COLLECTION OF BIOLOGICAL EVIDENCE FROM PLASTIC USING VARIOUS TYPES OF SWABS

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COLLECTION OF BIOLOGICAL EVIDENCE FROM

PLASTIC USING VARIOUS TYPES OF SWABS

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Abstract:

DNA can be left behind at a crime scene when a person comes in contact with a surface. However, not all DNA samples will result in a DNA profile because the amount of DNA recovered from the sample may not be adequate. Any increase in the yield of DNA recovered from crime scene samples improves the chances of producing probative DNA profiles for court. DNA results presented in court can be used to help determine a suspect's guilt or innocence.

Because of DNA's attraction to the silica in glass-fiber material, an increased adsorption of DNA to glass-fiber swabs may improve the collection and recovery of DNA from plastic evidence. The efficiency of glass-fiber swabs compared to polyester-tipped and cotton-tipped swabs for recovering DNA from plastic remained unknown. Since many common items are now made from plastic, further study was needed in order to determine which swab type was more effective for recovering DNA from plastic evidence.

This study was designed to compare glass-fiber swabs with polyester-tipped and cotton-tipped swabs to determine if glass-fiber swabs would improve the recovery of DNA from plastic evidence. Human blood was spotted on and collected from a plastic Rubbermaid® lid. DNA was isolated from the collection swabs, amplified by polymerase chain reaction, and quantified with the Q-TAT multiplex assay. The quantity of DNA recovered by the different types of swabs was then compared.

Glass-fiber swabs, polyester-tipped swabs, and cotton-tipped swabs do differ in their respective effectiveness for recovering DNA from blood stains spotted and dried onto a plastic Rubbermaid® lid. In the final analysis, cotton-tipped swabs were more effective than glass-fiber swabs and polyester-tipped swabs for recovering DNA from plastic. Further study showed that cotton-tipped swabs recovered more DNA from glass than polyester-tipped swabs and were equal, compared to polyester-tipped swabs, in recovering DNA from tile and wood.

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CHAPTER I

INTRODUCTION

Research problem

Could you commit the perfect crime? Realistically, a perfect crime would be hard to commit since DNA can be found at almost any crime scene that involves physical contact.[1] DNA can be left behind at a crime scene when a person comes in contact with a surface. Therefore, anything you touch can and most likely would be used against you.[1] DNA can be recovered from blood stains, skin cells, or other biological samples from a crime scene and used to generate the DNA profile of a suspect.[1,2] However, not all DNA samples will result in a DNA profile because the amount of DNA recovered from the sample may not be adequate. About 300 picograms (pg) of DNA is needed to generate a DNA profile from biological samples. Different types of swabs are often used to collect DNA samples. In order to obtain a probative DNA profile from evidentiary samples, maximum recovery of DNA is critical, especially from small samples such as are typical of touch DNA evidence.[3] For this reason, this study focused on the comparison efficiency of the collection and recovery of DNA using different types of swabs.

Review of literature

Previous studies indicate that cotton-tipped swabs are the least expensive and most commonly used swabs for collecting DNA samples.[3,4] According to Puritan® Medical Products (Guilford, ME) polyester-tipped swabs are more costly than cotton-tipped swabs, but polyester-tipped swabs have better collection and release properties.[5] The number of cells recovered from evidence is often less than the number needed to produce 0.3 nanograms (ng) of DNA.[6] For a full DNA profile, most short tandem repeat (STR) kits have been optimized to use 0.5-1.0 ng of DNA.[6] In order to generate a full DNA profile, the yield of DNA recovered from crime scene samples must be optimized.[6] For this reason, swabs and collection methods have been studied in an attempt to improve the collection and recovery of DNA from various objects. Some studies have examined the recovery of DNA from glass, fabric, metal, brick, plastic, and wood by using various collection methods such as the tape-lifting method, the cutting method, and the swabbing method.[7–11] Studies have also examined a variety of different swab materials for use as DNA recovery devices.[3,7] Swabs used in previous studies have had tips made of different materials: cotton, polyester, rayon, nylon, foam, and flocked.[3,7] The comparative efficiency among these different types of swabs was shown to vary.[7-10] Some swab materials were found to be more effective than others for recovering DNA from specific types of objects.[7] For example, Verdon et al. found that polyester swabs were more efficient for recovering DNA from glass while foam swabs were found to be more efficient for recovering DNA from wood.[7]

Deficiencies in literature

Although several studies have compared the efficiency of different swab types to other swab types, only a few studies have examined the efficiency of glass-fiber swabs to other swab types.[3,7-10] Glass-fiber swabs were first evaluated in a study conducted by Wilkins.[8] This new

swab collection device consists of a swab head made from glass-fiber material.[8] These glass-fiber swabs are made individually by hand and are not currently available commercially. Three studies have compared glass-fiber swabs to other types of swabs for collecting DNA from glass, metal, and cloth.[8-10] The efficiency of the glass-fiber swabs varied among the studies.[8-10] In studies conducted by Tucker and Burgei, glass-fiber swabs were shown to be comparable to cotton-tipped swabs and polyester-tipped swabs for collecting DNA, while the study conducted by Wilkins showed that glass-fiber swabs recovered more DNA when 10 ng of purified DNA was spotted onto glass slides.[8-10] Although all three studies compared swab types and examined the efficiency of glass-fiber swabs for collecting DNA from metal, glass, and cloth, the efficiency of glass-fiber swabs for collecting DNA from plastic was not studied. Therefore, the efficiency of glass-fiber swabs compared to polyester-tipped and cotton-tipped swabs for collecting DNA from plastic remained unknown.

Significance of study

DNA is a powerful tool used to help solve crimes.[12] Forensic laboratories use DNA to match evidentiary items with a suspect when a probative DNA profile is generated, and the information can then be used in court to prosecute a defendant. The DNA results presented in court can be used by the judge and jury to help determine a suspect's guilt or innocence. Any increase in the yield of DNA collected from crime scenes improves the chances of producing probative DNA profiles for court. Glass-fiber swabs may prove to be a better way to recover DNA from plastic over the normally used cotton-tipped and polyester-tipped swabs. Since many common items such as drinking bottles, dishes, food product containers, trash bags, buckets, toys, and light switches are made from plastic, further study was needed in order to determine which swab type was more effective for recovering DNA from plastic evidence.

Purpose statement

The purpose of this experimental study was to compare glass-fiber swabs with polyester-tipped and cotton-tipped swabs to determine if glass-fiber swabs would improve the collection and recovery of DNA from plastic evidence. Glass-fiber swabs were studied to determine if they would adsorb more blood than polyester-tipped or cotton-tipped swabs. An increase in the adsorption of blood to the glass-fiber swabs would allow more DNA to be recovered from a plastic material. The increased amount of DNA recovered from the glass-fiber swab would then increase the chance that a DNA profile would be obtained from the evidence.

Human blood was spotted onto and collected from a plastic Rubbermaid® lid. DNA was isolated from the collection swabs, amplified by polymerase chain reaction (PCR), and quantified with the Q-TAT multiplex assay. The quantity of DNA recovered by the swabs was then compared.

Glass-fiber swabs were used in the collection method because DNA is attracted to the silica in glass-fiber material. Glass-fiber swabs were hypothesized to recover more DNA from plastic than polyester-tipped or cotton-tipped swabs.

CHAPTER II

OVERVIEW OF LITERATURE

The purpose of this study was to determine if glass-fiber swabs would increase the yield of DNA recovered from blood spotted and dried onto plastic. DNA is a valuable tool in obtaining evidence left behind at a crime scene.[13] The amount of DNA recovered from an evidentiary item may affect the probability of obtaining a usable DNA profile.[3] Therefore, it is critical to recover the maximum amount of DNA possible from each item.[3] This study focused on comparing the collection and recovery efficiency of glass-fiber swabs to polyester-tipped and cotton-tipped swabs when collecting blood from plastic. The objective of this literature review is to determine if other literature exists on the comparative use of glass-fiber swabs to polyester-tipped and cotton-tipped swabs to collect crime scene samples from plastic evidence.

DNA

DNA can be recovered from blood spots, skin cells, or other biological samples left behind at a crime scene.[13] For example, skin cells are left behind when a person touches an object because a person can shed about 400,000 skin cells each day.[13] Even if a person leaves only 6-8 skin cells behind on a surface, scientists can use those skin cells in an attempt to generate a DNA profile.[12] Due to improvements in analytical techniques and the availability of different swab materials, the amount of DNA required for profiling has been reduced from ng to pg.[7] Crime

laboratories are receiving more and more requests for DNA analysis because DNA can provide investigative leads and also lead to successful prosecutions of the guilty.[14]

DNA Collection

Previous studies indicate that cotton-tipped swabs are the least expensive and most commonly used swabs for recovering DNA samples.[3,4] Swabs can be used dry, or moistened with sterile water or phosphate saline solutions.[4] Previous studies also describe different ways to recover DNA at crime scenes.[7,11] Differences in the recovery of DNA include the types of swabs used, the types of substrates the biological evidence is found on, and the different techniques used to increase adsorption of biological evidence to swabs.[5,11] Although biological samples are normally collected with cotton swabs, they may not always be the best type of swab to use for DNA recovery.[7, 12] According to Puritan® Medical Products (Guilford, ME), polyester-tipped swabs have better collection and release properties than cotton-tipped swabs.[5]

In a study conducted by Verdon et al., different types of swabs were examined in the collection of biological fluids from different substrates.[7] Nylon-flocked, rayon, polyester, cotton, and foam swabs were used to recover DNA from pitted plastic, glass, brick, and wood. In this study, the different swab types had an impact on the quantity of DNA recovered from biological fluids. The material of the swab, the length and thickness of the swab tip, and how the tip is wound around the applicator may influence the effectiveness of the swab.[7] Also, the different types of substrates had an effect on the ability of the swab to recover the DNA. According to Verdon et al., the Medical Wire and Equipment® (MWE) polyester swab was ranked the best swab for sampling from pitted plastic; the Puritan® Filtered Air Breathable cotton swab (FABSwab) was the best for glass; the MWE rayon swab was the best for brick; and the 1 Puritan® Foam swab (1 PF foam) was the best for wood.[7]

