The Development and Use of Mock Fingerprints in the Analysis of Touch DNA Evidence

BY

Jennifer Waranauskas
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THESIS

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Defense Committee:

Dr. Ashley Hall, Chair and Advisor, Biopharmaceutical Sciences
Dr. Karl Larsen, Biopharmaceutical Sciences
Dr. William Beck, Biopharmaceutical Sciences

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LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
LCN	Low copy number
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
SDS	Sodium dodecyl sulfate
STR	Short tandem repeat

SUMMARY

Touch DNA can be described as DNA left behind on an item or surface due to a person coming into contact with the object. During this time of contact, skin cells containing DNA are deposited and can be collected for analysis. These types of samples do not contain a large amount of DNA and this can lead to problems in downstream DNA analysis. In order to better represent touch DNA evidence we have developed mock fingerprints that mimic an eccrine fingerprint to be used as positive controls in touch DNA experiments. A unique feature of these mock fingerprints is that a known quantity of DNA can be added to them, and therefore variability is reduced and the percent recovery after DNA extraction for each sample can be determined. This was utilized in the optimization of collection and extraction protocols, which were then used in subsequent experiments.

In order to track the loss of DNA throughout the collection and extraction process, we deposited mock fingerprints directly into extraction tubes, onto swabs, and onto glass slides. Samples in which the mock fingerprints were pipetted directly into the tube demonstrated how much DNA is lost due to extraction. The amount of DNA left on the swab was exhibited by the mock fingerprints pipetted onto swabs. Finally, the amount of DNA lost during the entire collection and extraction process was demonstrated using mock fingerprints pipetted and dried on class slides. This allowed the percent loss at each step to be evaluated and demonstrated which steps may need to be optimized further in order to maximize the amount of DNA recovered from touch DNA evidence.

Finally, we also used the mock fingerprints, containing known quantities of DNA, to generate a standard curve for comparison with true fingerprints. After adjusting the upper and lower limits of the range for the standard curve, we plotted true fingerprints on the curve. True

SUMMARY (continued)

fingerprints deposited on various surfaces, which included a countertop, phone case (plastic), glass, and tile. The true fingerprints were plotted on the standard curve and the quantity of DNA deposited was interpolated. All of the true fingerprints collected from various surfaces fell within the range of the standard curve and the amount of DNA deposited was interpolated. This result provided information about the nature of true fingerprints and the initial quantity of DNA in a fingerprint.

While depositing true fingerprints onto surfaces or items is effective for research experiments, true fingerprints are variable and the initial quantity of DNA in the sample is unknown. For this reason, we developed mock fingerprints that contained a known quantity of DNA. Using mock fingerprint samples is a novel method for conducting touch DNA research. In this project, the mock fingerprints were effectively used as positive controls and therefore it is recommended that they be utilized in future experiments.

Chapter I: Literature Review

Introduction

      The purpose of this project was to develop mock fingerprints that can be used as positive controls in touch DNA experiments. Through experimentation, we tested the hypothesis that the mock fingerprints could be generated and used as positive controls in touch DNA experiments. Mock fingerprints will be a useful research tool for the field of forensic science because they serve two purposes. One purpose is to reveal information about the nature of a true fingerprint. Since the mock fingerprints contain a known quantity of DNA, they can be used to learn about the initial quantity of DNA in a true fingerprint. When a person deposits a fingerprint on a surface, the amount of DNA in that fingerprint is unknown. The amount of DNA recovered after collection and extraction is not equivalent to the initial quantity of DNA deposited because there is an inevitable amount of DNA lost during the process[1, 2, 3, 4]. The second purpose of the mock fingerprints is to gather information regarding the efficiency of collection and extraction protocols. As mentioned previously, DNA will be lost during the collection and extraction process but being able to measure how much DNA is lost at each step will help to minimize this loss. Mock fingerprints are well suited for this because they contain a known quantity of DNA and therefore it is easier to quantify the amount of DNA lost throughout the process. Using the mock fingerprints in this way will highlight areas of the collection and extraction protocols that need to be optimized so that the maximum amount of DNA is recovered.

      In this project, we used mock fingerprints as positive controls to quantify the amount of DNA deposited in true fingerprints and track the loss of DNA during the collection and extraction process. In order to be representative of true fingerprints, the mock fingerprints contained components such as salts, water and epithelial cells[5, 6]. The cells were counted in order to add a specific quantity of DNA to the mock fingerprint samples. Since a known quantity of

DNA is present in a single cell, the initial amount of DNA present in each sample is known and therefore allowed the percent recovery to be calculated. The values for percent recovery were used to compare various modifications to collection and extraction protocols and establish the most efficient protocols for further experimentation. Once the protocols were optimized, a standard curve for mock fingerprints on glass slides was generated. Using the mock fingerprints as standards, the total DNA extracted and DNA added were plotted against each other to generate the standard curve. This standard curve revealed where true fingerprints lie on the curve in relation to the mock fingerprints, and gave a better understanding of the nature of true fingerprints because the amount of DNA deposited in the fingerprints could be estimated. The use of mock fingerprints also allowed the collection and extraction steps to be evaluated and determine where DNA is lost during this process. We pipetted mock fingerprint samples directly into extraction tubes, onto swabs and onto glass slides in order to isolate each step within the collection and extraction process. By isolating each part, the steps could be evaluated individually and indicate which areas may need to be optimized further.

In order to understand the impact mock fingerprints will have on research in the field of forensic science, it is important to review the physiology of touch DNA samples as well as the various aspects of DNA analysis. Touch DNA is becoming more prevalent in forensic laboratories, and therefore it is necessary to conduct research on these types of samples so that they are better understood by researchers.

The Structure and Power of DNA

DNA is a powerful tool in the field of forensic science due to its high power of discrimination. Since each human being, besides identical twins, has a unique DNA sequence

that can be analyzed, forensic analysts can use this information to link a person to a crime scene or eliminate them as a suspect. The actual sequence of DNA being analyzed is a series of nucleotides that pair with one another to form the DNA double helix. This structure was discovered by Watson and Crick[7]. Nucleotides consist of three components: a phosphate group, a sugar molecule and a nitrogenous base[8]. The phosphate group and sugar molecule are connected through a phosphodiester bond in a 5' to 3' direction and make up the phosphate sugar backbone of DNA[8]. There are four nitrogenous bases in DNA, which include adenine, thymine, guanine, and cytosine. These bases bind to the sugar molecule and pair with another nucleotide to make DNA double stranded[8]. The pairings for the bases are as follows: adenine with thymine and guanine with cytosine. These sequences of paired nucleotides are unique and are the basis for the genetic code[8]. In forensic science, these sequences of bases are analyzed through STR profiling and capillary electrophoresis [9]. The short tandem repeats, or STRs, used for forensic analysis are found in noncoding regions of the genome, and are characterized by 2-5 base pair repeating sequences in arrays ranging from approximately 100 – 500 base pairs [10]. At each locus two alleles have been inherited; one from the mother and the other from the father. These alleles contain the short repeating sequences that can be counted to determine what alleles a person has at that locus[11]. For example, if a person has one allele that contains 11 repeats of the same sequence and 12 repeats of the sequence for the other allele, they are considered to be an 11, 12 at that specific locus. When this type of examination of alleles is conducted on multiple loci, the combination of each locus is the STR profile [8, 10]. Since an individual's STR profile is unique, it provides valuable information to forensic scientists because it can help match an individual to an unknown profile.

Clearly, DNA is a powerful tool in forensic science, but understanding the sources of DNA is also critical. There are various body fluids that contain DNA, which can be collected in order to extract the DNA from them. The concentration of DNA varies for each body fluid but some of the common sources include blood, semen, and saliva [12, 13]. Some or all of these body fluids are likely to be present at a crime scene and therefore can be collected for analysis. Another source of DNA is human skin, which can shed epithelial cells and result in DNA being left behind on an object or surface [14, 15]. This idea of "touch DNA" will be discussed later. There are numerous aspects to DNA analysis that can be investigated, but this project will focus on touch DNA samples and how they should be collected and extracted.

Touch DNA in the Field of Forensic Science

Touch DNA has become a widely used term in the field of forensic science, and as knowledge on this topic continues to expand it is important to understand why this type of evidence has become an integral part of forensic investigations. The term "touch DNA" has been described as shed epithelial cells from a person that came into contact with an object [16]. These epithelial cells contain DNA that can be extracted and analyzed. This idea of touch DNA is supported by Locard's Principal, which states that an exchange of material will occur when two objects come into contact [17]. This material may include fibers, blood and, in the case of touch DNA, epithelial cells. Although these samples contain a small amount of DNA, consistent results can be obtained using as little as 100 pg of purified DNA and a validated STR multiplex [14]. Research has shown that this type of DNA can be recovered from various types of evidence such as firearms [18]; weapon handles [19] and clothing [20]. Testing items such as these can give indications regarding who may have handled the weapon or wore the article of clothing when the crime was

committed, but not necessarily who was in possession of the item last [21, 22]. This information can be used as an investigative lead and support the theory of a case in court, but it cannot be used to confirm that a person used the item to commit the crime. For example, the DNA profile of a man may be found on the handle of a knife used in a murder. While the presence of his DNA says that he did handle the knife at some point, it does not confirm he used it in the murder; he may have used to prepare food during a previous occasion.

Physiology of Fingerprints

While all types of evidence can contain critical information to a case, fingerprints can provide two modes of identification. One is through the examination of the ridge details such as bifurcations, ending ridges and dots [23]. By comparing the questioned print with a reference print, an expert latent print examiner may be able to make an identification. For many years this was the only way fingerprints were helping investigators identify suspects, but currently fingerprints can also be used for DNA analysis [15]. This can be particularly valuable in cases where latent prints that are not suitable for fingerprint examination can be submitted for DNA analysis.

In humans, the skin is the largest organ in the body and it contains multiple layers. As the skin cells mature they travel through these layers until they reach the outermost epidermal layer[24]. From the epidermal layer, dead epithelial cells are shed and can be deposited onto items that a person comes into contact with or touches. These cells contain DNA that can be extracted and used for forensic analysis. This deposition of cells can occur from any part of the body including the fingertips.

Along with shed epithelial cells, fingerprints contain eccrine sweat which is secreted through eccrine glands. Two types of sweat glands are found in the human body: eccrine and apocrine glands. Although both of these glands secrete sweat, they differ in several ways. First, apocrine glands secrete sweat through the canal of the hair follicle and therefore are only found in areas of the body that have hair such as armpits, scalp and genital areas [25]. Apocrine glands are activated by stress or excitement after puberty, and secrete fatty acids, sulfanyl alkanols and odiferous steroids that are consumed by bacteria on the surface of the skin [26]. In contrast, eccrine glands are all over the body. They are responsible for maintaining body temperature and secreting sweat comprised mostly of water through the pores of the skin [24]. Although a large portion of eccrine sweat is water it also contains some organic and inorganic electrolytes such as sodium, potassium and lactic acid [27]. There are two components of a fingerprint: the water soluble component and the water insoluble component, which includes fats, lipids, microbial DNA and epithelial cells [5, 6, 28]. These epithelial cells are dead cells shed from the outer layer of the skin and are deposited along with the eccrine sweat. The fingerprint solution we have used in this project was designed to include some of the components of the eccrine fingerprint as well as epithelial cells to make the mock fingerprints representative of a true eccrine fingerprint. We also quantified the total DNA extracted throughout the project in order to account for the presence of microbial DNA in true fingerprints. Although these components are present in most latent prints, it has been found that the composition varies between age groups [6]. Variations in the composition of a latent print can also be due to the environment, which includes factors such as air, light, temperature and humidity [29, 30, 31]. For example, the squalene levels in the prints decrease faster in

light compared to being in the dark [32]. Other research has been conducted in relation to the effects of temperature, which revealed that water levels in latent prints decreases as the storage temperature increases[29]. Since eccrine sweat is comprised of mostly water, this factor will also affect the levels of the water soluble components in the fingerprints. Despite the variability in composition, eccrine sweat is present in most latent prints and plays an important role in the formation of a fingerprint because it is part of the residue left behind on the surface.

Regarding the mock fingerprints that will be developed for this project, a fingerprint solution that mimics eccrine sweat will be added to each sample. This fingerprint solution will need to contain specific components that are known to be present in an eccrine fingerprint. Studies that have focused on determining the composition of eccrine fingerprints have found that they are of 98% water with the remaining portion including urea, inorganic salts, and organic acids [5, 33]. Keeping this in mind, the fingerprint solution will be designed to contain some of these compounds. Other studies have also found that lipids and fats can be present in true fingerprints, but that this component is likely from normal human activity such as touching of the face or hair [6, 32]. Therefore, the lipids and fats present in the true fingerprints are not being secreted by the eccrine glands, but are being deposited onto the fingertip by other activity. Since the purpose of this project is to design mock fingerprints to mimic an eccrine fingerprint, lipids and fats will not be included in the fingerprint solution.

Limitations of Low Level DNA Samples

Although we did not test mock fingerprints with STR profiling in this project, it is important to understand the impact low level DNA samples can have on the work conducted by crime laboratories. The goal for forensic scientists conducting DNA analysis is to obtain a complete STR profile from the evidence, but utilizing samples that contain low quantities of DNA may hinder this process. This is because the amount of DNA present may be insufficient for successful analysis, which could lead to inaccurate or incomplete results. Since it is important that DNA analysts obtain a complete STR profile during their analysis, it is necessary to conduct research on these types of samples to help forensic scientists achieve this goal. Low-level DNA samples may have initially contained large quantities of DNA, but due to the environment or other conditions the DNA has become degraded [34]. Touch DNA samples also fall into the category of low-level DNA samples because they contain less than 100 pg of DNA[35, 36, 37, 38]. Even though polymerase chain reaction (PCR) can be utilized to make many copies of the DNA present in the sample, there are still instances where the quantity of DNA is too low. In cases involving degraded DNA, the DNA is fragmented and may prevent the PCR primers from binding to the template DNA [39]. This would result in fewer copies of the DNA being made during PCR and could cause problems in downstream analysis such as STR profiling.

Successful STR analysis with low-copy DNA has been performed [40], yet there are some issues that arise when using small amounts of DNA. For instance, a profile may exhibit allelic drop in or drop out. Drop in alleles may be due to sporadic contamination [11] while drop out alleles are a result of stochastic events during PCR [41]. Since there is

such a small sample of DNA, the primers for a given locus may be unable to bind

properly or not at all which causes the allele to drop out. One issue regarding allelic drop

out is that it may result in a false homozygote [42]. In this scenario, one of the alleles would

not be present in the profile leaving only one interpretable allele at the locus, giving the

impression that the donor is a homozygote at that location. This would alter the allele

calls at the locus and yield a false profile that does not match the actual donor of the

sample.

Another characteristic seen with low-copy DNA samples is excessive stutter,

which is caused by misaligning PCR primers. The primers used in PCR anneal, or bind,

to single stranded DNA at specific locations so that a designated region of DNA is

duplicated. These areas contain the short tandem repeat sequences mentioned earlier.

When primers misalign in these regions, the result is a copy of the DNA with either one

repeat longer or shorter than the true DNA fragment[43]. When many copies of this

incorrect fragment are made during PCR, they are visible on an STR profile either before

or after the true allele peak[44]. This is an artifact of STR profiling, in which a small peak is

visible next to the parent peak. Usually a stutter peak can be ignored because it is a

known artifact, but when it is greater than 20% of the parent peak it may appear to be a

callable allele. If the stutter peak is called as an actual allele then the STR profile would

be inaccurate and not demonstrate the true profile of the donor. Stutter can also cause

problems when analyzing a mixture because it can be difficult to determine if the peak is

due to stutter or if it is a minor allele [38]. Finally, heterozygote peak imbalance can be

observed when using low-copy DNA. In the optimal scenario if a person is heterozygous

at a locus, the two peaks representing their two alleles will be equal in size. This is not

the case when heterozygote peak imbalance is observed, because one of the allele peaks at the given locus is much larger than the peak for the other allele. Although this stochastic event can occur with any DNA sample, it is more commonly seen in LCN DNA because the low molecular weight allele is preferentially amplified during PCR [45]. Each of the potential problems described are likely to affect low level DNA samples. It is important to conduct research on low level samples in order to better understand these issues. Since the goal of this project was to develop and validate the mock fingerprints, problems seen in STR profiles were not be tested, but will definitely be the focus of future experiments because STR profiling is a significant aspect of DNA analysis. In terms of this project, we have optimized protocols to maximize DNA recovery. Forensic researchers and analysts should always strive to use methods that maximize the amount of DNA obtained because it limits potential issues observed in the STR profile. As the use of touch DNA becomes more prominent in forensic laboratories, it is crucial to work towards developing efficient protocols so that the risk of encountering these problems is minimized.

Creation of Mock Fingerprints

Positive Controls

One of the critical components to determining the efficiency of an experiment or protocol is the use of a positive control. Positive control samples are a necessity for any experiment because they serve as a sample that gives a known result [46]. Since the goal in most experiments is to change one variable at a time, the control sample or group gives information about what will happen without any experimental changes. Positive control

samples are also included throughout the process of analysis in order to confirm that the test worked successfully [47]. For example, a buccal swab may be extracted with unknown samples and then quantified to demonstrate that the reagents used during the extraction are working properly. Since it is known that the buccal swab contains DNA, a positive control sample yielding no DNA reveals a problem during the extraction. By including positive controls during each step of analysis, it proves that the experiment is working properly and means that the results obtained from the analysis can be trusted.

