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BOSTON UNIVERSITY

ARAM V. CHOBANIAN & EDWARD AVEDISIAN SCHOOL OF MEDICINE

Thesis

THE OPTIMIZATION OF EXTRACTING FOREIGN DNA FROM FINGERNAILS

by

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B.S., Monmouth University, 2022

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Master of Science

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THE OPTIMIZATION OF EXTRACTING FOREIGN DNA FROM FINGERNAILS

CAROLINE REVERENDO

ABSTRACT

Fingernail samples are submitted to a forensic laboratory for the analysis of endogenous deoxyribonucleic acid (DNA) for purposes of human identification or for the analysis of exogenous, or foreign, DNA on the nail surface. The presence of foreign DNA on nail samples may be a result of physical contact between two individuals, for example a victim and their attacker. The purpose of this study was to optimize the recovery of foreign DNA from fingernails. Phase 1 focused on length of incubation for fingernail samples to evaluate recovery of endogenous DNA; this informed Phase 2, which aimed at comparing the recovery of foreign DNA from fingernail samples when using a soaking or swabbing collection method.

In Phase 1, fingernails of three donors were used to assess the average recovery of DNA in nanograms per milligram of nail (ng DNA/mg nail) after extraction incubation times of 15, 30, 45, and 60 minutes (min). The recovery of DNA in nanograms per microliter of whole blood (ng DNA/µl whole blood) after extraction was also evaluated using neat blood controls. The objective was to select an incubation time for nail samples that would effectively extract foreign DNA with minimal release of endogenous DNA from the sample. An incubation time of 15 minutes was chosen for Phase 2 to minimize the potential of endogenous DNA recovery from the nail samples.

In Phase 2, the comparison of DNA recovery was assessed between the soaking and swabbing collection methods using nails spiked with various volumes and dilutions of blood. Results indicate that the soaking method recovers more total DNA (both nail DNA and blood DNA) present in the sample compared to the swabbing method; however, the difference in DNA recovery between the collection methods was not statistically significant.

The DNA profiles of the blood-spiked nail samples were interpreted to compare each collection method's efficiency at extracting foreign DNA while also minimizing the recovery of endogenous DNA. The difference in average peak height from unique nail alleles and unique foreign alleles, observed across loci where there were no shared alleles between nail and blood donors, were both not statistically significant between collection methods. However, the difference in the average percent recovery of unique nail alleles for nail samples spiked with 10, 5, and 2 microliters (µl) of whole blood was statistically significant between collection methods (p= 0.017), with 69.1% recovery using the soaking method and 32.7% recovery using the swabbing method. These results suggest that the potential for recovering endogenous DNA from samples is lower when using the swabbing method as compared to the soaking method.

However, there was no statistical significance in the difference of percent recovery of unique nail alleles for the samples spiked with the second set of blood volumes and concentrations (2 µl of whole blood, 2 of µl 1:2 blood dilution, and 2 µl of 1:5 blood dilution). Furthermore, similar to the first set of blood-spiked nails, both the soaking and swabbing collection methods recovered 100% of foreign DNA alleles from the fingernail

samples. As a result, no conclusion was made on the efficiency of recovery foreign DNA between collection methods.

The percent recovery of unique foreign alleles was 100% across all samples of Phase 2, indicating that the amount of whole blood and blood dilution samples chosen to be aliquoted onto the nail samples may have been too large to effectively assess the recovery of foreign DNA from nail samples between collection methods. As a result, no conclusion was made on the effectiveness of the soaking and swabbing collection methods on the recovery of foreign DNA from fingernail samples.

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LIST OF ABBREVIATIONS

α……………………………………….. Alpha

ANOVA……………………………...... Analysis of Variance

Avg……………………………………...... Average

β………………………………………. Beta

CA…………………………………… California

°C…………………………………… Degrees Celsius

DNA…………………………… Deoxyribonucleic acid

DTT…………………………………… Dithiothreitol

EDTA………………………Ethylenediaminetetraacetic acid

GA…..……………………...………………Georgia

g ….………………………………………. Gram

Hi-Di………………………………… Highly deionized

IRB……………………………… Institutional Review Board

MA…..…………………………………... Massachusetts

mg…………………………………….…….. Microgram

µl…………………………………………..…. Microliter

mg………………………………………..…………… Milligram

ml………………………………………………Milliliter

mg/ml…………………………….. Milligram per milliliter

mm…………………………………………... Millimeter

mM…………………………………………….. Millimolar

min………………………………………………………………………………… Minute

ng……………………………………………………………………………. Nanogram

ng DNA/mg nail…………………………….. Nanogram of DNA per milligram of nail

ng/µl….……………………………………………………… Nanogram per microliter

NY……………………………………………………………………………… New York

pg…………………………………………………………………………….. Picagrams

PCR……………………………………………………...Polymerase Chain Reaction

PK…………………………………………..………………………… Proteinase K

PNF………………………………………………….…………….... Proximal Nail Fold

qPCR……………………………………… Quantitative Polymerase Chain Reaction

RFU………………………………………………….……… Relative Fluorescence Units

rpm. ……………………………………………………..... Revolutions per minute

n……………………………………………………………………………… Sample Size

SANE……………………………………………..… Sexual Assault Nurse Examiner

DT…..…………………………………..………………………………… Soaking Method

Std. Dev…………………………….……………………………Standard Deviation

SOP……………………..………………………………... Standard Operating Procedures

SW…..………………….………………………………..……………… Swabbing Method

TE…..………………..………………………………………….………… Tris-EDTA

Tris……….……………………………………….. Tris(hydroxymethyl)aminomethane

V……….……………………………….………………………………………. Voltage

1. INTRODUCTION

1.1 Fingernail Samples in Forensic Casework

Fingernail samples are one of the various evidentiary sample types submitted to forensic laboratories for deoxyribonucleic acid (DNA) analysis. Fingernail samples may be submitted for the isolation of endogenous DNA from the nail for the identification of an individual. Nails are routinely used for human identification, particularly with highly decomposed remains, due to the durability and stability of the nail structure [1]. Fingernail samples may also be submitted for the detection of foreign, or exogenous, DNA found on the nail surface, which may have resulted from contact between a victim and their attacker [2]. For example, in 2000, the body of Mary Sullivan, the last known victim of the Boston Strangler, was exhumed, and her fingernails were sent for analysis of exogenous DNA in an attempt to identify the serial killer that had terrorized the greater Boston area during the 1960s [2]. However, the extraction of exogenous DNA may present complications. There may be a low quantity or no exogenous DNA present on the nail samples or a large quantity of endogenous DNA may mask the detection of foreign DNA. In the analysis of Mary Sullivan's fingernail clippings, the only DNA that was isolated and detected was that of Sullivan [2].

Numerous studies have evaluated the significance of isolating foreign DNA from fingernails. In 2000, Oz and Zamir had volunteers scratch each other's forearms, and extracted their fingernail clippings using a phenol-chloroform extraction method [3]. Oz and Zamir found that only the victim's DNA was isolated from the fingernail clippings, and, in turn, determined that routine typing of fingernails for foreign DNA recovery was

unnecessary [3]. However, in 2011, Matte et al. analyzed casework data from the Centre of Forensic Sciences and found that 33% of fingernail samples submitted for analysis contained foreign DNA, with 63% of these samples having foreign DNA detected at five or more loci [4]. Similar to Oz and Zamir, Matte et al. further conducted an experiment for detecting foreign DNA from fingernail clippings that involved volunteers scratching each other, followed by a modified organic extraction protocol using a Proteinase K (PK) lysis solution. [4]. Their results showed that 33% of the volunteers' fingernail clippings had foreign DNA detected, leading to the conclusion that the extraction of fingernails for foreign DNA is of limited significance [4]. However, there are limitations to studies involving the use of scratching experiments for evaluating the recovery of foreign DNA from fingernails. For example, volunteers in a controlled environment may not scratch with the amount of force that is used by victims during violent crimes [4].

Although numerous studies have highlighted the difficulties of recovering foreign DNA from fingernails, other experiments have shown that the collection and extraction methods used for fingernail samples can have a significant effect on the yield of foreign DNA [5-7]. For example, Hebda et al. recovered 96% of exogenous DNA from blood-spiked fingernails by adding the samples directly to lysis buffer for extraction, as opposed to swabbing or scrapping the samples, which resulted in 61% and 33% recovery, respectively [7].

1.2 Sampling of Fingernails

The collection of fingernail samples may vary greatly depending on the standard operating procedures (SOPs) of the medical examiners, Sexual Assault Nurse Examiners

(SANE Nurses), and forensic scientists that are receiving the evidence. To collect foreign DNA from fingernail samples, medical examiners will clip the victim's fingernails whereas SANE nurses may use wooden scrapers or swabs on the victim's fingernails that are then submitted for the detection of foreign DNA. Fingernail clippings that are sent for analysis may be swabbed, scraped, or placed directly into a lysis buffer, which is also known as the soaking method, for the isolation of exogenous material (Figure 1) [7].

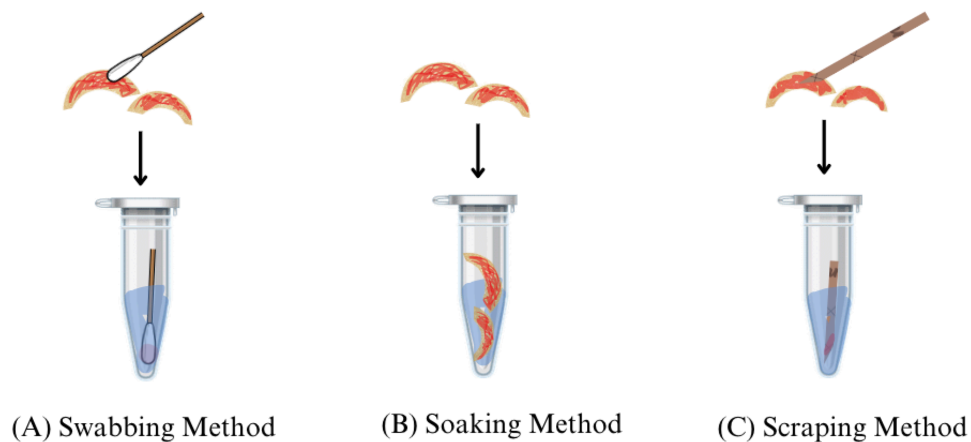

Figure 1. Collection methods for foreign DNA from fingernail samples. (A) The swabbing method involves a swabbing intermediary device to collect any biological material present on the nail; the swab is then placed into the sample tube containing a lysis buffer. (B) The soaking method involves fingernail clippings being placed directly into the sample tube containing a lysis buffer. (C) The scraping method involves the use of a wooden scraper to collect any foreign sample present on the nail; the scraper is then placed in the sample tube containing a lysis buffer.

1.2.1 Swabbing Method

The method of swabbing an evidentiary item for biological material is commonly used for various sample types, including fingernail samples [8]. International protocols suggest using a damp, thin-tipped swab to reach under the surface of the nails for the

collection of foreign DNA [9]. The double swabbing method proposed by Sweet et al., which involves the use of a moistened swab followed by a dry swab, is commonly implemented in the SOPs of laboratories for the collection of exogenous DNA from fingernails [10-11]. An advantage of using the swabbing method is a decreased potential for carryover of inhibitory agents present within the nail matrix [8]. Additionally, the swabbing technique may result in decreased likelihood of nail DNA masking any foreign DNA, commonly a minor component present in the sample [7].

Numerous studies have evaluated the effect of the material of the swabs used for the collection of biological material on the recovery of DNA from the sample [12-18]. Common materials used for swabbing devices include cotton, nylon, rayon, foam, and polyester [12]. Cotton swabs are widely used around the world due to their availability and low cost [9, 18]. However, many studies have shown that there is a loss of DNA that remains entangled in the cotton material of the swab after extraction [12]. Adamowicz et al. compared the recovery of DNA from samples using cotton swabs to control buccal cell suspension and liquid blood samples and concluded that over 50% of recoverable DNA is retained in the cotton material of the swab or lost during the extraction process [8]. Results of a similar study conducted by van Oorschot et al. found that 20% to 76% of DNA collected by a cotton swab is retained in the material's matrix or lost during extraction [19]. Other swab materials, such as nylon and polyester, have also been evaluated for their efficiency in recovering and releasing DNA.

However, there have been mixed results on which swab material is best to use for collection. Malwood et al. used a 0.5 microliter (μl) saliva sample to compare DNA

recovery using nylon-flocked swabs and cotton swabs [16]. Their results showed that the nylon flocked swabs had a higher average yield of 750 picograms (pg) compared to the average yield of 250 pg using the cotton swabs [16]. Conversely, multiple studies found that cotton swabs outperformed nylon flocked swabs for DNA recovery [15, 20]. Results of a study aimed at comparing nylon flocked swabs and cotton swabs conducted by Al-Snan and Shabbir showed no statistically significant difference in the recovery of DNA from biological fluids between the two materials [17]. A study that compared cotton, foam, nylon, polyester, and rayon swabs for the collection of DNA found that although the nylon-flocked swabs showed the highest extraction efficiency at 48% recovery and cotton swabs with the second highest efficiency at 21.5% recovery, no swab resulted in an extraction efficiency above 50%, which suggests that the majority of the DNA sample was retained on all swab types [18].

Overall, these studies suggest that DNA recovery using different swabbing materials may be dependent on sample type, surface, or item [14]. Moreover, many of these studies concluded that no one type of swabbing material is more effective than another [14, 20]. However, these studies also suggest that modifications may be made to the swabbing technique to increase the efficiency of this collection method. For example, Adamowicz et al. found that specific alterations to extraction protocols can affect the efficiency of DNA recovery [8]. Their results indicated an increase in DNA yield from swab samples using 3- and 18-hour incubations as compared to swabs that were incubated for 1 hour [8]. Although there has been no consensus on which swabbing material is

optimal for the collection of DNA, nylon-flocked swabs were chosen for this study to evaluate the swabbing method of foreign DNA collection from fingernails.

The Copan 4N6FLOQSwab (4N6), a proprietary nylon-flocked swab, was chosen in this study as the intermediary device for the swabbing method (Copan Diagnostics, Murrieta, CA). These nylon-flocked swabs were created with the purpose of maximizing DNA collection, as well as increasing the release of DNA during the extraction process [39]. A comparative study of different swabbing materials for the recovery of trace DNA found a statistically significant difference between the DNA yield of touch DNA samples using the 4N6 swabs and cotton swabs for various touch DNA samples. For example, there was an average DNA yield of 0.65 ng/µl from shirt collars using the 4N6 swabs compared to an average yield of 0.13 ng/µl using cotton swabs. These results suggest that nylon flocked swabs have the potential to recover biological samples of low quantities more effectively than swabs made of cotton [13]. One study conducted by Al-Snan and Shabbir that aimed to compare the 4N6 swabs to cotton swabs for various sample types found that the 4N6 swabs outperformed cotton swabs for the collection of DNA from wet samples of biological fluids, whereas cotton swabs were more efficient in collecting trace DNA from solid surfaces, such as light switches and sunglasses [17]. However, numerous studies have found that cotton swabs have recovered DNA from samples as effectively as other swabbing materials, and, in some cases, have exceeded the other materials' ability to recover and release DNA [14-15].

1.2.2 Soaking Method

Fingernail clippings that are submitted for the analysis of foreign DNA may be directly added to a sample tube with lysis buffer for a certain incubation time, depending on the protocol of the laboratory. This method is commonly referred to as the soaking method. An advantage of this method is that potential DNA loss during the collection process is minimized compared to when using intermediary devices, such as swabs or wooden scrapers. Conversely, when samples, such as fingernail clippings, are added directly into the sample tube with lysis buffer, inhibitory agents may remain in solution. These inhibitory molecules may interfere with the polymerase chain reaction (PCR) amplification of DNA by binding to the Taq polymerase, blocking necessary reagents, interacting directly with the DNA, or interacting with the Taq polymerase during primer extension [21].

Another complication that arises when using the soaking method of sampling fingernail clippings is the masking effect [35]. During the analysis of DNA mixtures, a masking effect may occur when one contributor's DNA is in a higher proportion than the other contributors' in the mixture, resulting in the major contributor obscuring the minor contributors. The masking effect may lead to difficulty in analyzing and identifying minor contributors to mixtures. This is ubiquitous when extracting DNA from fingernail clippings that have been sampled using the soaking method; the nail donor's DNA will commonly be present in a higher quantity than the foreign contributor(s), resulting in taller peak heights for the nail donor's alleles. The nail donor's taller allele peaks may obscure the smaller allele peaks from the foreign DNA present from the sample, affecting the

analysis of results. Hebda et al. found that the soaking method largely resulted in profiles where the major contributor was the nail donor or profiles where the nail donor and the foreign DNA contributed equally [7].