In another study conducted by Daly et al., the transfer of touch DNA was examined on the substrates of fabric, glass, and wood.[11] DNA was recovered from these objects with minitape-lifts and extracted with a Qiagen® QIAamp DNA mini kit. Minitapes are acetate strips with a double sided adhesive section at one end of the strip. The extracted DNA was quantified by a Quantifiler® kit assay and amplified with PCR. The ABI PRISM® 3130xl Genetic Analyzer was used to generate the DNA profile. In this study, DNA profiles were obtained from all three objects. Tape-lifts from wood produced the largest yield of DNA followed by fabric. Glass produced the smallest yield of DNA.[11]

Both of these studies show that the type of swab used, as well as the type of substrate a stain resides on, affects the quantity of DNA recovered.[7,11] The quantity of DNA recovered from substrates is also influenced by the type of swabbing solutions used on swabs during collection.[6] Swabs are often moistened with water or other solutions to help increase the collection and adsorption of biological samples. Adsorption or binding of DNA to the swab may help increase DNA yields.[6] In a study conducted by Thomasma et al., swabbing solutions were examined to determine their influence on DNA recovered from touch samples.[6] The study concluded that detergent-based swabbing solutions, such as sodium dodecyl sulfate (SDS) and Triton X-100, produced the best DNA yields over wetting swabs with water alone. Detergents cause organic elements that make up cells to become suspended in the solution, resulting in the enhanced recovery of DNA.[6]

DNA's ability to bind to silica in glass-fiber material has been a topic of interest for some time.[15] In a study conducted by Melzak et al., DNA's adsorption to silica was shown to be controlled by three competing effects: weak electrostatic repulsion, hydrogen bond formation, and dehydration.[15] A common method for purifying DNA is chaotropic-salt-induced adsorption.

This method aids in the adsorption of DNA to silica. In some cases, the binding reaction is carried out with silica-glass fibers. Melzak's study investigated the binding reaction sensitivity of DNA and silica to variations in temperature, pH, and ionic strength. According to the data, the competing effects controlling DNA's adsorption to silica enable DNA to bind more strongly to silica surfaces.[15] Thus, based upon the study by Melzak, an increase in adsorption of DNA to silica in the form of glass-fiber material may help to increase the amount of DNA that can be recovered when using glass-fiber swabs on plastic evidence.

Although several of these studies have compared the efficacy of DNA recovery by different swab types, only three studies have compared the yield of DNA recovery from glass-fiber swabs to other swab types.[8–10] These three studies focused on the recovery of DNA from metal, glass, and cloth.[8–10] In a study conducted by Tucker, different types of swabs and buffers were compared to determine which combination would work best for recovering DNA from different types of metals.[10] The study compared cotton-tipped swabs, polyester-tipped swabs, glass-fiber swabs, Scotch tape, and polymerase chain reaction squares. Tucker found that there was no one significant combination that was more efficient than the others for collecting DNA from metal.[10]

In a study conducted by Wilkins, polyester-tipped swabs and glass-fiber swabs were used to collect low concentrations of DNA spotted and dried onto glass.[8] This study concluded that glass-fiber swabs recovered more DNA from glass when 10 ng of purified DNA was spotted and dried. When DNA was spotted at lower concentrations of 5 ng and 2 ng, there was no significant difference in the amount of DNA recovered.[8] Therefore, glass-fiber swabs were more efficient at DNA recovery when more DNA was available. Due to the chaotropic lysis buffer used during the collection process and the glass substrate, it is possible that these results may have

been biased. The buffer may have caused the glass-fiber swabs and glass slides to compete for DNA adsorption.[8]

In a study conducted by Burgei, five collection methods were compared to determine the recovery efficiency of DNA spotted onto cloth.[9] This study compared glass-fiber swabs, polyester-tipped swabs, cotton-tipped swabs, extracting DNA from a cutting of the cloth, and a tape-lift method. The amount of DNA collected from assorted clothing articles varied. Although polyester-tipped swabs appeared to collect more DNA than cotton-tipped or glass-fiber swabs, the results concluded that the tape-lift and fabric cutting methods recovered more DNA from cloth than any of the swab methods.[9]

Although all three of these studies examined the efficiency of glass-fiber swabs for collecting small amounts of DNA from metal, glass, and cloth, the efficiency of glass-fiber swabs for collecting DNA from plastic was not studied.[8–10] Therefore, the efficiency of glass-fiber swabs compared to cotton-tipped and polyester-tipped swabs for recovering DNA from plastic remains unknown.

DNA Extraction

A significant amount of DNA can be collected using swabs, but later lost during processing.[3] The amount of DNA recovered is critical to be able to obtain a usable profile.[3] Adamowicz et al. conducted a study that evaluated methods of extraction and recovery of DNA.[3] Their goal was to try to increase the yield of DNA recovered from cotton swabs. The DNA extracted was quantified and short tandem repeat (STR) analysis was used to assess the quality of the extracted DNA. In this study, protocols for incubation and temperature were altered to test for increases in DNA recovery. The Qiagen® QIAamp DNA Investigator extraction kit protocol was

used as a base line in the study. The standard protocol for the Qiagen® QIAamp DNA Investigator kit calls for extraction of a sample for one hour at 56°C. Differences in the DNA yield were almost indistinguishable between 56°C and 65°C for 3 hours of incubation. Although temperature variations to the extraction protocol did not have a significant impact on DNA yields, incubation times proved to be important. An increase in the incubation time to 3 hours yielded equal or increased quantities of DNA when compared with 1 hour of incubation. Incubation times increased from 3 to 18 hours did not yield significant increase in DNA recovery. A drop in the yield of DNA actually occurred with incubation times of 24 hours. The study concluded that increasing the extraction incubation time to 3 hours at either 56°C or 65°C may increase the yield of DNA.[3]

In addition to the method described above, a manual extraction method or the Promega® DNA IQ™ (Madison, WI) extraction system can be used to extract DNA.[16,17] The manual extraction method uses proteinase K digestion followed by extraction with phenol: chloroform organic solvents to partition DNA away from contaminating cellular elements.[16] Ethanol and salts are used to precipitate DNA from the organic phase or, alternatively, DNA can be recovered from the extract using one of a number of DNA clean-up kits that typically involve binding the DNA to silica. Phenol chloroform extraction is time consuming, requires sample transfer among tubes, and uses hazardous reagents.[16]

An alternative to phenol chloroform extraction is the Promega® DNA IQ™ System.[17] In this system, DNA is isolated and captured with a high capacity DNA binding resin. The Promega® DNA IQ™ System protocol consists of extracting the DNA using a chaotropic salt solution at high concentration, capturing the DNA on magnetic beads coated with silica, washing the beads containing the bound DNA, and then eluting the DNA from the beads in a low salt buffer or in

reagent grade water. Since the Promega® DNA IQ™ System does not use reagents as hazardous as phenol or chloroform, this system may be a better alternative for DNA extraction than the phenol chloroform extraction method.[17] The Promega® DNA IQ™ System was used in this study to extract DNA from the three types of swabs. After DNA is extracted, a quantitation method can be used to quantify the DNA.

DNA Quantification

DNA quantitation determines the amount of DNA in an extracted sample. Real-time quantitative polymerase chain reaction (qPCR) was developed in the mid 1900's and uses fluorescence-detecting thermocyclers to amplify DNA and quantify fluorescence emitted from PCR amplicons as they accumulate in the qPCR reaction, which can be used to measure the DNA's concentration.[18] Target DNA sequences are quantified following each cycle of amplification using one of two basic methods to label amplified DNA fragments. The quantity estimates of the amplified DNA sequences are then compared with a standard curve prepared from amplifications of known quantities of genomic DNA, and the curve is used to estimate the amount of DNA in an unknown.[18]

The Plexor® HY System (Promega® Madison, WI) is another real-time polymerase chain reaction (PCR) system used to quantify DNA in samples.[19] Plexor® HY System's technology uses the interaction between two modified nucleotides for quantification. One PCR primer has a modified nucleotide (iso-dC) that is linked to a fluorescent label, while the second PCR primer is not labeled. Deoxynucleotides and iso-dGTP modified with the quencher dabcyl is opposite the iso-dC in the primer. At this position, the dabcyl-iso-dGTP results in a reduction in fluorescence by quenching the fluorescent dye on the complementary strand which allows for quantification. With Plexor® HY technology, quantification is accomplished during amplification.[19]

An end-point PCR based method used to quantify human genomic DNA is the Q-TAT multiplex assay.[20] A study conducted by Wilson et al. developed and validated the Q-TAT multiplex assay.[20] This assay uses fluorescent primers to quantify DNA by amplifying the sex-determining region Y (SRY) gene on the Y chromosome and the amelogenin locus on the X and Y chromosomes. After amplification, capillary electrophoresis is used to separate the products. The products produced from samples of unknown DNA concentration are then compared against a standard curve prepared from known amounts of DNA. This method uses post PCR quantitation, as opposed to a real time method where quantification is followed by measuring fluorescence in PCR products after each cycle of the thermal cycling program. The Q-TAT multiplex assay is a reliable DNA quantitation method and has been used by the Tulsa Police Laboratory for many years.[20]

The Q-TAT method uses capillary electrophoresis to separate DNA fragments. During electrophoresis, fluorescently labeled DNA fragments migrate toward the anode through capillaries filled with a polymer, separating the DNA fragments based on size.[21] Shorter DNA fragments move faster through the polymer than larger DNA fragments.[21] According to Butler, capillary electrophoresis coupled with fluorescent detection provides a reasonable measure of the amount of DNA in a sample.[22] Since post PCR quantitation and capillary electrophoresis are used with the Q-TAT multiplex assay, this quantitation method was used to quantify the DNA recovered from the different types of swabs used in this study.

Overview Conclusion

Recovery of the maximum amount of DNA available from evidentiary samples is critical.[3] The amount of DNA recovered may determine whether a profile will be produced from a given DNA extract that can be used to investigate a crime.[3] The type of swabs used, the type of

substrates the DNA is deposited on, and different techniques used to increase adsorption of DNA to swabs can affect the amount of DNA recovered.[5,11] Research shows that some swabs can be better than others for recovering DNA from particular substrates.[7]

Glass-fiber swabs may maximize the quantity of DNA recovered from plastic evidence. Although studies have compared the efficiency of glass-fiber swabs for collecting DNA from metal, glass, and cloth, the efficiency of glass-fiber swabs compared to cotton-tipped and polyester-tipped swabs for recovering DNA from plastic remains unknown.[8–10] The silica surface of glass-fiber swabs may allow DNA to bind more strongly to the swab and increase the amount of DNA recovered from plastic. Therefore, there was a need of further study using glass-fiber swabs in order to determine the swab's efficiency for recovering DNA from plastic evidence.

