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Trace DNA from Fingernails: Increasing the Success Rate of Widely Collected Forensic Evidence

2010-DN-BX-K224

David Foran PhD, Lisa Hebda MS, and Ashley Doran MS

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Abstract

During a physical assault, biological evidence may be transferred between victim and assailant. For instance, if the victim scratches the assailant, cells can be trapped beneath fingernails, thus nail evidence is regularly collected by practitioners such as sexual assault nurse examiners and medical examiners, and sent to the crime laboratory. However, the best techniques for collecting and processing such evidence have never been established. In this study, multiple methods for isolating exogenous cells from nail evidence, for purifying DNA from it, and for producing STR profiles, were compared. First, a set amount of male blood was placed on female nails, allowing for easy quantification of exogenous cell recovery based on a Y chromosome assay. Then multiple aspects of nail evidence processing were examined, including nail evidence collection methods used by practitioners (clipping nails, swabbing nails, or scraping nails), DNA isolation (standard organic extraction versus a commercially available kit), and DNA analysis (autosomal STRs versus Y-chromosome STRs). Based on these results, a protocol was developed that was applied to scratchings, wherein females scratched male volunteers on the forearm using a set amount of force, and scratchings were processed using the most effective procedures. The prevalence of cell loss or cross contamination using certain nail processing techniques was also examined, as was cell loss and cross contamination during nail transport and the influence of nail polish.

Results showed that the three methods for collecting exogenous DNA from nail evidence produced statistically significant differences in DNA yields, with the soaking method generating the most exogenous DNA. However, it also generated the most endogenous (nail) DNA, thus autosomal STR analysis produced large peaks from the nail and minimal peaks from the exogenous material. When Y-STR analysis of those DNAs was conducted, strong exogenous

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profiles were produced. Swabbing nails produced intermediate results, while scraping resulted in the least exogenous DNA, the fewest Y-STR results, but also the least DNA from the nail itself. Both DNA purification methods were effective, with slight alterations influencing which produced higher DNA yields. Transporting and processing nails together, as is often done in a forensic setting, was advantageous considering effort and reagents, and while no cross contamination occurred during transport, it did occur during nail processing. Further, exogenous cells were lost during both procedures. Overall, this study identified strengths and weaknesses in each step of fingernail evidence processing, resulting in recommendations that should be very useful to the forensic practitioners at which it was aimed.

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Executive Summary

Each day in hospitals and morgues across the country, evidence from assault victims is collected. Among these is fingernail evidence if it is possible or likely that a victim scratched an assailant. Various methods are used to collect fingernail evidence, which can include clipping the nail, swabbing beneath the nail using a small, moistened swab, or scraping beneath the nail, generally using a wooden applicator and collecting the debris. Once collected, nail evidence is packaged and transported to the crime laboratory, where it may or may not be processed. Microscopic examination may take place, looking for obvious signs of exogenous material such as blood. If nails are to undergo DNA testing, cells are collected using any of a variety of methods, including swabbing the nails, scraping nails and collecting debris, or placing nails directly into a tissue digestion buffer. DNA isolation then occurs, typically via an organic or commercial kit-based extraction. Finally, DNA analysis is undertaken using standard procedures a given laboratory utilizes.

In spite of this scenario taking place daily, there has been minimal effort to optimize or standardize methods for collecting, processing, and analyzing nail evidence. Our conversations with sexual assault nurse examiners, forensic pathologists, and crime laboratory personnel revealed that several cell collection methods are used on nail evidence and that practitioners do not receive feedback about whether these methods are optimal, or even adequate. Given this, we proposed a detailed study of collecting and processing nail evidence in order to better understand where difficulties occur, and to develop best practices for nail evidence analysis. Our goals included determining:

- What is the best method for collecting nail evidence so as to maximize DNA yields?

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- What is the best method for collecting nail evidence so as to maximize DNA retrieval from exogenous cells?
- What is the best method for collecting nail evidence so as to minimize DNA retrieval from the nails themselves?
- What is the best method for purifying DNA from nail evidence?
- What is the best method for obtaining STR data from collected nail DNA?
- What is the best method for obtaining STR data from only exogenous material on nails?
- What is the effect of processing nails as a group (cumulatively) versus individually?
- What is the source of endogenous DNA from a nail (the nail, or cells beneath the nail)?
- What is the effect of transporting nails as a group?
- What is the influence of common adulterants (i.e., fingernail polish) on processing nails, given the results above?