Touch DNA research has been conducted using different methods for mimicking a touch DNA sample. One way is to obtain blood and saliva standards from donors. The DNA from these blood or saliva samples was extracted and quantified so that the initial amount of DNA in the sample was known, and then the samples were diluted to mimic low level DNA[48]. While this is an effective method for knowing the initial amount of DNA in the samples, it is does not completely represent true fingerprints. The mock fingerprints developed in this project will have a known quantity of DNA, but will also contain a fingerprint solution to make the sample more representative of a touch DNA sample compared to a diluted saliva or blood sample. Another method for creating touch DNA samples in research is to have donors deposit fingerprints onto surfaces or to handle objects for a certain amount of time[22, 49, 50]. While this method effectively deposits true touch DNA samples, it does not allow for the initial quantity of DNA to be known. Since the quantity of DNA in these samples varies between individuals, it is critical to control for this variability. Again, the mock fingerprints will resolve this issue because the amount of DNA put into the sample is controlled by the researcher and can be replicated

in multiple samples using the protocol we developed for collecting and counting epithelial cells.

In the present project, mock fingerprints were developed to serve as a positive control for touch DNA samples. The mock fingerprints are considered positive controls because they should limit the variability seen in true fingerprints and should provide a known result that can be compared to unknown samples. These two factors are accounted for in the mock fingerprints because a known quantity of DNA will be added to each sample. This eliminates variability because the same amount of DNA can be put into multiple samples and any changes in DNA recovery will be due to the modification being tested, not the variability of the sample. By controlling for variability, the results for experiments with mock fingerprints are known, and therefore can be used for comparison to true fingerprints. With touch DNA on the rise, it is crucial to use positive controls that represent the type of evidence being analyzed. For this reason, mock fingerprints were developed and used throughout this project.

Cell Counting

An important aspect to the mock fingerprints is that they contain a specific and known quantity of cells. This eliminates variability that is seen in true fingerprints and allows for the percent recovery after extraction to be analyzed for each sample. In order to add in a specific number of cells into the mock fingerprints, a cell suspension must be generated and the cells counted to determine the concentration of the suspension. A common method for counting cells is using a Neubauer chamber or hemocytometer [51]. Cell counting has many applications such as performing blood cell counts, sperm cell

counts and processing samples for cell culture [52, 53, 54, 55], but in this experiment cell counting served to determine the concentration of the cell suspension. Since each square on the hemocytometer has a known volume, the concentration can be calculated after counting the cells [53]. This is an inexpensive method for cell counting which makes it desirable for use in research laboratories.

This manual method of counting cells is effective, but modern technology has advanced to include automatic cell counters. These instruments have been validated and proven as effective as the manual counting method [56, 57, 58]. Automated cell counters reduce the variability between cell counts obtained from different researchers. It also allows results to be reproduced reliably in other labs, and it reduces the time it takes to count the cells in the sample. All of these factors contribute to the reason why automated cell counters are a desirable research tool. In this project both the manual and automated cell counting methods will be tested and evaluated for their use in generating the mock fingerprints.

Efficiency of Protocols

Forensic scientists strive to use the most efficient protocols for the analysis of samples to ensure that the results being presented are accurate and reliable. Although there are numerous protocols used throughout the world, each one must be validated and tested thoroughly before it can be used with confidence. In terms of DNA analysis, the amount of DNA recovered and the viability of the STR profile are commonly used as gauges for the effectiveness of the protocol being tested. For this project, the two steps that will be focused on for increasing DNA recovery are the collection and extraction steps. Both of these steps are critical in maximizing the amount

of DNA obtained from a sample, and therefore mock fingerprints can be employed in the development of the most efficient protocols.

Collection Methods for Touch DNA

Collecting DNA is a critical step in DNA analysis because during this time DNA is removed from the item or surface it is on so that it can be moved to an extraction tube. This step affects all subsequent processes because if little to no DNA is collected at this point, there may not be enough DNA present to conduct downstream processing. The current methods for touch DNA collection include double swabbing, cutting, scraping and lifting [14]. Each method works best for a specific type of material. The double swab technique is often used for non-porous items because it allows for more of the sample to be collected off the surface [59]. This technique requires a wet swab to be used to swab the sample area followed by a dry swab. The purpose of the dry swab is to collect any liquid or DNA left behind on the surface in order to collect the maximum amount of sample. Cutting is generally used for porous materials in which a stain or sample may have been absorbed into the material. This method allows the entire sample to be obtained and used for extraction [14, 41]. Contrastingly, both of these methods are limited by the fact that a cotton swab or stained material is placed into the reaction tube, which may result in complications with extraction or analysis [60]. This may include DNA left behind on the substrate or swab, resulting in low levels of DNA recovery after extraction. Recently, tape lifting has become a more common procedure for sample collection in the laboratory because it can be used over a large surface area. This maximizes the chance of collecting the DNA from the object so that genetic analysis can be conducted. Adhesive tape has

been used to collect touch DNA from objects such as fabrics [61, 62, 63] and firearms [63]. These previous studies have found that taping recovers more DNA than a swabbing method for some substrates[61] and that using adhesive tape can reduce the amount of PCR inhibitors collected from the evidence [63]. Although we did not test taping as a method for the collection of touch DNA in this project, it can be tested in future experiments.

Another aspect to the collection of DNA that must be considered is the device that is used for the collection. Swabs are one of the more popular devices used for the collection of DNA. Since there are many different types of swabs available, some studies have been conducted in order to compare them[64, 65, 66, 67]. While a standard cotton swab is the most common swabbing device used, many studies have found flocked swabs to be more effective for the collection of DNA. Flocked swabs are swabs in which small fibers are attached perpendicularly to an adhesive-coated surface through a process called "flocking" [68, 69]. The sample is easily released because the swab does not have an internal core and therefore the sample stays close to the surface of the swab[68]. These flocked swabs focus on efficiently releasing the DNA so that the entire sample can be extracted, and only minimal amounts of DNA are left behind on the swab. There have also been studies that examine special swabs designed to allow the analyst to skip the extraction, and directly amplify the DNA in the sample [49]. Devices such as these pose an interesting potential for the future of DNA analysis because it eliminates extraction, which can help minimize the amount of DNA lost.

As mentioned before, swabbing is one of the more commonly used techniques for collecting DNA evidence. Before swabbing the sample containing DNA, a collection solution is added to the swab in order to help collect cells from the surface or item. Sterile

water is a common swabbing solution and has effectively been used in the collection of touch DNA samples [59, 70], but isopropanol[71] and sodium dodecyl sulfate (SDS)[72] have also been used in experiments. Another study was conducted to determine if water was the most efficient solution for swabbing. In this study, fingerprints were collected with swabs wetted with detergent-based solutions as well as water. The results showed that the detergent-based solutions, such as SDS, outperformed water as a collection solution for touch DNA samples [73]. Other research has also supported this result by revealing that complete STR profiles can be obtained when using SDS as the swabbing solution[72]. Although it is unknown why SDS is an effective swabbing solution, it may be because it is a detergent, which plays a role in cell lysis. It is possible that because the SDS is on the swab during collection, it begins to lyse some of the cells before extraction and therefore increases the amount of DNA recovered.

There has been limited research efforts to identify an optimal swabbing solution[74], and therefore we have tested various solutions in this project in order to optimize a collection protocol. In all of the studies that have been mentioned, various results have been obtained and this is because each experiment was designed differently. While some studies recommend the use of detergents as a collection solution, other results indicate that water is an acceptable swabbing solution. Variability is expected in these scenarios, but the mock fingerprints can be used to help reduce these differences by directly comparing various swabbing solutions. In this project, we employed mock fingerprints in experiments that test the collection method and solution for touch DNA. The double swab and single wetted swab collection methods were compared to evaluate which one is more effective. Different swabbing solutions were tested including sterile water, ethanol and

2% SDS to determine which solution results in higher DNA recoveries. Using the results from these experiments, we established a baseline so that future experiments can change a single variable at a time and allow the change in results to be evaluated.

Extraction

Extraction is a critical part of the process of DNA analysis because it is during this step that the DNA is isolated and purified from other components in the sample. This may include inhibitors or cellular components. For DNA extraction, there are numerous protocols and kits used by forensic laboratories. Some of the more common extraction methods include magnetic separation, organic and silica-based extractions. The result for all of these extraction methods is a purified DNA product, but some may be better suited for touch DNA samples compared to others. Various studies have compared these different kits and protocols to help determine which are most effective [3, 75, 76, 77]. The types of extractions included silica-based methods, magnetic bead separations and phenol-chloroform extraction. DNA was successfully extracted in all of these studies, but when comparing extraction methods, a silica-based extraction proved to be the most effective in most scenarios [75, 77]. For this reason, the Qiagen QIAamp® DNA Blood Mini Kit was selected for use in this project since it is a silica-based extraction method. In this project, we used the mock fingerprints to modify the manufacturer's protocol for this kit in order to establish an optimized extraction protocol. It is important to remember that previous studies may not have been designed with touch DNA samples in mind. For example, just because one type of extraction is the most effective for bone samples does not mean that it is the most effective for touch DNA samples. The components in touch

DNA samples may cause yield results that do not agree with previous studies, and therefore it is necessary that the mock fingerprints be used to test an extraction protocol for this project.

Another approach in testing the efficiency of extraction protocols is to test a single protocol and alter individual steps within the protocol. There are certain aspects within an extraction protocol that may affect the recovery of DNA. Some examples of these include the incubation phase, the elution volume and the reagents used to wash or purify the DNA. One study was conducted specifically to evaluate modifications to the incubation phase of the extraction protocol [48]. Some of these alterations included longer incubation times, higher incubation temperatures, resuspension of the sample, and the addition of shaking during incubation. Although many conclusions were made from this study, two of the significant points were that an extended incubation time and a higher incubation temperature increased DNA recovery.

Using mock fingerprints, we developed an optimized extraction protocol. Since the initial amount of DNA for each sample is known, the percent recovery for each sample can be determined. This allows each modification to be evaluated individually for its effect on DNA recovery. Being able to quantify this amount will provide confidence in the results obtained and allow an optimized protocol to be developed for use in subsequent experiments.

Loss of DNA

It has been made clear through previous studies that the amount of DNA recovered from fingerprints is minimal which is why there is a need for optimized protocols. Some forensic

research studies have recovered between 0.04ng and 1.23ng of DNA from touch DNA samples [35, 36, 37]. Although determining the amount of DNA extracted from an unknown sample is important, it does not reveal how much DNA was lost since the initial amount present in the sample is unknown. Knowing how much DNA was lost provides meaningful information regarding the protocols being used. For example, if 1 ng of DNA is recovered from a sample, but the initial quantity was 100 ng, a better protocol may need to be used. Yet, without knowing this initial amount of DNA, there is no way to make this determination regarding how much DNA is lost. Research regarding the initial quantity is critical in order to learn about the true nature of touch DNA samples. While other studies can report the amount of DNA recovered, data regarding how much was lost cannot be given. Since there is an inevitable amount of DNA lost during processing [1, 2, 3, 4], further experimentation needs to be conducted in order to identify which areas account for the largest amount of DNA loss. This loss may occur during the collection or extraction steps but isolating these areas to identify where the most DNA is lost can be difficult.

Few research studies have been done in this area, so this present project should provide valuable information regarding the loss of DNA during DNA processing. The mock fingerprints are well suited for this type of experiment because they eliminate variability between samples. If true fingerprints were used in this experiment, it would be difficult to discern if the differences in results for the samples was actual loss of DNA or due to donor variability. Mock fingerprints reduce this possibility and make a researcher more confident in the results obtained. The results from this specific experiment should which aspects of DNA processing are responsible for the largest amount of DNA loss. This information will reveal those steps that need to be optimized further, and push future experimentation to focus on these areas.

Standard Curve

A standard curve demonstrates the relationship between two quantities and is graphically represented by plotting the quantities against each other. The most important aspect to a standard curve is that the quantities being compared are known. Comparing these quantities can display trends for the data and allow for inferences about other samples to be made. The samples in which both quantities are known are considered standards, and in this instance the standards are the mock fingerprint samples. One of the most common uses of a standard curve in forensic laboratories is to quantify DNA using quantitation polymerase chain reaction (qPCR). This method estimates the DNA concentration of an unknown sample using a standard curve generated by samples with known DNA concentrations or standards [78, 79]. We employed a similar idea in order to compare mock and true fingerprints, which will ultimately provide information about the initial quantity of DNA in a true fingerprint. Since this value is not well studied or well known, this present project should provide significant insight regarding the nature of true fingerprints. The standard curve can also be used as a baseline in which future studies can be compared. This will serve as the foundation of experimentation with mock fingerprints, and future studies will continue to build from the information gathered through the generation of the standard curve.

Synopsis of Project

Since touch DNA is becoming more prevalent in forensic laboratories, it is important that research efforts focus on ways to improve the way in which these samples are analyzed. In this project, mock fingerprints were developed to mimic eccrine fingerprints. Mock fingerprints included the various components of a true fingerprint to give a more realistic representation of a

touch DNA sample. Since there may be components in a fingerprint that affect the collection or extraction of DNA, these mock fingerprints will include urea, sodium chloride, potassium chloride, lactic acid and water to represent eccrine sweat. Using mock fingerprints as a positive control helped increase the efficiency of the analysis process for touch DNA samples because they are more representative of a true fingerprint.

Once a protocol for generating mock fingerprints was developed, the collection and extraction protocols could be optimized. These protocols were used in subsequent experiments in this project to ensure that the maximum amount of DNA is being recovered. Additionally, the mock fingerprints were used to evaluate where DNA is lost throughout the collection and extraction process. As mentioned previously, touch DNA begins with low levels of DNA and therefore maximizing its recovery is critical. Then the mock fingerprints could be used to create a standard curve. By plotting true fingerprint samples on a standard curve, comparisons between the mock and true fingerprints could be made. This provided insight regarding the initial amount of DNA in a true fingerprint. True fingerprints collected from various surfaces were also analyzed to examine the differences in DNA recovered from them. Overall, using the mock fingerprints in this project permitted optimized protocols to be developed, tracked the loss of DNA and revealed how true fingerprints behave.

Chapter II: Improving Mock Fingerprint Preparation and Cell Counting

Introduction

It is important for researchers to have positive controls throughout experiments in order to confirm that the test is working properly. Positive controls are samples in which the results are known and therefore can be compared to unknown test samples. This comparison can lead to inferences regarding the effects of certain variables. Understanding how variables affect the results of an experiment is valuable, which is why having positive control samples is critical. One area in forensic science that is subjected to numerous variables is touch DNA. Touch DNA is DNA that is left behind on an object or surface due to contact with a person's skin. Specifically, shed epithelial cells are deposited onto the item or surface. The cells contain DNA which can be collected for DNA analysis.

When conducting research for touch DNA, there are different approaches for replicating a touch DNA sample. Volunteers can handle an item or wear an article of clothing in order to deposit DNA [20, 37, 80, 81]. Another method is to have donors place their fingertips or hands onto a surface, such as glass, so that the DNA left behind can be collected later [49, 50, 82]. Although these methods are effective for depositing touch DNA samples onto a substrate, they do not eliminate the variability between donors. Using these methods also does not allow for the initial amount of DNA in the sample to be quantified. These are important factors to consider when testing touch DNA samples. In order to control these elements we have developed mock fingerprints. By adding a specific number of cells to the mock fingerprint the initial amount of DNA was known, which allowed for data analysis regarding percent recovery to be conducted. The mock fingerprints also eliminated variability because the number of cells in each sample is controlled by the researcher. For these reasons, we developed and tested mock fingerprint samples.

Developing a mock fingerprint requires an understanding of the components that make up a true fingerprint. The mock fingerprints used in the following experiments mimic an eccrine fingerprint. Eccrine glands secrete sweat that is comprised of 98% water with the remaining portion including urea, inorganic salts, and organic acids [5]. Although fingerprints also contain fats, oils, and microbial DNA[83, 84], we chose to mimic an eccrine fingerprint because those components were easy to make in solution. Future studies will work to add in the organic components of a fingerprint. In order to represent an eccrine fingerprint, the fingerprint solution used in this series of experiments contained urea, sodium chloride, potassium chloride, lactic acid and water. Eccrine glands are found on hairless parts of the body including the palms of the hands and the soles of the feet [28]. When an individual touches an object with their hand or foot, eccrine sweat is transferred to the item. Besides these eccrine sweat components, the fingerprint left behind will also include shed epithelial cells that contain DNA. These materials can be collected from the substrate and then processed for DNA.

The purpose of this series of experiments was to improve the protocol for generating the mock fingerprints. Some experiments with the mock fingerprints yielded over 100% recoveries, which indicated that the cells may have been clumping in solution. Clumping causes an inaccurate amount of cells to be added to each sample and adds an element of uncertainty to the mock fingerprint. In order for the percent recovery values to be accurate, the concentration of the cells in suspension must be consistent throughout the sample. Therefore, we developed an improved protocol for generating mock fingerprints using Accumax, which is a cell dissociation solution that prevents clumping by mimicking trypsin and collagenase[85]. We also compared an automated cell counting method to the manual hemocytometer counting method to determine the

accuracy of the concentration values. Finally, we tested white blood cells, isolated from whole blood, for their use as the source of DNA in the mock fingerprints.