1.2.3 Scraping Method

The third collection method for foreign material from fingernails involves the use of a wooden applicator to scrape the underside of the nail. The scraping can be performed over a sterile weigh paper in which the materials collected on the weigh paper are transferred to the sample tube for extraction or the end of the wooden scraper can be cut off using a disposable scalpel and placed in the sample tube for extraction [7]. Due to previous studies that have found scraping as the least effective collection method of foreign DNA from fingernail samples, it was not included in the comparison of collection methods evaluated in this study [5, 7].

1.2.4 Comparison of Sampling Methods

Numerous studies have been conducted to compare the swabbing, scraping, and soaking methods for the isolation of foreign DNA from fingernails. Hebda et al. found that the soaking method had an average foreign DNA recovery of 96%, compared to the swabbing and scrapping methods that resulted in average yields of 61% and 33%, respectively [7]. However, the profiles of both the soaked and swabbed nails generally resulted in the detection of all possible foreign alleles [7]. Specifically, the soaking method for fingernail clippings that were either spiked with blood or collected after scratching

resulted in DNA profiles where the nail donor was the major contributor or the nail donor and foreign blood donor were equivalent contributors; conversely, the swabbing method produced profiles with the foreign DNA as the major contributor [7]. The scraping method resulted in profiles containing mostly exogenous alleles; however, drop-out—in which foreign DNA alleles were not detected—did occur [7]. Similarly, Foran et al. compared the swabbing, scraping, and soaking methods, and their results showed that while the soaking method isolated the most exogenous DNA, it also isolated the most endogenous DNA [5]. Furthermore, they determined that swabbing nail samples collected the second most exogenous DNA, followed by the scraping method [5]. Overall, these studies showed that the soaking method results in the most exogenous DNA isolated from nail samples; however, this method also results in the most endogenous DNA extracted from the nail itself. Conversely, the swabbing method is advantageous for extracting less endogenous DNA from fingernail samples; however, the yield of foreign DNA extracted from the sample is less than that of the soaking method. There are advantages and disadvantages to the swabbing and soaking methods foreign DNA recovery from fingernail samples, and modifications may be made to these methods for the optimization of extracting exogenous DNA from fingernails.

1.3 The Human Nail: Structure and Function

In addition to the collection and extraction methods used for extracting foreign DNA from fingernails, other factors that may affect analysis include the age of the samples

from collection to analysis, the condition or friability of the nail samples, and the length of the fingernails [5].

1.3.1 Biological Structure and Function of Fingernails

The human nail has been well studied by researchers in various fields, including dermatology, oncology, and forensic science. The main function of human fingernails is to prevent the skin of the finger from receding, as well as assisting in the manipulation of objects, such as gripping, scratching, and prying [22]. The general structure of the nail is made up of the nail plate, the proximal nail fold, the nail matrix, the nail bed, and the hyponychium (Figure 2) [23]. The nail plate is made up of layers of keratin and provides protection for the distal phalanx [24]. The nail plate appears to originate from a fold of skin located at the dorsum of the finger, known as the proximal nail fold (PNF). The nail matrix is located directly beneath the PNF and is composed of germinative tissue. It is the main site for differentiation of onychocytes, epithelial cells that specialize in producing the nail plate [24]. The nail rests on a vascular bed known formally as the nail bed [25]. Lastly, the hyponychium is the layer of skin lying beneath the nail plate's free edge; its main function is to prevent bacteria and other foreign materials from entering the nail bed [25].

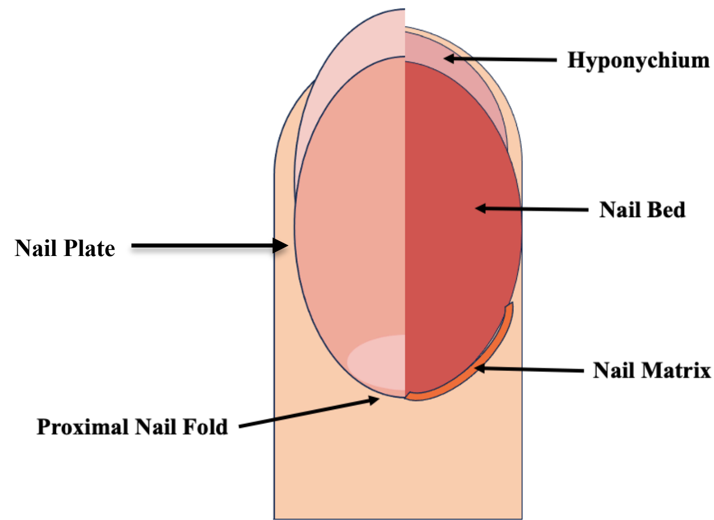

Figure 2. Biological structure of the nail unit.

Biological material, such as exogenous DNA from a perpetrator, accumulates under the nail's hyponychium, making it a significant site for the collection of evidentiary samples [9]. Dowlman et al. swabbed the nails of 80 volunteers to evaluate the accumulation of DNA under individuals' fingernails after daily activities. Their results suggest that DNA can collect under the nail's hyponychium after common, everyday activity, with mixed DNA profiles obtained for 41% of the samples [26]. In addition to the physical structure of the fingernail, its molecular composition may also provide information on the breakdown of fingernails using lysis buffers and reducing agents.

1.3.2 The Molecular Composition of Fingernails

A family of water insoluble proteins known as keratins are found in most types of epithelial tissue, with over 20 keratin polypeptides identified in various human epithelia [27]. Keratin is made up of fibrous protein, consisting of an abundance of sulfur [23].

Keratin can be found in various structures of the human body, including hair shafts and roots, skin cells, fingernails, and toenails. The keratinized structure of the nail plate provides stability and durability essential for common, daily activities such as buttoning a shirt [22].

The polypeptide chains that form the keratin matrix exist in two forms: a curved helical conformation (alpha-keratin, or α-keratin) or a pleated side-by-side conformation (beta-keratin, or β-keratin). The covalent disulfide bonds produced by the abundance of cysteine residues in the keratin matrix form cross-links between the polypeptide chains, providing stability and strength [23]. Specifically, the stability of the nail is due to the gauche-gauche-gauche formation of the disulfide bonds present in the keratin matrix, with the amount of disulfide bonds present in this formation, directly resulting in the nail protein's resistance to denaturation [28]. Furthermore, the keratin filaments present in the nail are arranged in a transverse orientation across the nail, with the purpose of resisting bending [22].

Alpha-keratin, found in nails, hair, and the epidermal layer of skin in mammals, is further characterized into hard α-keratin and soft α-keratin [22]. The composition of the human nail is made up of 80% hard α-keratin and 20% soft α-keratin [29]. Soft α-keratin is the primary component of the epidermis, whereas hard α-keratin is the main component of cornified mammalian tissues such as nails and horns [23]. Another difference between soft and hard α-keratin is the amount of sulfur present in their compositions. Soft α-keratin contains a lower quantity of sulfur, resulting in fewer cross links within its matrix [22]. Due to the stability of the nail structure provided by keratin, the keratin matrix must be

disrupted to break down nail samples for the purposes of isolating endogenous DNA. Conversely, if the goal of analysis is the extraction of exogenous DNA from fingernail samples, measures should be taken to minimize the breakdown of the keratin matrix.

1.4 Extraction of DNA from Fingernails

1.4.1 Reagents for Keratin Denaturation

The keratin matrix can be disrupted using reagents such as Proteinase K (PK). Proteinase K, given its name based on its ability to digest keratin, is a serine protease that was first isolated from the fungus *Engyodontium album* [30]. This protease effectively breaks down hard α-keratins with numerous cut sites in their polypeptide chain matrix. For example, a hard α-keratin filament coded by the gene KRT17 and known to be expressed in the human nail plate, has a sequence of 432 amino acids; PK has 215 cut sites within this sequence [31-32]. This suggests an increased potential of releasing endogenous DNA from the nail when using PK during the extraction process. Conversely, when aiming to extract exogenous DNA from nail samples, decreasing or eliminating the amount of PK used during the extraction process may increase the potential of detecting foreign DNA in the sample's profile.

Another reagent that can be used to disrupt the keratin matrix is dithiothreitol (DTT). DTT is commonly used as a reducing agent during DNA extraction from samples, including fingernail clippings, due to its ability to disrupt protein bonds. Specifically, DTT is routinely used to denature disulfide bonds present between the cysteine residues in the keratin matrix of nail samples [1, 33]. For example, a study conducted by Inkret et al.,

which was aimed at evaluating fingernail samples as a source of DNA for decomposed human remains, used DTT in their extraction protocol for endogenous DNA from nail samples [1]. Their results showed an average DNA yield of 36 micrograms (μg) of DNA per gram (g) of nail, suggesting nail samples can serve as an alternative source of DNA for human identification [1]. Furthermore, they show the potential of DTT to effectively disrupting the keratin matrix of nails for the purposes of releasing endogenous DNA.

Although there are no published studies comparing the efficiency between PK and DTT for the extraction of endogenous DNA from fingernails, there have been experiments with protocols that used both reagents to extract DNA from various samples. Ferreira et al. evaluated a phenol-chloroform extraction protocol for the isolation of DNA from bone samples with the addition of 340 μl of 20 milligrams per milliliter (mg/ml) PK and 360 μl DTT for the lysis of cells in 2 g of powdered bone [34]. The DNA yield from the bone samples using this extraction method was between 0.2 and 1.0 nanograms per microliter (ng/μl) for 8 samples and fell below 0.2 ng/μl for the remaining 12 bone samples [34]. Full profiles were obtained for 58% of these samples [34]. These results suggest that the modification of protocols, such as the addition of proteases and reducing reagents, for the isolation of DNA may have the potential to increase DNA recovery from samples.

1.4.2 Extraction of Endogenous and Exogenous DNA

The extraction method for the isolation of DNA from fingernail samples may vary depending on the context of the case. The extraction of fingernail clippings for the purposes of human identification is focused on the isolation of endogenous DNA from the

nail itself. Numerous studies aimed at extracting endogenous DNA from nails have concluded that fingernail samples are an effective source of DNA for decomposed human remains [1-2, 35]. Allouche et al. compared the extraction of endogenous DNA from fingernails of living volunteers and human corpses, as well as compared the DNA recovery of samples that were incubated in a solution of lysis buffer and PK overnight versus one hour (hr) [35]. Their results found the DNA concentration extracted from 5 milligram (mg) of nail from living volunteers ranged from 4.5 to 173.6 ng/µl for the overnight incubation and 4.5 to 36.2 ng/µl for samples that were incubated for 1 hr [35]. For the fingernail samples recovered from 10 human remains, the DNA concentration extracted from 5 mg of nail ranged from 2.5 to 222.7 ng/µl for the standard 16-hr incubation and 0.6 to 47.5 ng/µl for the samples that were incubated for 1 hr [35]. Their results suggest that the endogenous DNA from fingernails is well preserved, although the recovery of DNA from nail samples may vary depending on living status of the nail donor. An increased incubation time may be more advantageous for nail samples of human corpses. However, full profiles were obtained for all human remain nail samples across both incubation times. Their results indicate that an incubation time of one hour is as effective at obtaining full DNA profiles from nails than the standard 16-hour incubation time [35].

Conversely, the extraction of nails for the recovery of exogenous material, such as a perpetrator's DNA, is focused on the isolation of foreign DNA present on the nail surface. The collection method of exogenous DNA from fingernails, including the soaking, swabbing, and scraping methods, affect the extraction protocol used for nail samples. Studies show that fingernail clippings that are directly added to a sample tube with lysis

buffer have the highest potential for the recovery of exogenous DNA as well as the highest potential for the isolation of DNA from the nail itself [5, 7]. Modifications can be made to minimize the quantity of endogenous DNA recovered from nail samples. For example, decreasing the amount of protease or reducing agent used in the lysis solution, adjusting the incubation time of the sample in the lysis solution, and altering the collection method of the exogenous material from the nail, such as using the swabbing technique, may affect the quantities of exogenous and endogenous DNA recovered from fingernail samples. As previously stated, studies evaluating cotton swabs have suggested that the majority of DNA recovered from a sample is retained within its cotton material; however, other swabbing materials can be used to evaluate the recovery and release of exogenous DNA from fingernail samples [8].

1.5 Research Objectives

The objective of this research project was to identify and optimize the most effective method for extracting exogenous DNA from fingernails samples. This study was divided into two facets. Phase 1 involved the evaluation of various incubation times for fingernail samples to maximize the amount of foreign DNA recovered from nail samples, while simultaneously minimizing the amount of DNA recovered from the nail itself. The incubation times explored in this study were 15-minute (min) intervals (i.e., 15, 30, 45, and 60 min). These time lengths were determined based on the protocols of commonly used extraction kits, such as the PrepFiler™ Forensic DNA Extraction Kit and the ChargeSwitch® Forensic DNA Purification Kit, with recommended incubation times

varying from 20 min to 1 hr for samples such as nail clippings and swabbings [36-37]. Furthermore, data gathered by a previous thesis project, which was focused on the recovery of exogenous DNA from fingernails using various direct cell lysis methods and proteases, compared the extraction of endogenous DNA from fingernails using an incubation length of 1 hr versus 24 hrs [38]. Using a DNA purification method (i.e., QIAGEN QIAamp DNA Investigator kit) on cleaned fingernails, their results found a lower average concentration of nanograms of DNA per milligram of nail (ng DNA/mg Nail) isolated from the samples that were incubated for a longer time period [38]. The difference in DNA recovery between the two incubation times was not statistically significant, suggesting that an incubation period longer than one hour may not directly correlate with increased recovery of DNA from nail samples [38]. The purpose of Phase 1 was to identify the time period in which endogenous DNA from fingernails begins to release into the lysis solution. This may identify an incubation time for fingernail samples that would be a sufficient duration of time for the extraction of exogenous DNA but insufficient for the isolation of the nail donor's DNA.

Phase 2 of this study was focused on comparing the collection methods of exogenous material from fingernail samples. Specifically, the soaking and swabbing methods used for collecting foreign DNA from fingernails were evaluated using donated nail clippings that were spiked with various blood volumes and concentrations. The spiking of various concentrations and volumes of blood on the nail samples was used to evaluate the efficiency of each method at recovering foreign DNA that was present in varying proportions to the nail samples. Furthermore, the efficiency of the collection

methods was evaluated via the resulting DNA profiles of the blood spiked nail samples. The DNA profiles of the samples that were extracted using the soaking method or the swabbing method were analyzed for the presence of alleles belonging to the nail donor and the blood donor, which served as the "foreign DNA donor."

The optimization of the extraction of foreign DNA from fingernail samples will be determined by the identification of an incubation time that maximizes the extraction of exogenous DNA from blood-spiked nails while minimizing the release of endogenous DNA from the nail itself, as well as by the comparison of efficiency of the soaking and swabbing methods. The results of this study have the potential to lead to modifications in the protocols used in forensic crime laboratories to analyze fingernail samples for foreign DNA.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Biological sample kits designed for the collection of fingernail samples were made with unique donor ID numbers assigned to each kit. Simple instructions were included in every kit on how donors should properly collect their samples. Nail clippings and buccal swabs were collected from 8 volunteers in compliance with the Boston University Chobanian & Avedisian School of Medicine Institutional Review Board (IRB) guidelines. The volunteers were first asked to wash their hands thoroughly for a minimum of 20 seconds (sec) with soap and water and to use the alcohol pad included in the kit to sterilize their personal nail clippers. Donors were asked to clip fingernails that measured at least 2 millimeters (mm) in length and to gather the clippings into the glassine envelope. Samples of 8 volunteers were stored at room temperature for approximately 1 to 3 months prior to Phase 1, and for approximately 1 to 2 months prior to Phase 2. Lastly, donors were asked to provide a buccal swab to serve as a reference sample, which was also stored at room temperature.