Our first objective was to determine which of several commonly used methods to collect fingernail evidence—clipping, swabbing, or scraping—results in the best yield of exogenous cells (i.e., those from the assailant) and DNA from those cells, taking into consideration recovery of endogenous DNA (i.e., that from the nail itself, or from the nail donor). All processes were submitted to and approved by the Michigan State University Institutional Review Board. In preliminary experiments, volunteers scratched the palm side of the forearm of other volunteers. In order to help lessen variability inherent to this process, a standard amount of force was used for scratchings by having the volunteer place their arm on a scale, which was then zeroed and the

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individual scratching placed the three middle fingers of a hand on the forearm until the scale read two pounds, at which point they dragged their nails along the forearm, maintaining the two pounds of force.

As this work was conducted, it became clear that the amount of exogenous cells/DNA collected from nails following scratching was highly variable, hindering attempts to optimize methods for its collection. Furthermore, minimal alleles were recovered from the individual being scratched. Therefore we moved to a process wherein one microliter of male blood was spread onto the underside of a clipped female nail and allowed to dry. These nails were then either soaked directly in a tissue digestion buffer, double swabbed (Sweet et al. 1997) until all blood was visually removed and the swabs placed in digestion buffer, or scraped over a piece of weigh paper and the debris placed in digestion buffer. DNAs were purified using a standard phenol/chloroform extraction and Amicon® column filtration. DNAs were quantified using a Quantifiler® Y Human Male DNA Quantification Kit. The DNA quantification results are shown in Figure 1. As is apparent, the soaking method resulted in significantly more DNA than the swabbing method, which itself resulted in significantly more DNA than the scraping method. Owing to this, the soaking method was incorporated into our standard protocol for cell/DNA isolation.

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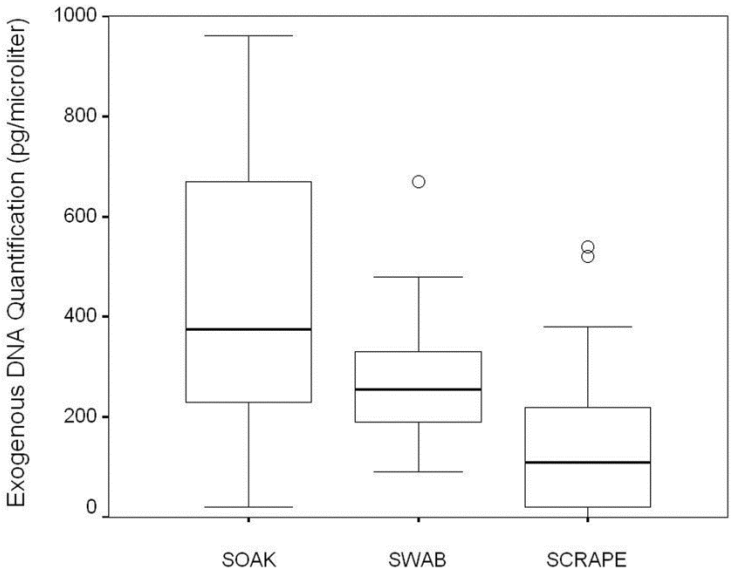

Figure 1: Boxplot of Exogenous (Male) DNA Quantities from Collection Methods. The y-axis is DNA quantity reported as pg/µL. The heavy line in the middle of the box represents the median. Whiskers extend to the minimum and maximum values that are not outliers. Open circles indicate mild outliers. The soaking method recovered the most exogenous DNA, whereas scraping recovered the least.

Next, a subset of DNAs included in Figure 1 was subjected to autosomal STR analysis using an Identifiler® PCR Amplification Kit. This resulted in mixed DNA profiles for the soaked and swabbed nails, as exemplified in Figure 2. The majority of soaked nails produced major DNA profiles of the nail (Figure 2a), or equal contributions from the nail and exogenous material. On the other hand, swabbed nails often produced major DNA profiles of the exogenous material (Figure 2b). Scraped nails usually recovered single source DNA profiles from the exogenous material (Figure 2c); however, profiles were sometimes incomplete.

When the same DNAs were analyzed using a Yfiler® PCR Amplification Kit, a strong and clean male profile was developed from all soaked and swabbed nails (e.g. Figure 2d). Most scraped nails also produced a clean male profile, although some had allelic dropout. Thus, Y-STR analysis was incorporated into our standard protocol for cell/DNA analysis.