CHAPTER III

METHODOLOGY

The design of this study was to focus on the potential increase in yield of DNA from glass-fiber swabs used to recover DNA from blood stains spotted onto plastic compared to the amount of DNA recovered when using cotton-tipped and polyester-tipped swabs. Blood was collected from plastic using various types of swabs in order to compare the recovery of DNA from the different swab types. Glass-fiber swabs were hypothesized to recover more DNA from plastic than cotton-tipped or polyester-tipped swabs. Because of DNA's attraction to the silica in glass-fiber swabs, an increase in adsorption of DNA to glass-fiber swabs was expected to improve the recovery of DNA from plastic. Human blood was spotted onto and collected from a plastic Rubbermaid® lid. DNA was isolated from the blood with the Promega® DNA IQ™ System (Madison, WI), and quantified by the Q-TAT multiplex assay. The quantity of DNA recovered by the different types of swabs was then compared.

In this study, DNA was extracted from human blood. One 6 mL sample of human blood was obtained from Oklahoma State University Center for Health Sciences (OSU-CHS) in Tulsa, Oklahoma. The blood sample was from an unidentified female source. The Institutional Review Board (IRB) of the OSU-CHS determined that this study does not qualify as human subject research and is not subject to IRB oversight.

Preparation of Glass-Fiber Swabs

Prior to DNA collection, the glass-fiber swabs were constructed. Normally, pre-packaged polyester-tipped swabs are used for buccal swab collections by the DNA Human Identity Testing Laboratory at OSU-CHS. In 2015, OSU-CHS developed a new type of swab constructed with glass-fiber filter paper. These glass-fiber swabs are individually made by hand.

The swabs used in this study were constructed in a manner similar to those that had been used in previous studies. As described by Wilkins, each glass-fiber swab was constructed on a polyester-tipped applicator on the end opposite that of the polyester swab's head.[8] For this study, the head of the glass-fiber swab was made from a 3.4 cm x 1 cm strip of Whatman™ 934-AH™ glass microfiber filter paper. One end of the glass microfiber filter paper was glued to the end of the applicator stick with a drop of Krazy Glue®. The strip was then wrapped around the applicator stick and fastened in place with a second drop of Krazy Glue®. Ultraviolet (UV) radiation was used to sterilize the swabs for about 12 seconds at ~3000 μwatts/cm^2. Figure 1 shows a side-by-side tip comparison of the hand-constructed glass-fiber swab and the commercially available polyester-tipped and cotton-tipped swabs used in this study.

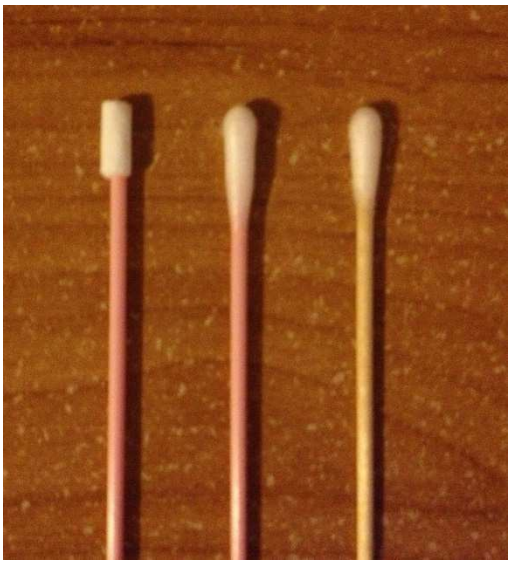

Figure 1. *Side-by-side tip comparison of swabs: (left to right) glass-fiber, polyester-tipped, and cotton-tipped swabs.*

Collection of Samples

Blood was collected from a plastic Rubbermaid® lid that was 34 cm long by 21 cm wide. The Rubbermaid® lid was made of polypropylene and was chosen for ease of use. Prior to spotting blood, the plastic Rubbermaid® lid was cleaned with bleach and pre-marked with a Sharpie to label and number the areas where blood would be spotted. Each blood spot was about 3 cm apart. The blood sample was diluted 1:10 with a TNE buffer composed of 10 mM Tris Chloride, 0.2 M Sodium Chloride (NaCl), and 1 mM Ethylenediaminetetracetic acid (EDTA) prior to dispensing. A pipette was used to spot 50 µL blood spots on the plastic Rubbermaid® lid. Each blood spot was spread out with the pipette tip to allow for a faster drying time. The Rubbermaid® lid was then placed in a cabinet to dry overnight.

After the blood had dried, polyester-tipped swabs, cotton-tipped swabs, and glass-fiber swabs were used to collect the blood from the plastic Rubbermaid® lid, as shown in Figure 2.

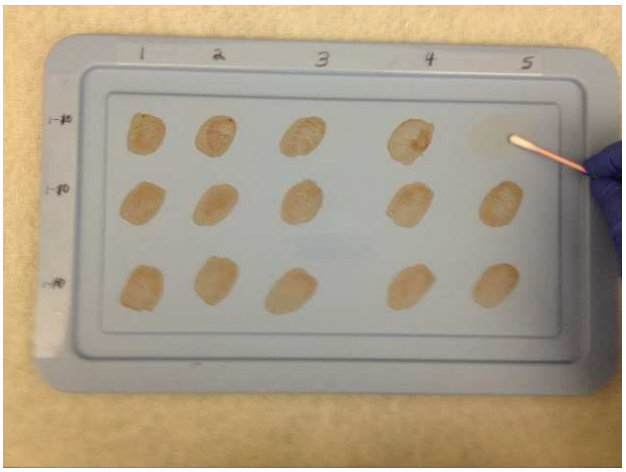

Figure 2. *Collection of blood from plastic Rubbermaid® lid.*

For each swab type, a pre-wetting solution was used to moisten the swab's head. Two different solutions, one for the glass-fiber swabs and another for the polyester and cotton-tipped swabs, were used in the pre-wetting process. Because of DNA's ability to bind to the silica in glass-fiber material in the presence of a chaotrope, glass-fiber swabs were moistened with a 75 µL chaotropic salt solution composed of 6-8 M guanidinium thiocyanate (GTC) and 10 mM of dithiothreitol (DTT).[8] This lysis/DTT buffer was provided as a reagent supplied with the Promega® DNA IQ™ System (Madison, WI).

In a study conducted by Melzak et al., DNA adsorption to silica was shown to be controlled by three competing effects that aid in the adsorption of DNA to silica.[15] According to the data, changes in adsorption isotherms for DNA on silica particles establish ionic strength, pH, and temperature sensitivity. These competing effects that control DNA absorption include weak electrostatic repulsion, hydrogen bond formation, and dehydration. As a result of these competing effects, DNA is able to bind more strongly to the silica surface.[15] Therefore, the chaotropic salt solution may aid in the recovery of DNA from the glass-fiber swabs by creating a high salt environment.

Cotton-tipped and polyester-tipped swabs were moistened with a 100 µL TE^{-4} buffer composed of 10 mM of Tris Chloride and 0.1 mM of EDTA. Prior to DNA collection, each swab was moistened with the appropriate pre-wetting solution.

The glass-fiber swabs were moistened with 75 µL of lysis/DTT buffer.[8] The polyester-tipped and cotton-tipped swabs were moistened with 100 µL TE^{-4} buffer.[8] Each swab was used to collect one blood spot. For collection of the dried blood spots, each pre-moistened swab was rubbed and rolled over a designated blood spot on the plastic Rubbermaid® lid. DNA was then isolated from the blood on each swab.

Extraction of DNA

For extraction of DNA from the blood, the Promega® DNA IQ™ System (Madison, WI) was used to isolate the DNA from all three types of swabs. This system uses a silica coated paramagnetic resin (magnetic beads) to capture DNA.[17] The Promega® DNA IQ™ System (Madison, WI) protocol consists of solubilizing the DNA containing cells with the chaotrope, capturing the liberated DNA onto the surface of the magnetic beads, washing the magnetic beads to remove any contaminating cellular materials, and then eluting the DNA from the magnetic beads in a low salt buffer.[17]

Following transfer of the blood stain to the wetted swab head, DNA was extracted from the blood. The extraction protocol varied slightly among the swabs. For glass-fiber swabs, scissors were used to clip off the head of a swab into a 0.2 mL centrifuge tube. A sterile thumb tack was used to puncture a hole in the bottom of the 0.2 mL centrifuge tube which was then placed into a 0.6 mL centrifuge tube. Each 0.6 mL centrifuge tube containing the wet swab head was then incubated at 70^0C for 30 minutes to complete dissolution of cellular elements in the blood stain.

For cotton-tipped and polyester-tipped swabs, scissors were used to clip off the head of a swab into a 0.6 mL centrifuge tube. Cotton-tipped and polyester-tipped swab heads were then saturated with 200 µL of lysis/DTT buffer composed of GTC and the reducing agent DTT. Each 0.6 mL centrifuge tube was then incubated at 70^0C for 30 minutes. Figure 3 shows a centrifuge tube containing the head of a polyester-tipped swab.

Figure 3. *Centrifuge tube containing head of polyester-tipped swab.*

The remainder of the extraction protocol was the same for all types of swabs. A sterile thumb tack was used to puncture a hole in the bottom of each 0.6 mL centrifuge tube. Each 0.6 mL centrifuge tube was then placed into a 1.5 mL centrifuge tube. These tubes were then centrifuged for 1 minute at 10,000 rpm to recover the liquid containing dissolved cellular elements and genomic DNA mixture from the swab heads. The flow-through containing the DNA was collected in the bottom of the 1.5 mL centrifuge tubes. The 0.2 mL and 0.6 mL centrifuge tubes containing the swab heads were then discarded. The concentrated chaotropic salt in the lysis buffer solubilized the cell membranes and released the DNA. The extracted DNA is recovered in the flow-through following centrifugation.