To effectively assess the recovery of endogenous DNA from fingernail clippings, a protocol for cleaning the nail samples was used to remove any potential skin cells and biological fluids from the surface of the nails [38]. This protocol involved the use of an Infinity Rechargeable electric toothbrush with interchangeable toothbrush heads (CVSHealth, Woonsocket, RI). During the cleaning process, the nail sample was held in place in a weigh boat using sterilized tweezers. The toothbrush head was dipped in 10% bleach solution and then 70% ethanol before every nail sample. The toothbrush head was

tapped to release any remaining ethanol on the toothbrush bristles, and then immersed in TE (Tris-EDTA) buffer, a solution of 10 millimolar (mM) Tris(hydroxymethyl) aminomethane (Tris) and 0.1 mM Ethylenediaminetetraacetic acid (EDTA) for approximately 10 sec, and used to scrub the entire surface of the nail for 30 sec. The fingernail was then rinsed in an additional 100 µl of TE buffer and dried using a Kimwipe (Kimberly-Clark Professional, Roswell, GA). This cleaning process was repeated for all donated nail samples.

The cleaned fingernails were individually weighed using a calibrated Denver Instrument Analytical Semi-Micro Balance (Bohemia, NY). Each nail sample for Phase 1 had a target weight of approximately 40 mg. Nail samples for Phase 2 had a target weight of 5 to 20 mg of nail.

2.1.2 Preparation of Blood-spiked Nails for Phase 2

The sample preparation for Phase 2 included spiking nails with whole or diluted blood; the same male blood donor (commercially purchased) was used for all samples for consistency. This involved the preparation of nail samples spiked with 10, 5, and 2 µl of whole blood, and nail samples spiked with 2 µl of a 1:2 blood dilution and a 1:5 blood dilution. The 1:2 blood dilution was prepared using one part whole blood with one part TE buffer; the 1:5 blood dilution was prepared using one part whole blood with four parts TE buffer. The nail samples were placed in individual weigh boats and held securely with sterilized tweezers as the appropriate volumes of blood sample were added to the underside

of the nails. The blood-spiked nail samples were then left to dry in a ventilated fume-hood overnight before the extraction process.

Specifically, for each nail donor, a nail sample weighing approximately 5 to 20 mg was spiked with 10 µl whole blood. The spiked nail sample was dried overnight and then placed in a sample tube with lysis solution to assess the soaking collection method of exogenous DNA. Another nail sample from each donor, weighing approximately 5 to 20 mg, was again spiked with 10 µl whole blood to assess the swabbing method of sample collection. The spiked nail was dried overnight and then swabbed using a Copan 4N6FLOQSwab nylon-flocked swab (Copan Diagnostics, Murrieta, CA). Five microliters of DNA-free water was added to the tip of the 4N6 swabs, to serve as a wetting agent, and the swab was held at an approximate 60º angle against the surface of the nail during collection of foreign material, which Hedman et al. found was the most efficient way of sampling with a swabbing device [14]. The underside of the nail was swabbed for approximately 30 sec, and the head of the swab was then added to the sample tube. This protocol was repeated for nails spiked with 5 µl and 2 µl of whole blood and 2 µl of a 1:2 blood dilution and a 1:5 blood dilution.

2.2 Phase 1: Incubation Time

2.2.1 Extraction Method

The ChargeSwitch® Forensic DNA Purification Kit, which utilizes magnetic bead technology for the purification of DNA, was used to identify when endogenous DNA begins to release from the nail in solution (ChargeSwitch® Forensic DNA Purification Kit,

Invitrogen Life Technologies, Carlsbad, CA). The original ChargeSwitch® protocol suggests the incubation of samples in a solution of 1 ml of lysis buffer and 10 µl of PK for 1 hr on a thermal shaker set to 55 degrees Celsius (°C). A modification made to the ChargeSwitch® protocol was to remove an aliquot of 100 µl from the sample tube at 15-min intervals, up to and including 60 min (Figure 3). The original sample was briefly vortexed before the removal of each aliquot. Furthermore, the addition of shaking at 250 revolutions per minute (rpm) during incubation and a modified lysis solution (0.5 ml of lysis buffer and 5 µl of PK) was implemented to the new protocol. The original ChargeSwitch® protocol was then followed for the remainder of the extraction, and the samples were eluted in 150 µl [37]. Approximately 40 mg of cleaned nail samples of two donors, Donor A and Donor B, were extracted for the recovery of endogenous DNA using the modified ChargeSwitch® protocol. The sample extracts were then stored at -30°C until quantification was carried out. The extraction of nail samples from these donors was performed in duplicate to assess the repeatability of the extraction protocol.

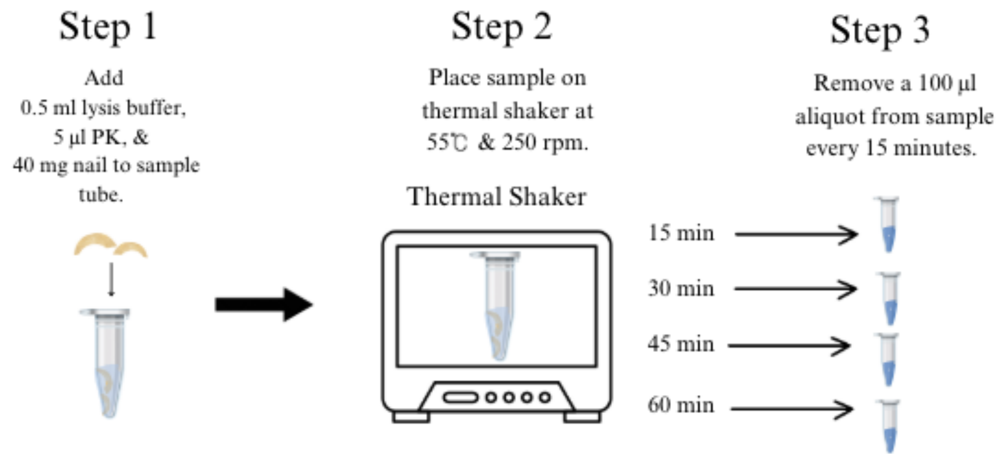

Figure 3. Modified protocol for the ChargeSwitch® Forensic DNA Purification Kit. Step 1 of the modified protocol was to add the nail sample and the lysis solution to the sample tube. The sample tube was then placed on a thermal shaker at 55°C and 250 rpm (Step 2). An aliquot of 100 µl sample was then removed from the sample tube at 15 min intervals from the initial start of incubation (Step 3).

Due to the lack of continued availability of ChargeSwitch® Forensic DNA Purifiction kits, a PrepFiler™ extraction protocol, which also uses magnetic bead technology for the purification of DNA, was used for extraction of the remaining samples of Phase 1 and the blood-spiked nails of Phase 2 (PrepFiler™ Forensic DNA Extraction Kit, Applied Biosystem, Waltham, MA). The original PrepFiler™ protocol suggests an incubation of 20 to 40 minutes for dried stains or samples on swabs in a solution of 300 µl lysis buffer and 3 µl DTT. The sample tube was then placed on a thermal shaker set at 70°C and 900 rpm. The modification that was made for the ChargeSwitch® extraction method was also made to the PrepFiler™ extraction protocol, which involved the removal of a 100-µl aliquot from the sample tube every 15 min (Figure 4). The original PrepFiler™ protocol was then followed for the remainder of the extraction, and the samples were eluted in 50 ul [36]. An additional set of control samples of whole blood were extracted using the

modified PrepFiler™ protocol. The sample extracts were then stored at -30°C until quantification was carried out.

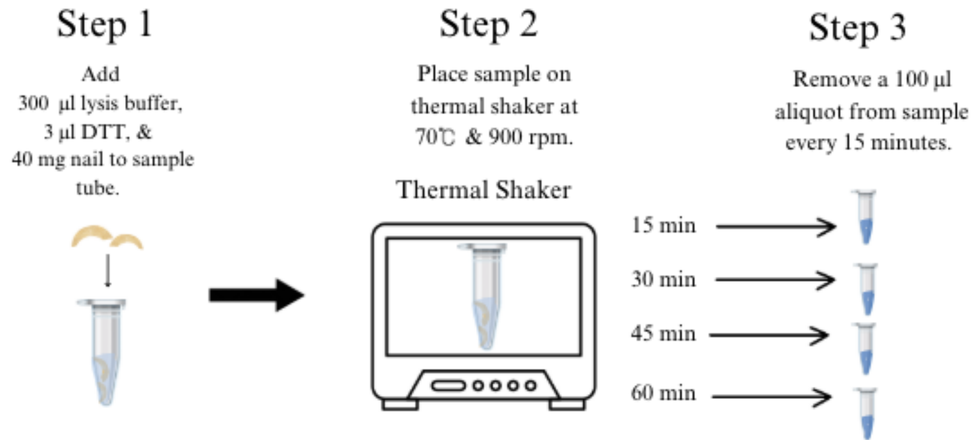

Figure 4. Modified protocol for the PrepFiler™ Forensic DNA Extraction Kit. Step 1 of the modified protocol was to add the nail sample and the lysis solution to the sample tube. The sample tube was then placed on a thermal shaker at 70°C and 900 rpm (Step 2). An aliquot of 100 µl sample was then removed from the sample tube at 15 min intervals from the initial start of incubation (Step 3).

2.3 Phase 2: Comparison of Collection Methods for Exogenous DNA from Fingernail Samples

2.3.1 Extraction

The evaluation of the swabbing and soaking methods first involved the comparison of DNA recovery from nail samples spiked with 10, 5, and 2 µl of whole blood. Cleaned nail samples from Donors D, E, and F weighing approximately 5 to 20 mg were spiked with whole blood and dried overnight. They were then added to the lysis solution directly or collected with the nylon-flocked swabs. The nail clipping or swab was extracted using

the modified PrepFiler™ Forensic DNA Extraction kit protocol with an incubation time of 15 minutes. There was a total of six spiked nails from each donor: one nail for each whole blood volume for each collection method (Figure 5). Nail samples from each donor were extracted using the modified PrepFiler™ protocol to assess the approximate amount of endogenous DNA extracted from that specific donor's nails and to serve as a reference for the nail donor's alleles during interpretation of the resulting profiles.

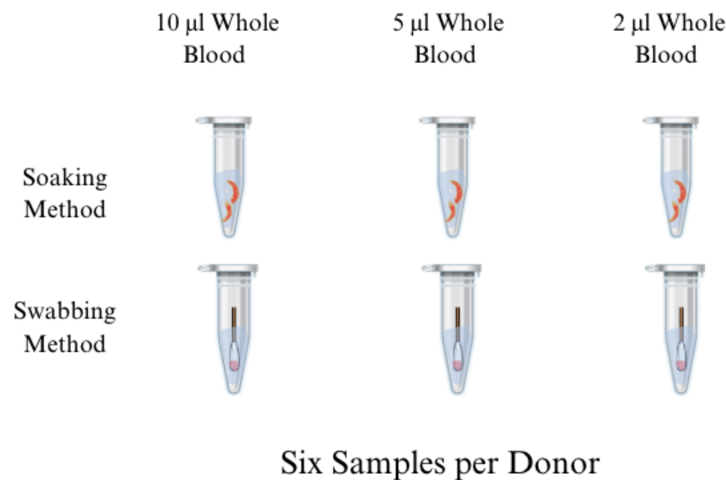

Figure 5. Schematic of total samples from each donor. Two nail samples from each donor were spiked with 10 µl whole blood. One of these blood-spiked nail samples was added to a sample tube with lysis solution to assess the soaking collection method, and the other was swabbed using a 4N6 swab to assess the swabbing method. This protocol was repeated for the 5 µl and 2 µl whole blood-spiked samples. In total, there were six samples from each donor.

The comparison of the soaking and swabbing methods was further assessed using nail samples spiked with blood dilutions. Cleaned nail samples from two donors, Donor E and G, weighing approximately 5 to 20 mg were spiked with 2 µl of whole blood, a 1:2 blood dilution, or a 1:5 blood dilution. The spiked nails were dried overnight, added to the

lysis solution (soaking method) or collected using the nylon-flocked swabs (swabbing method), and then extracted using the PrepFiler™ protocol with an incubation of 15 min. There was a total of six spiked nails from each donor (Figure 6). Nail samples from each donor were also extracted using the PrepFiler™ protocol with the 15 min incubation to assess the approximate amount of endogenous DNA extracted from that specific donor's nails and to serve as a reference for that donor during profile interpretation. The resulting sample extracts were stored at -30°C until quantification was carried out.

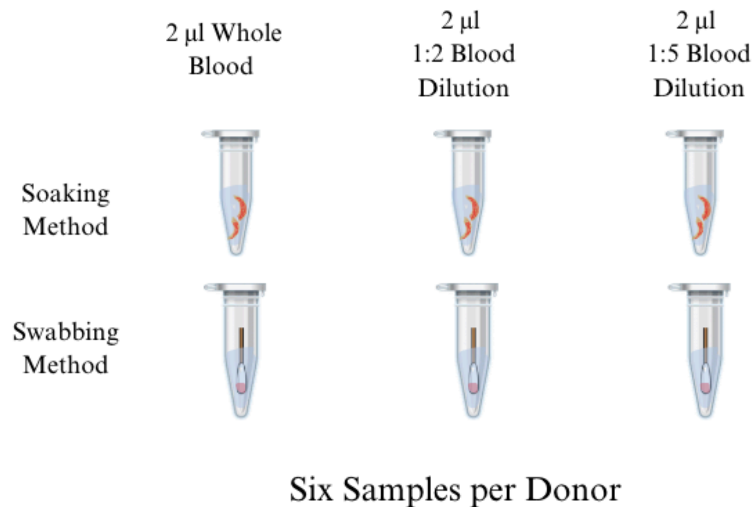

Figure 6. Schematic of total samples from each donor. Two nail samples from each donor were spiked with 2 µl whole blood. One of these blood-spiked nail samples was added to a sample tube with lysis solution to assess the soaking collection method, and the other was swabbed using a 4N6 swab to assess the swabbing method. This protocol was repeated for the 1:2 and 1:5 blood dilution-spiked samples. In total, there were six samples from each donor.

Additionally, four liquid whole blood aliquots (2 samples of 5 µl whole blood and 2 samples of 2 µl whole blood) were extracted using the PrepFiler™ Forensic DNA Extraction Kit and an incubation of 15 min to assess the average recovery of DNA per

microliter of blood (ng DNA/ µl whole blood). The sample extracts were stored at -30°C until quantification was carried out.

2.4 Quantification

Sample extracts from Phases 1 and 2 were quantified using the Quantifiler Trio DNA Quantification Kit (Applied Biosystems, Foster City, CA) with the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Sample extracts from Phase 1 were quantified in duplicate. Data tables show the average of the duplicate quantitative PCR (qPCR) measurements. Sample extracts from Phase 2, which evaluated the recovery of foreign DNA from fingernails using the soaking and swabbing collection methods, were quantified once.

2.5 Amplification

Sample extracts from Phase 2 were amplified using the GlobalFiler PCR Amplification Kit (Applied Biosystems, Foster City, CA). A target mass of 0.75 nanograms (ng) was used for all samples. Sample dilutions were prepared using TE buffer if sample extracts exceeded the target mass in 15 µl. One positive control of a GlobalFiler DNA standard and a negative control of GlobalFiler master mix was also prepared. A GeneAmp PCR System 9700 was used for amplification with the following cycling parameters: 1 min at 95°C, 28 cycles of 94°C for 10 sec and 59°C for 90 sec, followed by 60°C for 10 min (Applied Biosystems, Foster City, CA). The samples were then removed from the thermal cycler and stored at -30°C until capillary electrophoresis was carried out.

2.6 Capillary Electrophoresis

Ten microliters of master mix containing highly deionized (Hi-Di) formamide (Life Technologies, Woburn, MA) and GeneScan 700 LIZ Size Standard (ThermoFisher Scientific, Waltham, MA) was added to the wells of a 96-well plate. One microliter of amplified sample was added to the appropriate wells, and one microliter of GlobalFiler Allelic Ladder (Applied Biosystems, Foster City, CA) was added to the appropriate wells. The amplified positive control and a negative control containing LIZ Size Standard and Hi-Di were also added to their appropriate wells. After sample denaturation and cooling, capillary electrophoresis was performed on the SeqStudio™ Genetic Analyzer (Applied Biosystems, Foster City, CA) using a 100/240 voltage (V), a run time of approximately 30 min per sample, and a 10 second injection. The electropherograms were then analyzed using GeneMapper® ID-X v1.5 software (Applied Biosystems, Foster City, CA).

2.7 Data Interpretation

2.7.1 Phase 1: Incubation Time

Comparisons were made between the recovery of endogenous DNA from nail samples that were incubated for 15, 30, 45, or 60 min. The yield of endogenous DNA from nail samples for these incubation times were compared between the modified ChargeSwitch® extraction method and the modified PrepFiler™ method. Statistical analysis using two-tailed *t*-tests, Analysis of Variance (ANOVA) tests, and linear regression was performed using Microsoft® Excel v16.94 (Microsoft Corporation, Redmond, WA) and JMP Pro v18.0.2 statistical software (JMP Statistical Discovery, LLC,

Cary, NC). Results with a probability value, or p-value, of less than 0.05 were considered statistically significant (significance level of 0.05).