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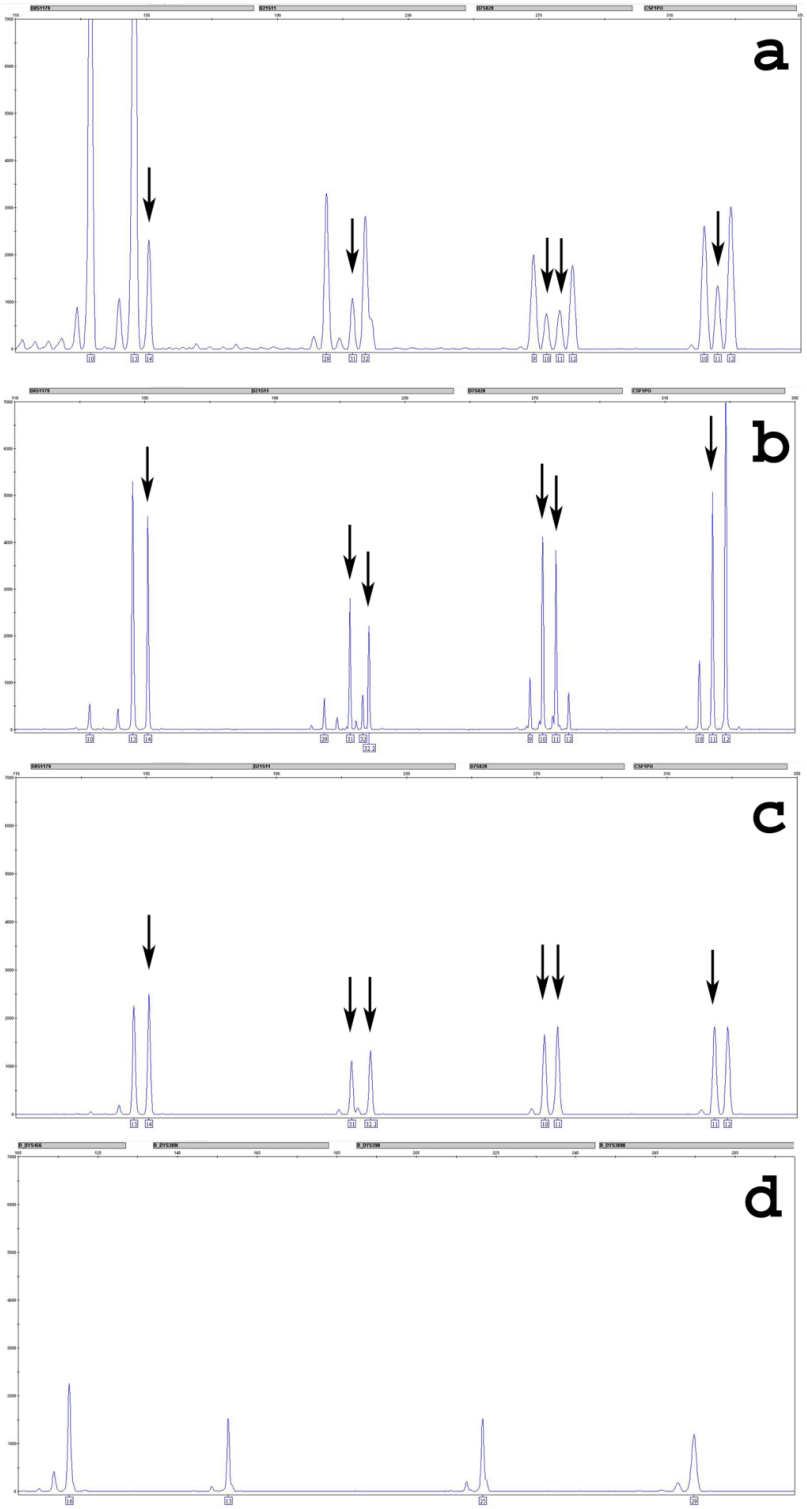

Figure 2: STR Analysis of Nails Harboring Blood for Each Collection Method. Autosomal STR profiles from a soaked (a), swabbed (b), and scraped (c) nail. Alleles specific to the exogenous profile are indicated by an arrow; large peaks in (b) and (c) without arrows are shared

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alleles. Soaked and swabbed nails resulted in mixtures, while scraped nails were typically single source profiles of exogenous material, which occasionally exhibited allelic dropout. (d) Y-STR profile of the swabbed nail in (b). Complete Y-STR profiles of exogenous material were obtained from soaked and swabbed nails, with scraped nails again showing dropout.