Materials and Methods

Generation of Mock Fingerprints

Cell Counting

Throughout this series of experiments an improved Neubauer hemocytometer, was used. After adding 10 µl of the cell suspension to the hemocytometer, every cell in all 9 squares was counted. After counting 3 squares, the original suspension was gently pipetted up and down to prevent large cells clumps from forming before returning to counting the cells. Once each square was counted, the concentration (cells/µl) of the suspension was determined using the equation below:

$$\frac{Total\ \#\ of\ cells}{\#\ of\ squares\ counted} \times \frac{1\ square}{0.1\mu l} = cells/\mu l$$

The total number of cells counted on the hemocytometer was divided by the number of squares counted, and then divided by 0.1 µl. Since the volume for a single square in a Neubauer chamber is 0.1 µl, it is used to convert the number of cells per square into cells per microliter. This calculation gives the concentration of the suspension. Then this value can be used to calculate the volume needed to add a specific quantity of cells to the mock fingerprint. By dividing the desired quantity of cells by the concentration of the suspension, the required volume of cell suspension is determined. This calculation is shown below:

$$Desired\ \#\ of\ cells \times \frac{1\ \mu l}{\#\ of\ cells\ in\ suspension} = Volume\ (\mu l)\ needed\ for\ desired\ \#\ of\ cells$$

Fingerprint solution

We developed a fingerprint solution to mimic an eccrine fingerprint. It contained 0.197M urea, 0.195M sodium chloride, 0.086M potassium chloride, 0.0678M lactic acid in 98% sterile water.

Composition of Mock Fingerprints

Epithelial cells were collected from human subjects by swabbing the inside of the donor's cheek. Samples were collected using protocols approved by the University of Illinois at Chicago Institutional Review Board (IRB Protocol #2016-0431). Each twenty microliter mock fingerprints contained epithelial cells, fingerprint solution and 1X PBS. For each sample, 2 µl of the fingerprint solution was added, and the remaining 18 µl was made up of a cell suspension and PBS. We added a known quantity of epithelial cells, the source of DNA for each sample, based on the calculated concentration of the cell suspension. This concentration was determined through cell counting. After calculating the volume of cell suspension that was needed, we subtracted this value from 18 µl to determine the volume of PBS required to bring the volume of the mock fingerprint up to 20 µl.

Improving the Preparation of the Cell Suspension

Cell clumping was suspected to be occurring in the cell suspension when making mock fingerprints. In a preliminary study, PBS was used to make four replicates of mock fingerprints,

each containing 0, 200 or 8,000 cells, and only used Accumax for the volume of cells used for counting. Therefore, this protocol needed to be modified to prepare the entire suspension in Accumax. Accumax is a cell dissociation solution that contains enzymes to prevent clumping of cells. To test a protocol using Accumax for counting and suspension preparation, we generated four replicates of mock fingerprints with cell quantities of 0, 500 and 1,000 cells according to the following protocol. The 0 cell mock fingerprint served as a negative control and was used to check for DNA contamination. An 8,000 cell mock fingerprint, used in the preliminary study, was not used in this experiment in an effort to make the mock fingerprint more like a true fingerprint. Since 8,000 cells will likely contain much more DNA than a fingerprint, an 8,000 cell mock fingerprint would not provide useful information to the researcher.

Preparing the Cell Suspension

We collected epithelial cells using a buccal swab. The swab was cut and placed into a microcentrifuge tube with 500 µl of Accumax. The sample was incubated at room temperature for 30 minutes with manual shaking every 5 minutes. In order to prevent cell lysis, the sample was not vortexed. After the incubation period, the swab was moved to a spin basket, which was placed back into the tube. The tube containing the spin basket was centrifuged on high for 5 minutes to collect any liquid in the swab and to pellet the cells. The swab and basket were discarded, and then the pelleted cells at the bottom of the tube were re-suspended by gently pipetting the solution up and down. Five hundred microliters of Accumax was added to the tube, bringing the volume to 1 mL, and again the sample was mixed by pipetting the contents up and down. Ten microliters of the prepared cell suspension was pipetted onto a hemocytometer in order count the cells and

to determine the concentration of the suspension. This protocol was used to generate

mock fingerprints in the remaining experiments, but in order to determine if this new

protocol reduced clumping, we generated 4 replicates of mock fingerprints with 500 and

1,000 cells and pipetted them directly into an extraction tube.

Extraction

During preliminary studies, and before an optimized extraction protocol was

established, mock fingerprint samples were extracted using the QIAamp® DNA Blood

Mini Kit (Qiagen, Hilden, Germany). The extraction protocol is outlined in the

QIAamp® DNA Mini and Blood Mini Handbook and is titled "DNA Purification from

Buccal Swabs (Spin Protocol)". The manufacturer's protocol is outlined here. Four

hundred microliters of 1X PBS and 20µl of Qiagen protease were added to the samples in

the extraction tubes. All samples were vortexed for approximately 2 seconds. Then 400µl

of Buffer AL was added, and each sample was vortexed for 15 seconds. The samples

were then incubated at 56°C for 10 minutes. After the incubation period, 400 µl of

absolute ethanol was added and the samples were mixed on a vortex. In order to remove

any liquid from the lid, the samples were briefly centrifuged. Then the swabs were

removed from the extraction tube and placed into a spin basket. The spin baskets were

then placed back into the extraction tube and centrifuged on high for 5 minutes in order to

collect any liquid retained by the swab. The swab and spin basket were discarded and

then 750 µl of the liquid collected in the extraction tube was applied to the QIAamp Mini

spin column. These samples were centrifuged for 1 minute at 6,000xg to move the liquid

through the column. This step was repeated for the remainder of the liquid in the

extraction tube so that the entire sample was run through the column. Next, 500 µl of Buffer AW1 was added to the samples followed by a 1 minute centrifugation period at 6,000xg. Then 500µl of Buffer AW2 was added to the column and each sample was centrifuged on high for 3 minutes. The liquid collected in the tube after this step was discarded and the column was centrifuged for one more minute on high. In order to elute the DNA, the spin column was moved to a 1.5mL tube and 30µl of Buffer AE was added to the column. The samples were centrifuged for 1 minute at 6,000xg. Controls for the extraction were also included during this process. A buccal swab was used as the positive control to ensure that the extraction method was working properly, and therefore was expected to yield DNA after extraction. The negative control was included in the extraction process, but no DNA was added or expected to be in the sample. If DNA was detected during quantification, it would indicate that DNA was introduced during the extraction process and that the results for the experiment would be invalid.

Quantification

After extraction, we quantified all samples using the Qubit® Fluorometer 3.0 (Thermo Fisher Scientific, Waltham, MA). The Qubit High Sensitivity dsDNA kit was used for all samples, which utilizes PicoGreen® fluorescent dye that targets double stranded DNA[86]. This method of quantification is not specific for human DNA and therefore the result given by the fluorometer is the total DNA in the sample. In these experiments, the total DNA was quantified because fingerprints contain bacteria and other microbes that contribute to the amount of DNA present in a fingerprint [83, 84]. The Qubit® Fluorometer 3.0 was calibrated according the manufacturer's protocol using the

two standards included in the kit. Both standards are contained in TE buffer with Standard 1 having a concentration of 0 ng/µl and Standard 2 having a concentration of 10 ng/µl. A 1:200 dilution of dye with the Qubit Buffer was made for each sample, then 195 µl of this solution was used in combination with 5µl of DNA extract for analysis. Positive and negative controls were also quantified to ensure that the instrument was working properly. The positive control consisted of a male genomic sample with a concentration of 1ng/µl, which was expected to yield this value when quantified with the Qubit. The negative control contained only 200µl of the Qubit Buffer and dye solution. It was expected that this sample would result in a concentration value of 0ng/µl, which is indicated on the Qubit as "TOO LOW".

Percent Recovery

Once the quantification values were obtained for each sample, we calculated the percent recovery. Knowing that one diploid cell, in this case the epithelial cells, contain 6pg of DNA[87, 88, 89] the initial amount of DNA in each sample was determined. For example, a 500 cell mock fingerprint contains 3 ng of DNA because 500 cells multiplied by 6 pg is 3,000 pg or 3 ng. Then the total DNA extracted was calculated by multiplying the concentration (ng/µl) by the elution volume (30 µl). The percent recovery was calculated as shown below:

$$\% \ Recovery = \frac{Total \ DNA \ extracted \ (ng)}{Initial \ DNA \ in \ sample \ (ng)} \times 100$$

Comparison of Counting Methods

In order to test the accuracy of the cell concentrations obtained from the manual counting method, we tested a second counting method. This utilized the LUNA™ Automated Cell Counter (Logos Biosystems, South Korea). Three separate cell suspensions in Accumax were prepared as previously described. Ten microliters were placed on a hemocytometer for counting and the concentration was determined. Ten microliters were also added to the reusable slide and placed into the automated cell counter. This was repeated for each of the three prepared cell suspensions. The cell suspension was not dyed with Trypan Blue, as suggested by the manufacturer, when using the automated counting method and the following counting settings were used:

- Dilution – 1

- Noise Reduction – 6

- Live detection sensitivity – 2

- Roundness – 40

- Min. cell size – 5

- Max cell size – 60

- Declustering level – Medium

The focus knob was used to bring the cells on the slide into focus and then the "count" option was selected. A concentration for the cell suspension was produced in cells per mL. This was converted into cells/µl by dividing the value by 1,000. This concentration was then compared to the concentration obtained by the manual cell counting method.

Testing of White Blood Cells

We tested white blood cells for their use in the mock fingerprints as a source of DNA. First, whole blood was collected by a trained phlebotomist at the University of Illinois at Chicago Center for Clinical and Translational Science in accordance with the protocol approved by the Institutional Review Board (IRB Protocol #2016-0431). Then, 4 mL of whole blood was placed into a 50 mL conical tube followed by the addition of 8.5 mL of cold 0.5% acetic acid. The sample was inverted to mix the contents followed by an incubation period of five minutes at room temperature. Then, 8.5 mL of cold 1.8% sodium chloride was added. After gently mixing by inversion, the sample was centrifuged for 10 minutes at 4°C at 252xg. This pelleted the cells and allowed for the supernatant to be easily removed. Next, 8.5 mL of cold, sterile water was added to the tube, and then the sample was incubated at room temperature for five minutes. After incubation, 8.5 mL of 1.8% sodium chloride was added and the sample was mixed by inversion. Again, the sample was centrifuged at 252xg for 10 minutes at 4°C. The white blood cells were pelleted at the bottom of the tube and the supernatant was removed. 2 mL of a freezing solution was added to the white blood cells. This solution consisted of 0.017M sodium phosphate monobasic, 0.020M sodium phosphate dibasic, 0.093M citric acid trisodium salt, 40% glycerol, and water. The isolated white blood cells, in freezing solution, were stored at -80°C until use.

The isolated white blood cells were prepared for counting by taking 50 µl of the isolated white blood cells in freezing solution and diluting them with 500 µl of 1X PBS. Five hundred and fifty microliters of Accumax were added to the diluted sample to prevent clumping. Then 10 µl of the suspension was placed into a 1.5 mL extraction tube and 10 µl of Trypan Blue Stain (0.4%) (Life Technologies, Carlsbad, CA) was added. The sample was vortexed for 5 seconds to

mix thoroughly. Ten microliters of the dyed cell suspension were placed onto a hemocytometer for counting.

Once the cells were counted and the concentration of the suspension was determined, we prepared four replicates of mock fingerprints with cell quantities of 0, 200, 500 and 1,000. Two replicates for each quantity were pipetted directly into an extraction tube and the other two replicates were pipetted directly onto a cotton swab. All samples were extracted using the extraction protocol optimized in Chapter 3. The extraction protocol is also described in this chapter for this experiment. Any time this extraction protocol was used, two control samples were included. A buccal swab was used as a positive control to ensure that the extraction was working properly, and a blank sample with no DNA was used as the negative sample to ensure there was no contamination occurring throughout the process. Samples were extracted using the QIAampDNA Blood Mini Kit (Qiagen, Hilden, Germany). The samples were placed into 2 mL extraction tubes with the addition of 400 µl of PBS and 20 µl of Qiagen protease. All samples were incubated overnight at 56°C for no more than 18 hours. After incubation, 400 µl of ethanol was added to the tube. Six hundred fifty microliters of this solution was added to a QIAamp mini spin column and centrifuged for 1 minute at 6,000xg. Swabs in the remaining solution were removed and placed into a spin basket and centrifuged for 5 minutes on high in order to obtain any liquid retained by the swab. Once the samples were spun down the remaining liquid was added to the QIAamp mini spin column and centrifuged for 1 min at 6,000xg. The filtrate was discarded and 500µl of AW1 Buffer was added to the column and spun down at 6,000xg for 1 minute. Again the filtrate was discarded and then 500µl of AW2 Buffer was added to the column and centrifuged on high for 3 minutes. The samples were centrifuged again on high for 1 minute after the filtrate was removed from the collection tube. The spin columns were then placed into

1.5mL tubes and 30µl of AE Buffer was added to the column. After incubating at room temperature for 1 minute, samples were centrifuged at 6,000xg for 1 minute in order to elute the DNA. After extraction all samples were quantified using the Qubit® Fluorometer 3.0 and the total DNA extracted was calculated followed by the percent recovery value.

Results

AVERAGE PERCENT RECOVERY FROM MOCK FINGERPRINTS CONTAINING WHITE
BLOOD CELLS

Samples	Average % Recovery
200 cell direct	0.00
500 cell direct	47.8
1,000 cell direct	47.7
200 cell swab	0.00
500 cell swab	0.00
1,000 cell swab	18.7

Table I. Average recoveries from mock fingerprints with white blood cells as the source of DNA

(n=2). Mock fingerprints with cell quantities of 200, 500 and 1,000 were pipetted directly into an

extraction tube or directly onto a swab. After extraction, the recovery value for each sample was

calculated to determine if white blood cells could be used in the mock fingerprints.
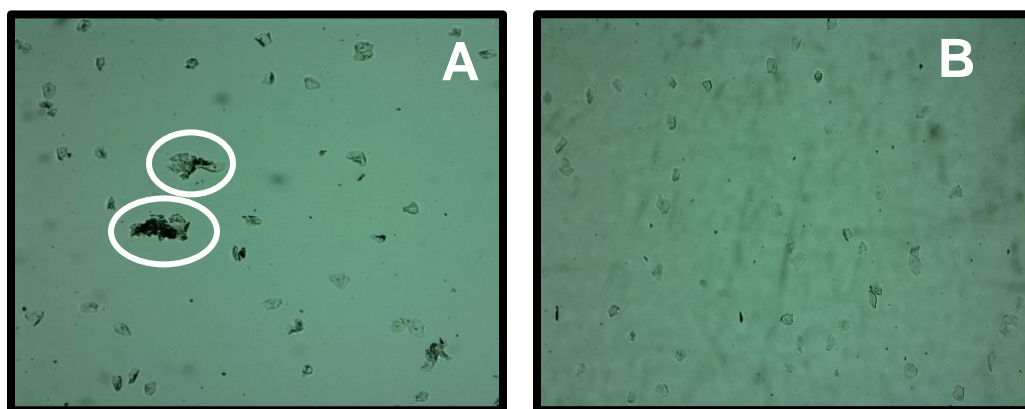
Figure 1. Visual comparison between epithelial cells suspended in PBS and in Accumax. Figure

1A demonstrates epithelial cell clumping in PBS. Figure 1B is an example of a suspension with

no clumping using Accumax.

AVERAGE PERCENT RECOVERY FROM MOCK FINGERPRINTS WITH CELLS IN PBS

Sample	Average % Recovery
500 cell direct	125% ± 35.3%
8,000 cell direct	104% ± 14.5%

Table II. Average percent recoveries for mock fingerprints containing 500 and 8,000 epithelial cells before optimization (n=4). Mock fingerprints were pipetted directly into a tube and then extracted. These samples utilized Accumax for cell counting, but not for the original cell suspension.

AVERAGE PERCENT RECOVERY FROM MOCK FINGERPRINTS WITH CELLS IN ACCUMAX

Sample	Average % Recovery
500 cell direct	71.1% ± 7.64%
1,000 cell direct	67.0% ± 4.19%

Table III. Average percent recoveries for mock fingerprints when Accumax was used to prepare and count the cell suspension (n=4). The mock fingerprints contained 500 or 1,000 epithelial cells and were pipetted directly into a tube for extraction.