2.7.2 Phase 2: Comparison of Collection Methods for Exogenous DNA from Fingernail Samples

The percent recovery of total DNA (both nail and blood) from blood-spiked nails using the swabbing and soaking collection methods was calculated by taking the observed total DNA (ng) recovered from the samples over the expected total DNA yield. The expected total DNA yield for each sample collected using the soaking method was calculated using the sum of the expected DNA (ng) recovered from the volume of whole blood (µl) added and the expected DNA (ng) recovered from the amount of nail (mg) used in the sample. The amount of DNA expected to be recovered from one microliter of whole blood, 39.11 ng, was calculated by taking the average recovery of DNA per microliter of whole blood from 2 blood control samples of 5 µl and 2 blood control samples of 2 µl extracted with the PrepFiler™ protocol (incubation time of 15 min) with an elution volume of 50 µl (Table 3). The average ng DNA/mg nail recovered from the nail control sample for each donor was used to calculate the expected DNA recovered from the amount of nail sample used from each donor (expected ng DNA of experimental nail= ng DNA/mg nail control x mg of nail used). Furthermore, the percent recovery of total DNA from the blood-spiked samples that were collected using the swabbing method was calculated using only the expected DNA (ng) recovered from the volume of whole blood (µl) added to the nails. However, it is important to note that the expected total DNA from the nail samples was

underestimated for all samples. This is because the nail donor's DNA that remained on the nail's surface after the cleaning process was not included in the calculation.

The resulting DNA profiles from the blood-spiked nail samples were assessed using an analytical threshold of 30 relative fluorescence units (RFU). The unique nail and foreign alleles, which were the alleles not shared between the nail and blood donors, were identified by comparing the DNA profile of the donor nail control and the DNA profile of the blood sample to the resulting profiles. The nail donor mixture proportion, or proportion of peak height from nail alleles in the electropherograms, which were only assessed across loci where no alleles were shared between the nail and blood donors, was calculated by taking the peak height sum of the nail alleles present at a locus over the peak height sum of all alleles present at that locus. These nail mixture proportions were then averaged across loci for each profile. The foreign mixture proportion at each of these loci were calculated by subtracting the nail mixture proportion from 1. The percent recovery of unique nail and foreign alleles detected was also used to assess the detection of endogenous and exogenous DNA from the blood-spiked nail samples between collection methods. The percent recovery of unique nails was calculated by taking the number of observed unique nail alleles over the number of total possible unique nail alleles in the profile.

Statistical analysis using Analysis of Variance (ANOVA) tests was performed to calculate the statistical significance of the difference in total DNA yield, percent recovery of nail and foreign unique alleles, and nail and foreign mixture proportions between collection methods. Results with a probability value, or p-value, of less than 0.05 were considered statistically significant (significance level of 0.05).

3. RESULTS & DISCUSSION

3.1 Phase 1: Incubation Time Study

3.1.1 Modified ChargeSwitch® Extraction Method

Cleaned nail samples from two volunteers, Donor A and Donor B, were extracted using the modified ChargeSwitch® protocol to assess the quantity of endogenous DNA that is extracted from fingernails after an incubation period of 15 minutes, 30 minutes, 45 minutes, and 60 minutes. The repeatability of the extraction was assessed by performing the extraction in duplicate, using two sets of nails per incubation period from each donor. The average amount of DNA (ng) /nail (mg) recovered from the samples (n= 2) of each donor was used to evaluate the amount of endogenous DNA released from nail samples at varying incubation times.

There was an increase observed in the amount of DNA (ng) per milligram (mg) of nail recovered from nail samples as the incubation length increased, with an average across all donors of 0.315, 0.497, 0.674, and 0.707 ng DNA/mg nail (n=.4), for incubation times of 15, 30, 45, and 60 minutes, respectively (r^2= 0.934) (Table 1). However, this difference in DNA recovery between incubation periods was not statistically significant (p = 0.54). Both nail samples for Donor B had a decrease in the amount of ng DNA /mg nail recovered as the incubation length increased from 30 to 60 minutes (Table 1). These results suggest potential variation that may exist in the extraction of DNA from fingernail samples (Figure 6).

Table 1. Recovery of DNA from nail samples of Donors A & B using modified ChargeSwitch®. Nail samples were extracted using the ChargeSwitch® extraction protocol and an elution volume of 150 µl. The table displays the average DNA recovery from nails for each incubation time. Two nails were extracted from each donor (n= 2).

Donor	Incubation Time (mins)	Average Mass of Nail (mg)	Average DNA Concentration (ng/µl) in Eluent	Average Total DNA (ng)	Average ng DNA/mg Nail ± 1 Std. Dev.
A	15	39.85	0.0588	8.813	0.221 ± 0.09
A	30	39.85	0.0694	10.410	0.261 ± 0.05
A	45	39.85	0.187	28.005	0.703 ± 0.52
A	60	39.85	0.213	31.898	0.801 ± 0.57
B	15	39.25	0.107	16.106	0.410 ± 0.20
B	30	39.25	0.194	29.003	0.739 ± 0.69
B	45	39.25	0.169	25.403	0.647 ± 0.55
B	60	39.25	0.162	24.270	0.618 ± 0.57

3.1.2 Modified PrepFiler™ Extraction Method

The recovery of endogenous DNA from fingernail samples of two donors, Donor B and Donor C, was evaluated using the modified PrepFiler™ extraction method. The average amount of ng DNA /mg nail recovered from samples with incubation times of 15, 30, 45, and 60 min was used to assess the recovery of endogenous DNA extracted from fingernails after varying incubation times. A set of samples from the same donor also used during the modified ChargeSwitch® experiment, Donor B, was used with the PrepFiler™ extraction protocol to compare the recovery of endogenous DNA between the extraction

methods. Those results were used to assess the repeatability of the extraction of endogenous DNA from one donor's nails between different extraction methods.

There was an increase observed in the average recovery of ng DNA/mg nail for incubation times from 15 to 45 minutes, followed by a decrease at 60 min for both donors (Table 2). The average DNA (ng)/ nail (mg) recovered from nail samples of Donor C increased from 2.082 to 6.877 ng/mg nail for incubation times of 15 to 45 minutes, respectively (r^2= 0.987) (Table 2). Similarly, the average ng DNA /mg nail recovered from nails of Donor B increased from 0.117 to 0.354 ng/mg for incubations of 15 to 45 minutes (r^2= 0.986) (Table 2). These results suggest that the release of endogenous DNA from the nail increases as incubation time increases from 15 to 45 minutes (Figure 7). However, for both donor nail samples, there was a decrease in DNA (ng) per milligram of nail observed at 60 minutes, suggesting that increased incubation time does not result in increased DNA recovery. However, the amount of DNA (ng) released from the nail samples did not decrease at 60 min. An explanation for the lower value observed at 60 min is that the majority of endogenous DNA from the nail samples was recovered within the first 45 min of incubation, and then the release of nail DNA plateaued. Another explanation may be due to error in the weighing of the nail samples, and, as a result, an incorrect measurement for the mass of the nail sample was used to calculate the amount of DNA (ng) recovered from 1 mg nail. Furthermore, there was a distinct difference in the recovery of ng DNA/mg nail for Donor C compared to the other donors. The increased recovery of DNA (ng) from Donor C's nail sample may be due to the biological structure of the donor's nails, i.e., lack of stability in the keratin matrix of the nails.

Table 2. Recovery of DNA from nail samples of Donors B and C using the modified PrepFiler™ method. Nail samples were extracted using the PrepFiler™ extraction protocol and were eluted in 50 µl. The table displays average DNA recovery of donor nails for each incubation time. One nail sample was extracted for each donor (n =1), and quantified in duplicate, with values averaged for each sample.

Donor	Incubation Time (min)	Mass of Nail (mg)	Avg. DNA Concentration (ng/µl) in Eluent	Avg. Total DNA (ng)	Avg. ng DNA/ mg Nail
C	15	33.8	1.408	70.39	2.082
C	30	33.8	3.354	167.68	4.961
C	45	33.8	4.649	232.45	6.877
C	60	33.8	3.813	190.67	5.641
B	15	38.4	0.090	4.48	0.117
B	30	38.4	0.200	9.98	0.260
B	45	38.4	0.272	13.58	0.354
B	60	38.4	0.221	11.05	0.288

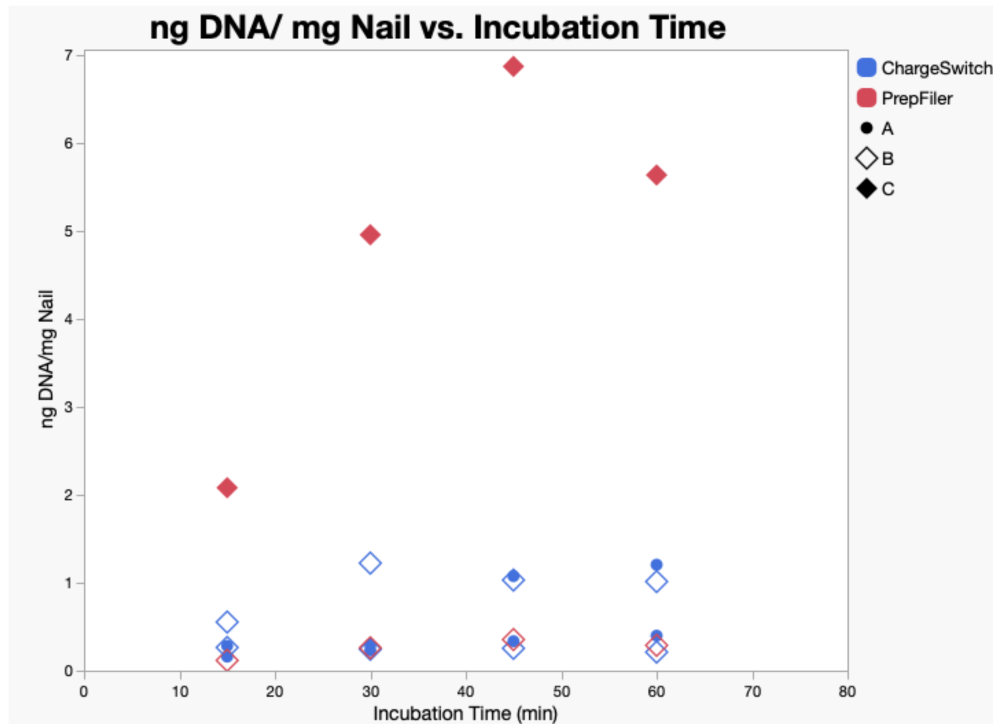

Figure 7. The recovery of endogenous DNA from nail samples using the modified ChargeSwitch® and PrepFiler™ extraction methods. A comparison of the average DNA (ng)/nail (mg) recovered from samples at varying incubation times between two donors (one sample from each donor, n= 2) using the PrepFiler™ method (Red) and two donors (two samples from each donor, n= 4) using the ChargeSwitch® protocol (Blue).

3.1.3 Comparison of Endogenous DNA Recovery using ChargeSwitch® and PrepFiler™ Protocols

The nail samples from Donor B that were extracted using both the modified ChargeSwitch® and PrepFiler™ protocols showed a greater average recovery of DNA across all incubation times using the ChargeSwitch® protocol compared to that of the PrepFiler™ protocol. The recovery of DNA per nail ranged from 0.410 to 0.739 ng/mg using the ChargeSwitch® protocol (Table 1) and 0.117 to 0.354 ng/mg using the PrepFiler™ protocol (Table 2). However, the difference in DNA recovery between the

extraction methods for samples from Donor B was not statistically significant ($p = 0.148$). Furthermore, a pattern was observed for both extraction methods: there was an increase in average recovery of DNA (ng)/ nail (mg) from samples that were incubated for 15 minutes to samples incubated at 30 minutes. These results suggest a potential variation in the amount of DNA extracted from nails between incubation times, as well as a potential variation in DNA recovery between extraction methods (Figure 8). Fifteen minutes was chosen as an optimal length of incubation for nail samples to minimize the recovery of endogenous DNA from fingernail samples. The PrepFiler™ protocol with an incubation of 15 min was used for the extraction of samples in Phase 2.

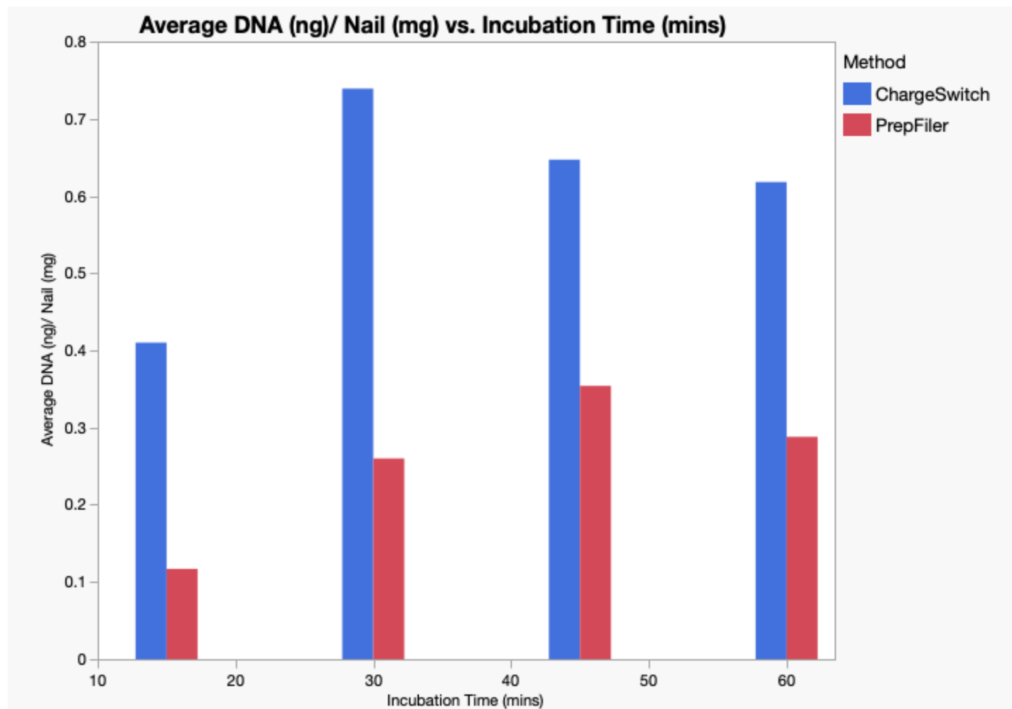

Figure 8. Comparison of DNA recovery of Donor B nail samples between ChargeSwitch® and PrepFiler™ extraction methods. A comparison of the average DNA (ng)/ nail (mg) recovered from samples at incubation times of 15, 30, 45, and 60 min using the ChargeSwitch® (blue) (n= 2) and the PrepFiler™ method (red) (n= 1).

3.2 Phase 2: Comparison of Collection Methods for Exogenous DNA from Fingernail Samples

3.2.1 Recovery of DNA from Blood-Spiked Nails for the Comparison of Collection Methods: Experiment 1

To compare the efficiency of collection methods of exogenous DNA from fingernails, nail samples from three volunteers, Donor D, Donor E, and Donor F, were spiked with whole blood volumes of 10, 5, or 2 µl and were sampled using either the soaking method or swabbing method.

First, the collection methods were assessed on their efficiency at recovering DNA from samples using percent recovery of total DNA from the blood-spiked samples. The observed yield of total DNA recovered and the expected yield of total DNA recovered were used to calculate the percent recovery of all available DNA from each sample. The total DNA for these samples represents the combined expected total DNA from the nail and the blood sample, as previously explained in Section 2.7.2 of the Materials and Methods section. The amount of DNA expected to be recovered from one microliter of whole blood, 39.11 ng, was calculated using the average recovery of DNA per microliter of whole blood from blood control samples of 5 µl and 2 µl using the PrepFiler™ extraction protocol with an incubation time of 15 min and an elution volume of 50 µl (Table 3).

Table 3. Recovery of DNA (ng)/ (ul) from whole blood using control samples of 5 µl and 2 µl liquid whole blood and the PrepFiler™ extraction protocol. The average recovery of DNA per microliter of whole blood was calculated to be 39.11 ng DNA/ ul blood using blood control samples of 5 µl (n= 2) and 2 µl (n= 2) that were extracted using the PrepFiler™ extraction protocol with an elution volume of 50 µl.