Next, two methods of DNA purification were compared on soaked nails harboring blood: the same organic extraction and a silica-based QIAamp® DNA Investigator Kit. The commercial kit recovered significantly more exogenous DNA than did the organic extraction (Figure 3), thus the kit was incorporated into our standard protocol for cell/DNA purification. However, subsequent testing showed that if the Amicon® column was pretreated with yeast RNA (Doran and Foran, 2014), the organic extraction produced somewhat higher yields (data not shown).

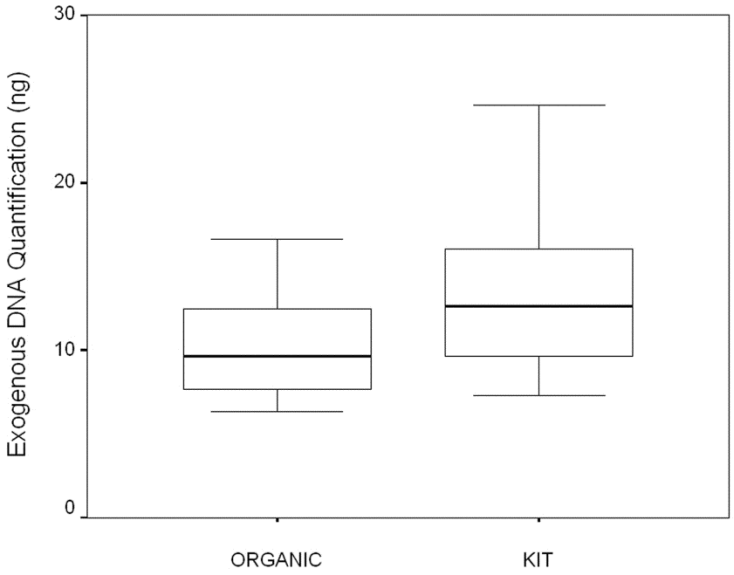

Figure 3: Boxplot of Exogenous (Male) DNA Quantities from Organic and Silica-Based Kit Extractions. The y-axis is DNA quantity in ng. The heavy line in the middle of the box represents the median. Whiskers extend to the minimum and maximum values. The commercial kit recovered a greater amount of exogenous DNA than organic extraction, although this was not the case in subsequent testing when the Amicon® column used in the organic extraction was pretreated (Doran and Foran 2014).

With an optimized protocol for cell/DNA collection, purification (based on the commercial kit), and analysis developed, we returned to scratchings. For these tests, female volunteers scratched male volunteers' forearms three times using the middle three fingers of a hand, again

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applying two pounds of force. Nails were then clipped, soaked, processed using the kit, and Y-STR profiles developed (Table 1). Far more usable data were produced than in preliminary experiments, including multiple full profiles, with an average of 69% of the male Y-STR alleles recovered.

Sample #	Percent of Exogenous Y-STR Profile
1	100%
2	65%
3	100%
4	35%
5	100%
6	100%
7	65%
8	88%
9	76%
10	100%
11	53%
12	82%
13	24%
14	12%
15	41%
16	65%
17	71%
18	94%
19	0%
Average	69%

Table 1: Percent of Y-STR Alleles Recovered from the Scratched Individual. Y-STR analysis of nails after scratching produced both partial and full profiles consistent with the scratched individual, with an average of 69% of the profile recovered.

We next undertook several ancillary tests examining factors that could influence results of nail evidence analysis. The first of these was transport of nails, as would occur when such evidence is transferred to the crime laboratory. One question addressed was whether cross contamination between nails occurs when they are transported together. We also examined if

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exogenous material is lost during transport. Sets of one female nail harboring male blood and two ‘clean’ nails were placed in coin envelopes. Envelopes were transported for five days, after which all three nails were processed via soaking and organic extraction. DNA yields are shown in Figure 4. Most of the ‘clean’ nails had little or no exogenous DNA. Y-STR analysis was then performed on the five ‘clean’ nails with the highest exogenous DNA yields in order to investigate its origin. One of these produced no results, while the other four produced full Y-STR profiles inconsistent with the blood, and with any laboratory personnel. Thus there was no substantial cross contamination of exogenous material between nails during transport.