COMPARISON OF THE HEMOCYTOMETER AND AUTOMATED CELL COUNTER

Replicate	Hemocytometer concentration (cells/µl)	LUNA™ concentration (cells/µl)
1	68	66
2	76	62
3	184	194

Table IV. Comparison of epithelial cell suspension concentrations obtained from a manual counting method and an automated counting method. Each prepared cell suspension was counted using both methods.
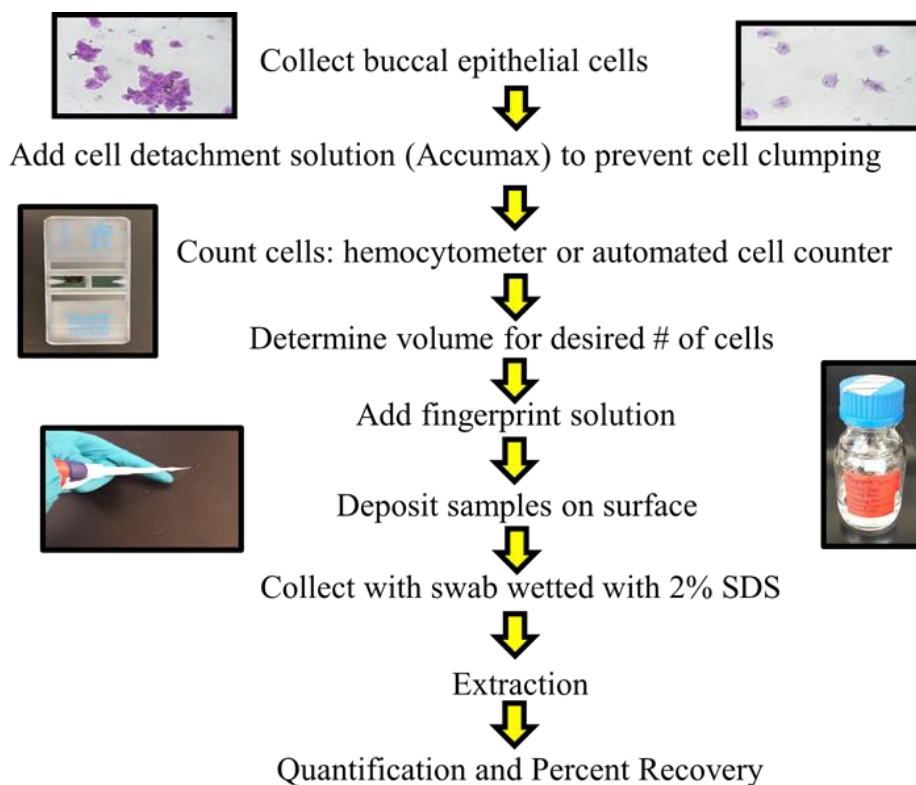
Figure 2. Flow chart depicting the process of generating mock fingerprint samples followed by collection, extraction and quantification.

<u>Discussion</u>

As touch DNA evidence becomes more prominent in forensic laboratories, research on this type of evidence will need to advance in order to continue to provide valuable information to researchers. One potential area that can be improved upon is how positive controls are used for touch DNA samples. Current methods of depositing touch DNA are effective, but do not control for variability and the initial amount of DNA in the sample is unknown [49, 50, 82]. In this present series of experiments, we optimized mock fingerprints for their use as positive controls in future experiments.

When a person comes into contact with an object there is a transfer of material which includes the eccrine sweat as well as shed epithelial cells. Based on this fact, epithelial cells were chosen as the source of DNA in the initial development of the mock fingerprints. Although the epithelial cells had been successfully used in the mock fingerprints, it was important to test white blood cells as another possible source of DNA. This is because in the event that using the white blood cells improved DNA recoveries, the source of DNA in the mock fingerprints would need to be reconsidered. White blood cells were isolated and mock fingerprints containing 200, 500, or 1,000 cells were pipetted directly into the extraction tube or directly onto a swab. Since the range for the mock fingerprints had not been tested at this point, these cell quantities were chosen in an effort to represent samples with low, medium and high quantities of DNA. Regarding the mock fingerprints pipetted directly into the tube, the 200 cell samples did not yield any DNA, and the average recovery for the 500 and 1,000 cell samples were 47.8% and 47.7% respectively (Table I). When looking at the mock fingerprints pipetted onto swabs the 200 and 500 cell samples did not yield any DNA, while the average recovery value for the 1,000 cell

samples was 18.7%. These results revealed that mock fingerprints containing 200 cells do not yield any DNA after processing for both the direct and swab samples. This is important to take into consideration since true fingerprints contain low quantities of DNA [14, 36, 37, 60]. If white blood cells cannot accurately represent these low DNA quantities in mock fingerprints then they should not be used in subsequent testing. Based on the results, epithelial cells were chosen to be the source of DNA for the mock fingerprints. Not only do the epithelial cells make the mock fingerprints more representative of a true fingerprint, but they also allow smaller cell quantities to be added to the mock fingerprints. The mock fingerprints need to mimic a true fingerprint, and because the white blood cells were not providing sufficient recoveries, they were not used in subsequent experiments.

In this experiment, mock fingerprints were developed to mimic an eccrine fingerprint. They had a total volume of 20 µl that consisted of 2 µl of a fingerprint solution (urea, sodium chloride, potassium chloride, lactic acid and water) and 18 µl of PBS and cell suspension. The volume of the PBS and cell suspension added was dependent upon the concentration of the cell suspension. Before beginning experimentation with the mock fingerprints, a protocol for cell counting needed to be developed. One aspect of cell counting that we evaluated was the presence of cell clumping during the counting process. When cells are clumped together it can become difficult to accurately count them, and therefore Accumax was tested for its ability to reduce clumping. Accumax is a cell dissociation solution that can assist in increasing the accuracy of cell counts by reducing clumps of cells in the suspension [85]. After creating cell suspensions in PBS and in Accumax, it was evident that Accumax reduced the number of clumps in the sample (Figure 1). When epithelial cells were suspended in PBS, clumps of cells were visible and discerning the number of cells in the clump was difficult (Figure 1A), but when the cells were

suspended in Accumax there was little to no clumping and the cells were evenly distributed (Figure 1B). For this reason, Accumax was added to the cell suspension for the counting portion of the protocol in order to increase the accuracy of the cell counts.

To test the effectiveness of using Accumax to count the cells, we generated mock fingerprints containing either 500 or 8,000 cells. The samples were pipetted directly into a tube to be extracted. The results revealed that mock fingerprint samples generated in this fashion yielded recovery values over 100%. On average, mock fingerprints with 500 cells pipetted directly into extraction tubes had a 125% recovery value while the 8,000 direct samples were at 104% (Table II). Assuming that the correct number of cells were initially added to the sample, a recovery value over 100% would be impossible. These high values indicate that there was either more DNA in the sample than initially accounted for, or DNA was being added by contamination. Contamination was ruled out as a possibility because the 0 cell mock fingerprint and the negative control for the extraction both resulted in no DNA. Taking this into consideration the possibility of cell clumping was likely and needed to be eliminated.

In order to decrease the DNA recoveries for the mock fingerprints, Accumax was used to create the cell suspensions instead of using PBS. Mock fingerprints containing 500 and 1,000 cells suspended in Accumax were generated and pipetted directly into extraction tubes. Each sample was extracted and quantified to calculate the percent recovery. The 500 cell mock fingerprints pipetted directly into the tube yielded an average recovery of 71.1%, while the 1,000 cell samples exhibited a 67.0% recovery rate (Table III). The visual reduction of clumping in Figure 1 and the reduction of the percent recovery values indicate that cell clumping was eliminated. Since the loss of DNA during extraction is well-documented [3, 4, 48], it was not expected that recovery values would be nearly 100% and therefore the results were as expected.

Based on these results it was determined that the mock fingerprints were suitable for use in further experimentation.

The purpose of using the mock fingerprints in research was to have a known initial quantity of DNA, which is why cell clumping needed to be eliminated for these experiments. Knowing the initial quantity of DNA in the mock fingerprints is advantageous because it allows the percent recovery values to be calculated. In this experiment, these values were necessary in order to evaluate the precision of the mock fingerprints. If cell clumping was present in the mock fingerprints, the results obtained when using them would be inaccurate and could not be used as valid data. It was critical to decrease cell clumping in the suspension because subsequent testing relied on the accuracy of the mock fingerprints. By re-evaluating the protocol for the preparation of the cell suspension, cell clumping was eliminated and the mock fingerprints became more accurate.

Another aspect of the protocol for generating mock fingerprints that needed to be tested was the cell counting method. While a hemocytometer is a trusted method for cell counting, there is a chance for human error [51, 57]. In order to add a secondary method of counting, and to help make the experiments repeatable in other labs, we tested the LUNA[TM] Automated Cell Counter. Three cell suspensions were made and then counted using both the manual and automatic counting methods and then compared to one another. The largest difference was seen in the third replicate in which there was a difference of 10 cells/µl (Table IV). Replicate #2 had a difference of 6 cells/µl, while the smallest difference was 2 cells/µl seen in replicate #1 (Table IV). It is important to note that when comparing the concentrations from both methods, the values were not exactly the same. It was not expected that the methods would have perfectly matching values because there was always some level of variability within the sample, but not to

the same degree of variability as seen when there is cell clumping. If the two methods yielded drastically different concentrations this would cause concern and the methods would need to be re-evaluated. These results provided confidence that the researcher manually counting the cells on the hemocytometer was using the method correctly and obtained accurate results. It also revealed that both the hemocytometer and LUNA[TM] Automated Cell Counter could be used to effectively count epithelial cells, and therefore either method could be used in subsequent experiments and replicated in different labs

After using the LUNA[TM] Automated Cell Counter, it was evident that there were many benefits to using the instrument. The amount of time required to obtain the cell suspension concentration is minimal and the preparation for counting is simple. It is also user-friendly, which makes it easier to implement in the lab. Another key feature is the clustering report, which lists the number of clumps exhibited in the sample as well as an approximate amount of cells in each clump. This is useful in monitoring the clumping of a cell suspension because if a high clustering level is evident, a new cell suspension can be made. Even though it may require a new sample to be prepared, it ultimately saves time and resources. If the experiment were to be carried out, with high clumping in the cell suspension, it is likely that inaccurate recovery values will be obtained and the experiment would need to be repeated. Overall, the features of the LUNA[TM] Automated Cell Counter make it a useful tool in counting cells and allow reliable results to be obtained.

Overall, these experiments successfully developed mock fingerprints that mimic true eccrine fingerprints. After improving the way the mock fingerprints were generated and counted, further experimentation could be conducted. A summary of the processing the mock fingerprints is depicted in Figure 2. Since true eccrine fingerprints contain epithelial cells and other chemical

compounds, these components were added to the mock fingerprints. The mock fingerprints contained a known quantity of DNA, and by reducing clumping in the cell suspension the quantity of DNA being added became more accurate. This was a crucial step in this project because the mock fingerprints needed to be accurate for subsequent experiments that rely heavily on knowing the initial quantity of DNA in the samples. The results in this experiment also showed that two different methods of cell counting can be used to count cells, and that an accurate concentration for the cell suspension can be obtained. All of these factors provide confidence in the methods employed and reveal that the results obtained when using them is accurate.

Future Studies

There are future studies that need to be done in relation to these experiments. The next step regarding the fingerprint solution is to add organic components. Lipids and amino acids are also found in true fingerprints[28] due to natural human activities. Touching of the face or hair can add oils to the fingertips, which is transferred in touch DNA samples. These components also come from natural debris that humans encounter every day. If a fingerprint solution that contains these elements was developed, it could be tested in the mock fingerprints and be compared to the original fingerprint solution as well as true fingerprints. While the current fingerprint solution is a good representation of an eccrine fingerprint, a solution that includes all of these components will be more beneficial and provide valuable information to researchers.

Chapter III: Optimization of Collection and Extraction Protocols

Introduction

We developed mock fingerprints that mimic true fingerprints so that researchers have control over their experiments because a known amount of DNA can be put into a sample that is representative of touch DNA evidence. In order to reduce the number of variables in later experiments, collection and extraction protocols were optimized. This allowed consistent methods to be used and provided confidence that the maximum amount of DNA was being recovered. There have been many studies that work to improve the recovery of DNA, which have focused on the collection and extraction methods because they are critical steps in the process of DNA analysis [3, 48, 49, 59, 73, 77, 90, 91]. The collection phase is where the DNA sample is removed from a substrate on which it has been deposited so that it can be analyzed. This step is not specific to DNA and therefore will collect other components and miscellaneous debris in addition to the sample, which is why the sample must also be extracted. Studies that focused on the optimization of DNA collection indicated that detergents were the most effective swabbing solution[73], while a double swabbing technique was the most effective collection method for non-porous items[59]. During extraction, DNA is isolated from elements such as lipids, proteins and other cellular components. The purified DNA product is free from inhibiting components that may affect downstream DNA processing. Through the process of extraction, the DNA is purified so that there are no other inhibiting components present in the sample. In studies conducted to compare silica-based methods, magnetic bead separations and a phenol-chloroform extraction, all methods recovered DNA. Yet, the results indicated that a silica-based extraction was the most effective in most instances [75, 77]. Both collection and extraction have multiple parts that may be altered or modified in order to improve the amount of DNA that is recovered. For this reason, they have been the focus of many papers that concentrate on maximizing DNA recovery.

There is not one single method for extracting DNA, and there are many variations in protocols that are used in forensic laboratories throughout the world. Some of the more common extraction methods include organic and silica-based extractions. It is evident that there are many aspects to a DNA extraction that can be altered based on the numerous commercial kits available for use. Even though these kits may utilize different reagents and procedures, the result is a purified DNA product. The amount of DNA recovered from these kits can vary and it is important to use one that is best suited for the type of evidence being examined. Researchers have compared various kits for their DNA recovery abilities [76, 77, 91, 92] as well as automated extraction robots [64, 90] . Not all of these studies tested the extractions for touch DNA specifically, but rather across many different types of forensic evidence. These studies focused on comparing the kits or protocols, and some altered the parameters in order to increase DNA yields. Some of the parameters that have been tested include longer incubation times, resuspension steps, and shaking of samples during incubation [48]. Making small adjustments to the extraction protocol can help increase the recovery of DNA, but it is important to test one parameter at a time so that it can be evaluated individually. By doing this, any change in the recovery of the DNA can be attributed to the modification being tested and not any other variable. While there are various aspects of DNA extraction that can be modified to increase DNA yields, the following study focused on the incubation phase. During this phase of extraction, the DNA must be released from the device used to collect it from the substrate. If the DNA is not successfully released, there may be little to no DNA obtained after extraction, which is why the incubation phase is critical when optimizing an extraction protocol. Mock fingerprints are well suited for testing modifications to the incubation phase because the initial quantity of DNA in them is known, and therefore the percent recovery was be calculated and compared to other samples. This

comparison allowed a researcher to identify which parameters were the best at increasing the recovery of DNA.

Another aspect that is often adjusted for DNA processing is the collection protocol. The amount of DNA obtained from a substrate varies upon many different components. One of them is the method used to collect the sample, which may include taping [61, 63, 93], cutting [87, 94], or using a swab [18, 67] on the substrate. In relation to the swabbing method, there are even more modifications that can be made. Some studies have tested the swabbing technique [59] or the swabbing solution [73] used. Swabbing technique refers to the number of swabs used and if they are wet or dry swabs. One example of a collection technique is referred to as the double swab method. When using this technique, a wet swab is used first to loosen and free the cells from the substrate, and then it is followed by a dry swab in order to collect any cells or material left behind. Another technique that can be used requires an analyst to utilize a single wet swab in order to collect the DNA from the substrate. Both of these methods require a wet swab, and the solution that is used to moisten the swab may play a significant role in the collection of touch DNA samples. In regard to the swabbing technique and solution, previous studies have found that a double swab method and the use of an SDS solution is the most effective for collecting DNA [59, 70, 73]. Various solutions can be tested for their effectiveness in collecting touch DNA samples using mock fingerprints. In the present study, we evaluated swabbing solutions and technique for their ability to increase DNA recoveries from touch DNA samples in order to optimize a collection protocol.

The purpose of this series of experiments was to test different collection and extraction parameters in order to optimize the protocols. The mock fingerprints allowed a single variable to be evaluated, and then a custom protocol was designed with the variables that have proven to be

advantageous in recovering DNA. Once each parameter had been tested and compared to the others, the collection and extraction protocols were defined and used as the baseline for further experimentation with the mock fingerprints. These optimized protocols provided confidence that the maximum amount of DNA is being recovered throughout each step.

Materials and Methods

Extraction Protocol

We generated four replicate sets of mock fingerprints according to the protocol outline in Chapter 2 (pg. 27-28) with the following cell quantities: 500, 2,000, and 8,000. These samples were either pipetted directly into a 2 mL extraction tube ("direct" samples) or pipetted onto a glass slide and allowed to dry for 1 hour ("slide" samples). Slide samples were collected with 50µl of sterile water on a cotton swab (Puritan, Guilford, ME). The purpose of pipetting mock fingerprints directly into the extraction tube was so that the efficiency of the extraction alone could be evaluated. In these samples, any DNA lost can only be attributed to the extraction process and no other variable. The slide samples functioned to represent how a true fingerprint would be collected from a substrate and then extracted. These samples were more representative of touch DNA evidence, but also include more variables that may contribute to the loss of DNA during processing. We also generated control mock fingerprints that contained 0 cells. For each sample type, direct or slide, a 0 cell mock fingerprint was tested to check for contamination at that step. All samples were extracted using the QIAampDNA Blood Mini Kit (Qiagen, Hilden, Germany).