The magnetic beads supplied with the Promega® DNA IQ™ System (Madison, WI) were then added to capture the DNA. Before being added to the DNA extract, magnetic beads were subjected to vortex mixing for approximately 3 seconds to produce a uniform suspension of the magnetic beads. Afterwards, 1 µL of the beads were dispensed into each 1.5 mL centrifuge tube containing the DNA extract recovered by centrifugation. Beads were incubated with the DNA extract for 5 minutes at room temperature with intermittent vortex mixing. During this step, DNA binds to the magnetic beads. Each extract was then vortexed for 3 seconds and then placed into a tube rack with a magnet to separate the beads harboring bound DNA from the liquid in the extract. The liquid portion was then removed with a pipette and discarded, leaving only the magnetic beads with the bound DNA.

The magnetic beads were washed to remove impurities with a wash buffer supplied with the Promega® DNA IQ™ System (Madison, WI). The magnetic beads were washed once with 50 µL of lysis/DTT buffer and then 2 times with 50 µL of wash buffer, composed of 30% TE^{-4}, 35% ethanol alcohol, and 35% isopropanol alcohol supplied with the Promega® DNA IQ™ System (Madison, WI). After adding wash solution, the tubes were mixed briefly and then placed on a magnetic tube stand where the liquid mixture was immediately separated from the magnetic beads. A pipette was used to aspirate the liquid. After the final wash and aspiration, the magnetic beads were allowed to air dry for 5 minutes at room temperature to partially evaporate the alcohols. After drying, 30 µL of TE^{-4} buffer was added to the magnetic beads to rehydrate and elute the DNA from the magnetic beads. These tubes were incubated at 65°C for 5 minutes and then briefly vortexed. After incubation, the DNA was released from the magnetic beads. Each tube was vortexed for 3 seconds and placed on a magnetic tube stand. The tubes remained on the magnetic stand until the solution containing the DNA was collected with a

pipette and placed into 0.2 mL PCR tubes. The DNA was then ready for amplification and quantification.

Amplification and Quantification of DNA

The Q-TAT multiplex assay was used to quantify the DNA recovered from the stains. The Q-TAT multiplex assay uses end-point multiplex PCR and human specific primers to amplify the amelogenin locus (a gene locus on the X and Y chromosomes) and the sex-determining region Y gene (SRY) to quantify DNA.[20] A cloned non-human DNA template, a pRL plasmid, was included in the Q-TAT PCR reactions and served to detect PCR inhibition. This assay has been shown to be a reliable DNA quantitation method.[20] Since the source of the DNA was female, only the X allele was visible in Q-TAT amplifications.

PCR Setup and DNA Amplification

For purposes of amplifying DNA, the end-point multiplex PCR known as Q-TAT was used to quantify DNA recovery from all samples. DNA was amplified with the GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems Inc., Foster City, CA). A reaction master mix consisting of 1.75 µL water, 1.25 µL of 10X PCR primer mix, 7.5 µL of GoTaq® DNA polymerase (Promega Corp, Madison, WI), and 1 µL of pRL plasmid (at 0.5 pg/reaction) (Promega Corp, Madison, WI) was prepared for each reaction. The pRL plasmid was co-amplified with added human genomic DNA and served as an internal amplification control to detect possible PCR inhibitors that may co-extract with human DNA from an evidentiary sample.[20] Sufficient master mix was prepared for the number of DNA samples to be quantified and then the appropriate aliquot of the master mix was dispensed into each reaction tube.

One µL of extracted DNA and 11.4 µL of master mix were pipetted into 0.2 mL centrifuge tubes in triplicate. A positive control containing a known amount of human genomic DNA and a negative control lacking human genomic DNA were included with each amplification run. Positive controls received 1 µL (equal to 100 pg of genomic DNA) of AmpFlSTR® Control DNA 9947A (Applied Biosystems Inc., Foster City, CA) and 11.4 µL of the master mix. Negative controls received 1 µL of distilled water and 11.4 µL of the master mix. All tubes were vortexed for about 3 seconds and then placed in the GeneAmp® PCR System 9700 (Applied Biosystems Inc., Foster City, CA) thermal cycler for amplification. The Q-TAT PCR cycling parameters are listed in Table 1.

Table 1. Q-TAT PCR cycling parameters.

Hold	30 Cycles			Hold	Hold
98°C	98°C	55°C	72°C	60°C	4°C
2 minutes	10 seconds	1 minute	30 seconds	20 minutes	∞

After PCR amplification was complete, the amplicons (PCR products) were electrophoresed using the ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) so the amount of human DNA could be quantified.

Electrophoresis and DNA Quantification

Separation of DNA fragments and quantitation of fluorescence associated with each fragment was performed using capillary electrophoresis.[20] One µL of amplified PCR products was mixed with 10 µL of formamide containing LIZ 500 internal sizing standards labeled with LIZ® dye (Applied Biosystems Inc., Foster City, CA). The ABI PRISM® 3130xl Genetic Analyzer

(Applied Biosystems Inc., Foster City, CA) was used for electrophoresis and separation of the fluorescently labeled PCR fragments in the samples. The amount of fluorescence emitted from the dye was proportional to the amount of PCR product contained in each unknown sample which, in turn, was proportional to the amount of human DNA template present in the DNA extract. Data collection and genotyping software (GeneMapper® ID ver. 3.2, Applied Biosystems Inc., Foster City, CA) were then used to identify each of the amplicons in the PCR reactions and to quantify the fluorescence associated with each of those amplicons.

Data Analysis

For the determination of DNA recovery for each of the swabs compared in this study, the quantity of fluorescence associated with each Q-TAT amplicon was determined and plotted on a standard curve of fluorescence versus the known input quantity of standardized DNA used to create the curve to estimate the amount of DNA in unknown samples. GeneMapper® ID software (version 3.2, Applied Biosystems Inc., Foster City, CA) was used to analyze the data from the ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). This software provided the relative fluorescent units (total area) associated with the quantity of DNA in each sample. The presence of PCR products produced from the pRL plasmid template in the electropherogram showed that there were no PCR inhibitors in the DNA extract. Therefore, the quantity estimate for unknowns reflected the actual amount of DNA recovered. Fluorescence associated with the positive and negative controls were evaluated to assess the quality of each reaction. The data was then entered into a Microsoft® Excel spread sheet.

Standard Curve

Standard curves were used to quantify the concentrations of DNA recovered from blood stains spotted on the plastic. A standard curve plots relative fluorescence associated with each PCR product amplified with the Q-TAT assay against known amounts of input DNA so that fluorescence in products from samples of unknown DNA concentration can be plotted to estimate DNA amounts. For this study, a working standard curve was generated by averaging fluorescence associated with Q-TAT products from 10 replicate standard curves. The dynamic range of the standard curve ranges is from 37 pg to 1000 pg of input genomic DNA as shown in Table 2. A DNA stock of 100 ng/µL was used to generate the standard curve DNA dilution series. A standard curve was created from a 3-fold dilution series with 4 DNA concentrations.

Table 2. DNA dilution series for the standard curve.

Standard	DNA	Water	DNA Concentration
1	1 µL of undiluted DNA	99 µL	1000 pg
2	10 µL of standard 1	20 µL	333.33 pg
3	10 µL of standard 2	20 µL	111.11 pg
4	10 µL of standard 3	20 µL	37 pg

One µL of each diluted DNA sample and 11.4 µL of master mix were dispensed into each 1.5 mL centrifuge tube. The tubes were vortexed for 3 seconds to mix the contents. The tubes were then centrifuged for 1 minutes at 10,000 rpm and placed in the GeneAmp® PCR System 9700 (Applied Biosystems Inc., Foster City, CA) thermal cycler to be amplified. The PCR cycling parameters follow those described in Table 1. After PCR cycling was complete, amplification products were electrophoresed using the ABI PRISM® 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) as described above.

Statistical Analysis

A standard curve was generated using the peak area fluorescence of each Q-TAT amplification product from each amount of input DNA. The relative fluorescent units (total areas) were proportional to the amount of DNA in the PCR product. An R^2 value shows how closely data points fit the ideal standard curve regression line. Based on literature, an R^2 value greater than 0.9 indicates a good fit of the data points to the standard curve. Figure 4 represents the standard curve created for this study.

Total Human		Slope	132.4141988
Y-Intercept	9529.327316	R Square	0.960830758

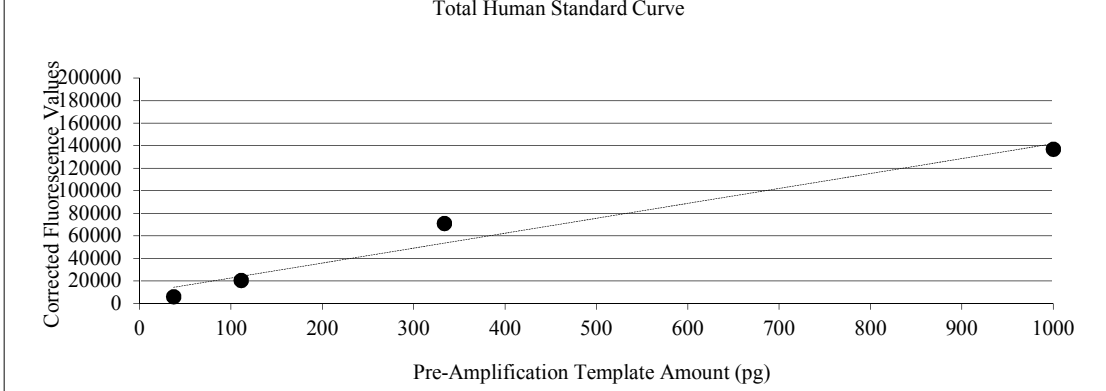

Figure 4. *The standard curve with R^2 value.*

The R^2 value of 0.96 indicates a good fit of the data points to a linear regression and suggests the standard curve will yield reasonably accurate quantity estimates for unknowns. The statistics for the DNA quantities were calculated in Microsoft® Excel based on this standard curve comparison.

The amount of DNA recovered from each swab type was estimated using this standard curve to determine each swab type's ability to recover DNA from plastic. Independent samples t-tests were then used to determine if there were any significant differences between the swab

types. An independent samples t-test compares the means of two independent samples or groups to determine if they are statistically different from each other.[23] A p-value indicates if there is a significant difference in the means between samples that are compared. A significant difference is indicated with a p-value of ≤ 0.05.[23]

CHAPTER IV

RESULTS

The amount of DNA recovered from a crime scene may vary depending on the type of swab used to collect biological evidence from a surface. It is imperative to collect as much sample as possible from crime scene evidence to increase the opportunity of obtaining a probative DNA profile from that evidence. Glass-fiber, cotton-tipped, and polyester-tipped swabs were compared in this study to determine which swab was more effective for recovering DNA from plastic evidence.