Volume of Whole Blood (µl)	DNA Concentration (ng/µl)	DNA (ng)	DNA (ng)/ Blood (µl)
5	4.149	207.4	41.49
5	4.437	221.9	44.37
2	1.259	62.97	31.48
2	1.564	78.19	39.10

As previously mentioned, the percent recovery of nail samples from three donors was used to assess and compare the DNA recovery efficiency from the blood-spiked nail samples after the use of the soaking or the swabbing collection methods. Although it was expected that the soaking method would result in higher percent recovery of total DNA (both nail and foreign DNA) due to the addition of the entire fingernail sample into the lysis solution during extraction, the overall difference in average total DNA recovery between the soaking and swabbing methods was found to be not statistically significant across all donors (Table 4) ($p = 0.85$). However, it is important to note that the percent total recovery was calculated for the soaking method using the maximum amount of DNA that could have been extracted from the nail itself, and the recovery of endogenous DNA may vary between nails of the same donors.

Specifically, for blood-spiked nail samples from Donor E, there was a percent yield of total DNA recovery ranging from 29.7% to 42.3% using the soaking method and 42.7% to 75.8% using the swabbing method (Table 4). For blood-spiked nail samples from Donor

F, there was a percent yield of total DNA recovery ranging from 34.8% to 53.6% using the soaking method and 31.2% to 34.5% using the swabbing method (Table 4). Lastly, the blood-spiked nail samples from Donor D had a percent yield of total DNA recovery ranging from 45.1% to 84.6% using the soaking method and 42.9% to 53.6% using the swabbing method (Table 4). The average percent recovery of total DNA recovered from nail samples was less than 50% of the sample's total DNA using either the soaking or swabbing method. These results suggest that the majority of DNA is lost during the collection of DNA from nail samples regardless of the collection method. However, it is important to note the limitations when comparing the percent recovery of total DNA between collection methods due to the use of estimated values for expected recovery of DNA for the blood volumes and nail samples. Specifically, the potential of nail donor DNA remaining on the surface of the nail after the cleaning process was not accounted for. As a result, the measurements of nail and foreign DNA mixture proportions and the percent recovery of unique nail and foreign alleles were used for the comparison of collection methods for foreign DNA recovery.

Table 4. DNA recovery from Donor F, E, and D nail samples spiked with 10 µl, 5 µl, & 2 µl whole blood and collected using the soaking or swabbing method. The table displays the DNA concentration (ng/µl) of eluent and the total DNA (ng) recovered from each sample, as well as the calculated values for the expected total DNA yield and the percent total yield. The average amount of DNA (ng) recovered from 1 µl whole blood, 39.11, and the amount of ng DNA/mg nail from the donor, were used in the below calculations for expected total DNA (ng). (n = 1)

Donor	Sample Type	Volume of Whole Blood (µl) Added to Sample	Nail Mass (mg)	DNA Concentration (ng/µl) of 50 µl Eluent	Total DNA (ng)	Expected Total DNA (ng) Yield	Percent Total Yield (Observed/ Expected)
F	Soaking	10	9.3	4.141	207.0	452.8	45.7%
F	Soaking	5	7.2	2.608	130.4	243.3	53.6%
F	Soaking	2	6.7	0.855	42.73	122.7	34.8%
F	Swabbing	10	9.0	2.695	134.8	391.1	34.5%
F	Swabbing	5	6.7	1.221	61.07	195.6	31.2%
F	Swabbing	2	5.5	0.518	25.89	78.22	33.1%
F	Nail Control	N/A	8.0	1.062	53.10	53.10	N/A
E	Soaking	10	13.0	3.787	189.4	555.6	34.1%
E	Soaking	5	9.9	2.713	135.7	320.8	42.3%
E	Soaking	2	9.6	1.186	59.30	199.7	29.7%
E	Swabbing	10	13.2	3.341	167.0	391.1	42.7%
E	Swabbing	5	10.6	2.964	148.2	195.6	75.8%
E	Swabbing	2	10.8	1.077	53.84	78.22	68.8%
E	Nail Control	N/A	13.3	3.366	168.3	168.3	N/A

D	Soaking	10	10.4	3.535	176.8	393.2	45.0%
D	Soaking	5	11.9	3.153	157.6	198.0	79.6%
D	Soaking	2	10.4	1.359	67.95	80.35	84.6%
D	Swabbing	10	11.1	3.357	167.9	391.1	42.9%
D	Swabbing	5	10.8	2.098	104.9	195.6	53.6%
D	Swabbing	2	11.7	0.823	41.15	78.22	52.6%
D	Nail Control	N/A	14.6	0.0596	2.982	2.982	N/A

3.2.2. Analysis of DNA Profiles from Extracts of Blood-Spiked Nails for the Comparison of Collection Methods: Experiment 1

The DNA profiles of the blood-spiked nail samples were interpreted, using an analytical threshold of 30 RFU, to assess the efficiency of exogenous DNA recovery between the swabbing and soaking methods. The unique nail alleles, or the nail alleles that were not shared with the “foreign” blood donor, and the unique foreign alleles, or the alleles from the blood donor that were not shared with the nail donor, were identified by comparing the DNA profile of the nail control for each donor with the DNA profile of the blood sample. The proportion of peak height from nail alleles in the mixture profiles were assessed across loci where there were no shared alleles between donors. The percent recovery of unique foreign alleles was calculated to evaluate the efficiency of detecting foreign DNA from fingernail samples using the soaking and swabbing methods.

3.2.2.1 Donor E Nail Samples Spiked with 10 µl, 5 µl, and 2 µl Whole Blood

There was a total of 27 unique nail alleles identified from Donor E. The profiles from the blood-spiked nail samples that underwent the soaking method for this donor had a high average percent recovery of unique nail alleles, ranging from 77.8% to 100% recovery; however, the nail mixture proportion for the blood-spiked samples did not exceed 25% using the soaking method (Table 5). Conversely, the blood-spiked samples that were swabbed had a low average percent recovery of unique nail alleles, ranging from 0%, to 14.8%, and a proportion of peak height from nail alleles of 1.14% from the nail sample spiked with 10 µl blood (Table 5). The difference in recovery of unique nail alleles between

collection methods was statistically significant ($p = 0.0006$). Figure 9 and Figure 10 show a comparison of the yellow dye loci of the electropherogram (D2S441, D19S433, TH01, and FGA) for the blood-spiked nails of Donor E that were collected by the soaking method and swabbing methods, respectively. These results suggest that the soaking method had a higher percent recovery of endogenous DNA from the blood-spiked samples of Donor E as compared to the swabbing method. The percent recovery of unique foreign alleles was 100% across all blood-spiked samples for Donor E.

Table 5. Recovery of unique nail and foreign alleles from nail samples of Donor E spiked with whole blood and collected the soaking and swabbing methods. The table displays the percent recovery of unique nail and foreign alleles, and the proportion of peak height from nail and foreign alleles detected in the electropherograms. There were 27 unique nail alleles identified from Donor E and 30 unique blood alleles (Tables A & B). Standard deviation and sample size were equivalent for nail and foreign mixture proportions for each sample. (n= # of loci used to calculate nail and foreign mixture proportions)

Blood Aliquot (µl)	Collection Method	Nail Mass (mg)	% of Nail Alleles Observed	% of Foreign Alleles Observed	Nail Mixture Proportion ± 1 Std. Dev	Foreign Mixture Proportion
10	Soaking	13	77.80%	100.00%	0.047 ± 0.03 (n= 3)	0.953
5	Soaking	9.9	100.00%	100.00%	0.245 ± 0.05 (n= 3)	0.755
2	Soaking	9.6	100.00%	100.00%	0.179 ± 0.07 (n= 3)	0.821
10	Swabbing	13.2	14.81%	100.00%	0.0114 (n= 1)	0.989
5	Swabbing	10.6	0.00%	100.00%	0	1.0
2	Swabbing	10.8	11.11%	100.00%	0	1.0

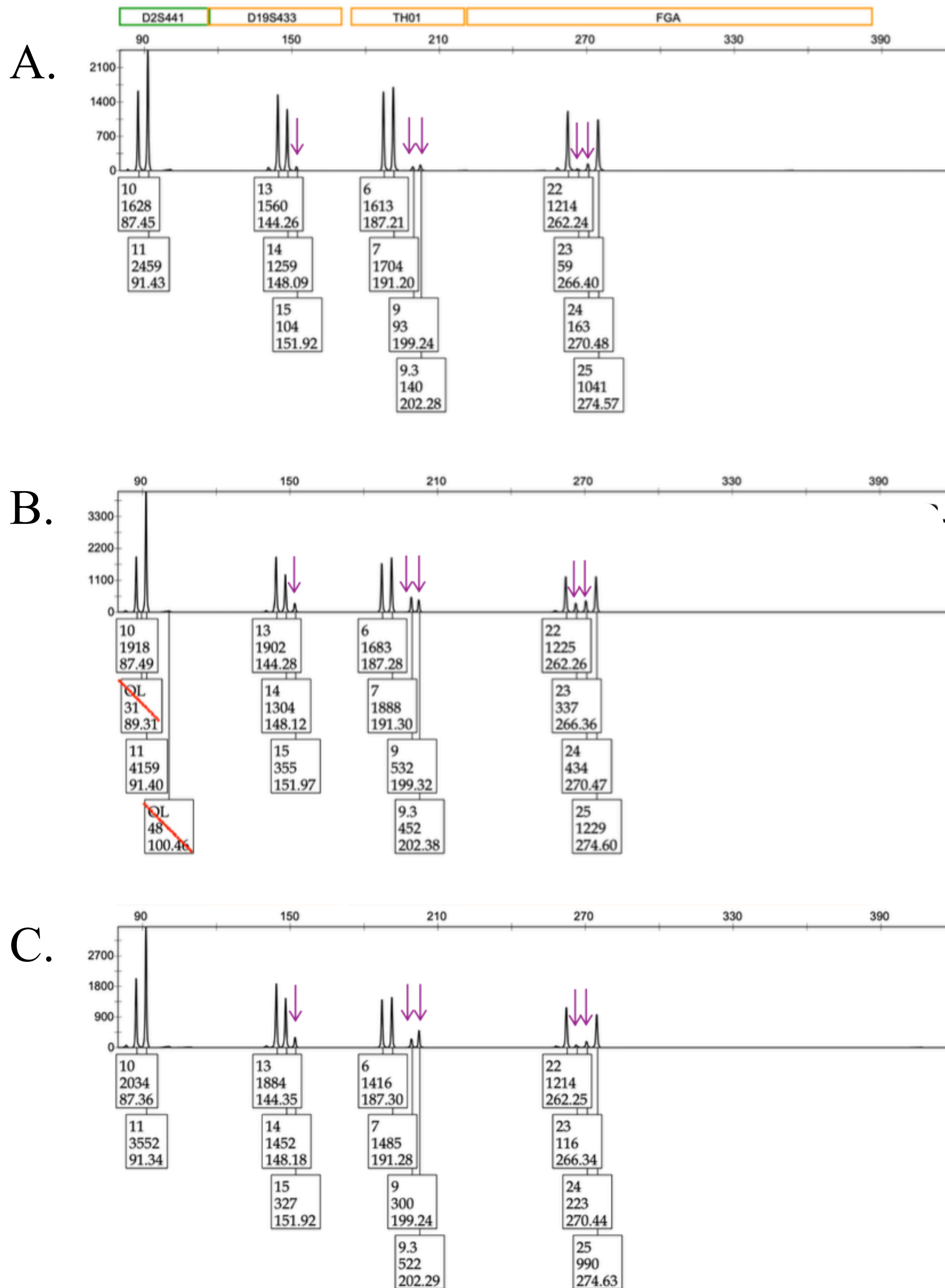

Figure 9. DNA profiles of Donor E nails spiked with 10 µl (A), 5 µl (B), and 2 µl (C) of whole blood collected with the soaking method. Data for the yellow dye channel is shown for blood spiked nail samples from Donor E using the soaking method (referenced in Table 5). Purple arrows were used to indicate alleles unique to the sample nail. (Off Ladder (OL) artifact detected in electropherograms)

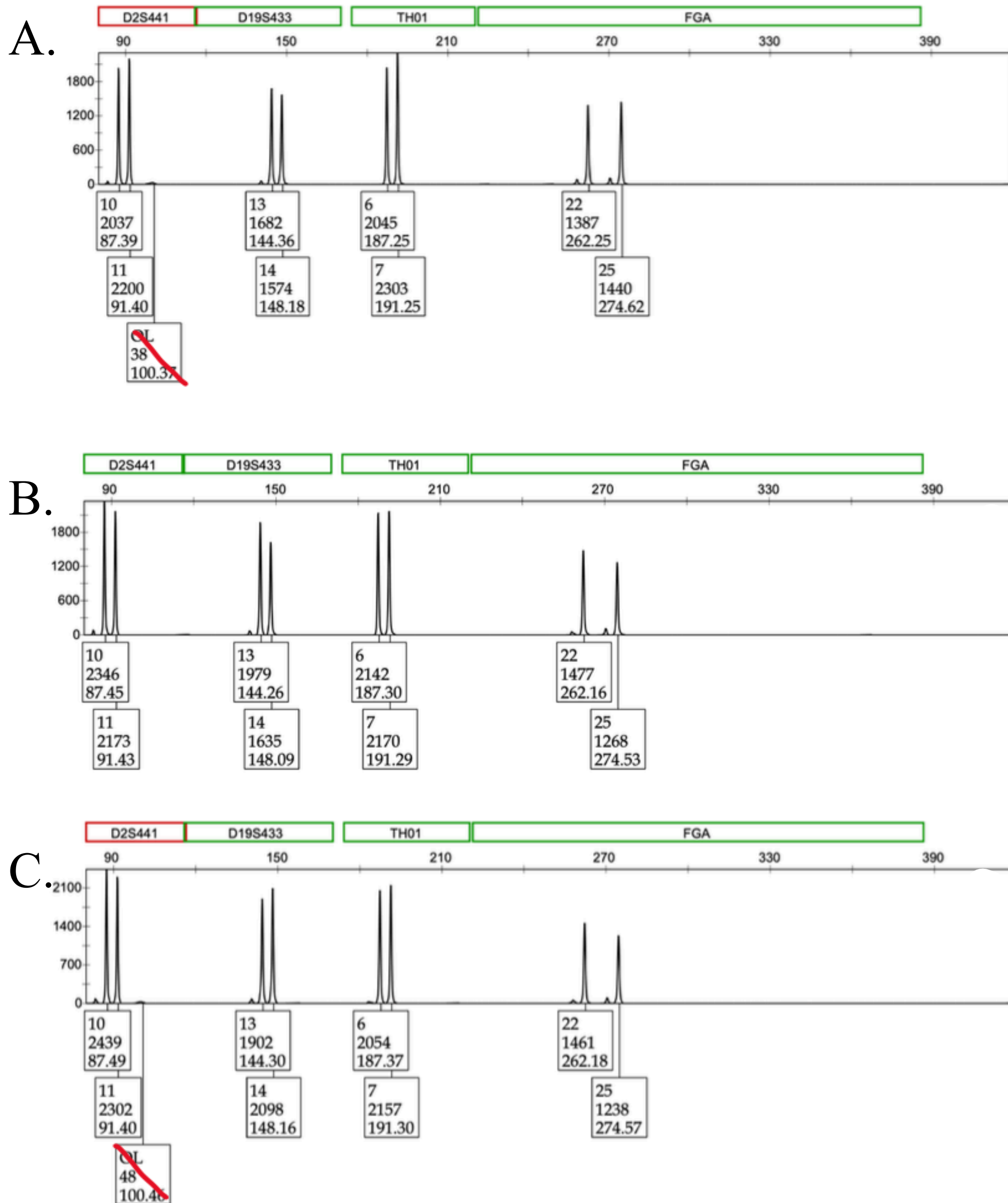

Figure 10. DNA profiles of Donor E nails spiked with 10 µl (A), 5 µl (B), and 2 µl (C) of whole blood collected with the swabbing method. Data for the yellow dye channel is shown for blood spiked nail samples from Donor E using the soaking method (referenced in Table 5). No unique nail alleles were observed in the yellow dye loci for the Donor E nails spiked with 10µl, 5µl, and 2 µl whole blood and collected using the swabbing method. (Off Ladder (OL) artifact detected in electropherograms)

3.2.2.2 Donor F Nail Samples Spiked with 10 µl, 5 µl, and 2 µl Whole Blood

There was a total of 27 unique nail alleles identified from Donor F. Similar to Donor E, the blood-spiked nail samples from this donor had a higher average percent recovery of unique nail alleles using the soaking method as compared to the swabbing method, with the exception of the soaked nail sample spiked with 5 µl blood (Table 6). However, the difference between collection methods was found to be not statistically significant ($p = 0.711$). Furthermore, the average nail mixture proportion in the profiles was not significantly different between collection methods, with the maximum proportion of nail observed to be 21% using the soaking method and 12.5% using the swabbing method (Table 6). The results of the blood-spiked samples from Donor F showed variation in the recovery of endogenous DNA across the soaking and swabbing methods, indicating no significant advantage in using one collection method over the other to minimize nail DNA recovery. There was 100% recovery of the unique foreign alleles and a proportion of peak height from foreign alleles greater than 79% across all samples, regardless of collection method (Table 6).