The extraction protocol was optimized by evaluating different incubation times. Samples were placed into 2mL extraction tubes with the addition of 400 µl of PBS and 20 µl of Qiagen

protease. All samples were incubated at 56°C for either 10 minutes, 12 – 18 hours, 1 hour with hand-mixing every 10 minutes, or 1 hour with re-suspension every 20 minutes. To re-suspend, the swab was removed to a sterile spin basket and the tube centrifuged for 5 minutes on high to remove a majority of the liquid. The dry swab was placed back into the same extraction tube, and the used spin basket discarded. After incubation, 400 µl of ethanol was added to the tube. Six hundred and fifty microliters of this solution was added to a QIAamp mini spin column and centrifuged for 1 minute at 6,000xg. Swabs in the remaining solution were removed and placed into a spin basket. The spin basket was placed back into the tube and centrifuged for 5 minutes on high in order to collect any liquid retained by the swab. Once the samples were spun down, the remaining liquid was added to the QIAamp mini spin column and centrifuged for 1 min at 6,000xg. The filtrate was discarded and 500 µl of AW1 Buffer was added to the column and spun down at 6,000xg for 1 minute. Again, the filtrate was discarded and then 500 µl of AW2 Buffer was added to the column and centrifuged on high for 3 minutes. The samples were centrifuged again at on high for 1 minute after the filtrate was removed from the collection tube. The spin columns were then placed into 1.5mL tubes and 30 µl of AE Buffer was added to the column. After incubating at room temperature for 1 minute, samples were centrifuged at 6,000xg for 1 minute in order to elute the DNA.

After extraction, all samples were quantified using the Qubit Fluorometer 3.0 according to the protocol outline in Chapter 2 (pg. 31-32). The sample concentration was used to calculate percent recovery.

Collection Protocol

To determine which swabbing solution would be used in the collection of mock fingerprints, we generated 12 replicates of mock fingerprints containing 600 cells, and 12 replicates containing 1,200 cells (Chapter 2; pg. 27-28). Solutions tested included water, 2% SDS and 100% ethanol. The mock fingerprints were pipetted onto clean, glass slides and allowed to dry for 1 hour. Fifty microliters of the appropriate swabbing solution were added to each swab, and the mock fingerprint was collected. The swabs were cut and placed into a 2mL tube and extracted using the previously optimized protocol (pg. 35-36). Following extraction, all samples were quantified using the Qubit Fluorometer 3.0 protocol discussed in Chapter 2 (pg. 31-32). Once the concentration for each sample was obtained, the total DNA extracted and percent recovery was determined for all samples.

The final aspect that we tested was the swabbing technique for the collection of mock fingerprints. Four replicates of mock fingerprints were generated to contain 600 cells, while 4 other replicates were made to contain 1,200 cells. These samples were pipetted onto clean, glass slides. After drying for 1 hour, all samples were collected using the double swab technique. This required a wet cotton swab, wetted with 50µl of 2% SDS, to be used to swab the glass slide containing the mock fingerprint. Following this step, a dry cotton swab was used to swab the same area. For each cell quantity, 2 replicates had the wet and dry swab extracted in separate tubes, while the other 2 replicates required the wet and dry swabs to be extracted together in the same tube. Once all of the swabs were placed into their appropriate tubes, all of the samples containing only 1 swab in the tube were extracted according to the optimized extraction protocol (pg. 35-36). In samples where the wet and dry swabs were extracted together, 400µl of 1X PBS, 800µl of AL Buffer and 40µl of Qiagen protease were added to the samples. This was done in

order to account for the fact that there were two swabs in the tube instead of only one. These samples were then incubated overnight at 56°C and the remainder of the optimized extraction protocol was followed. After extraction, all samples were quantified using the Qubit Fluorometer 3.0 according to the protocol outline in Chapter 2 (pg. 31-32). Once the concentration for each sample was obtained, the total DNA extracted and percent recovery was determined.

Results

<div align="center">COMPARISON OF MODIFIED INCUBATION PHASE PARAMETERS</div>

Sample	DNA Added (ng)	Standard Qiagen Extraction	Overnight incubation	1 hour incubation with swab resuspension	1 hour incubation with shaking every 10 mins
500 cell direct	3	0.00%	0.00%	0.00%	0.00%
2,000 cell direct	12	23.0%	16.9%	0.00%	8.5%
8,000 cell direct	48	12.4%	20.9%	5.28%	18.6%
500 cell slide	3	0.00%	32.4%	0.00%	0.00%
2,000 cell slide	12	0.00%	11.0%	0.00%	5.80%
8,000 cell slide	48	13.5%	17.1%	3.03%	16.1%

Table V. Evaluation of the percent recoveries when different extraction parameters are utilized.

Direct and slide samples were prepared with three different cell quantities (500, 2,000, and

8,000). One sample set was subjected to the manufacturer's protocol. One set was subjected to an

overnight incubation, and the other two sample sets had a resuspension step added or were

shaken during incubation. Shown in this table are the percent recoveries for each sample.

EVALUATION OF SWABBING SOLUTIONS

Sample	Average Total DNA Extracted (ng)	Average Percent Recovery
600 cell slide H_2O	0.99	27.5% ± 6.40%
1200 cell slide H_2O	0.92	12.8% ± 4.47%
600 cell slide EtOH	1.37	38.1% ± 3.40%
1200 cell slide EtOH	1.55	21.5% ± 7.12%
600 cell slide 2% SDS	1.90	52.8% ± 9.23%
1200 cell slide 2% SDS	2.64	36.7% ± 4.08%

Table VI. Average total DNA extract and average percent recoveries of DNA from mock fingerprints on glass slides (n=4). Various swabbing solutions, sterile water, ethanol, and 2% SDS, were tested for their ability to collect mock fingerprints from glass.

COMPARISON OF WET AND DOUBLE SWABBING COLLECTION METHODS

Sample	Total DNA Extracted (ng)	% Recovery
600 cell wet swab #1	0.97	27.0
600 cell dry swab #1	0.00	0.00
600 cell wet swab #2	0.90	25.0
600 cell dry swab #2	0.00	0.00
600 cell wet+dry swabs #1	1.24	34.3
600 cell wet+dry swabs #2	1.06	29.3
1200 cell wet swab #1	2.52	35.0
1200 cell dry swab #1	0.00	0.00
1200 cell wet swab #2	2.34	32.5
1200 cell dry swab #2	0.00	0.00
1200 wet+dry swabs #1	1.32	18.3
1200 cell wet+dry swabs #2	2.34	32.5

Table VII. Determination of swabbing technique to be used in subsequent testing. A single wet

swab method and a double swab (wet and dry swabs) method are compared using mock

fingerprints containing either 600 or 1,200 cells. The mock fingerprints were pipetted onto glass

slides and then collected using the appropriate collection method.

Discussion

Mock fingerprints were used in order to establish an extraction protocol because they

begin with a known quantity of DNA, and can therefore reveal what percentage of DNA is

recovered after extraction. While there are many different types of extractions available for use

in forensic samples, we chose the QIAamp DNA Blood Mini Kit because of it is commonly used

in operational laboratories, yields high quantities of DNA. We experimented with the incubation

time to maximize sample release from the swab in order to establish a baseline protocol that

could be used in subsequent experiments. We also optimized the collection technique and

swabbing solution. The swabbing solutions tested included sterile water, ethanol and 2% SDS.

Additionally, we tested a double swabbing method for the collection of DNA. By testing these

components, we developed an optimized collection and extraction protocols that were used as a

baseline.

The extraction protocol was the first to be optimized through the testing of different

modifications to the incubation phase [48, 75]. Mock fingerprints containing either 500, 2,000 or

8,000 cells were generated and pipetted directly into extraction tubes or onto glass slides to be

collected with a moistened cotton swab. These cell quantities were selected to represent a wide

range of DNA quantities so that the efficiency of each protocol alteration could be evaluated for

low, medium and high DNA quantities. In this experiment, the parameters that were tested

include an extended incubation time, shaking of the samples and the addition of a resuspension

step during the incubation phase. The results in Table V demonstrate that the addition of an

overnight incubation increased DNA recoveries for all samples except the 2,000 cell direct

sample. The 500, 2,000 and 8,000 cell slide samples, had percent recovery values of 32.4%,

11.0% and 17.1% respectively (Table V). All of these values were higher than those obtained

from the standard extraction protocol which were 0.00% for both the 500 and 2,000 cell slide

samples, and 13.5% for the 8,000 cell slide sample (Table V). For the samples that had the swabs

re-suspended during the incubation, the only samples to yield any results were the 8000 direct

and 8,000 slide samples, which were 5.28% and 3.03% respectively (Table V). These recoveries

were lower than the corresponding 8,000 direct and slide samples subjected to the unmodified

protocol, which had recovery values of 12.4% and 13.5% (Table V). Finally, the results for the

samples subjected to an incubation period with shaking outperformed the incubation with swab

resuspension and unmodified protocol, but did not perform better than the sample set subjected to an extended incubation period. The 500, 2,000 and 8,000 direct samples had recovery values of 0.00%, 8.50% and 18.6% respectively, while the slide samples for these cell quantities exhibited percent recoveries of 0.00%, 5.80% and 16.1% respectively (Table V). Based on the results from these replicate sample sets, we determined that an overnight incubation would be added to the extraction protocol. This was because it helped increase the DNA recovery the most out of all the parameters tested, and it required no extra work to be performed by the analyst. It was also chosen based on the increase in DNA recovery for the 500 cell slide sample. Since there is very little DNA found in true fingerprints [35, 36, 37], we expected that later experiments would require the use of mock fingerprints with low quantities of cells. The 500 cell slide sample is representative of a true fingerprint because it contains a low amount of DNA and is on a substrate. Therefore, this increase in recovery, for the 500 cell slide sample, revealed that an overnight incubation will aid in the recovery of DNA from true fingerprints. While the addition of manual shaking increased DNA recoveries for some samples when compared to the unmodified protocol, it was not added to the optimized protocol because manually shaking the samples every 10 minutes during an overnight incubation was not feasible. Future studies could test an overnight incubation with automatic shaking to determine if the combination of these modifications increases the DNA recoveries even more. Other future studies should be conducted in order to explore other extraction methods to determine if other protocols are more efficient than the optimized QIAamp DNA Blood Mini Kit protocol described here. This may include the testing of an organic extraction or other commercially available kits. Ideally, these mock fingerprints will be used to determine what type of extraction is most efficient in the extraction of touch DNA samples.

Once an extraction protocol was established, we could optimize the collection method. A crucial part in the processing of touch DNA samples is the ability to collect the DNA, and the collection solution may play a critical role in this step. Different solutions may interact with the DNA on the substrate differently and thus result in more or less DNA being collected. By using mock fingerprints on glass slides, various swabbing solutions could be evaluated for their collection capabilities. We generated four replicates of mock fingerprints containing either 600 or 1,200 cells and dried them on clean, glass slides. A cotton swab moistened with either sterile water, ethanol or 2% SDS was used to collect the sample from the slide, and then each sample was extracted and quantified so that the percent recovery could be calculated. The results from this experiment demonstrate that swabbing with the sterile water resulted in the lowest percent recovery on average, which was 27.5% for the 600 cell mock fingerprint samples and 12.8% for the 1200 cell samples (Table VI). Ethanol was the second best swabbing solution yielding average recoveries of 38.2% and 21.5% for the 600 cell and 1200 cell samples respectively (Table VI). Finally, the samples collected with 2% SDS displayed the highest average percent recoveries, which were 52.8% for the 600 cell samples and 36.7% for the 1200 cell samples (Table VI). The results of this experiment cannot explain why swabbing with the 2% SDS solution provided better recovery values, but one possibility is that the molecular interactions between the SDS and the DNA are favorable for DNA collection. If the DNA molecules are more attracted to the SDS molecules they will be drawn away from the substrate and collected by the swab moistened with 2% SDS. Another possible reason for this is that the SDS, a detergent, begins to break down the epithelial cells that contain the DNA. This would make the DNA more accessible and easier to collect resulting in more DNA being recovered. The 2% SDS swabbing solution not only outperformed the other solutions tested, but it also displayed the highest

recovery for the 600 cell samples which is important when working with touch DNA and low level DNA samples. Since there is already a limited quantity of DNA present in touch DNA samples, the amount of DNA collected must be maximized and in this experiment the 2% SDS swabbing solution was the most effective. For this reason, the 2% SDS was selected as the swabbing solution for subsequent experiments. Future studies could include more swabbing solutions or alter the amount of swabbing solution added to the swab in order to determine if these parameters will increase the recovery of DNA.

The final parameter that we tested in the establishment of the collection and extraction protocol was the use of a double swab technique. In the previous experiments, a single wetted swab was used to collect the mock fingerprints from glass slides, but another possible technique utilizes two swabs, one wet and one dry swab[59, 70]. In this experiment, four replicates mock fingerprints were generated that contained either 600 or 1,200 cells, which were then dried on glass slides. All of the samples were collected using the double swab technique, which utilized both a wet and dry swab. Two of the replicates had the wet and dry swabs extracted together in the same tube. The other two replicates had the wet and dry swab for that sample extracted in individual tubes. After each mock fingerprint sample was collected, extracted and quantified, the percent recovery for each sample was calculated.

When looking at the 600 cell mock fingerprint samples that had the wet and dry swabs extracted separately, the wet swab for the first replicate had a 27.0% recovery value while the second replicate exhibited a recovery value of 25.0% (Table VII). The dry swab in both of these replicates did not yield any DNA after extraction (Table VII). When evaluating the 600 cell mock fingerprint samples that had the wet and dry swabs extracted in the same tube, the recovery values for the first and second replicates were 34.3% and 29.3% respectively (Table VII).

Regarding the mock fingerprint samples containing 1200 cells, which had the wet and dry swabs extracted separately, the results revealed that the wet swab in the first replicate had a 35.0% recovery value while the wet swab in second replicate exhibited a recovery value of 32.5% (Table VII). The dry swab in both of these replicates did not yield any DNA after extraction (Table VII). When examining the 1200 cell mock fingerprint samples that had the wet and dry swabs extracted in the same tube, the recovery values for the first and second replicates were 18.3% and 32.5% respectively (Table VII). In this experiment, the dry swab that followed the wet swab during collection did not yield any DNA after extraction for all replicates in which the wet and dry swabs were extracted separately. This may be because the amount of DNA recovered by the dry swab is below the detection level of the Qubit® Fluorometer 3.0, which is 0.2 ng for the high sensitivity dsDNA assay [95] . For example, the wet swab used to collect the 600 cell mock fingerprint (replicate #2) recovered 0.97 ng of DNA, while 1.06 ng of DNA was recovered from a 600 cell mock fingerprint when the wet and dry swab were extracted together. This is a difference of 0.08 ng, which is below the level of detection for the Qubit assay used. The additional recovered DNA from the samples that had the swabs extracted together may be due to the second dry swab, but this cannot be confirmed from this experiment on its own. Based on these results from the swab technique experiment, we determined that a single swab wetted with 2% SDS would be sufficient for collecting touch DNA samples. Since swabs are not the only device that can be used for collecting touch DNA samples, future studies should test other methods. A common method is to tape the object in order to collect any material on the substrate so that it can be analyzed for DNA [20, 63, 96]. There are many different types of tape being used in crime laboratories and therefore a study that examines these tapes may provide useful insight regarding their use in the field.

It is important to remember that we designed these experiments as an ideal situation that served as the starting point for research with the mock fingerprints. Subsequent experiments relied on these optimized protocols to generate a standard curve and evaluate the loss of DNA during collection and extraction. Having optimized protocols gives confidence to the researcher that the maximum amount of DNA is being recovered, which is especially important when dealing with touch DNA that contains low quantities of DNA. In this series of experiments, the mock fingerprints allowed for single variables in the collection and extraction protocols to be evaluated. The ability to add a specific amount of DNA is a critical component of the mock fingerprints, and this characteristic will continue to be useful in subsequent experimentation.

Chapter IV: Tracking the Loss of DNA

Introduction:

We conducted the previous experiments to validate and optimize the mock fingerprints for their use in research pertaining to touch DNA samples. Testing proved that the mock fingerprints mimicked true fingerprints and could be used as positive controls in further experimentation. Other forensic research studies have been conducted, and the results indicated that between 0.04 ng and 1.23 ng of DNA could be recovered from touch DNA samples [35, 36, 37]. This is a limited quantity of DNA and therefore efforts should be made to recover as much DNA as possible. The purpose of the experiments described in this chapter was to track the loss of DNA during the collection and extraction process, and demonstrate which area(s) account for the largest loss of DNA. Furthermore, knowing which steps exhibit the largest loss of DNA would eveal which areas should be the focus of future research and be optimized further.

There is an inevitable amount of DNA lost during processing, which may occur during the collection and extraction steps [1, 2, 3, 4]. Although this is a known fact about DNA processing, it can be difficult to isolate each individual step in order to identify where most of the DNA is lost. This is where mock fingerprints were well suited for this experiment. First, being able to add a specific number of cells to the sample eliminated the variability between donors. If true fingerprints were used in this experiment it would be difficult to discern if the differences in results for the samples was actual loss of DNA or due to donor variability. Mock fingerprints eliminated this possibility and made the researcher more confident in the conclusions drawn from the results. Adding a known number of cells also allowed the percent recovery to be evaluated. This gave a result which was easy to compare to other samples. Having these values made it easier for us to evaluate where DNA is being lost throughout the process. For these reasons, the mock fingerprints were an ideal tool for this experiment.