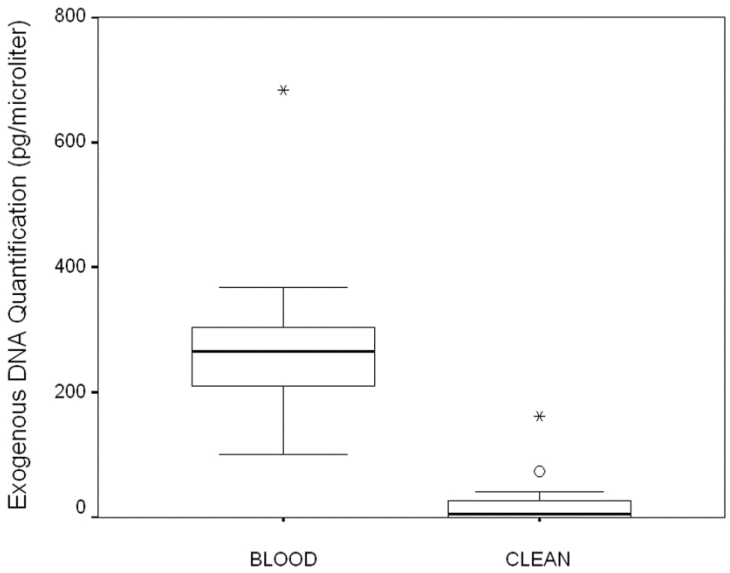

Figure 4: Boxplot of Exogenous (Male) DNA Quantities from Nails Harboring Blood Following Transport. The y-axis is DNA quantity in pg/µL. The heavy line in the middle of the box is the median. Whiskers extend to the minimum and maximum values that are not outliers. Open circles indicate mild outliers, while extreme outliers are shown by an asterisk. Minimal to no exogenous DNA was present on the ‘clean’ nails after transport.

Nails from scratchings were then transported, along with one nail not used for scratching, to see if exogenous cells likely to be looser than those from dried blood were lost from nails. The inside bottom of the envelope used for transport was also swabbed to note if any loose cells were recovered. No cell transfer between nails used for scratching and the ‘clean’ nail was found, nor

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was there detectable loss to the envelope. However, exogenous material was lost from the nails based on a significant ($p < 0.001$) decrease in Y-STR alleles recovered after transportation (avg. 25%, Table 2) as compared to the percent from nails not undergoing transport (69%, Table 1).

Sample #	Percent of Exogenous Y-STR Profile
1a	0%
1b	6%
1c	0%
2a	59%
2b	6%
2c	0%
3a	82%
3b	0%
3c	0%
4a	41%
4b	100%
4c	0%
Average	25%

Table 2: Percent of Exogenous Y-STR Profile Recovered from Post-Scratching Transported Nails. Note that several nails generated no alleles. Compared to post-scratching nails that were not transported, significantly fewer Y-STR alleles were recovered (25% vs. 69%, Table 1).

Nails in the crime laboratory are often swabbed as a group, resulting in one 'cumulative' swab for a set of nails. The goal of this is to save time and resources, and to accumulate as much exogenous material as possible for a single assay. However, cumulative swabbing also provides a chance to cross contaminate nails, and to lose exogenous cells collected on an earlier swabbing to nails swabbed next. For this test, nails harboring blood were first swabbed, followed by 'clean' nails, in a blood-clean-blood-clean pattern. Then, a second swab was used on the two nails harboring blood, to determine how much blood had been left behind. Finally, the two 'clean' nails were re-swabbed using a third swab pair to determine if blood had been deposited on them. The results from this are shown in Figure 5. The two important findings were that

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exogenous cells were left behind on the nails harboring blood by the first swab, and that exogenous cells were deposited on the 'clean' nails, including enough to develop full Y-STR profiles for the subset tested.

