2	1.00	0	0	0	0	0.27	0	0	0	0
	2.09	0	0.11	0	0	0	0	0	0	0
	1.35	0	0	0	0		0	0	0	0
Cotton 3	1.9	0	0	0	0	0	0	0	0	0
	1.51	0	0	0	0	0	0	0	0	0
	3.03	0	0	0	0		0	0	0	0
Cotton 4	2.11	0	0	0	0	0	0	0	0	0.10
	2.95	0.14	0	0	0	0	0	0.25	0	0.15
	2.59	0	0	0	0		0	0	0	0
Cotton 5	1.35	0	0	0	0	0	0	0	0	0
	2.09	0	0	0	0	0	0	0	0	0
	2.12	0	0	0	0		0	0	0	0

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Denim neg	0.12	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0.39	0	0	0	0
	0	0	0	0	0		0	0	0	0
Denim 1	3.28	0	0	0	0	0	0	0	0	0.14
	3.65	0	0	0	0	0	0	0	0	0
	3.68	0	0	0	0		0	0	0.24	0
Denim 2	2.95	0	0	0	0.29	0	0	0	0.31	0
	2.46	0	0	0	0	0	0	0	0.12	0
	1.96	0	0	0	0		0	13.36	7.17	0.18
Denim 3	2.03	0.06	0	0	0	0	0	0.15	0	0
	3.45	0	0	0	0	0	0	0	0.10	0
	2.34	0	0	0	0		0	0	0	0
Denim 4	2.95	0	0	0	0	0	0	0	0	0
	2.55	0	0	0	0.37	0	0.13	0	0	0
	2.45	0	0	0	0		0	0	0	0
Denim 5	3.43	0	0	0	0.12	0	0.47	0	0	0
	2.54	0	0	0	0	0	0.16	0	0	0
	2.82	0	0	0	0		0	0	0	0

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Poly neg	0	0	0.14	0	0	0	0	0	0	0
	0	0	0.13	0	0	0	0	0	0	0
	0	0	0.13	0	0		0	0	0	0
Poly 1	0.67	0	0	0	0	0	0.31	0	0	0
	0.70	0	0	0	0	0	0.22	0	0	0
	0.74	0	0	0	0		0.50	0	0	0
Poly 2	0.02	0.32	0	0	0	0	0	0	0	0
	0.40	0.32	0	0.06	0	0	0	0	0	0
	0.29	0	0	0	0		0	0	0	0
Poly 3	0	0	0	0	0	0	0	0	0	0
	0.23	0.26	0	0	0	0	0	0	0	0
	0.13	0	0	0	0		0	0	0	0
Poly 4	0.16	0	0	0	0	0	0	0	0	0
	0.48	0	0	0	0	0	0	0	0	0
	0.20	0	0	0	0		0	0	0	0
Poly 5	0	0	0	0	0.92	0	0.09	0	0.34	0
	0	0	0	0	0.22	0	0	0	0	0
	0	0	0	0	0.79		0	0	0	0

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Silk neg	0	0	0	0	0	0	0	0	0	0
	0.13	0	0	0	0	0	0.01	0	0	0
	0.16	0	0	0	0		0	0	0	0
Silk 1	2.18	0.20	0	0	0	0	0	0	0	0
	1.89	0.10	0	0	0	0	0	0	0	0
	1.34	0	0	0	0		0	0	0	0
Silk 2	2.41	0	0	0	0	0	0	0	0	0
	3.68	0	0	0	0	0	0.16	0	0	0
	2.86	0	0	0	0		0	0	0	0
Silk 3	1.49	0	0	0	0	0	0	0	0	0
	2.23	0	0	0	0	0	0	0	0	0
	3.06	0	0	0	0		0	0	0	0
Silk 4	2.50	0	0	0	0	0	0.34	0	0	0
	4.50	0	0	0	0	0	0	0	0	0
	3.58	0	0	0	0		0	0	0	0
Silk 5	1.58	0	0	0	0	0	0	0	0	0
	1.59	0	0	0	0	0	0	0	0	0
	2.09	0	0	0	0		0	0	0	0