Recovery of DNA from glass-fiber and polyester-tipped swabs

Glass-fiber swabs, prepared manually using glass-fiber paper were compared to commercially available polyester-tipped swabs for their ability to recover DNA from bloodstains spotted onto plastic. DNA was extracted from both types of swabs, amplified by PCR, and quantified using the Q-TAT multiplex assay. GeneMapper® ID software and Microsoft® Excel were used to analyze the data. The raw data obtained using glass-fiber and polyester-tipped swabs is shown in Appendix 1. A one-tailed t-test assuming unequal variances was used for this comparison due to the small number of observations (56) used in this comparison and the large variance difference (4.2 fold) between the 2 types of swabs. The mean of total pg recovered for polyester-tipped swabs was about 1,984 pg compared to about 658 total pg recovered for glass-

fiber swabs. A p-value of 0.003 indicated a significance difference in recovery of DNA from glass-fiber versus polyester-tipped swabs. A p-value >0.05 would indicate no significant difference.[23] Based upon the results shown in Table 3, polyester-tipped swabs were determined to be better than glass-fiber swabs at collecting DNA from biological samples spotted onto the plastic Rubbermaid® lid.

Table 3. Comparison of polyester-tipped and glass-fiber swabs.

t-Test: Two-Sample Assuming Unequal Variances		
	Polyester-tipped swabs	*Glass-fiber swabs*
Mean	1984.033333	657.6923077
Variance	5098375.947	1219458.462
Observations	30	26
P(T<=t) one-tail	0.003358768	

A one-tailed t-test was used for each comparison in this study, since all DNA recoveries were either positive or zero, and the sample sizes were small. The mean is the average of DNA recoveries for the number of observations (i.e., biological replicates of the recovery experiment) in the study. The variance is the squared deviation of the DNA recoveries used to calculate the mean. An equal variance was used in each t-test, unless one sample variance was 4 or more times greater than the magnitude of the other sample variance.

Change in Protocol

Each 50 µL blood spot should have contained about 150 ng or 150,000 pg of DNA. Due to the low recovery amounts of DNA observed in the previous swab comparison, protocol changes were made in the volume of elution buffer that was used to elute the bound DNA from the magnetic beads. Assuming that the volume was inadequate to completely elute the bound DNA, the amount of elution buffer was increased from 30 µL to 50 µL. To accommodate this

change, the composition of the Q-TAT PCR master mix was also adjusted in hopes of improving the reproducibility of the experimental results by increasing the amount of DNA quantitated in each assay. Water was removed as a component to the PCR reaction mix, allowing for the addition of 2.75 µL of DNA template instead of just 1 µL. The new master mix, therefore, consisted of 1.25 µL of PCR primer, 7.5 µL of GoTaq® DNA polymerase, and 1 µL of (0.5 pg/µL) pRL plasmid. 9.75 µL of the master mix was added to each PCR tube, along with an increased amount of 2.75 µL of recovered DNA.

There were no noticeable changes in the amount of DNA recovered after increasing the volume of the elution buffer from 30 µL to 50 µL. Therefore, after completing the comparison of glass-fiber and polyester-tipped swabs, an additional protocol change was made. In addition to the increase in elution buffer from 30 µL to 50 µL, the amount of magnetic beads used to capture DNA in the initial extracts was increased. Assuming that perhaps the amount of DNA in an extract exceeded the binding capacity of the magnetic beads, the amount of magnetic beads was increased from 1 µL to 5 µL for the remainder of the comparisons.

During the extraction protocol, tubes were centrifuged for 1 minute at 10,000 rpm to separate the DNA mixture (flow-through) from the swab heads. Some of the DNA extract still remained in the swab heads. In order to collect all of the flow-through during centrifugation, the tube contents of the 1.5 mL centrifuge tubes were placed into new 2.0 mL centrifuge tubes and then centrifuged again for 1 minute at 10,000 rpm. A pipette was used to combine the flow-through contents of the 2.0 mL centrifuge tubes into the 1.5 mL centrifuge tubes. The remainder of the extraction protocol was unchanged.

Completing our comparison of glass-fiber and polyester-tipped swabs, we turned our attention to comparing polyester-tipped and cotton-tipped swabs. Initially, we chose to use

widely available and very typical cotton-tipped swabs attached to a wooden applicator stick. However, we discovered a cotton-tipped swab with a very small tip that we included in the study because of the occasional need for a crime scene investigator to collect evidence from crevices or other small, hard to access areas. The protocol for the small cotton-tipped swabs followed the same protocol as the large cotton-tipped swabs, except the small-cotton tipped swabs were pre-wet with 50 µL of TE^{-4} buffer instead of 100 µL of TE^{-4} buffer used to pre-wet large cotton-tipped swabs. The difference in tip size for the large and small cotton-tipped swabs is shown in Figure 5.

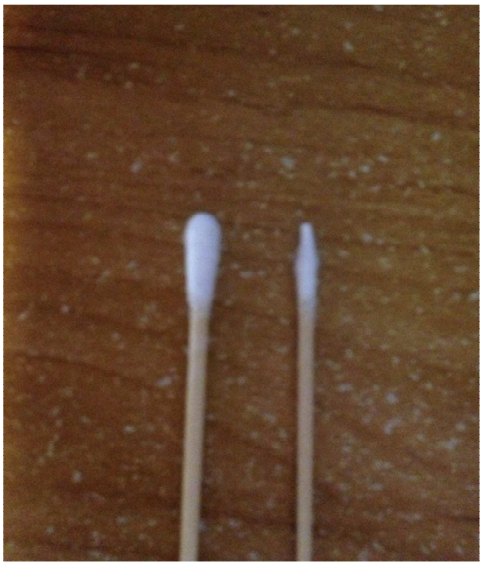

Figure 5. *Side-by-side tip comparison of large and small cotton-tipped swabs.*

Recovery of DNA from polyester-tipped and cotton-tipped swabs

In the preceding sections, a significant difference in the recovery of DNA from blood stains spotted onto plastic using glass-fiber swabs versus polyester-tipped swabs was demonstrated, with polyester-tipped swabs performing significantly better than glass-fiber swabs. In the next comparisons, polyester-tipped swabs were compared to cotton-tipped swabs. The same

protocol was used to prepare the stains, to harvest the stains using the different swabs, and to extract, recover, and quantify DNA collected with the different swabs using the Q-TAT multiplex assay. GeneMapper® ID software and Microsoft® Excel were also used to analyze the data. The raw data used for the comparisons of polyester-tipped and cotton-tipped swabs is shown in Appendix 2. A one-tailed t-test assuming equal variances was used for the comparison, due to the small number of observations (55) used in the comparison and the similar variances (1.6 fold) between the 2 types of swabs. Table 4 shows the results of the statistical analysis used to compare the raw data from the polyester-tipped and cotton-tipped swabs.

Table 4. Comparison of polyester-tipped and cotton-tipped swabs.

t-Test: Two-Sample Assuming Equal Variances		
	Polyester-tipped swabs	*Cotton-tipped swabs*
Mean	8640.227273	13432.57576
Variance	10655015.42	5942123.627
Observations	22	33
P(T<=t) one-tail	3.88172E-08	

The total recovery of DNA from polyester-tipped swabs was about 8,640 pg compared to about 13,433 total pg recovered from cotton-tipped swabs. A p-value of 3.881×10^{-8} indicated a significant difference in the mean recovery of DNA from cotton-tipped versus polyester-tipped swabs. Cotton-tipped swabs recovered more DNA than polyester-tipped swabs from blood samples spotted onto plastic. This conclusion was also supported by raw data in the electropherogram comparison shown in Figures 6 and 7. Both electropherograms show the amplicons produced from the amplification of the amelogenin locus using the Q-TAT multiplex assay. Figure 6 shows the results of the polyester-tipped swabs, and Figure 7 shows the results of the cotton-tipped swabs. The presents of the pRL amplicon in each electropherogram shows that neither cotton-tipped nor polyester-tipped swabs released inhibitors of the Q-TAT reaction.