Table 6. Recovery of unique nail and foreign alleles from nail samples of Donor F spiked with whole blood and collected using the soaking and swabbing methods. The table displays the percent recovery of unique nail and foreign alleles and the proportion of peak height from nail and foreign alleles detected. There were 27 unique nail alleles identified from Donor F and 30 unique blood alleles (Tables C & D). Standard deviation and sample size were equivalent for nail and foreign mixture proportions for each sample. (n= # of loci used to calculate nail and foreign mixture proportions)

Blood Aliquot (µl)	Collection Method	Nail Mass (mg)	% of Nail Alleles Observed	% of Foreign Alleles Observed	Nail Mixture Proportion ± 1 Std. Dev.	Foreign Mixture Proportion
10	Soaking	9.3	92.60%	100.0%	0.077± 0.007 (n= 4)	0.923
5	Soaking	7.2	25.90%	100.0%	0.018 (n= 1)	0.982
2	Soaking	6.7	100.00%	100.0%	0.210 ± 0.01 (n= 3)	0.790
10	Swabbing	9	59.26%	100.0%	0.029± 0.008 (n= 2)	0.971
5	Swabbing	6.7	88.89%	100.0%	0.125± 0.03 (n= 3)	0.875
2	Swabbing	5.5	37.04%	100.0%	0.018 (n= 1)	0.982

3.2.2.3 Donor D Nail Samples Spiked with 10 µl, 5 µl, and 2 µl Whole Blood

Lastly, there were a total of 27 unique nail alleles identified from Donor D. Similar to Donors E and F, the profiles from the Donor D blood-spiked nail samples that were soaked showed a higher percent recovery of nail alleles as compared to the samples that were swabbed, with the exception of the swabbed nail sample spiked with 2 µl blood (Table 7). However, similar to Donor F, the difference in recovery of unique nail alleles between the collection methods was not statistically significant (p = 0.230). Furthermore,

the average proportion of peak height from nail alleles in the profiles was similar across collection methods, with the proportion of nail component not exceeding 4% for either the soaking or swabbing method (Table 7). Consistent with the results of Donor F, the results suggest no significant difference in the recovery of endogenous DNA between collection methods. There was a 100% recovery of the unique foreign alleles and a proportion of peak height from foreign alleles greater than 96% across all samples, regardless of collection method (Table 7).

Table 7. Recovery of unique nail and foreign alleles from nail samples of Donor D spiked with whole blood and collected using the soaking and swabbing methods. The table displays the percent recovery of unique nail and foreign alleles, and the proportion of peak height from nail and foreign alleles detected. There were 27 unique nail alleles identified from Donor D and 34 unique blood alleles (Tables E & F). Standard deviation and sample size were equivalent for nail and foreign mixture proportions for each sample. (n= # of loci used to calculate nail and foreign mixture proportions)

Blood Aliquot (µl)	Collection Method	Nail Mass (mg)	% of Nail Alleles Observed	% of Foreign Alleles Observed	Nail Mixture Proportion ± 1 Std. Dev.	Foreign Mixture Proportion
10	Soaking	10.4	37.04%	100.00%	0.005 ± 0.0007 (n= 2)	0.995
5	Soaking	11.9	48.15%	100.00%	0.014 ± 0.006 (n= 2)	0.986
2	Soaking	10.4	40.74%	100.00%	0.023 ± 0.02 (n= 2)	0.977
10	Swabbing	11.1	14.81%	100.00%	0.034 (n= 1)	0.966
5	Swabbing	10.8	11.11%	100.00%	0.007 (n= 1)	0.993
2	Swabbing	11.7	48.15%	100.00%	0.021± 0.003 (n= 3)	0.979

3.2.2.4 Overall Comparison of Nail Samples Spiked with 10 µl, 5 µl, and 2 µl Whole Blood Between Collection Methods for Donors D, E, and F

The average percent recovery of unique nail alleles of blood-spiked nails across all three donors and all three amounts of blood was 69.1% using the soaking method and 31.7% using the swabbing method (Table 8). Although the differences in percent recovery of unique nail alleles was found to be statistically insignificant for the samples of Donors D and F for both collection methods, the overall difference in the average recovery of unique nail alleles of blood-spiked samples was statistically significant (p= 0.017) between collection methods. These results suggest that the swabbing method is more advantageous for minimizing the potential recovery of endogenous DNA from fingernail samples. However, the 100% recovery of unique foreign alleles of blood-spiked nails of the three donors was consistent between collection methods (Table 8). These results suggest that the amount of whole blood chosen to be aliquoted onto the nail samples in this experiment may have been too large to effectively assess the recovery of smaller amounts of foreign DNA from nail samples. As a result, this experiment was repeated and an additional set of blood dilutions was used.

Table 8. Average recovery of unique nail and foreign alleles from Donors F, E, and D nail samples spiked with whole blood using the soaking or swabbing method. The table displays the average percent recovery of unique nail and foreign alleles detected across all three donors and blood volumes.

Collection Method	Percentage of Unique Nail Alleles Observed	Percentage of Unique Foreign Alleles Observed
Soaking (n= 9)	69.1%	100%
Swabbing (n= 9)	31.69%	100%

3.2.2.5 Determination of Blood Dilutions for Second Set of Blood-Spiked Nails

The average amount of DNA extracted from one microliter of blood was calculated to be 39.11 ng using whole blood control samples (n= 4) from the previous experiment. The average amount of DNA (ng) extracted from one milligram of nail was calculated to be 6.49 ng from the nail controls of Donors D, E, and F (n= 3). These values were used to determine the new concentrations of blood that would be used to spike the fingernail samples (Table 9). It was determined that fingernail samples with a mass of 5 to 20 mg of nail would be spiked with a 1:2 blood dilution and a 1:5 blood dilution to assess the recovery of unique foreign alleles from blood-spiked samples that were either sampled using either the soaking or swabbing method. These blood dilutions would potentially result in a mixed DNA profile in which the nail donor and the foreign blood donor were of equivalent contribution, or the foreign DNA was the major contributor.

Table 9. Theoretical yield of DNA from blood samples. The average amount of DNA extracted from one microliter of blood, calculated as 39.11 ng, was used to calculate the theoretical yield of DNA (ng) from 1 µl of a 1:2 blood dilution and a 1:5 blood dilution. This was used to determine the blood dilutions for the second set of blood-spiked nails.

Blood Sample/Dilution	Theoretical DNA (ng) Yield in 1 µl of Blood Sample
Whole Blood	39.11
1:2 Blood Dilution	19.56
1:5 Blood Dilution	7.822

3.2.3 Recovery of DNA from Blood-Spiked Nails for the Comparison of Collection Methods: Experiment 2

Fingernail samples with aliquots of the adjusted blood dilutions were used to assess the recovery of unique foreign alleles from blood-spiked samples that were sampled using either the soaking or swabbing method. The following volumes and concentrations of blood were used to spike sets of nails from two donors, Donor G and Donor E: 2 µl of whole blood, 2 µl of a 1:2 blood dilution, and 2 µl of a 1:5 blood dilution.

There was no significant difference in the average percent yield of total DNA recovery for blood-spiked nail samples from both donors using the swabbing or soaking methods. Donor G had a range of 25.5% to 95.0% recovery of total DNA using the soaking method and 48.7% to 239% using the swabbing method (Table 10). For Donor E, the average percent yield of total DNA for the blood-spiked samples ranged from 16.4% to 47.7% using the soaking method and 62% to 202% using the swabbing method (Table 10). The values for percent recovery of total DNA yield over 100% for the swabbed samples may reflect the underestimate of expected total DNA recovery from the blood-spiked nails. Specifically, the estimated expected total DNA recovery from the swabbed samples did not account for any nail donor DNA remaining on the nail's surface after the cleaning process. This may provide an explanation for the detection of nail alleles in these samples' profiles. As previously mentioned, due to the limitations in the significance of the percent recovery of total DNA yield results, the measurements of nail and foreign DNA mixture proportions and the percent recovery of unique nail and foreign alleles were used for the comparison of collection methods for foreign DNA recovery.

Table 10. DNA recovery from Donor G and E nail samples spiked with whole blood and diluted blood and collected using the soaking or swabbing method. The table displays the DNA concentration (ng/ µl) in eluent and total DNA (ng) recovered from each sample (n =1), as well as the calculated values for the expected total DNA yield and the percent total yield. The average amount of DNA (ng) recovered from 1 µl of whole blood, 1:2 blood dilution, and 1:5 blood dilution, and the amount of ng DNA/mg nail, determined using nail controls for each donor, was used in the below calculations for expected total DNA (ng).

Donor	Sample Type	Nail Mass (mg)	Blood (µl) Added to Sample	DNA Concentration (ng/µl) in Eluent	Total DNA (ng)	Expected Total DNA (ng) Yield	Percent Total Yield (Observed/ Expected)
G	Soaking	16.5	2 µl Whole Blood	0.734	36.69	143.9	25.5%
G	Soaking	18.8	2 µl 1:2 Blood Dilution	2.164	108.21	113.9	95.0%
G	Soaking	16.5	2 µl 1:5 Blood Dilution	1.408	70.39	81.31	86.6%
G	Swabbing	26	2 µl Whole Blood	0.762	38.10	78.22	48.7%
G	Swabbing	23.6	2 µl 1:2 Blood Dilution	1.874	93.70	39.12	239%
G	Swabbing	20.3	2 µl 1:5 Blood Dilution	0.436	21.82	15.64	139%
G	Nail Control	15.4	N/A	1.227	61.35	N/A	N/A
E	Soaking	12.6	2 µl Whole Blood	2.266	113.3	237.6	47.7%
E	Soaking	16.5	2 µl 1:2 Blood Dilution	0.812	40.60	247.8	16.4%
E	Soaking	11.8	2 µl 1:5 Blood Dilution	0.850	42.50	164.9	25.8%

E	Swabbing	13.7	2 µl Whole Blood	1.119	55.94	78.22	71.5%
E	Swabbing	15.5	2 µl 1:2 Blood Dilution	0.485	24.27	39.12	62.0%
E	Swabbing	11.5	2 µl 1:5 Blood Dilution	0.635	31.74	15.64	202%
E	Nail Control	13.3	N/A	3.366	168.3	N/A	N/A

3.2.4. Analysis of DNA Profiles from Extracts of Blood-Spiked Nails for the Comparison of Collection Methods: Experiment 2

The percent recovery of nail alleles and nail mixture proportions was assessed across loci where there were no shared alleles between donors in order to evaluate the recovery of nail DNA from the blood-spiked nail samples using both collection methods. Furthermore, the percent recovery of unique foreign alleles was assessed to evaluate the efficiency of detecting foreign DNA from fingernail samples using the soaking method and the swabbing method.

3.2.4.1 Donor G Nail Samples Spiked with 2 µl Whole Blood, 1:2 Blood Dilution, and 1:5 Blood Dilution

The average percent recovery of unique nail alleles of Donor G was consistent between the soaking and swabbing methods (p = 0.51), with the exception of the swabbed nail sample spiked with whole blood, which had 80% recovery (Table 11). Furthermore, the nail mixture proportions were similar across both collection methods, with the

proportion exceeding 60% for the samples that were spiked with a 1:2 blood dilution for both collection methods. The high percent recovery of nail alleles observed in the profiles of the swabbed samples may indicate that nail donor DNA remained on the nail's surface after the cleaning process (Table 11). The results may also suggest that as the proportion of nail DNA in the sample becomes more equivalent to the proportion of foreign DNA in the sample, the potential of unique nail allele recovery in the resulting profiles increases.

Table 11. Recovery of unique nail and foreign alleles from Donor G nail samples spiked with blood dilutions and collected using the soaking and swabbing methods. The table displays the percent recovery of unique nail and foreign alleles and the nail mixture proportion and foreign mixture proportion of the samples. There were 20 unique nail alleles identified from Donor G and 25 unique blood alleles (Tables I & J). Standard deviation and sample size were equivalent for nail and foreign mixture proportions for each sample. (n= # of loci used to calculate nail and foreign mixture proportions)

Blood Dilution	Collection Method	Nail Mass (mg)	% of Nail Alleles Observed	% of Foreign Alleles Observed	Nail Mixture Proportion ± 1 Std. Dev.	Foreign Mixture Proportion
Whole Blood	Soaking	16.5	95.0%	100.00%	0.0917 ± 0.03 (n= 3)	0.908
1:2 Blood Dilution	Soaking	18.8	100.0%	100.00%	0.662 ± 0.04 (n= 3)	0.338
1:5 Blood Dilution	Soaking	16.5	100.0%	100.00%	0.557 ± 0.07 (n= 3)	0.443
Whole Blood	Swabbing	26.0	80.00%	100.00%	0.0341 ± 0.02 (n= 3)	0.966
1:2 Blood Dilution	Swabbing	23.6	100.00%	100.00%	0.611± 0.1 (n= 3)	0.389
1:5 Blood Dilution	Swabbing	20.3	100.00%	100.00%	0.207± 0.01 (n= 3)	0.793

3.2.4.2 Donor E Nail Samples Spiked with 2 µl of Whole Blood, 1:2 Blood Dilution, and 1:5 Blood Dilution

Contradictory to the data from Donor G, there was an increase in the percentage of unique nail alleles detected in the electropherograms of Donor E for the swabbing method as compared to the soaking method (Table 12). However, this difference between collection methods was not statistically significant (p= 0.35). As previously mentioned, the high percent recovery of nail alleles may be attributed to endogenous DNA that remained on the nail's surface after cleaning. Furthermore, Donor E had an increased proportion of peak height from nail alleles observed in the resulting profiles for the soaking method as compared to the swabbing method (Table 12). Similar to the data of Donor G, this may suggest that nail donor DNA on the nail's surface was collected by the swab. The results may also suggest that as the proportion of nail (DNA) in the sample becomes more equivalent to the proportion of foreign DNA in the sample, the potential of unique nail allele recovery in the resulting profiles increases. The increase in the proportion of peak height from nail alleles observed in the profiles using the soaking method was expected, due to the entirety of the sample being added to the lysis solution.

Figures 11 and 12 show the comparison of the yellow dye loci of the electropherograms (D2S441, D19S433, TH01, and FGA) for the blood-spiked nails from Donor E that were collected by the soaking method and the swabbing method, respectively. For all of the blood-spiked samples extracted in this experiment, 100% of the unique foreign alleles were recovered in the resulting profiles (Table 12). Similar to the first experiment, these results suggest that the blood dilutions used to spike the nail samples in

this experiment were not effective at assessing the recovery of foreign DNA from nail samples between collection methods. Future research using lower blood concentrations, i.e., a 1:10 or 1:20 blood dilution, may lead to significant results in the comparison of collection methods on foreign DNA recovery from fingernail samples [15, 38].