In order to track the loss of DNA throughout the collection and extraction process, we utilized three different types of samples. The first was a "direct" sample in which the mock fingerprints were pipetted directly into the extraction tube. Through the use of the direct mock fingerprint samples, the percent loss of DNA due to extraction was determined. In these samples, there was no other factor that affected the recovery of DNA, and therefore the results revealed how much DNA is lost from extraction alone. The second was a "swab" sample, which required that the mock fingerprints be pipetted directly onto the cotton swab. Since the mock fingerprints were pipetted directly onto the swab, these samples were not testing the swab's ability to collect DNA from a surface, but rather the capability to release DNA. Finally, a "slide" sample referred to mock fingerprints pipetted onto glass slides, dried and then collected. These samples imitated how a true fingerprint would be collected from a surface. By using mock fingerprints in this fashion, it indicated how well DNA was being collected on the swab, and if some of the sample was being left behind on the surface. The data from all three of these samples was compared to one another in order to learn more about where DNA is lost during processing.

Since there is a very low quantity of DNA available in touch DNA samples it is important to utilize protocols that maximize its recovery. Mock fingerprints can aid in identifying which areas may need to be optimized. Similar to the way mock fingerprints were used in Chapter 3 to optimize collection and extraction protocols, we used them here to determine the recovery values for each sample. Comparisons between the recovery values for the direct, swab and slide mock fingerprint samples helped explain which steps caused the most DNA to be lost. This allows a specific area to be the focus of future experiments, with the end goal being to maximize DNA recovery from touch DNA evidence.

Materials and Methods:

      Mock fingerprints were generated according to the protocol outlined in Chapter 2 (pg. 27-28) using the LUNA^{TM} Cell Counter (Logos Biosystems, South Korea). The cell quantities in the mock fingerprints included 250, 300, 475, 650, 825, 1,000 and 1,100 cells, which were the same quantities used in generating the standard curve in Chapter 4 excluding the 1,500 cell samples. In total, 12 replicates for each cell quantity were made and then they were divided up into three types of samples: direct, swab and slide. Four replicates of direct, swab and slide samples were generated for each cell quantity. Each cell quantity was used in direct, swab and slide samples, and there were 4 replicates for each type of sample. In order to monitor contamination throughout the experiment, negative controls were also included for each type of sample. Three 0 cell mock fingerprints that contained 2µl of fingerprint solution and 18µl of 1X PBS were generated. For each type of sample, direct, swab and slide, we also tested a 0 cell mock fingerprint. The 0 cell direct sample ensured that there is no contamination regarding the mock fingerprint, and the 0 cell swab sample tested for contamination from the swab. Finally, the 0 cell slide sample checked for contamination during the collection process, which would include contamination of the surface.

      To generate direct samples, mock fingerprints were pipetted directly into a 2mL extraction tube. Swab samples had the mock fingerprints pipetted directly onto a cotton swab (Puritan, Guilford, ME), and then the swab was cut and placed into a 2mL extraction tube. Lastly, we pipetted slide mock fingerprint samples onto clean, glass slides and dried for 1 hour. Then they were collected with a cotton swab moistened with 50µl of 2% SDS. The swab was cut and placed into a 2mL tube for extraction. Once all of the direct, swab and slide samples for each cell quantity were ready for extraction, the optimized extraction protocol from Chapter 3 (pg. 35-

36) was performed on all of the samples. Then the samples were quantified using the Qubit®

Fluorometer 3.0 (pg. 31-32) to obtain the concentration of DNA in each individual sample. The

total DNA extracted was calculated for each sample followed by the calculation of the percent

recovery. Since this experiment focused on the loss of DNA during the collection and extraction

process, the percent recovery was converted into percent loss. This was accomplished by

subtracting the percent recovery from 100%. Then the average percent loss was determined for

the 4 replicates for direct, swab and slide samples containing each cell quantity.

Results:

AVERAGE PERCENT LOSS DURING COLLECTION AND EXTRACTION

Average % Loss			
Sample	Direct	Swab	Slide
250 cells	42.6% ± 9.18%	100.% ± 0.00%	60.8% ± 9.38%
300 cells	36.2% ± 5.90%	70.0% ± 6.36%	60.7% ± 3.81%
475 cells	57.8% ± 6.81%	77.3% ± 6.12%	71.2% ± 3.25%
650 cells	66.0% ± 1.82%	81.3% ± 7.73%	81.2% ± 2.15%
825 cells	68.9% ± 3.15%	88.2% ± 4.48%	77.0% ± 0.92%
1,000 cells	75.9% ± 4.19%	86.7% ± 2.65%	81.9% ± 2.13%
1,100 cells	76.2% ± 2.20%	90.2% ± 0.61%	84.2% ± 2.23%

Table VIII. Average percent loss for direct, swab and slide samples (For each quantity, n=4).

Mock fingerprints with cell quantities of 250, 300, 475, 650, 825, 1,000 and 1,100 cells were

generated and then pipetted directly into an extraction tube, directly onto a cotton swab or dried

on a glass slide and collected. The percent recovery for each sample was calculated to determine

the average percent loss for each sample type and cell quantity.

AVERAGE PERCENT OF DNA LEFT ON SWAB

Sample	Average percent of DNA left on swab
250 cells	57.4%
300 cells	33.8%
475 cells	19.5%
650 cells	15.3%
825 cells	19.4%
1,000 cells	10.9%
1,100 cells	14.0%

Table IX. Average percent of DNA left on the swab after extraction. This value was calculated for each cell quantity by subtracting the average percent loss for the direct sample from the average percent loss for the corresponding swab sample. The result is the percentage of the DNA sample left on the swab for that cell quantity.

Discussion:

The goal of this experiment was to identify the aspects of the collection and extraction process that account for the highest amounts of DNA loss. We accomplished this by generating mock fingerprints with various cell quantities and pipetting them directly in an extraction tube, onto a swab or drying on a glass slide. By utilizing the mock fingerprints in this way, each step of DNA processing was isolated and could be examined individually. Table VIII shows the average percent loss for each cell quantity for each type of sample: direct, swab and slide. One noticeable trend in the data is that the percent loss generally increased as the number of cells in the sample increased, and this is true for direct, swab and slide samples. When looking at the

direct mock fingerprints the 250 cell samples had an average percent loss of 42.6% while the 1,100 cell samples exhibited an average loss of 76.2% (Table VIII). In terms of the swab samples, the 300 cell mock fingerprints had an average percent loss of 70.0% and 90.2% for the 1,100 cell samples (Table VIII). The slide samples exhibited an average percent loss of 60.8% with 250 cells and 84.2% with 1,100 cells (Table VIII). While this trend is not a great concern in this experiment, since the mock fingerprints work with small cell quantities, it may be useful to investigate this further with mock fingerprints that contain larger cell quantities. Discovering the cause of this trend may allow a more efficient procedure to be developed.

The previously described trend also revealed that as the number of cells in the mock fingerprints increased, more of the loss was due to extraction than the swab. The percent loss values for the swab samples included the loss due to extraction and the DNA left on the swab. By subtracting the average percent loss due to extraction from the average percent loss for the swab samples, the percent of DNA left on the swab was calculated. Table IX revealed that the 250 cell samples lose 57.4% of DNA due to the swab, but as the cell quantity increased to 1,000 cells only 10.9% of the loss can be attributed to the swab. This demonstrated that more DNA was being lost solely due to extraction in samples with higher cell quantities compared to the mock fingerprints with lower cell quantities. Other studies, that have focused on tracking the loss of DNA, have found varying percent loss values. These values range between 43.0% and 65.3% [3, 4]. Extraction is a critical step in DNA processing and since the results from this experiment displayed higher percent loss values than other published studies, the protocol may need to be re-evaluated to improve DNA recoveries. Based on this trend, future experiments should be conducted to test different extraction methods to determine if it resolves the issue of losing more DNA due to extraction as the initial quantity of DNA increases.

In terms of analyzing where DNA is lost during the collection and extraction steps comparisons between the direct, swab and slide samples for the same cell quantity must be made. For example, with samples containing 300 cells the average sample lost 36.2% due to extraction, 70.0% was lost when extraction and the swab are taken into consideration, and finally 60.7% was lost during the entire collection and extraction process (Table VIII). It is interesting that more DNA is lost when the mock fingerprints were pipetted directly onto the swab than when they were dried on glass slides. Since there is a possibility that DNA is left behind on the surface, it may be expected that the slide samples would have a higher percent loss compared to the swab samples. Yet, this trend of the swab samples having an average percent loss higher than the slide samples was exhibited by all cell quantities (Table VIII). These results gave confidence to the researcher that all of the DNA was being collected off of the surface. A higher average percent loss for the swab samples revealed that releasing the DNA is the issue, not collecting it from the surface. It was important to take into consideration that the experimental design for this test may have played a role in the swabs samples having a higher percent loss than the slide samples. One potential reason for this difference is the way the DNA was deposited or collected on the swab. In the samples in which the mock fingerprints were pipetted directly onto the swab, the cells may have absorbed deeper into the swab making them more difficult to release during extraction. When collecting the mock fingerprints from the slide, the cells were likely to remain on the exterior of the swab, thus causing them to be released from the swab more easily. Based on these results it is reasonable to believe that testing for a more effective swab should be the focus of future experiments.

Future Studies

This experiment can serve as the foundation for future experimentation because it provided valuable information regarding the areas of DNA processing that need to be optimized further. First, the increased loss due to extraction as the cell quantities increased is a concern. Since every sample needs to be extracted, it is crucial that this step minimize the amount of DNA lost. Future studies should be performed by researchers to examine different types of extraction so that they can be evaluated with the mock fingerprints for their ability to efficiently recover DNA. Some of the potential methods that could be considered include an organic extraction, a magnetic bead extraction method or other commercially available silica based kits. Testing various extraction methods will help researchers discover which one may be the most efficient in recovering DNA from touch samples.

This experiment also suggested that different types of swabs should be an emphasis in future experimentation. Based on the results from this study the cotton swabs did not effectively release the DNA for all mock fingerprint samples. Although the use of the cotton swab is common practice in forensic laboratories, there are many other swabs being produced that may release the DNA more efficiently. For example, FLOQSwabs[TM], manufactured by COPAN, are designed to maximize the release of DNA during extraction and have been proven to be more efficient than the standard cotton swab [65, 66, 97]. Using the same experimental design used here, but with the FLOQ swabs would provide insight regarding which swab is more effective for touch DNA samples. Additionally, other collection devices, such as tape, could be tested with the mock fingerprints since swabs are not the only devices employed in the lab by forensic scientists. [20, 63, 96]. It is important to remember that this experiment was designed as a preliminary study and to be the foundation of future research. The results from this study have provided valuable

information and presented numerous new avenues for research. Testing with the mock

fingerprints should continue and work towards optimizing the processing of touch DNA samples.

Chapter V: Generation of a Standard Curve

Introduction:

Once we optimized the collection and extraction protocols, we could generate a standard curve using the mock fingerprints. A standard curve demonstrates the relationship between two quantities and is graphically represented by plotting the quantities against each other. In this instance, the two quantities were the total DNA added to the mock fingerprints and the total DNA extracted from the mock fingerprints. Since both of these quantities were known for each individual mock fingerprint, all 28 of the mock fingerprint samples could be plotted to generate a standard curve. The mock fingerprints used to generate the standard curve were collected from glass slides using a cotton swab. Glass is an easy substrate to collect DNA from, decontaminate, handle and store, which makes it an ideal surface for research. Therefore, we selected it as the substrate to generate the standard curve. Then we plotted true fingerprints on the standard curve. This was accomplished based on the DNA extracted values obtained from 10 true fingerprints. Since only one quantity was known for the true fingerprints, the total DNA extracted, the value for total DNA "added" was interpolated using the standard curve. This allowed for comparisons between the true and mock fingerprints to be made, and helped determine if any adjustments need to be made to the mock fingerprints.

The standard curve generated through these experiments can also be used as a baseline for the quantity of DNA in true fingerprints. Having a baseline is important because it gives a point of reference and allows results to be easily compared. Experiments can change a single variable and then be compared to the baseline to determine if the modification helped or hindered the recovery of DNA. Using the standard curve as a baseline allows for these types of comparisons to be made, and result in protocols becoming more optimized and effective for touch DNA evidence.

Studies involving touch DNA samples have demonstrated a wide range of DNA quantities recovered from fingerprints. Some studies have found extraction quantities as little as 0.04 ng or as high as 1.23 ng or somewhere in between [35, 36, 37] It is important to remember that the experimental design of touch DNA studies vary and therefore the DNA quantities obtained were not be the same. In this experiment, the first important aspect tested was the range of DNA found in true fingerprints. The purpose of the range experiment was to determine the lowest number of cells that could be put into a mock fingerprint and still recover DNA after extraction. It was also important to compare the DNA recovery values for the mock fingerprints to that of the true fingerprints to ensure that the true fingerprints fell within the range. This experiment also set the range for the cell quantities to be used in the standard curve. Since the amount of DNA in a fingerprint varies from person to person, it was crucial to consider this when determining a range for mock fingerprints. In this experiment, the range of the standard curve included the upper and lower limits of true fingerprints in order to include all possible DNA quantities in various fingerprints. This was accomplished by collecting true fingerprints from multiple donors in order to account for variability. Once the range for the quantity of cells in the mock fingerprints was set to reflect the quantities of DNA in true fingerprints, a researcher had more confidence in the mock fingerprints and knew that they are representative of a touch DNA sample.

In the following experiments, we also tested true fingerprints on various surfaces. True fingerprints were deposited onto nonporous surfaces, which included a laminate countertop, porcelain tile and a plastic phone case. These fingerprints were collected, extracted, quantified and then plotted on the standard curve to determine their relationship to one another and to true fingerprints on other surfaces. This provided information about differences between the selected

surfaces and the ability to collect DNA from them. Since touch DNA samples can be found on numerous different surfaces and in different quantities at a crime scene, it was imperative that more than one was analyzed.

Generating a standard curve provided valuable information about the performance of the mock fingerprints as well as information regarding the nature of true fingerprints. By adjusting the range of the cell quantities in the mock fingerprints, the standard curve served as a validation of the capabilities of the mock fingerprints to act as a positive control. If the true fingerprints fall within the adjusted range it demonstrates that the mock fingerprints are an accurate representation of touch DNA samples. The standard curve also allowed for comparisons between samples to be made and function as the baseline for future experiments. Overall, the standard curve is a useful tool in research involving the mock fingerprints, and therefore was generated in the following experiments.

Materials and Methods:

Range Determination

We made four replicates of mock fingerprints with cell quantities of 0, 100, 150, 200, 250, 300, 350, 400, 450, 500, 1,000 and 1,500 according to the protocol outlined in Chapter 2 (pg. 27-28). A 0 cell mock fingerprint was also generated which contained 18µl of 1X PBS and 2µl of fingerprint solution. This sample ensured that no contamination was exhibited in the mock fingerprint samples. Glass slides were decontaminated by soaking them in 10% bleach for 15 mins and then rinsing with 70% ethanol. Once the slides were dry, the mock fingerprints were pipetted onto the slides and allowed to dry for 1 hour. Then a single cotton swab (Puritan, Guilford, ME) moistened with 50µl of 2% SDS was used to collect each mock fingerprint. These

samples will be referred to as "slide samples" because they were pipetted and dried on the glass slides. All samples were then extracted according to the extraction protocol optimized in Chapter 3 (pg. 35-36). Positive and negative controls for the extraction process were included to ensure that the reagents were working properly and to check for contamination. After extraction, the samples were quantified using the Qubit® Fluorometer 3.0, and then we calculated the total DNA extracted for each sample. Then we evaluated the results for total DNA extracted to establish the lowest quantity of cells that could be used in a mock fingerprint for subsequent analysis.

Ten true fingerprints were deposited onto clean, glass slides by five different donors. Donors rolled their fingertip from end to end 6 times without lifting their finger. A cotton swab wetted with 50µl of 2% SDS was used to swab and collect the material from the slide. Each fingerprint was extracted according extraction protocol optimized in Chapter 3 (pg. 35-36), followed by quantification using the Qubit® Fluorometer 3.0 (pg. 31-32). Then the value for total DNA extracted was calculated. These results were compared to the mock fingerprints in the range experiment to determine if it is possible for a mock fingerprint to contain the proper number of cells in order to represent a true fingerprint.

Generation of a Standard Curve

Four replicates of mock fingerprints with cell quantities of 0, 250, 300, 475, 650, 825, 1,000, 1,100, and 1500 were generated according to the protocol outlined in Chapter 2 (pg. 27-28) and pipetted onto clean, glass slides. The 0 cell mock fingerprint served as a control sample to check for contamination as well as set the intercept for the standard curve at (0,0). The mock fingerprints were allowed to dry for 1 hour, and then collected using a cotton swab moistened

with 50μl of 2% SDS. Using the protocol optimized in Chapter 3 (pg. 35-36) all samples were extracted, followed by quantification using the Qubit® Fluorometer 3.0.