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Spandex neg	0.26	0	0	0	0	0	0.20	0	0	0
	0	0	0	0	0	0	0.33	0	0	0
	0	0	0	0	0		0.18	0	0	0
Spandex 1	0.99	1.03	0.65	0.25	0	0	0.79	0	0	0
	0.84	1.55	1.27	0	0	0.11	0	0	0	0
	0.73	1.18	1.02	0	0		1.38	0	0	0
Spandex 2	3.19	0	0.07	0	0.17	0.29	0	0	0	0.30
	4.83	0	0.15	0	0	0.28	0.16	0.16	0.06	0.39
	3.50	0	0.13	0	0		0	0	0	0.49
Spandex 3	2.29	2.41	1.39	0	0	0.20	1.26	0	0	0.17
	3.73	0	1.04	0	0.25	0.20	1.99	0	0.19	0.14
	2.48	0.73	0.87	0	0		1.89	0	0.41	0
Spandex 4	3.31	1.19	0	0	0.12	0	0.25	0	1.65	0
	6.30	0.49	0	0	0.26	0	0.35	0.13	7.32	0
	5.30	2.11	0	0	0		0.15	0	0.46	0
Spandex 5	2.93	0.53	0.11	0	0	0	0	0	1.04	0
	3.84	0.45	0.47	1.76	0.28	0	0	0	1.08	0
	4.53	0.90	0.63	0.60	0.39		0	0	1.49	0.10

	Cutting- 2cm^2	Cotton- TE	Cotton- Lysis	Dacron- TE	Dacron- Lysis	Glass- TE	Glass- Lysis	Taping- 1 lift	Taping- 10 lift	Taping- 25 lift
Wool neg	0.28	0	0	0	0	0	0.69	0	0	0
	0.38	0	0	0	0	0	0.37	0	0	0
	0.22	0	0	0	0		0.14	0	0	0
Wool 1	0.13	1.65	0	0.51	0.06	0	1.20	0	0	0.43
	0.14	1.66	0	0.33	0.10	0	1.88	0	3.17	0.13
	0.26	0.85	0.09	0.49	0		1.96	0	0.80	0.16
Wool 2	0.50	3.28	0.58	0.27	0.75	0	2.57	0	0.76	0
	0.43	3.14	0.65	0.33	0.33	0	5.99	0	0.92	0
	0.79	4.40	0.24	0.43	1.31		2.46	0	0.18	0
Wool 3	0.22	2.06	0.84	1.24	0	0	1.76	0	1.34	0
	0	1.32	1.44	0.72	0	0	3.87	0	1.43	0
	0.46	1.77	0.97	0.41	0		1.77	0	1.56	0
Wool 4	0	1.34	0.23	0.90	0	0	1.25	0	2.09	0.32
	0.75	3.10	0.26	2.20	0	0	1.25	0.13	3.82	0.78
	0	3.06	0.04	0.67	0		2.57	0.61	3.87	0.12
Wool 5	0.29	1.71	0.28	1.16	0	0	0	0	0	0.12
	0	1.51	0.12	0.81	0.49	0	0	0	0	0.39
	0.53	3.60	0.28	1.45	0.13		0	0	0	0.28

*Any results labeled as 0 actually reads as below the detection limit of 23pg

APPENDIX J: REAL WORLD RESULTS

	Cutting	Cotton-TE	Dacron-Lysis	Glass-Lysis	Taping
Hat neg		0	0	0	0
		0	0	0	0
		0	0	0.15	0
Hat pos	0.16	0.28	0.78	0	1.53
	1.03	0.32	0.84	1.95	1.96
	0	1.07	0.33	0.26	1.23
Hat fabric neg	0				
	0				
	0				
Hat padding neg	0				
	0				
	0.43				
Hat fabric	0.20				
	0.65				
	0.16				
Hat padding	0.87				
	4.16				
	2.54				

	Cutting	Cotton-TE	Dacron-Lysis	Glass-Lysis	Taping
Tee neg	0	0.46	0	0.18	0
	0	0	0	0	0
	0.11	0	0	0	0
Tee pos		0	1.28	0	0.50
		0.14	0.65	0.28	0.47
		0	1.43	0.13	0
Tee back of neck	0.11				
	0.66				
	0.43				
Tee arm	0.25				
	0				
	1.07				
Tee neck V	0.21				
	0.38				
	0.48				

	Cutting	Cotton-TE	Dacron-Lysis	Glass-Lysis	Taping
Cotton glove neg	0.14	0	0	0	0.14
	0	0.10	0	0	0.19
	0	0	0	0	0
C glove elastic	0	0	1.27	0.46	0
	0	0.11	0.78	0.71	0.06
	0	0.41	1.39	0.27	0.20
C glove palm	0				
	0				
	0				

	Cutting	Cotton-TE	Dacron-Lysis	Glass-Lysis	Taping
Leather glove neg	0.17	0	0	0	0
	0.16	0	0	0	0
	0.11	0	0.29	0	0
L glove elastic	0.19	0	0.02	0	0.82
	0.75	0.13	0.31	0	0.54
	0.71	0.24	0	0	0.30
L glove palm	0				
	0.95				
	0				

*Any results labeled as 0 actually reads as below the detection limit of 23pg

VITA

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