Table 12. Recovery of unique nail and foreign alleles from nail samples of Donor E spiked with blood dilutions and collected using the soaking and swabbing method. The table displays the percent recovery of unique nail and foreign alleles, and the proportion of peak height from nail and foreign alleles detected in the resulting profiles of the samples. There were 27 unique nail alleles identified from Donor E and 30 unique blood alleles (Tables G & H). Standard deviation and sample size were equivalent for nail and foreign mixture proportions for each sample. (n= # of loci used to calculate nail and foreign mixture proportions)

Blood Dilution	Sample Type	Nail Mass (mg)	% of Nail Alleles Recovered	% of Foreign Alleles Recovered	Nail Mixture Proportion ± 1 Std. Dev.	Foreign Mixture Proportion
Whole Blood	Soaking	12.6	88.9%	100.00%	0.147 ± 0.07 (n= 2)	0.853
1:2 Blood Dilution	Soaking	16.5	81.5%	100.00%	0.105 ± 0.3 (n= 2)	0.895
1:5 Blood Dilution	Soaking	11.8	100.0%	100.00%	0.423 ± 0.2 (n= 2)	0.577
Whole Blood	Swabbing	13.7	92.59%	100.00%	0.0651 ± 0.01 (n= 2)	0.935
1:2 Blood Dilution	Swabbing	15.5	100.00%	100.00%	0.0700 ± 0.02 (n= 2)	0.930
1:5 Blood Dilution	Swabbing	11.5	96.30%	100.00%	0.287± 0.06 (n= 2)	0.713

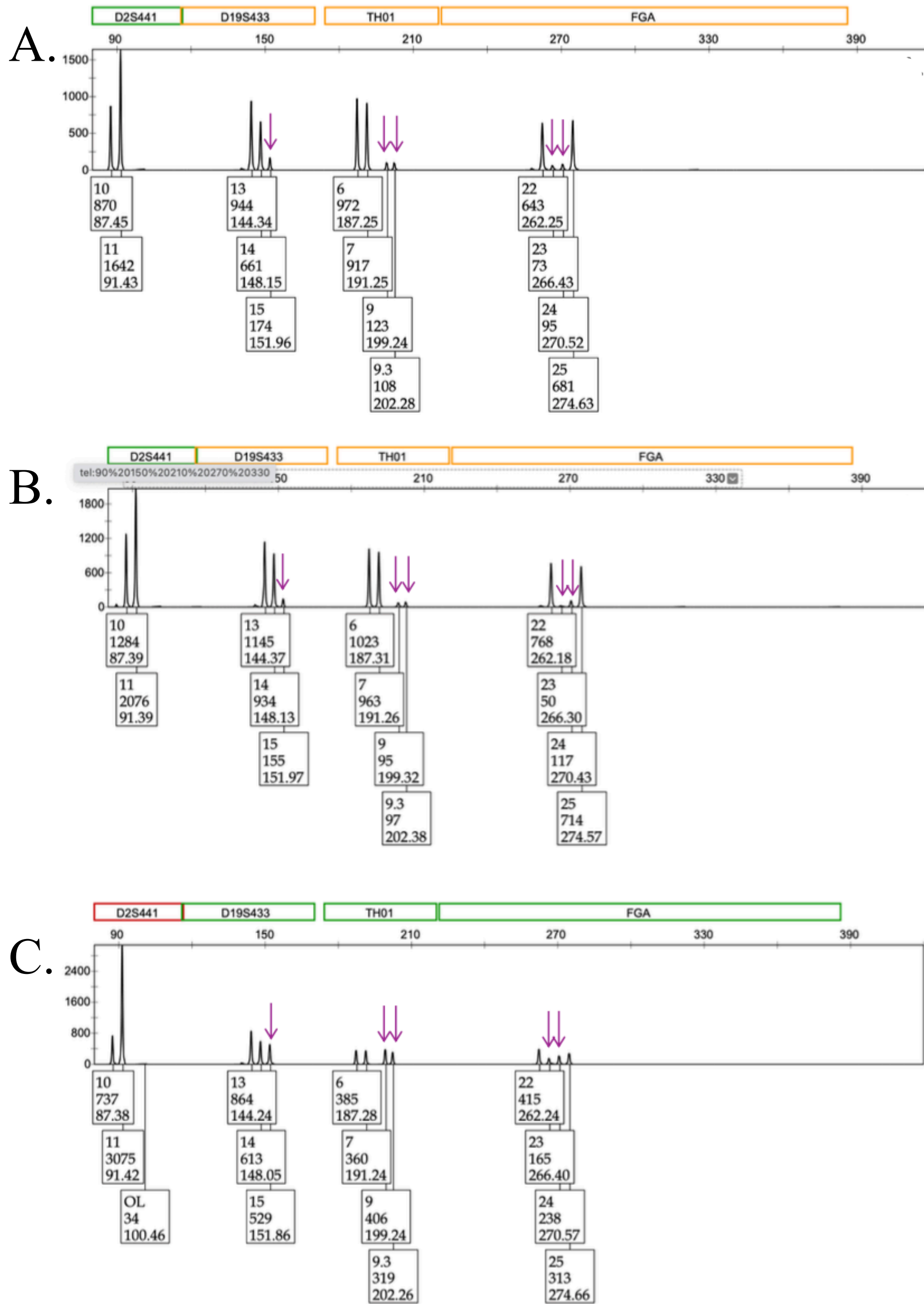

Figure 11. DNA profiles of nails (Donor E) spiked with whole blood (A), 1:2 Blood Dilution (B), and 1:5 Blood Dilution (C) collected with the soaking method. Data for the yellow dye channel is shown for blood spiked nail samples from Donor E using the soaking method (referenced in Table 12). Purple arrows were used to indicate unique nail alleles to the sample nail. (Off Ladder (OL) artifact detected in electropherograms)

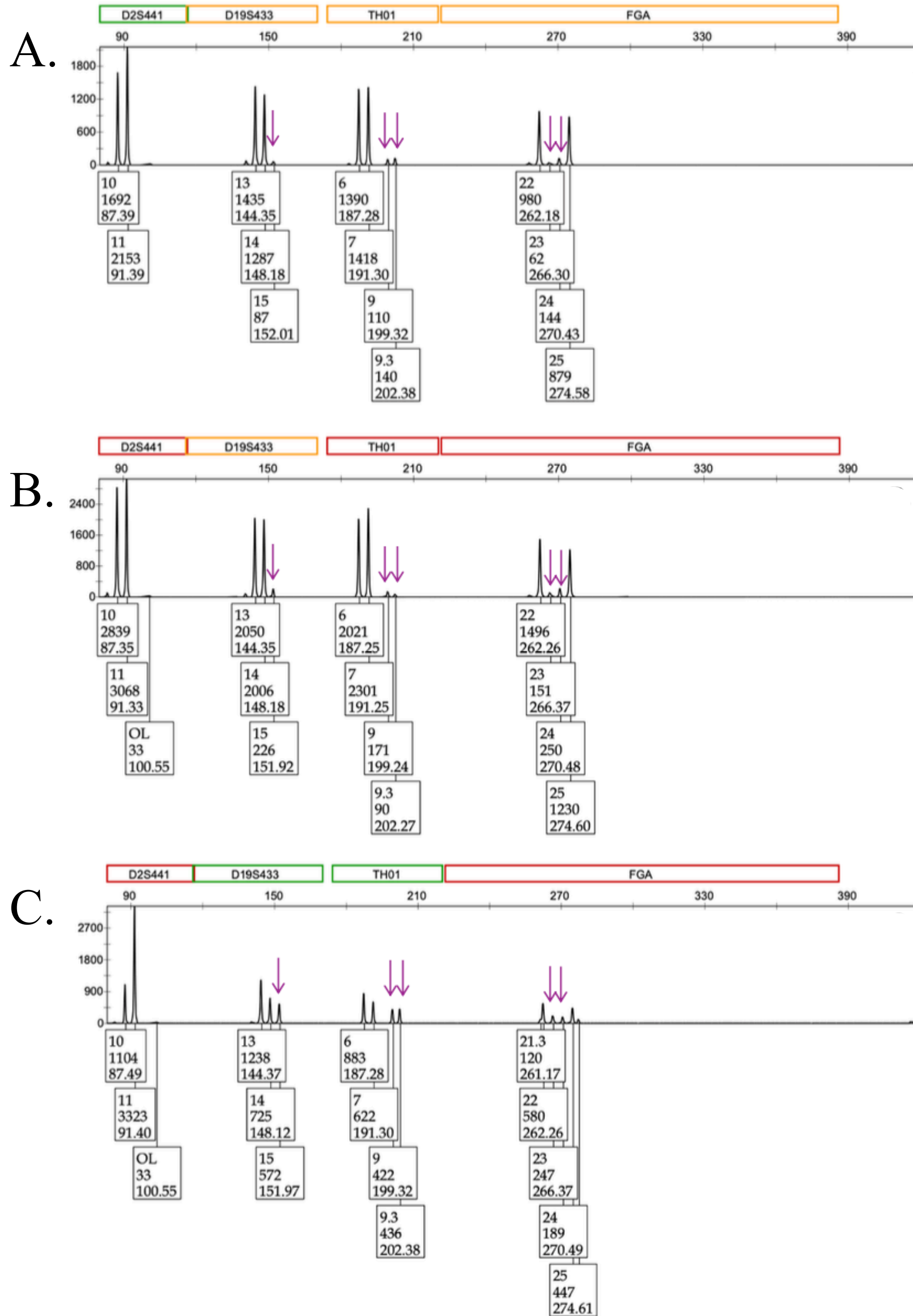

Figure 12. DNA profiles of nails (Donor E) spiked with whole blood (A), 1:2 Blood Dilution (B), and 1:5 Blood Dilution (C) collected with the swabbing method. Data for the yellow dye channel is shown for blood spiked nail samples from Donor E using the swabbing method (referenced in Table 12). Purple arrows were used to indicate unique nail alleles to the sample nail. (Off Ladder (OL) artifacts detected in electropherograms)

4. CONCLUSIONS & FUTURE DIRECTIONS

Numerous studies have evaluated the extraction of foreign DNA from fingernail samples; however, there is no consensus on the most effective method for exogenous DNA recovery from nail samples. The purpose of this study was to optimize the extraction of this sample type by assessing incubation time of samples in lysis solution and the effectiveness of two common collection methods used for foreign DNA recovery from fingernails.

4.1 Phase 1: Incubation Time

The objective of Phase 1 in the present study was to compare the amount of endogenous DNA recovered from cleaned nail samples that were incubated for 15, 30, 45, or 60 minutes in the ChargeSwitch® and PrepFiler™ lysis solution. Although the difference in DNA recovery between incubation periods was found to be not statistically significant, the average amount of DNA (ng)/ nail (mg) recovered from the nail samples extracted using the ChargeSwitch® protocol increased as the length of incubation increased from 15 to 60 minutes. For the nail samples extracted with the PrepFiler™ protocol, there was a similar increase in average DNA recovery observed for incubation lengths of 15 to 45 minutes. These results indicate variation in DNA recovery from nail for up to 60 minutes of incubation. The nail samples were cleaned, as previously stated in Section 2.1 of the Materials and Methods section, in an effort to minimize the variation in DNA recovered from nail samples. Specifically, fingernails were cleaned to decrease the amount of skin cells and biological fluids on the nails' surface in an attempt to only extract

DNA from within the nail. However, the variation in the recovery of endogenous DNA from fingernails observed in the results of Phase 1 agreed with data collected from a previous thesis study that also focused on the extraction of foreign DNA from fingernail samples [38]. There was an increased average endogenous DNA recovery observed from fingernail samples in this study, ranging from 0.117 to 6.877 ng/mg, compared to that of the previous thesis, which ranged from 0.005 ng/mg nail to 0.845 ng/mg [38]. Although no significant difference was observed in the average recovery of endogenous DNA between incubation times, an overall trend was observed as the amount of DNA (ng)/ nail (mg) increased from the 15-minute to 45-minute incubation. As a result, an incubation time of 15 min was used during Phase 2 in order to minimize the potential for endogenous DNA recovery from nail samples.

When comparing the recovery of endogenous DNA from Donor B nails between the ChargeSwitch® and PrepFiler™ extraction methods, there was a greater average recovery of DNA across all incubation times using the ChargeSwitch® protocol compared to the PrepFiler™ protocol. These results suggest that the ChargeSwitch® Forensic DNA Purification Kit extraction was more effective at isolating DNA from the nail samples. This may suggest an increased efficiency in DNA extraction with the solution of PK and other reagents in the ChargeSwitch® lysis buffer compared to the PrepFiler™ lysis solution with the addition of DTT (both lysis solutions have proprietary formulations). However, if the goal of analysis is the recovery of foreign DNA from fingernails, the recovery of endogenous DNA should be minimized, which would favor the use of the PrepFiler™ DNA Extraction kit.

4.2 Phase 2. Comparison of Collection Methods for Exogenous DNA from Fingernail Samples

The objective of Phase 2 was to evaluate the effectiveness of collection methods for the recovery of foreign DNA from nail samples spiked with whole blood and blood dilutions. The efficiency of total DNA recovery and the recovery of nail and foreign alleles in the profiles was assessed between the soaking and swabbing collection methods.

4.2.1 Total DNA Recovery between Collection Methods Using Nails Spiked with 10 µl, 5 µl, and 2 µl Whole Blood

There was no statistical significance to the difference in average total DNA recovered from the soaked and swabbed nails from each donor. However, there were limitations of comparing percent recovery between collection methods due to the underestimation of total DNA recovery. Specifically, the estimated total DNA that could be recovered from a blood-spiked nail sample did not account for nail donor DNA present on the nail's surface. The calculations of percent recovery of unique alleles and mixture proportions were more efficient in comparing foreign DNA recovery between collection methods.

4.2.2 Recovery of Foreign DNA Between Collection Methods Using Nails Spiked with 10 µl, 5 µl, and 2 µl Whole Blood

The objective of analyzing fingernail samples for the detection of foreign DNA is to effectively identify the foreign material while minimizing the recovery of endogenous

DNA from the nail. This was assessed using the recovery of unique nail alleles and unique "foreign" blood alleles, as well as examining the proportion of peak height from the alleles of the two contributors observed in the profiles of nails spiked with 10 µl, 5 µl, and 2 µl whole blood. The results for each donor showed higher average percent recovery of unique nail alleles using the soaking method as compared to the swabbing method. However, this difference was not statistically significant for Donors D and F but was statistically significant for Donor E (p = 0.0006). However, when the percent recovery of unique nail alleles was averaged across all donors between collection methods, there was a statistically significant difference between the soaking and swabbing methods (p = 0.017). These results suggest that the swabbing method is more advantageous for minimizing the potential recovery of endogenous DNA from fingernail samples. It is noted that a larger sample size is needed to confirm a pattern of percent recovery difference between the two collection methods.

Using as little as 2 µl of whole blood, all donor blood alleles were detected, and no conclusion could be made regarding the effectiveness of the collection methods for the recovery of foreign DNA from fingernail samples. As a result, the experiment was repeated using 1:2 and 1:5 blood dilutions and nail samples from Donors G and E.

4.2.3 Total DNA Recovery Between Collection Methods Using Nails Spiked with 1:2 and 1:5 Blood Dilutions

There was no statistical significance to the difference in average total DNA recovered from the soaked nails and the swabbed nails. There were limitations to the

significance of these results due to the underestimation of total DNA recovery. Values for percent recovery of total DNA yield that fell above 100% reflected the expected total DNA yield calculation not accounting for nail donor DNA present on the nail. The calculations of percent recovery of unique alleles and mixture proportions were more efficient in comparing foreign DNA recovery between collection methods.

4.2.4 Recovery of Foreign DNA Between Collection Methods Using Nails Spiked with 1:2 and 1:5 Blood Dilutions

The two donors used in this study showed varying results for the recovery of unique nail alleles between collection methods. The average percent recovery of unique nail alleles from Donor G was consistent between the soaking and swabbing methods ($p = 0.51$). Contradictory to Donor G and the results of the first set of blood-spiked samples, there was an increase in the percentage of unique nail alleles from Donor E for the swabbing method as compared to the soaking method. However, the release of endogenous DNA may have been limited during extraction due to the stability of the keratin matrix present in the donor's nail samples, leading to less DNA isolation, and ultimately, less total DNA recovered from the samples. Another explanation could be the presence of endogenous DNA on the surface of Donor G's nail samples. Furthermore, the nail mixture proportion observed in the resulting profiles of samples using the soaking method compared to the swabbing method varied between donors. This may be due to the variation in DNA shedding between individuals.

Analogous to the first set of blood-spiked nails, both the soaking and swabbing collection methods recovered 100% of unique foreign DNA alleles from the spiked fingernail samples. This may be due to the high volume of blood added to the samples, which may be larger than the amount of foreign material commonly found on fingernail samples. Furthermore, the absence of the masking effect of the nail DNA on the foreign DNA extracted from the blood-spiked samples, commonly seen in casework, may be due to the cleaning of the fingernail samples before the addition of foreign material, as well as the addition of excess blood added to the samples in this study. An individual's DNA may accumulate on the surface and under their fingernails, affecting the proportion of nail DNA to foreign DNA recovered from fingernail samples in casework.

However, when using loci where there are no shared alleles between the blood donor and the nail donors, the proportion of the peak height from the foreign donor alleles for samples spiked with whole blood and a 1:2 dilution was observed to be approximately the same with both collection methods. Conversely, there was an increase for both donors in the proportion of peak height from foreign alleles for the samples spiked with the 1:5 blood dilution when using the swabbing method (shown in Tables 13 & 14). This indicates that the swabbing method should be investigated further as an avenue for increased foreign allele recovery.