We plotted each mock fingerprint on a graph of total DNA extracted vs. DNA added using Excel (Microsoft, Redmund, WA). Using the concentration value, we calculated the total DNA extracted for each sample. Then the DNA added was calculated by multiplying the number of cells in the sample by 6 pg, since there are 6 pg of DNA in an epithelial cell. Then a best fit line with the intercept set to (0,0) was added to the graph representing all of the mock fingerprints, and the equation for this line was given. This line was considered the standard curve for the mock fingerprints.

The 10 true fingerprints used for comparison in the range experiment were plotted on the standard curve using Excel. Since these samples were from true fingerprints, the total DNA "added" was unknown and therefore needed to be interpolated from the standard curve generated by the mock fingerprints. Using the slope from the equation for the standard curve and the total DNA extracted, we calculated the amount of DNA "added". Once this value was determined, we plotted the true fingerprints were plotted on the standard curve.

$$Total\ DNA\ \text{added for true fingerprints (ng)} = \frac{Total\ DNA\ extracted(ng)}{Slope\ of\ standard\ curve}$$

Various Surfaces

We used true fingerprints deposited onto various nonporous surfaces for comparison against the true fingerprints collected from glass slides. The surfaces included porcelain tile, laminate countertop and a plastic phone case. Before depositing any fingerprints, the surfaces were cleaned with 10% bleach followed by 70% ethanol. The true fingerprints were deposited

onto the surface by rolling the fingertip from end to end six times without lifting the finger. In total, 10 true fingerprints were collected for each surface and 5 different donors were used in depositing these fingerprints. Each true fingerprint from each surface was collected using a cotton swab wetted with 50µl of 2% SDS. All samples were extracted using the optimized extraction protocol (pg. 35-36) and quantified (pg. 31-32) as previously described. The value for total DNA extracted was calculated and then exported to excel so that the true fingerprint could be plotted onto the standard curve. Similar to the true fingerprints collected from glass slides, we used the slope of the standard curve to interpolate the amount of DNA "added" for the true fingerprints from various surfaces. All 10 of the true fingerprints from each surface, including glass, were plotted on the standard curve for comparison.

Results:

AVERAGE TOTAL DNA EXTRACTED FROM MOCK FINGERPRINTS USED FOR
RANGE EXPERIMENT

Sample	DNA Added (ng)	Average DNA Extracted (ng)	Standard Deviation
0 cell slide	0	0.00	0.00
100 cell slide	0.6	0.00	0.00
150 cell slide	0.9	0.00	0.00
200 cell slide	1.2	0.20	0.41
250 cell slide	1.5	0.85	0.30
300 cell slide	1.8	0.64	0.88
350 cell slide	2.1	0.78	0.77
400 cell slide	2.4	0.85	0.26
450 cell slide	2.7	1.06	0.36
500 cell slide	3	0.98	0.51
1,000 cell slide	6	1.87	1.14
1,500 cell slide	9	2.66	1.15

Table X. Average DNA extracted from mock fingerprints used in the range experiment (n=4).
The cell quantities used included 0, 100, 150, 200, 250, 300, 350, 400, 450, 500, 1,000 and 1,500
cells. "Slide" samples required that the mock fingerprints be dried on a clean, glass slide, then
collected, extracted and quantified. The amount of DNA extracted from mock fingerprints was
compared to the amount of DNA extracted from true fingerprints.

TOTAL DNA EXTRACTED FROM TRUE FINGERPRINTS ON GLASS

True Fingerprint	Total DNA Extracted (ng)
1	1.49
2	1.07
3	0.71
4	0.82
5	0.86
6	0.00
7	1.38
8	0.96
9	0.99
10	0.00

Table XI. Total DNA extracted (ng) from 10 true fingerprints deposited on clean, glass slides. These samples were compared to the mock fingerprints extracted in the range experiment to determine the range of cells that would be appropriate for the standard curve.
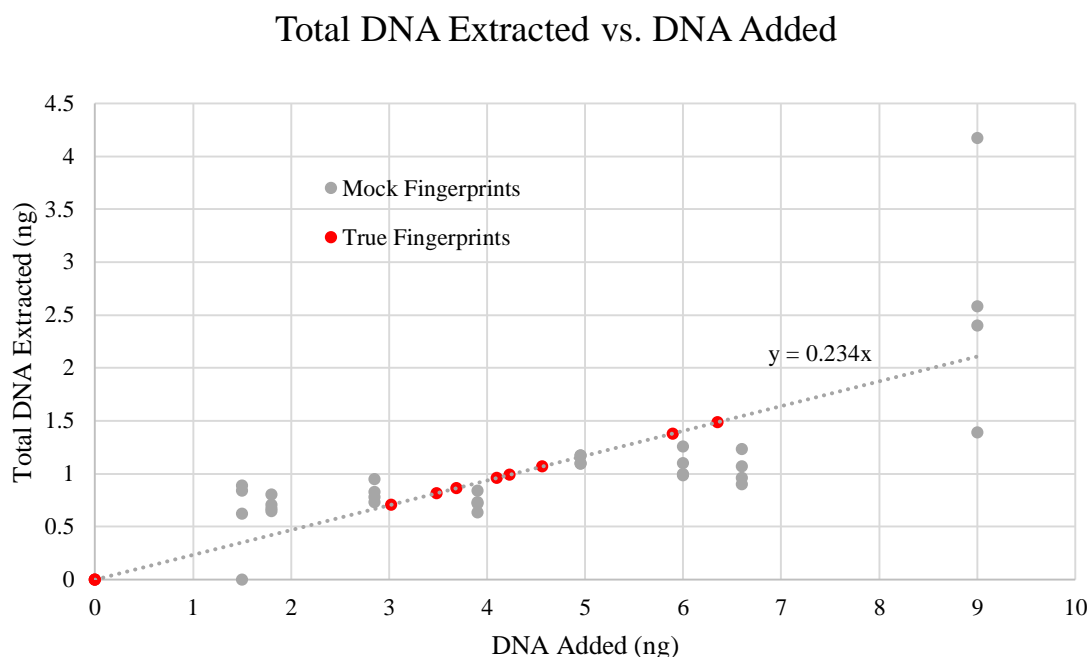
Total DNA Extracted vs. DNA Added

Figure 3. The standard curve generated by mock fingerprints with true fingerprints plotted on

the curve. Mock fingerprints with cell quantities of 0, 250, 300, 475, 650, 825, 1,000, 1,100 and

1,500 were generated and pipetted onto glass slides, and then collected, extracted and quantified

(n=4). Using the quantification values, the total DNA extracted was plotted against the DNA

added to generate the standard curve. True fingerprints were also plotted on the standard curve.

For these samples, the initial amount of DNA (DNA added) was based on the total DNA

extracted for each sample (n=10).

TRUE FINGERPRINTS COLLECTED FROM VARIOUS SURFACES

Sample	Countertop		Tile		Phone		Glass	
	DNA "added" (ng)	Total DNA Extracted (ng)	DNA "added" (ng)	Total DNA Extracted (ng)	DNA "added" (ng)	Total DNA Extracted (ng)	DNA "added" (ng)	Total DNA Extracted (ng)
1	3.07	0.72	4.71	1.10	7.28	1.70	6.35	1.49
2	0.00	0.00	3.23	0.76	0.00	0.00	4.56	1.07
3	0.00	0.00	2.82	0.66	3.98	0.93	3.02	0.71
4	0.00	0.00	0.00	0.00	0.00	0.00	3.48	0.82
5	4.97	1.16	6.51	1.52	4.00	0.94	3.69	0.86
6	4.77	1.12	5.28	1.24	4.20	0.98	0.00	0.00
7	0.00	0.00	3.18	0.74	5.23	1.22	5.89	1.38
8	0.00	0.00	2.72	0.64	0.00	0.00	4.10	0.96
9	3.43	0.80	3.13	0.73	2.87	0.67	4.23	0.99
10	3.33	0.78	3.79	0.89	2.82	0.66	0.00	0.00

Table XII. Total DNA extracted values for 10 true fingerprints deposited onto various surfaces. The surfaces included a laminate countertop, porcelain tile, a plastic phone case and glass. Since the initial amount of DNA in a true fingerprint is unknown, and therefore must be calculated. By calculating the total DNA extracted and using the slope of the standard curve the amount of DNA "added" for each true fingerprint could be determined.

AVERAGE DNA EXTRACTED FROM VARIOUS SURFACES

Surface	Average Total DNA extracted (ng)	Standard Deviation
Countertop	0.46	0.50
Tile	0.83	0.41
Phone Case	0.71	0.57
Glass	0.83	0.50

Table XIII. Average total DNA extracted and standard deviation values for true fingerprints collected from various surfaces (n=10). The surfaces included a laminate countertop, porcelain tile, plastic phone case and glass.
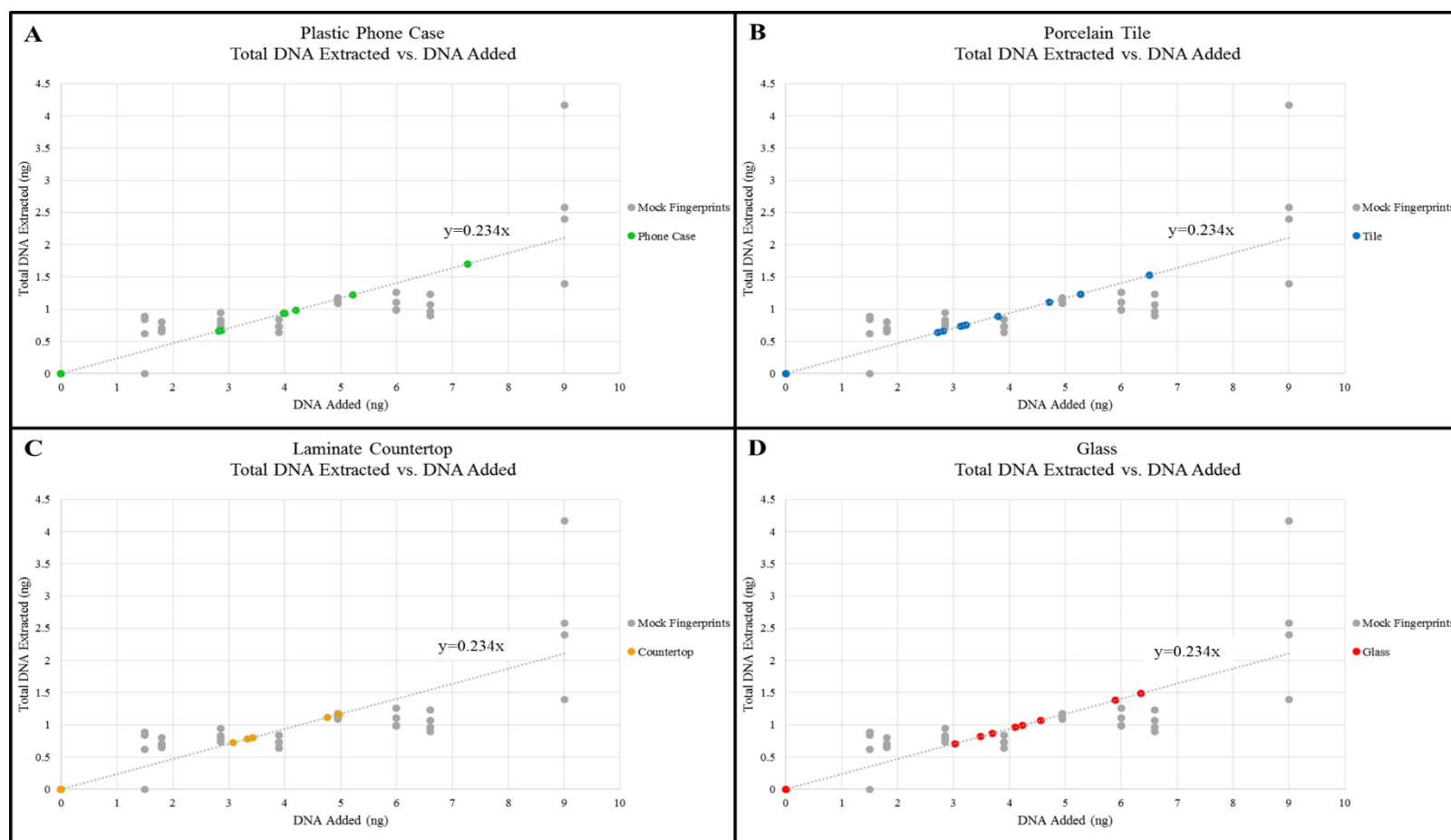
Figure 4. True fingerprints from various surfaces plotted on the standard curve generated with mock fingerprints. Figure 3A is the standard curve with true fingerprints collected from the plastic phone case shown in green. Figure 3B displays the true fingerprints collected from porcelain tile plotted on the standard curve in blue. Figure 3C is the standard curve with true fingerprints collected from the laminate countertop shown in orange. Figure 3D shows the true fingerprints collected from glass slides plotted on the standard curve in red.

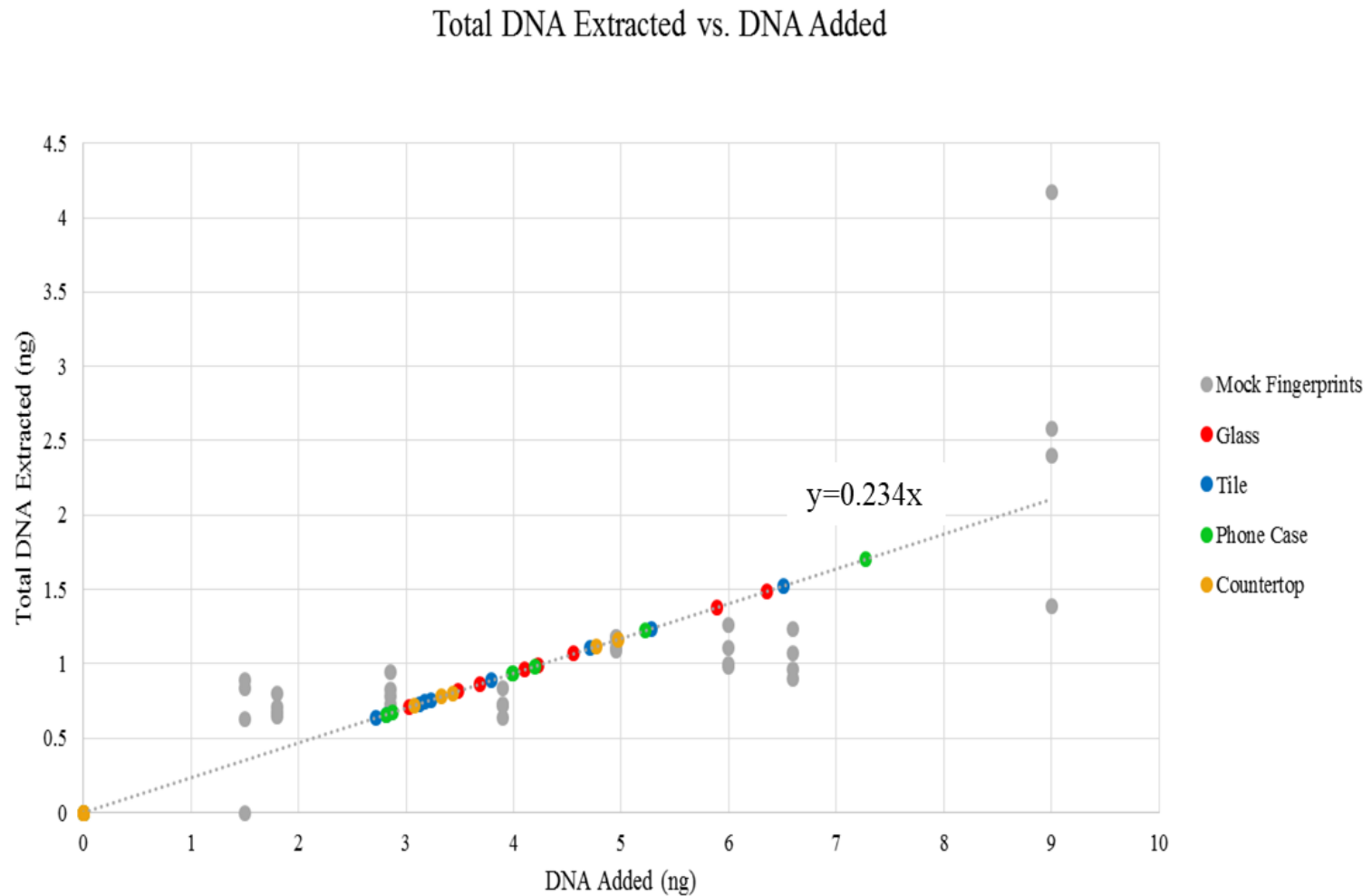**Figure 5**. True fingerprints from various surfaces plotted on the standard curve (n=40). Donors deposited fingerprints onto glass, porcelain tile, a plastic phone case and a laminate countertop. After collection, extraction and quantification each individual fingerprint was plotted on the curve. Plotting all of the true fingerprints on one curve allows for comparisons between each surface to be made.