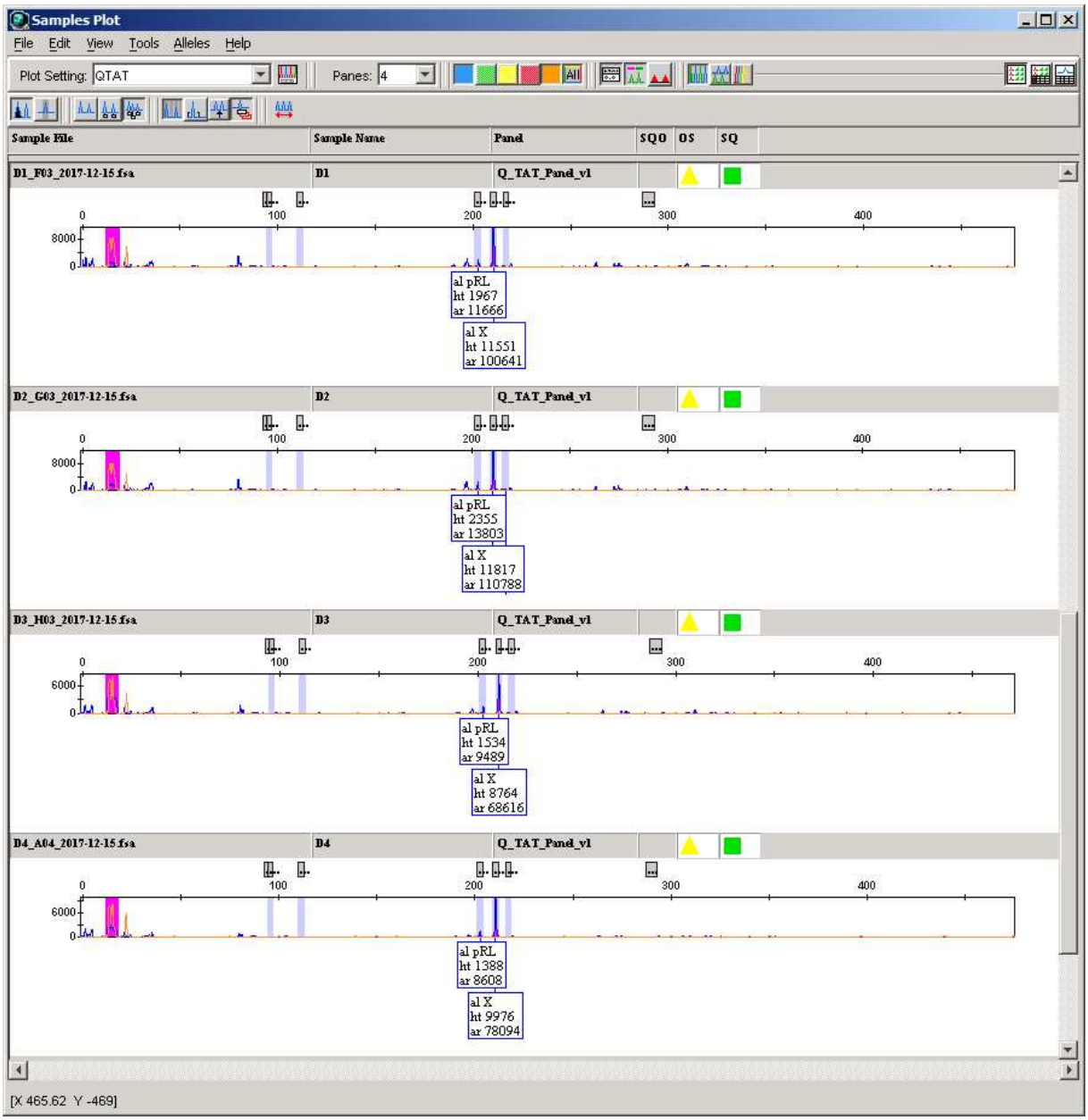

Figure 6. *Electropherogram of Q-TAT amplicons amplified from DNA recovered from polyester-tipped swabs.*

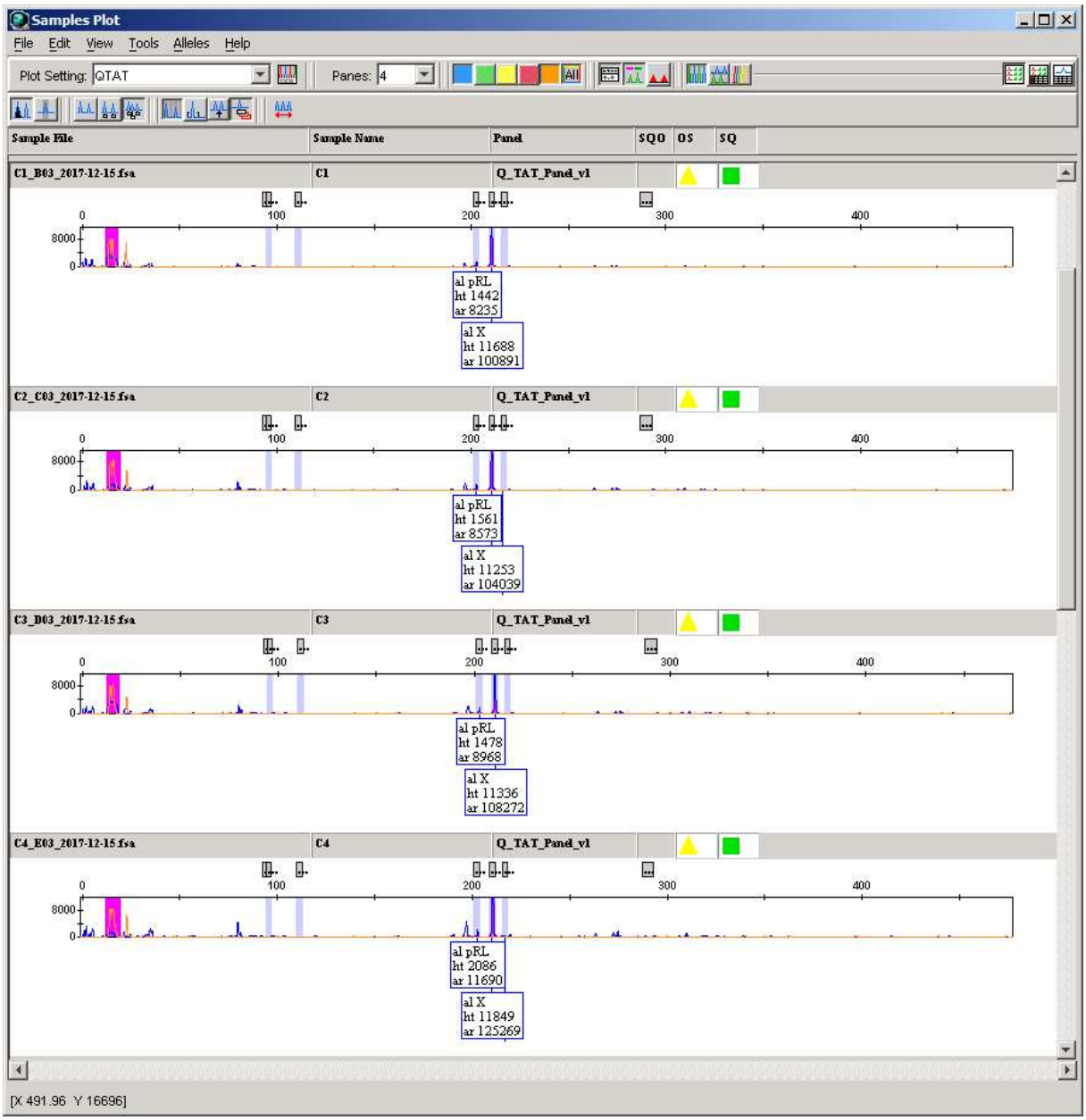

Figure 7. *Electropherogram of Q-TAT amplicons amplified from DNA recovered from cotton-tipped swabs.*

The average of the total areas of fluorescence for the amelogenin locus (labeled al X in the electropherogram) in Figure 6 is 89,535 pg for the polyester-tipped swabs labeled D1-D4. The average of the total areas of fluorescence for the amelogenin locus in Figure 7 is 109,618 pg for

the cotton-tipped swabs labeled C1-C4. Cotton-tipped swabs were shown to have larger and more consistent total peak areas (labeled ar in the electropherogram) than the polyester-tipped swabs.

Recovery of DNA from large and small cotton-tipped swabs

Finally, the recovery of DNA from large and small cotton-tipped swabs were compared. As stated above, the small cotton-tipped swabs could be useful recovering biological evidence present in hard-to-reach areas at a crime scene, so it was important to determine if there is a difference in DNA recovery between the large and small cotton-tipped swabs.

DNA was extracted and then amplified by PCR and quantified using the Q-TAT multiplex assay. GeneMapper® ID software and Microsoft® Excel were used to analyze the data. The raw data for the comparison of small cotton-tipped and large cotton-tipped swabs is shown in Appendix 3. A one-tailed t-test assuming equal variances was used for this comparison due to the small number of observations (33) used in this comparison and the similar variances (1.2 fold) between the 2 types of swabs. The mean DNA recovery for small cotton-tipped swabs was about 12,717 pg compared to about 14,865 total pg recovered for large cotton-tipped swabs. Table 5 shows the results of the statistical analysis used to compare the raw data from the large and small cotton-tipped swabs.

Table 5. Comparison of small and large cotton-tipped swabs.

t-Test: Two-Sample Assuming Equal Variances		
	Small Cotton-tipped swabs	*Large Cotton-tipped swabs*
Mean	12716.59091	14864.54545
Variance	5368160.444	4358272.273
Observations	22	11
P(T<=t) one-tail	0.007240983	

A p-value of 0.007 indicated a significant difference in the mean recovery of DNA from large cotton-tipped versus small cotton-tipped swabs. Large cotton-tipped swabs recovered more DNA from the blood stains on plastic than did the small cotton-tipped swabs. The significant difference may be due to the larger surface area of the large cotton-tipped swabs, which were able to absorb and recover more DNA from the plastic lid.

Based on the data in this study, large cotton-tipped swabs were found to be better for biological sample collection than glass-fiber, polyester-tipped, and small cotton-tipped swabs, at least when recovering DNA from blood stains deposited onto plastic. The final question asked in this study related to the performance of cotton-tipped swabs in the recovery of DNA from other substrates.

Proof of Concept

According to previous comparisons in this study, cotton-tipped swabs were shown to be more effective for recovering DNA from plastic when compared with glass-fiber and polyester-tipped swabs. Based upon the results from blood spotted onto plastic, an investigation was performed comparing the recovery of DNA using the large cotton-tipped swabs versus polyester-tipped swabs from blood stains spotted onto the substrates of wood, tile, and glass. DNA recovery and quantification from all substrates used the same protocols that were used previously in this study. Blood was spotted on ceramic tile, glass microscope slides, the wooden handle of a butcher knife, and on the plastic Rubbermaid® lid used in previous comparisons. Plastic was included as a control group for this set of comparisons. An illustration of these substrates is shown in Figure 8.

Figure 8. *Substrates of wood, tile, and glass.*

Wood

A one-tailed t-test assuming unequal variances was used to compare the collection efficiency of polyester-tipped and cotton-tipped swabs for recovering DNA from wood due to the small number of observations (6) and the large variance difference between the 2 types of swabs. The raw data indicated that cotton-tipped and polyester-tipped swabs collected and recovered about the same amount of DNA from wood. The mean total of recovered DNA was 14,491 pg for polyester-tipped swabs and 14,533 pg for cotton-tipped swabs. A p-value of 0.494 confirmed there was no significance difference in the mean recovery of DNA from polyester-tipped and cotton-tipped swabs.

Tile

A one-tailed t-test assuming unequal variances was also used to compare the collection efficiency of polyester-tipped and cotton-tipped swabs for recovering DNA from tile due to the small number of observations (6) used in this comparison and the large variance difference between the 2 types of swabs. Although the raw data indicated that cotton-tipped swabs collected and recovered slightly more DNA from tile than polyester-tipped swabs, a p-value of 0.115 indicated that a significant level of ≤ 0.05 was not achieved. Therefore, there is no significant difference in the mean recovery of DNA from polyester-tipped and cotton-tipped

swabs. The mean total of recovered DNA was 10,994 pg for polyester-tipped swabs and 14,897 pg for cotton-tipped swabs.