4.3 Future Directions

Ultimately, no conclusions could be drawn regarding the optimal collection method for fingernail samples. A larger sample size and varying concentrations of foreign material

are needed to effectively compare the efficiency of extracting foreign DNA from fingernails while minimizing the recovery of nail DNA. Furthermore, research should be considered on the use of nail samples without the addition of the cleaning step before analysis. Lastly, other swabbing materials, such as rayon, foam, or polyester, can be compared to the soaking method for fingernails to determine which technique is most effective at recovering foreign DNA from nail samples.

APPENDIX

Table A. Genotypes of Donor E and blood donor for samples spiked with whole blood and collected using the soaking method. The table displays the alleles detected from the nail samples of E that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the soaking method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl Whole blood	5 µl Whole blood	2 µl Whole blood
D3S1358	16, 17	14, 16	17	17	17
vWA	14, 18	17, 18	14	14	14
D16S539	9, 13	12, 13	9	9	9
CSF1PO	10, 11	12		10,11	10,11
TPOX	8, 11	8, 10		11	11
Y-Indel	N/A	2			
AMEL	X, X	X, Y			
D8S1179	13, 15	12, 14	13,15	13,15	13,15
D21S11	29, 30	29	30	30	30
D18S51	12, 15	15, 17		12	12
DYS391	N/A	11			
D2S441	11	10, 11			
D19S433	13, 15	13, 14	15	15	15
TH01	9, 9.3	6, 7	9, 9.3	9, 9.3	9, 9.3
FGA	23, 24	22, 25	23, 24	23, 24	23, 24
D22S1045	16	15, 16			
D5S818	12, 13	11	12, 13	12, 13	12, 13
D13S317	9, 11	10,11		9	9
D7S820	8, 12	7, 12	8	8	8
SE33	19, 27.2	18, 30.2	19, 27.2	19, 27.2	19, 27.2
D10S1248	14, 16	13, 14	16	16	16
D1S1656	12	11, 15	12	12	12
D12S391	18, 23	21, 22	18, 23	18, 23	18, 23
D2S1338	16, 25	20, 23	25	16, 25	16, 25
Total			21 of 27	27 of 27	27 of 27

Table B. Genotypes of Donor E and blood donor for samples spiked with whole blood and collected using the swabbing method. The table displays the alleles detected from the nail samples of Donor E that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the swabbing method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl Whole blood	5 µl Whole blood	2 µl Whole blood
D3S1358	16, 17	14, 16	17		
vWA	14, 18	17, 18			
D16S539	9, 13	12, 13			
CSF1PO	10, 11	12			
TPOX	8, 11	8, 10			
Y-Indel		2			
AMEL	X, X	X, Y			
D8S1179	13, 15	12, 14			13
D21S11	29, 30	29	30		
D18S51	12, 15	15, 17			
DYS391		11			
D2S441	11	10, 11			
D19S433	13, 15	13, 14			
TH01	9, 9.3	6, 7			
FGA	23, 24	22, 25			
D22S1045	16	15, 16			
D5S818	12, 13	11			12
D13S317	9, 11	10,11			
D7S820	8, 12	7, 12			
SE33	19, 27.2	18, 30.2	19		19
D10S1248	14, 16	13, 14			
D1S1656	12, 12	11, 15	12		
D12S391	18, 23	21, 22			
D2S1338	16, 25	20, 23			
Total			4 of 27	0 of 27	3 of 27

Table C. Genotypes of Donor F and blood donor for samples spiked with whole blood and collected using the soaking method. The table displays the alleles detected from the nail samples of Donor F that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the soaking method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl Whole blood	5 µl Whole blood	2 µl Whole blood
D3S1358	15, 17	14, 16	15, 17	17	15, 17
vWA	14, 16	17, 18	14, 16	14	14, 16
D16S539	12, 12	12, 13			
CSF1PO	9, 11	12	9		9, 11
TPOX	8, 11	8, 10	11		11
Y-Indel		2			
AMEL	X, X	X, Y			
D8S1179	10,16	12, 14	10,16	10,16	10,16
D21S11	31.2, 32.2	29	31.2, 32.2		31.2, 32.2
D18S51	16, 22	15, 17	16, 22		16, 22
DYS391		11			
D2S441	11, 13	10, 11	13	13	13
D19S433	13, 16	13, 14	16		16
TH01	8, 9	6, 7	8, 9		8, 9
FGA	20	22, 25	20		20
D22S1045	15, 16	15, 16			
D5S818	10, 11	11	10		10
D13S317	11, 12	10,11	12	12	12
D7S820	9, 12	7, 12	9		9
SE33	17	18, 30.2			17
D10S1248	14, 15	13, 14	15	15	15
D1S1656	15, 17.3	11, 15	17.3		17.3
D12S391	18, 22	21, 22	18		18
D2S1338	19, 24	20, 23	19, 24		19, 24
Total			25 of 27	7 of 27	27 of 27

Table D. Genotypes of Donor F and blood donor for samples spiked with whole blood and collected using the swabbing method. The table displays the alleles detected from the nail samples of Donor F that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the swabbing method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl Whole blood	5 µl Whole blood	2 µl Whole blood
D3S1358	15, 17	14, 16	17	15, 17	17
vWA	14, 16	17, 18	14	14, 16	16
D16S539	12, 12	12, 13			
CSF1PO	9, 11	12		9, 11	
TPOX	8, 11	8, 10	11	11	
Y-Indel		2			
AMEL	X, X	X, Y			
D8S1179	10,16	12, 14	10, 16	10,16	
D21S11	31.2, 32.2	29	31.2	31.2, 32.2	
D18S51	16, 22	15, 17	22	16, 22	
DYS391		11			
D2S441	11, 13	10, 11	13	13	13
D19S433	13, 16	13, 14	16	16	16
TH01	8, 9	6, 7	8, 9	8, 9	8, 9
FGA	20	22, 25	20	20	
D22S1045	15, 16	15, 16			
D5S818	10, 11	11			
D13S317	11, 12	10,11	12	12	12
D7S820	9, 12	7, 12	9	9	
SE33	17	18, 30.2			
D10S1248	14, 15	13, 14	15	15	15
D1S1656	15, 17.3	11, 15		17.3	17.3
D12S391	18, 22	21, 22	18	18	18
D2S1338	19, 24	20, 23		24	
Total			16 of 27	24 of 27	10 of 27

Table E. Genotypes of Donor D and blood donor for samples spiked with whole blood and collected using the soaking method. The table displays the alleles detected from the nail samples of Donor D that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the soaking method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl whole blood	5 µl whole blood	2 µl whole blood
D3S1358	17	14, 16	17	17	17
vWA	16	17, 18			
D16S539	9, 10	12, 13	10		10
CSF1PO	10, 11	12			10
TPOX	11	8, 10			
Y-Indel		2,			
AMEL	X, X	X, Y			
D8S1179	15, 16	12, 14	15	15, 16	15, 16
D21S11	30	29	30	30	30
D18S51	13, 14	15, 17	13	13	
DYS391		11			
D2S441	11,14	10, 11	14	14	14
D19S433	14, 15	13, 14		15	15
TH01	8, 9.3	6, 7	8	9.3	9.3
FGA	21, 22	22, 25			
D22S1045	16	15, 16			
D5S818	11	11		12	
D13S317	9, 12	10,11	12	12	12
D7S820	8, 11	7, 12	8	8	
SE33	22.2, 24.2	18, 30.2			
D10S1248	14	13, 14			
D1S1656	12, 14	11, 15	12	12	
D12S391	18, 22	21, 22		18	18
D2S1338	19, 20	20, 23			
Total			10 of 27	13 of 27	11 of 27

Table F. Genotypes of Donor D and blood donor for samples spiked with whole blood and collected using the swabbing method. The table displays the alleles detected from the nail samples of Donor D that was sampled with 10 µl, 5 µl , or 2 µl whole blood and collected using the swabbing method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	10 µl Whole blood	5 µl Whole blood	2 µl Whole blood
D3S1358	17	14, 16			17
vWA	16	17, 18			16
D16S539	9, 10	12, 13			
CSF1PO	10, 11	12	10		
TPOX	11	8, 10			
Y-Indel		2,			
AMEL	X, X	X, Y			
D8S1179	15, 16	12, 14	15	15	15, 16
D21S11	30	29	30	30	30
D18S51	13, 14	15, 17			
DYS391		11,			
D2S441	11,14	10, 11			14
D19S433	14, 15	13, 14			15
TH01	8, 9.3	6, 7			8, 9.3
FGA	21, 22	22, 25			
D22S1045	16	15, 16			
D5S818	11	11			
D13S317	9, 12	10,11			12
D7S820	8, 11	7, 12			11
SE33	22.2, 24.2	18, 30.2			
D10S1248	14	13, 14			
D1S1656	12, 14	11, 15	12	12	12
D12S391	18, 22	21, 22			18
D2S1338	19, 20	20, 23			
Total			4 of 27	3 of 27	13 of 27

Table G. Genotypes of Donor E and blood donor for samples spiked with whole blood and collected using the soaking method. The table displays the alleles detected from the nail samples of Donor E that was sampled with whole blood and diluted blood and collected using the soaking method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	2 µl whole blood	2 µl 1:5 dilution blood	2 µl 1:2 dilution blood
D3S1358	16, 17	14, 16	17	17	17
vWA	14, 18	17, 18	14	14	14
D16S539	9, 13	12, 13	9	9	9
CSF1PO	10, 11	12	10		10, 11
TPOX	8, 11	8, 10	11		11
Y-Indel		2,			
AMEL	X, X	X, Y			
D8S1179	13, 15	12, 14	13, 15	13, 15	13, 15
D21S11	29, 30	29	30	30	30
D18S51	12, 15	15, 17	12	12	12
DYS391	N/A	11			
D2S441	11	10, 11			
D19S433	13, 15	13, 14	15	15	15
TH01	9, 9.3	6, 7	9, 9.3	9, 9.3	9, 9.3
FGA	23, 24	22, 25	23, 24	23, 24	23, 24
D22S1045	16	15, 16			
D5S818	12, 13	11	12, 13	12, 13	12, 13
D13S317	9, 11	10,11	9	9	9
D7S820	8, 12	7, 12	8	8	8
SE33	19, 27.2	18, 30.2	19	19, 27.2	19, 27.2
D10S1248	14, 16	13, 14	16	16	16
D1S1656	12	11, 15	12	12	12
D12S391	18, 23	21, 22	18, 23	18, 23	18, 23
D2S1338	16, 25	20, 23	16,		16, 25
Total			24 of 27	22 of 27	27 of 27

Table H. Genotypes of Donor E and Blood Donor for samples spiked with whole blood and collected using the swabbing method. The table displays the alleles detected from the nail samples of Donor E that was sampled with whole blood and diluted blood and collected using the swabbing method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	2 µl Whole blood	2 µl 1:5 Dilution Blood	2 µl 1:2 Dilution Blood
D3S1358	16, 17	14, 16	17	17	17
vWA	14, 18	17, 18	14	14	14
D16S539	9, 13	12, 13	9	9	9
CSF1PO	10, 11	12	10	10, 11	10
TPOX	8, 11	8, 10	11	11	11
Y-Indel		2,			
AMEL	X, X	X, Y			
D8S1179	13, 15	12, 14	13, 15	13, 15	13, 15
D21S11	29, 30	29, 29	30	30	30
D18S51	12, 15	15, 17	12	12	12
DYS391		11			
D2S441	11	10, 11			
D19S433	13, 15	13, 14	15	15	15
TH01	9, 9.3	6, 7	9, 9.3	9, 9.3	9, 9.3
FGA	23, 24	22, 25	23, 24	23, 24	23, 24
D22S1045	16	15, 16			
D5S818	12, 13	11	12, 13	12, 13	12, 13
D13S317	9, 11	10,11	9	9	9
D7S820	8, 12	7, 12	8	8	8
SE33	19, 27.2	18, 30.2	19, 27.2	19, 27.2	19, 27.2
D10S1248	14, 16	13, 14	16	16	16
D1S1656	12	11, 15	12	12	12
D12S391	18, 23	21, 22	18, 23	18, 23	18, 23
D2S1338	16, 25	20, 23	25	16, 25	16, 25
Total			25 of 27	27 of 27	26 of 27

Table I. Genotypes of Donor G and Blood Donor for samples spiked with whole blood and collected using the soaking method. The table displays the alleles detected from the nail samples of Donor G that was sampled with 2 µl whole blood, 2 µl 1:2 blood dilution, or 2 µl 1:5 blood dilution and collected using the soaking method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	2 µl Whole blood	2 µl 1:5 Dilution Blood	2 µl 1:2 Dilution Blood
D3S1358	15	14, 16	15	15	15
vWA	15, 18	17, 18	15	15	15
D16S539	11	12, 13	11	11	11
CSF1PO	10, 12	12	10	10	10
TPOX	8, 12	8, 10	12	12	12
Y-Indel	2,	2,			
AMEL	X, Y	X, Y			
D8S1179	12, 14	12, 14			
D21S11	32.2	29, 29	32.2	32.2	32.2
D18S51	13, 21	15, 17	13, 21	13, 21	13, 21
DYS391	10	11	10	10	10
D2S441	11	10, 11			
D19S433	13	13, 14			
TH01	6, 9	6, 7	9	9	9
FGA	22	22, 25			
D22S1045	16	15, 16			
D5S818	11, 13	11	13	13	13
D13S317	11, 13	10,11	13	13	13
D7S820	8, 10	7, 12	8, 10	8, 10	8, 10
SE33	20	18, 30.2	20	20	20
D10S1248	13, 18	13, 14	18	18	18
D1S1656	13, 17.3	11, 15	13, 17.3	13, 17.3	13, 17.3
D12S391	21, 22	21, 22			
D2S1338	17, 19	20, 23	17	17, 19	17, 19
Total			19 of 20	20 of 20	20 of 20

Table J. Genotypes of Donor G and Blood Donor for samples spiked with whole blood and collected using the swabbing method. The table displays the alleles detected from the nail samples of Donor G that was sampled with 2 µl whole blood, 2 µl 1:2 blood dilution, or 2 µl 1:5 blood dilution and collected using the swabbing method. Highlighted loci were used for mixture proportion calculations.

			Unique Nail Alleles Present		
Locus	Nail Alleles	Blood Alleles	2 µl Whole blood	2 µl 1:5 Dilution blood	2 µl 1:2 Dilution blood
D3S1358	15	14, 16	15	15	15
vWA	15, 18	17, 18	15	15	15
D16S539	11	12, 13	11	11	11
CSF1PO	10, 12	12	10	10	10
TPOX	8, 12	8, 10	12	12	12
Y-Indel	2	2			
AMEL	X, Y	X, Y			
D8S1179	12, 14	12, 14			
D21S11	32.2	29	32.2	32.2	32.2
D18S51	13, 21	15, 17		13, 21	13, 21
DYS391	10	11		10	10
D2S441	11	10, 11			
D19S433	13	13, 14			
TH01	6, 9	6, 7	9	9	9
FGA	22	22, 25			
D22S1045	16	15, 16			
D5S818	11, 13	11	13	13	13
D13S317	11, 13	10,11	13	13	13
D7S820	8, 10	7, 12	8, 10	8, 10	8, 10
SE33	20	18, 30.2	20	20	20
D10S1248	13, 18	13, 14	18	18	18
D1S1656	13, 17.3	11, 15	13, 17.3	13, 17.3	13, 17.3
D12S391	21, 22	21, 22			
D2S1338	17, 19	20, 23	17	17, 19	17, 19
Total			16 of 20	20 of 20	20 of 20

LIST OF JOURNAL ABBREVIATIONS

Curr Probl Dermatol	Current Problems in Dermatology
Forensic Sci Int Genet	Forensic Science International: Genetics
Forensic Sci Int Genet Suppl Ser	Forensic Science International: Genetics Supplement Series
Int Congr	International Congress
Int J Cosmet Sci.	International Journal of Cosmetic Science
Interational J Leg Med	International Journal of Legal Medicine
J Cell Biol.	Journal of Cell Biology
J Dermatol Sci	Journal of Dermatological Science
J Forensic Sci.	Journal of Forensic Sciences
J Miner Met Mater Soc	Journal of The Minerals, Metals & Materials Society
Sci Justice	Science and Justice
Sci World J	Scientific World Journal
Soc Investig Dermatol	Society for Investigative Dermatology

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