Discussion:

The goal for this series of experiments was to validate and define the range of the mock fingerprints as well as generate a standard curve. Creating a standard curve with the mock fingerprints served as the baseline allowing for comparisons to be made between data generated in experiments with touch DNA. The standard curve was also used to evaluate the mock fingerprint's ability to accurately represent a true fingerprint. Developing this foundation with the mock fingerprints is crucial for future studies and therefore the standard curve was generated.

In order to generate a standard curve, we needed to determine the range for the mock fingerprints. This range experiment served as a way to validate the mock fingerprints and determine if the quantity of DNA in the mock fingerprints was representative of true fingerprints. The purpose of this test was also to discover the quantity of cells in a mock fingerprint at which no DNA was recovered or detected after extraction. To accomplish this, we generated four replicates of mock fingerprints to contain cell quantities including 0, 100, 150, 200, 250, 300, 350, 400, 450, 500, 1,000 and 1,500 cells. Each sample was dried on a glass slide. Then we collected, extracted and quantified each sample. We calculated the total DNA extracted in order to determine the optimal range. The first step in this experiment was to determine the lowest cell quantity that could be used in a mock fingerprint. Table X reveals that 200 cell mock fingerprints yielded an average of 0.20 ng of DNA, which was the lowest value out of all the mock fingerprints. It is important to note that out of the four replicates for the 200 cell samples, 3 did not yield any DNA after extraction. This reveals that 200 cell mock fingerprint samples were unreliable, and that 200 cells in a sample could be used to represent a true fingerprint. In addition, none of the mock fingerprints that contained 100 or 150 cells yielded any DNA (Table

X). Based on the results for the 100, 150 and 200 cell mock fingerprints, we excluded these cell quantities as the lower limit for the range. In contrast, the remaining mock fingerprints did yield DNA after extraction. The highest average quantity was 2.66ng, which was recovered from the 1500 cell mock fingerprints (Table X). Table X demonstrates that mock fingerprints with less than 250 cells did not yield reliable DNA quantities extraction. For this reason, 250 cells was established as the lowest cell quantity in the range for the standard curve. The goal for this experiment was met with the determination that 250 cells was the lowest possible quantity that could be used in a mock fingerprint.

The next step in the range experiment was to compare the results for DNA extracted from mock fingerprints to that extracted from true fingerprints. True fingerprints were deposited onto glass slides, collected, extracted and quantified so that the total DNA extracted from each individual fingerprint could be calculated. In this series of experiments, we analyzed the total DNA extracted instead of just human specific DNA. This was to help in establishing the baseline values for the mock fingerprints. Since there may be a combination of microbial and human DNA present in true fingerprint samples [83, 84], it is important to start by analyzing the total DNA extracted. Future studies should be conducted to look more closely at the amount of DNA specific to humans in true fingerprints. Once the total DNA extracted was calculated, for both the true and mock fingerprints, the values were compared. This comparison served two purposes. The first was to determine the range to be used in the generation of the standard curve. The amount of DNA in a true fingerprint is variable and therefore it was important that the standard curve covers the range of DNA quantities in true fingerprints. In this experiment, the range represented the values in which a true fingerprint could fall between. When looking at the total DNA extracted from true fingerprints shown in Table X, the lowest amount of DNA extracted

was 0.71ng (true fingerprint #3) and the highest was 1.49 ng (true fingerprint #1). These values were most accurately represented by mock fingerprints with 250 cells and 1,000 cells, which yielded an average of 0.85 ng and 1.87 ng respectively (Table X). Based on the data, the lower limit for the range was determined to be 250 cells and the upper limit to be 1,500 cells. Since 250 cells was the lowest quantity of cells for detection and because it resulted in similar DNA yields to some true fingerprints, it was used as the lower limit of the range. Although none of the 10 true fingerprints resulted in DNA yields similar to 1,500 cells, it was used as the upper limit of the range. This is because it was important that the upper limit of the range extend beyond the range for true fingerprints in order to include all possible DNA yields from any fingerprint. Using the upper and lower limits, the range was defined and then the cell quantities to be used for the standard curve were selected to be 250, 300, 475, 650, 825, 1,000, 1,100 and 1500 cells. These values allowed for an even distribution across the range of the mock fingerprints.

Another important aspect that was included in this comparison was the determination that the quantity of DNA in the mock fingerprints was representative of true fingerprints. Each of the true fingerprints in Table XI yielded total DNA values near or above the value for mock fingerprints with 250 cells. This excluded the two true fingerprints that yielded no DNA after extraction (Table XI; True fingerprints 6 and 10). Since 250 cells were previously determined as the lower limit for mock fingerprints, it was critical that true fingerprints did not fall below this threshold. If the DNA yields for true fingerprints fell drastically below this limit, the mock fingerprints would have been unable to contain the appropriate number of cells to accurately mimic an eccrine fingerprint, and therefore further experimentation could not continue.

A standard curve was a necessity because it allowed the mock and true fingerprints to be compared to one another, and it created a baseline for future experimentation. We generated the

standard curve by plotting two known quantities against one another. In this experiment the values are the total DNA extracted and the DNA added, which were both known quantities for the mock fingerprints. These quantities were valuable and necessary for this experiment because they allow the standard curve to be generated. Once the range for the standard curve was determined, four replicates of mock fingerprints containing 0, 250, 300, 475, 650, 825, 1,000 and 1,500 cells were analyzed. These samples were dried on glass slides, collected, extracted and then quantified. Then the total DNA extracted was measured and the two known values, DNA added and total DNA extracted, for each mock fingerprint could be plotted against one another on a graph. These data points are shown in grey in Figure 3. The standard curve for all the data points is also indicated and has a value of 0.234 for the slope (Figure 3). A positive value for the slope demonstrated that as one quantity increases the other quantity also increases. This trend was expected because as the initial DNA quantity in a sample increases it was anticipated that a higher total DNA extracted value will be obtained. In general, the more DNA that was present in a sample the more DNA there was available to be extracted. Figure 3 also shows the 10 true fingerprints plotted on the standard curve, which are displayed in red. Since the initial amount of DNA in the true fingerprints was unknown, the value was interpolated using the slope of standard curve. Doing this gave the desired value and resulted in the true fingerprints falling directly on the curve because the standard curve was used to obtain the value. Unlike the mock fingerprints, there was no way to measure the initial amount of DNA in a true fingerprint and therefore it was interpolated using the equation given. Once all of the true fingerprints were plotted, it was evident that they fell within the range of the mock fingerprints and demonstrated that true fingerprints have an initial quantity of DNA between 1.5 ng and 6 ng. This was an important aspect because it exemplified the power of the mock fingerprints, and revealed how

mock fingerprints could aid in making inferences about the characteristics of true fingerprints that were previously unknown. The standard curve was used to estimate the initial quantity of DNA in a true fingerprint, which helped researchers obtain a better understanding of the true nature of touch DNA. Overall, the generation of the standard curve proved that the mock fingerprints were representative of true fingerprints and provided confidence to the researcher that they could be used as positive controls in touch DNA testing.

The final step in this series of experiments was to deposit true fingerprints onto surfaces other than glass, and then plot them on the standard curve. The surfaces that we evaluated were nonporous, meaning smooth and nonabsorbent, which included porcelain tile, laminate countertop and a plastic phone case. We selected these surfaces based on their potential to contain touch DNA at a crime scene as well as the ease of adding and collecting DNA from them. Since they were nonporous, the DNA deposited on them could not be absorbed, leaving it close to the surface and easily accessible during collection. In order to obtain an accurate value for the total DNA extracted, there could not be any extraneous DNA on the surface that may contribute to this value. The nonporous surfaces were easy to bleach and clean so that contamination from an outside source was not possible.

Previously, the standard curve was only used for true fingerprints on glass slides, but because there are numerous different surfaces that can have touch DNA evidence on them more surfaces needed to be evaluated. The purpose of plotting true fingerprints from various surfaces was to determine if the range and standard curve were applicable to other surfaces besides glass. Thirty true fingerprints, 10 fingerprints for each surface, were deposited onto porcelain tile, laminate countertop and a plastic phone case by 5 donors. After collection, extraction and quantification these true fingerprints were plotted on the standard curve. As seen in Figure 4 and

Figure 5, all of the fingerprints from the countertop, tile and phone case were within the range of the standard curve. Figure 5 displays all of true fingerprints from the various surfaces plotted together on the standard curve, which makes for easy and direct comparison. Figure 3 includes the same data as Figure 5, but separates the fingerprints based on the surface they were collected from in order to examine the results from each surfaces individually. Both of these figures revealed that the baseline not only represents fingerprints from glass slides, but also those from other nonporous surfaces. It validated the mock fingerprints because it demonstrates how they are not limited to mimicking fingerprints on glass, and provides reason to use them in further experimentation with other surfaces. An important aspect to note about the true fingerprints on various surfaces is the number of fingerprints that yielded no DNA after extraction. Table XII displays the amount of DNA "added" for each true fingerprint and its corresponding value for total DNA extracted, but some fingerprints yielded no DNA. The laminate countertop samples resulted in five true fingerprints with no DNA after extraction, the porcelain tile had 1, the plastic phone case had 3 and the glass slides had 2 (Table XII). There are several potential reasons for this difference between surfaces, and one could be attributed to the fact that there is variability between donors, also known as shedder status. Shedder status describes an individual's ability to leave touch DNA behind. This ability varies between persons and cannot be controlled by the donor. A good shedder will leave a large amount of DNA and a non-shedder may leave behind little to no DNA on the item or surface [21, 22, 82]. In this experiment, it is possible that the samples that yielded no DNA were from a donor that is a non-shedder. The shedder status of the donors was not tested in this experiment, and therefore conclusions regarding this could be made from these results alone. Another reason there was variability between the samples collected from the different surfaces may be because certain surfaces retained more DNA than others. The

interactions between the DNA and the surface at the molecular level may have influenced the DNA that could be collected. When looking at the samples collected form the countertop, 5 true fingerprints did not yield any DNA after extraction, which is the most out of all the surfaces (Table XII). This may indicate that the laminate countertop retains more DNA and that is it more difficult to collect DNA from compared to the other surfaces. This idea was also supported by the fact that the samples collected from the laminate countertop had the lowest average total DNA extracted which was 0.46 ng (Table XIII). When looking at the average total DNA extracted for the true fingerprints collected from tile and glass, the values were similar. On average, the samples from the tile yielded 0.83 ng of DNA while the samples from glass yielded an average of 0.83 ng (Table XIII). These results may indicate that these surfaces are easier to collect DNA from since they yielded the higher average DNA yields. Again, the differences in the amount of DNA recovered from these surfaces may be due to the interactions of the fingerprint components with the surface, or it may be because of the shedder status of the donors. Although this series of experiments could confirm these possibilities, it was important to keep these types of interactions and factors in mind in future experiments.

Overall, the goals for this series of experiments were met. A range for the mock fingerprints was determined and used in the generation of a standard curve. By plotting true fingerprints on the standard curve generated by mock fingerprints it was demonstrated that the mock fingerprints accurately mimicked true fingerprints. The standard curve could also be used as a baseline, since it was generated under ideal conditions. The amount of DNA recovered from each mock fingerprints allowed researchers to better understand the initial quantity of DNA in true fingerprints. Any changes or modifications in future experiments can be compared to the baseline in order to evaluate the effects of those alterations. This series of experiments served as

a validation of the mock fingerprints and proved that they can be used in further experimentation.

Future Studies

These experiments surrounding the generation of a standard curve serve as the foundation for future experiments with mock fingerprints. In relation to the generation of the standard curve, a similar experiment could have a researcher pipette mock fingerprints directly onto the various surfaces and then collect them. The same range of cell quantities would be used and once the standard curve for each surface was generated, it could be compared to the standard curve for the glass surface. If the curve has a steeper slope or lies above the glass standard curve that would mean that more DNA is recovered from that surface compared to glass. If the new standard curve was below the glass standard curve, or its slope value was smaller, it would mean that for that surface less DNA is recovered compared to glass. These types of comparisons would give valuable information regarding which surfaces are easier to collect DNA from, and reveal which surfaces may need a different or more optimized collection and extraction protocol.

Regarding the various surfaces tested in this series of experiments, future studies should evaluate surfaces that are more commonly seen at a crime scene. While the surfaces tested here provided insight to the DNA recovery from nonporous surfaces, there are many other surfaces or objects that can be tested. For example, surfaces such as doorknobs, windowsills, steering wheels and car gear shifters should be the focus of future studies. All of these items or surfaces need to be touched or handled, thus testing them for touch DNA could provide probative information in a case. Testing these types of surfaces with the mock fingerprints will make the research more applicable to DNA casework and provide useful information regarding the more effective way to collect touch DNA samples.

It is important to remember that in this series of experiments, the tests were designed under ideal conditions. These experiments were successful and conducted as intended, but future experimentation should be designed to include real world factors. One example of these ideal conditions is that the surfaces were decontaminated before depositing any of the fingerprints, mock and true. By doing this, any DNA recovered after collection and extraction must have come from the fingerprint and not an outside source. This aspect is helpful in eliminating variables, but is not realistic to true touch DNA samples since many are mixtures of DNA from multiple donors. Another characteristic of these experiments that made the situation ideal was the way donors deposited true fingerprints onto the surfaces. By rolling their fingertip from end to end, it maximized the surface area for the touch DNA sample, which maximizes DNA yields. While this standardizes the deposition of fingerprints, it does not accurately mimic how a touch DNA sample is deposited on evidence. Future experiments should not only work with mixtures of DNA from various donors, but also have donors only touch or handle the item momentarily to more accurately mimic a touch DNA sample. Then the values for total DNA recovered for those true fingerprints could be plotted on the standard curve generated by mock fingerprints. Comparisons between the touched and rolled true fingerprints could be made to reveal any differences in their recoveries.

Chapter VI: Summary

The purpose of this project was to develop mock fingerprints to be used as positive controls in touch DNA research. In order to make the mock fingerprints more accurate we used Accumax as the cell suspension for preparing the samples to reduce clumping. By improving the accuracy of the mock fingerprints we were able to use them in further testing. This testing included the optimization of collection and extraction protocols for touch DNA samples. We used the mock fingerprints to determine that the addition of an overnight incubation and a single wet swab, wetted with 2% SDS, resulted in the largest percent of DNA recovered. By optimizing these protocols, it ensured that the maximum amount of DNA was being recovered throughout the remainder of the project. Using the mock fingerprints, we were also able to track the loss of DNA throughout the collection and extraction process. The results from this experiment showed that different extraction methods and different swabs should be tested in future studies. Finally, we were able to generate a standard curve by utilizing the mock fingerprints as standards. These standards were used for comparison against true fingerprints from various surfaces to learn more about the nature of true fingerprints and their initial quantity of DNA. In our hands, we determined that the initial quantity of DNA in a true fingerprint is between 1.5 ng and 6 ng, which was represented by mock fingerprints containing either 250 or 1,000 cells. Overall, we effectively used mock fingerprints as positive controls throughout this project, and therefore it is recommended that mock fingerprints continued to be used in research pertaining to touch DNA samples.

The work completed in this project will influence the broader research community because we have developed positive controls for touch DNA. These mock fingerprints can be recreated in other labs for use in other research projects. While true fingerprints can be used to conduct touch DNA research, the variability between donors can skew the results obtained from

these experiments. The mock fingerprints eliminate this variability and allow more accurate studies to be performed. Controlling the variability in samples is an important aspect for all researchers, and the mock fingerprints have successfully accomplished this goal. The use of the mock fingerprints we have developed has the potential to change the way touch DNA research is conducted because they effectively mimic an eccrine fingerprint. Touch DNA research will be streamlined and allow more accurate results to be obtained when using the mock fingerprints, and therefore they should be used in all studies pertaining to touch DNA.

Ideally, the mock fingerprints developed here can make a meaningful impact by creating optimized protocols that can be used in forensic crime labs. Due to the varying nature of surfaces, one collection and extraction protocol may not be effective for all surfaces. For this reason, different protocols may be needed for different surfaces. Although having completely optimized protocols for every possible surface is ideal, it is not realistic. In order to better understand the needs of crime labs, researchers should reach out to crime lab personnel to discuss what types of items or surfaces are most commonly submitted by agencies. The most common surfaces analyzed in crime labs should be the focus of future experiments so that the research conducted is relevant to the forensic science community. This connection between crime and research labs will also play an important role in updating protocols used in crime laboratories. Many crime labs do not have the time or resources to conduct research because casework is a priority, which results in many crime labs being unable to test and implement new protocols and methods. This is where research labs can contribute significantly because researchers can do the bulk of the experimentation for new protocols. Then crime lab personnel can perform the internal validation in order to implement the new protocol into the lab. This will

save time for crime labs, and the open discussions between research and crime lab personnel will highlight the needs of the crime lab.

The goals set forth in this project were successfully met, and the use of mock fingerprints in touch DNA research should be continued. Since touch DNA evidence is becoming more prominent, it will be important that the research continue to evolve to meet the needs of crime laboratories. Using mock fingerprints will aid in this because they act as positive controls, and therefore allow more streamlined experiments to be designed and conducted in the future.

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VITA

Name: Jennifer Marie Waranauskas

Education: B.S., Biology, The University of Arizona, Tucson, Arizona, 2015

Honors: W.E. van Doren Scholar, College of Pharmacy, University of Illinois at Chicago, 2016

Professional Memberships: American Academy of Forensic Sciences