Glass

In the comparison made in the recovery of DNA from blood stains deposited on glass using polyester-tipped and cotton-tipped swabs, a one-tailed t-test assuming equal variances was used due to the small number of observations (6) used in this comparison and the similar variances between the 2 types of swabs. The raw data indicated that cotton-tipped swabs collected and recovered more DNA from glass than polyester-tipped swabs. A p-value of 0.036 indicated there was a significant difference in mean recovery of DNA from glass between the two swab types. The mean recovery of DNA was 14,224 pg for polyester-tipped swabs and 16,903 pg for cotton-tipped swabs.

Plastic

Finally, in the control comparison between polyester-tipped and cotton-tipped swabs for recovering DNA from plastic, a one-tailed t-test assuming unequal variances was used due to the small number of observations (6) and the large variance difference between the 2 types of swabs. The mean recovery of DNA was 10,867 pg for polyester-tipped swabs and 15,679 pg for cotton-tipped swabs. Cotton-tipped swabs, as shown in earlier comparisons in this study, recovered more DNA from blood spotted onto plastic than the polyester-tipped swabs. In this control experiment, a p-value of 0.016 replicates the earlier observations indicating there is a significant difference in the mean DNA recovery of polyester-tipped versus cotton-tipped swabs from the plastic control. Figure 9 shows an electropherogram comparison of the recovery of DNA from plastic between polyester-tipped and cotton-tipped swabs. Note that the total area

of fluorescence for the amelogenin locus suggests a total DNA recovery of 99,951 pg for the polyester-tipped swab labeled PP1 and 122,166 pg for the cotton-tipped swab labeled PC1 indicating that cotton-tipped swabs recovered more DNA from the plastic.

Figure 9. *Electropherogram of Q-TAT amplicons produced using DNA recovered from polyester-tipped and cotton-tipped swabs. (Top to bottom) Positive control, polyester-tipped swab, and cotton-tipped swab electropherograms.*

Cotton-tipped swabs recovered as much or more DNA than polyester-tipped swabs from each of the substrates tested. Compared to the other substrates, the mean recovery of DNA using cotton-tipped swabs to lift blood stains from the plastic substrate was higher than the mean recovery of DNA from wood and tile. However, that was not the case for the glass substrate. The mean recovery of total DNA from the glass substrate, using a cotton-tipped swab, was higher compared to that of the plastic control. This difference may be due to the

smooth surface of the glass substrate compared to the somewhat rough texture of the plastic Rubbermaid® lid. Table 6 shows the comparison of the mean totals of recovered DNA by large cotton-tipped swabs and polyester-tipped swabs from blood stains spotted onto the substrates of wood, tile, glass, and plastic.

Table 6. Comparison of mean totals of recovered DNA from substrates.

Mean Totals of Recovered DNA in pg			
Substrate	**Polyester-tipped Swab**	**Cotton-tipped Swab**	**P-Value**
Wood	14,491	14,533	0.494
Tile	10,994	14,897	0.115
Glass	14,224	16,903	0.036
Plastic (Control)	10,867	15,679	0.016

In summary, the results of this study indicate that cotton-tipped swabs are more effective than polyester-tipped swabs at recovering DNA from both glass and plastic and are at least as effective in recovering DNA from wood and tile.

CHAPTER V

CONCLUSIONS

The purpose of this study was to investigate the effectiveness of different swab-based blood stain collection materials for the recovery of chromosomal DNA suitable for DNA profiling. Included in our investigation of their ability to recover DNA from a plastic substrate were glass-fiber swabs, polyester-tipped swabs, and cotton-tipped swabs. Previous studies from this laboratory have evaluated different DNA collection methods for glass, cloth, and metals,[8-10] however the recovery of DNA from a plastic substrate had not been investigated. Many items made of plastic, such as drinking bottles, flashlights, knife handles, and light switch covers are potentially present at crime scenes. DNA left behind on these items would need to be recovered for analysis in a crime laboratory. For this reason, a sample collection method that maximizes recovery of DNA from plastic needed to be studied. Therefore, plastic was included in this study as a substrate for investigation.

Three studies have compared glass-fiber swabs to other types of swabs for collecting DNA from glass, metal, and cloth.[8-10] The efficiency of the glass-fiber swabs varied among the studies.[8-10] In a study conducted by Tucker, different types of swabs and buffers were compared to determine which combination would work best for recovering DNA from different types of metals.[10] The study compared cotton-tipped swabs, polyester-tipped swabs, glass-fiber swabs,

Scotch tape, and polymerase chain reaction squares. Tucker found that there was no one significant combination that was more efficient than the others for collecting DNA from metal.[10]

In a study conducted by Wilkins, polyester-tipped swabs and glass-fiber swabs were used to collect small quantities of purified DNA spotted onto glass.[8] The results concluded that glass-fiber swabs recovered more DNA from glass when 10 ng was spotted, but did not demonstrate an improved DNA recovery when 5 ng or 2 ng were spotted.[8]

In a study conducted by Burgei, collection methods were compared to determine the recovery of DNA from cloth.[9] This study compared glass-fiber swabs, polyester-tipped swabs, cotton-tipped swabs, a method in which a stain was cut from fabric and processed for DNA extraction, and a tape-lift method. The amount of DNA collected from clothing articles varied. Although polyester-tipped swabs appeared to collect more DNA than cotton-tipped or glass-fiber swabs, the results concluded that the tape-lift and cutting the stain from the fabric methods recovered more DNA from cloth than any of the swab methods.[9]

Glass-fiber swabs were studied to determine if they would adsorb more DNA than the polyester-tipped or cotton-tipped swabs, due to DNA's ability to bind to the silica in glass-fiber material. An increase in the adsorption of DNA to swabs would allow more DNA to be recovered from plastic. This increased amount of DNA collected and then recovered via extraction and amplification from the swabs would increase the chance that a DNA profile would be obtained from the plastic evidence.

During the course of this study, it was determined that glass-fiber swabs were not as efficient as polyester-tipped and cotton-tipped swabs in recovering DNA from plastic. Previous predictions stated that an increase in adsorption of DNA to silica in glass-fiber material may help to increase the amount of DNA that can be recovered with glass-fiber swabs from plastic

evidence, but this was not the conclusion to emerge from this study. The absorption of DNA to silica failed to help the glass-fiber swabs collect and recover more DNA over the polyester-tipped and cotton-tipped swabs.

Using a different type of glue may allow the glass-fiber swabs to be more effective. The glued portion of the swab head was hard and prevented absorption of the blood in that area. Super glue, used in all of the previous glass-fiber studies, created the same problem as the Krazy Glue® used in this study. A thicker swab head may help counteract the unusable glued portion of the swab head. The effectiveness of the glass-fiber swab may also be improved by increasing the volume of magnetic beads, used to capture DNA in the initial extracts, from 1 µL to 5 µL.

Some problems were encountered with the use of the glass-fiber swabs. In several runs, glass-fiber swabs failed to produce any data whatsoever. The data was often more inconsistent for glass-fiber swabs and polyester-tipped swabs compared to the cotton-tipped swabs. Cotton-tipped swabs were the most consistent in providing data. As discussed above, the glued portion of the swab head made part of the swab head unusable. Also, the glass-fiber swab material tended to fall apart during the collection process. The integrity of the swab heads for both the polyester-tipped and cotton-tipped swabs remained intact during the collection process. Lastly, the construction of the glass-fiber swabs was time-consuming. Both polyester-tipped and cotton-tipped swabs were pre-packaged and ready for use. The construction and fragmentation of the glass-fiber heads made the polyester-tipped and cotton-tipped swabs easier to use for collections.

The fragmentation of the glass-fiber swab heads may have contributed to the inconsistencies seen in the data analysis. The results indicate that glass-fiber swabs would not be as effective as cotton-tipped swabs for recovering DNA from plastic. However, glass-fiber

swab recovery of DNA from a smoother surface may result in a more significant outcome. A smoother surface may allow the head of the glass-fiber swab to remain more intact and better able to recover DNA. For practical purposes, time and cost would most likely be considered by law enforcement when choosing a swab for sample collection purposes. The construction of the glass-fiber swabs would be time-consuming and cost more to make, but if the glass-fiber swab proved to be efficient then the idea could be sold to a manufacturer. It is also possible that the glass-fiber filter paper used in the study by Wilkins may have had a different composition than the glass-fiber filter paper used here, inasmuch as the paper used in the earlier study was very old and its formulation may have changed with the replacement material available today and used in this study.

Polyester-tipped and cotton-tipped swabs were chosen for comparison with glass-fiber swabs because of their popularity for biological sample collection among forensic laboratories. Previous studies indicate that cotton-tipped swabs are the least expensive and most commonly used swabs for collecting biological samples.[3, 4] However, according to Puritan® Medical Products, polyester-tipped swabs have better collection and release properties for DNA.[5] Normally, pre-packaged polyester-tipped swabs are used in the Forensic Science DNA Clinical Laboratory at OSU-CHS. About 150,000 pg of DNA was spotted on the plastic Rubbermaid® lid. None of the swabs were able to recover all of the DNA that was spotted. Cotton-tipped swabs recovered the most DNA compared to polyester-tipped and glass-fiber swabs. Further study is needed to determine why only a portion of the spotted DNA was recovered.

Comparisons of the effectiveness of recovering genomic DNA using polyester-tipped and cotton-tipped swabs were significantly different. Cotton-tipped swabs were more effective than polyester-tipped swabs at recovering DNA from blood spotted onto the plastic Rubbermaid® lid.

Further comparisons also showed cotton-tipped swabs to be more effective at collecting blood and recovering DNA from glass. The lack of significant results in the comparison made for recovering DNA from tile was surprising. The ceramic tile surface was slightly pocked. This irregular or slightly rough surface of the tile was similar to the slightly rough surface of the plastic lid. For this reason, it was expected that cotton-tipped swabs would be more effective than polyester-tipped swabs at recovering DNA from tile. Even though the p-value was above 0.05 indicating there was no significant difference in DNA recovery, the raw data indicated that cotton-tipped swabs recovered slightly more DNA from tile than polyester-tipped swabs. When it is recalled that the number of comparisons between polyester-tipped and cotton-tipped swabs was small, it may be that more repetitions of this experiment could lower the p-value into the range of significance. On the other hand, the lack of significant results for the swab comparison from wood was not unexpected. Both types of swabs collected and recovered about the same amount of DNA from wood. The finished wooden handle of the knife seemed to "soak up" or absorb some of the spotted blood. This absorption of blood may have affected the amount of dried blood available for collection from both types of swabs.

In a study conducted by Verdon et al., different types of swabs were examined in the collection of biological fluids from different substrates.[7] Nylon-flocked, rayon, polyester, cotton, and foam swabs were used to recover DNA from pitted plastic, glass, brick, and wood. In this study, the different types of substrates had an effect on the ability of the swab to collect DNA.[7] The MWE polyester swab was concluded to be the best swab for sampling from pitted plastic, the Puritan® FABSwab (cotton) was the best for glass, the MWE rayon swab was the best for brick, and the Puritan® 1 PF foam was the best for wood.[7] As shown similarly in my study, cotton-tipped swabs recovered more biological fluids from glass. On the other hand, polyester-tipped swabs, not cotton-tipped swabs, were shown to be the best swab for sampling from

pitted plastic in Verdon's study.[7] The difference in results could be due to the different types of plastics used in the two studies. In Verdon's study, foam-tipped swabs recovered more biological fluids from wood.[7] Although I did not use foam-tipped swabs in my study, both cotton and polyester-tipped swabs recovered about the same amount of DNA from wood.

According to the comparison of large and small cotton-tipped swabs, size does matter. Recovery of DNA was less when using the smaller cotton-tipped swabs. The smaller surface area on the small swab may have hindered the swabs ability to absorb as much of the blood stain as the larger surface area on the larger swab. When the head of the swab became saturated, I noticed that the small cotton-tipped swabs were unable to absorb all of the blood from the plastic tray. This limitation in absorption caused residual blood stain and, hence, DNA to be left behind on the plastic tray. The larger cotton-tipped swabs were able to collect all of the blood from the plastic tray. The difference in absorption abilities between the two swabs made a significant difference in the recovery results. Therefore, size of the swab heads does appear to make a difference in DNA recovery from cotton-tipped swabs. On the other hand, this was not the case between the small cotton-tipped swabs and the polyester-tipped swabs.

The raw data for the comparison of small-cotton tipped swabs and polyester-tipped swabs is shown in Appendix 4. A one-tailed t-test assuming equal variances was used to compare small-cotton tipped swabs to polyester-tipped swabs due to the small number of observations (44) used in this comparison and the similar variances (2 fold) between the 2 types of swabs. The mean DNA recovery for small cotton-tipped swabs was about 12,717 pg compared to about 8,640 pg for polyester-tipped swabs. Table 7 shows the results of the statistical analysis used to compare the raw data from the small cotton-tipped swabs and polyester-tipped swabs.

Table 7. Comparison of polyester-tipped and small cotton-tipped swabs.

t-Test: Two-Sample Assuming Equal Variances		
	Polyester-tipped swabs	*Small Cotton-tipped swabs*
Mean	8640.227273	12716.59091
Variance	10655015.42	5368160.444
Observations	22	22
P(T<=t) one-tail	1.09608E-05	

A p-value of 1.096×10^{-5} indicated a significant difference in the mean recovery of DNA from polyester-tipped versus small cotton-tipped swabs. Therefore, even small cotton-tipped swabs recovered more DNA from blood spotted onto the plastic lid than the polyester-tipped swabs.

Protocol changes were made after observing the low recovery amounts of DNA obtained from the comparisons made between glass-fiber swabs and polyester-tipped swabs. The low DNA recovery amounts prompted a change in the experimental protocol to increase the volume of elution buffer used to elute DNA from the magnetic beads. To assure complete elution of bound DNA, the amount of elution buffer was increased from 30 µL to 50 µL. To compensate for the higher volume of elution buffer, a higher volume of purified DNA was added to Q-TAT reactions. For the comparisons made between polyester-tipped and cotton-tipped swabs, the amount of magnetic beads added to the DNA extract to bind greater amounts of DNA was increased from 1 µL to 5 µL, in addition to the elution buffer increase. In order to collect all of the initial DNA extract from the swab heads during centrifugation, the tube contents were centrifuged again for 1 minute at 10,000 rpm. Changes made to this protocol created a large-four-fold increase in the amount of DNA recovered from swabs used in the remaining comparisons.

Future research on glass-fiber swabs should include the protocol changes mentioned above. An increase in the volume of magnetic beads and elution buffer may increase the recovery of DNA for glass-fiber swabs. Also, glass-fiber swabs may be more effective in recovering DNA from smoother surfaces. Different types of plastic could be used to determine if the smoothness of the surface makes a difference.

Future research on polyester-tipped and cotton-tipped swabs could include DNA collection using polyester-tipped swabs on polyester fabric and cotton-tipped swabs on cotton fabric. Each swab may be more effective at recovering DNA from fabric that is made of the same material that the swab head is made of. Both types of swabs could also be tested on a cotton/polyester mixed or blended cloth. Collecting biological samples from the same type of material that the swab head is made of may increase the amount of DNA recovered due to the lack of competition between the types of materials.

In conclusion, glass-fiber swabs, polyester-tipped swabs, and cotton-tipped swabs do differ in their respective effectiveness in recovering DNA from blood stains spotted and dried onto different substrates. In the final analysis, cotton-tipped swabs were more effective than glass-fiber swabs and polyester-tipped swabs for recovering DNA from plastic. Cotton-tipped swabs recovered more DNA from glass than polyester-tipped swabs and were equal, compared to polyester-tipped swabs, in recovering DNA from tile and wood.

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APPENDICES

APPENDIX 1

Comparison of total DNA and pRL raw data amounts for polyester-tipped swabs and glass-fiber swabs.

Polyester-tipped Swabs				Glass-Fiber Swabs			
Total recovered DNA (pg)	pRL (RFU)	Total recovered DNA (pg)	pRL (RFU)	Total recovered DNA (pg)	pRL (RFU)	Total recovered DNA (pg)	pRL (RFU)
930	1531	510	238	3660	3644	0	3374
0	2227	1410	970	1050	1514	0	2940
420	2891	3691	1237	0	2728	0	787
4950	3125	4091	1908	780	2561	450	1982
1980	3813	0	1918	1710	2516	0	2274
6090	2237	0	2607	0	2879	2340	2302
8010	1296	672.5	2390	2970	3399	0	1167
6420	3848	690	1231	2730	4444	0	2142
1890	3207	435	1157	0	4335	0	2260
3990	4038	0	1575	1320	4104	0	2898
4470	4579	1325	2181	90	6056	0	1943
3930	4333	945	831	0	6073		
0	987	36.5	1440	0	6289		
1350	1395	0	1184	0	2152		
450	1195	835	1955	0	5385		
1984.03	Average of Total Recovered DNA			657.69	Average of Total Recovered DNA		
2257.96	Standard Deviation Recovered DNA			1104.29	Standard Deviation Recovered DNA		

APPENDIX 2

Comparison of total DNA and pRL raw data amounts for polyester-tipped swabs and cotton-tipped swabs.

Polyester-tipped Swabs				Cotton-tipped Swabs			
Total recovered DNA (pg)	pRL (RFU)			Total recovered DNA (pg)	pRL (RFU)		
8220	2991			11145	1864		
4525	1657			16620	2165		
13725	2423			8875	2496		
12655	2173			16455	1918		
6565	1445			13420	1497		
9055	3726			7745	662		
4945	1148			13925	1184		
4545	1468			14800	1370		
4180	975			12565	2053		
4925	901			13600	2219		
5820	704			12925	1057		
8125	1052			9235	1160		
12945	2296			13090	1321		
7310	1390			14710	1905		
12510	1967			15165	1928		
13910	2355			12725	1394		
8110	1534			13925	1126		
9420	1388			12510	888		
7980	1248			11365	606		
6945	1400			12710	1298		
12145	1407			12510	1125		
11525	1848			9745	883		
				11875	1060		
				15000	2015		
				12545	1442		
				12980	1561		
				13565	1478		
				15890	2086		
				13980	842		
				15545	1267		
				16945	1483		
				16475	1791		
				18710	2380		
8640.23	Average of Total Recovered DNA			13432.58	Average of Total Recovered DNA		
3264.2	Standard Deviation Recovered DNA			2437.65	Standard Deviation Recovered DNA		

APPENDIX 3

Comparison of total DNA and pRL raw data amounts for small cotton-tipped swabs and large cotton-tipped swabs.

Small Cotton-tipped Swabs				**Large Cotton-tipped Swabs**			
Total recovered DNA (pg)	pRL (RFU)			Total recovered DNA (pg)	pRL (RFU)		
11145	1864			11875	1060		
16620	2165			15000	2015		
8875	2496			12545	1442		
16455	1918			12980	1561		
13420	1497			13565	1478		
7745	662			15890	2086		
13925	1184			13980	842		
14800	1370			15545	1267		
12565	2053			16945	1483		
13600	2219			16475	1791		
12925	1057			18710	2380		
9235	1160						
13090	1321						
14710	1905						
15165	1928						
12725	1394						
13925	1126						
12510	888						
11365	606						
12710	1298						
12510	1125						
9745	883						
12716.59	Average of Total Recovered DNA			14864.55	Average of Total Recovered DNA		
2316.93	Standard Deviation Recovered DNA			2087.65	Standard Deviation Recovered DNA		

APPENDIX 4

Comparison of total DNA and pRL raw data amounts for small cotton-tipped swabs and polyester-tipped swabs.

Small Cotton-tipped Swabs					**Polyester-tipped Swabs**			
Total recovered DNA (pg)	pRL (RFU)				Total recovered DNA (pg)	pRL (RFU)		
11145	1864				8220	2991		
16620	2165				4525	1657		
8875	2496				13725	2423		
16455	1918				12655	2173		
13420	1497				6565	1445		
7745	662				9055	3726		
13925	1184				4945	1148		
14800	1370				4545	1468		
12565	2053				4180	975		
13600	2219				4925	901		
12925	1057				5820	704		
9235	1160				8125	1052		
13090	1321				12945	2296		
14710	1905				7310	1390		
15165	1928				12510	1967		
12725	1394				13910	2355		
13925	1126				8110	1534		
12510	888				9420	1388		
11365	606				7980	1248		
12710	1298				6945	1400		
12510	1125				12145	1407		
9745	883				11525	1848		
12716.59	Average of Total Recovered DNA				8640.23	Average of Total Recovered DNA		
2316.93	Standard Deviation Recovered DNA				3264.2	Standard Deviation Recovered DNA		

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