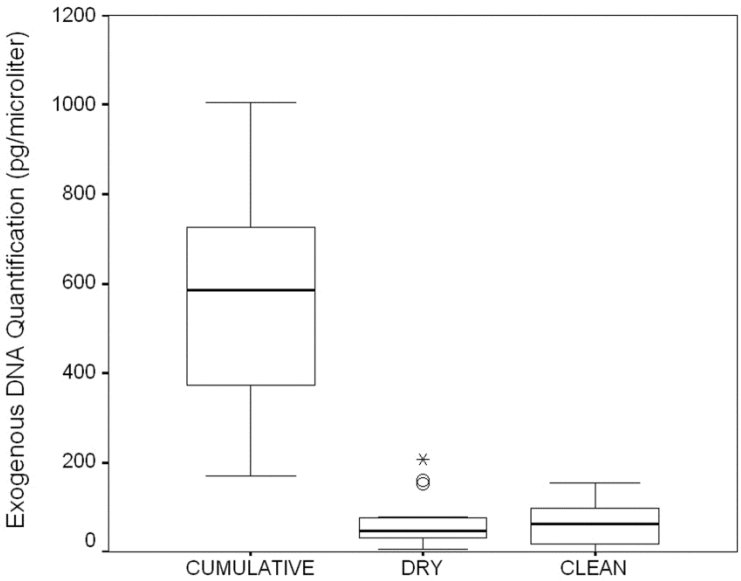

Figure 5: Boxplot of Exogenous DNA Quantities from Cumulatively Swabbed Nails Harboring Blood. The y-axis is DNA quantity in pg/µL. The heavy line in the middle of the box represents the median. Whiskers extend to the minimum and maximum values that are not outliers. Open circles indicate mild outliers, while extreme outliers are displayed as an asterisk. "Cumulative" is a wet swab of all four nails; "dry" is a dry swab of the two nails with blood; "clean" is a wet and dry swab of the nails without blood. The cumulative swab recovered the majority of the exogenous DNA, while the dry swab recovered DNA left behind by the first swab. The cumulative swab transferred exogenous cells to 'clean' nails.

The last set of experiments was used to examine if nail polish had any negative effect on the standard cell collection/DNA analysis protocol tested above. Three types and colors of polish were tested using both organic and kit extractions. From this small sample, no negative influence of polish on PCR amplification or STR analysis was seen.

The overall goal of this study was to examine different methods commonly used for nail evidence collection and processing, and to see if an optimal procedure could be developed that would allow genetic data to be more reliably attained from fingernails used for scratching, such

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as might be encountered when a victim attempts to fend off an assailant. The development of such a procedure was augmented immensely through placing a set volume of male blood on female fingernails, which greatly reduced the variability in exogenous cell quantity that inherently exists in scratching. Based on this, soaking nails directly in digestion buffer and isolating DNA using a commercial kit resulted in the highest exogenous DNA yields (although see notes above regarding column pretreatment and organic extraction). However, this method also resulted in large amounts of DNA from the nail itself, and mixtures were found when an autosomal STR kit was used, with the predominant peaks being endogenous in origin. Given this, a Y-STR kit was utilized, which resulted in clean profiles from the exogenous material.

We then used this protocol to once again test scratchings, and obtained highly improved results. Using the middle three fingers of a hand for three scratches and two pounds of force, almost all scratchings generated Y-STR results, including multiple full profiles (17 loci), with an average of 69% of loci having results. It is important to note that two pounds of force used in this study is enough to redden but not break the skin. However, it seems likely that in a violent struggle much more force could be applied through scratching, and recovering exogenous DNA would presumably be that much more successful.

A potential adulterant, nail polish, did not seem to have any negative impact on PCR amplification or DNA analysis when nails were soaked. However, two procedures often used—transporting nails together in a coin envelope and processing nails using a cumulative swab—did have undesirable results. While no cross contamination occurred between nails with and without exogenous material during transport, when a clean nail was processed with a swab that had just been used on a nail harboring blood, a substantial amount of DNA was transferred to the clean nail, enough to produce full Y-STR profiles. Not only is such cross contamination undesirable in

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general, but the results mean substantial amounts of DNA can be lost through cumulative swabbing. Therefore a tradeoff exists, where savings in time and resources, as well as the potential to combine small numbers of cells from multiple nails, must be weighed against transferring exogenous material between nails.

Transporting nails in an envelope did appear to result in DNA loss from nails used for scratching. Clearly this is disadvantageous; therefore other methods of transport may be desirable. For instance, placing dried nails into clear, sterile microcentrifuge tubes, either individually or as groups, would allow the crime laboratory analyst to view them microscopically without having to remove them from the transportation container. If desired, digestion buffer could be added directly to the tube, meaning that any exogenous material that had fallen off the nail would still be available for processing. Other options exist as well, any one of which could help negate the loss of exogenous cells during transport.

Finally, soaking a nail resulted in retrieving the most exogenous DNA, but it also released more endogenous DNA. This problem was overcome using Y-STR analysis in this study, but if autosomal STRs are tested following soaking, mixtures can be expected. Optimizations of the soaking technique, including soaking nails for shorter intervals, removing the nail before continuing the extraction, or use of different proteinases or detergents, may help to minimize levels of endogenous DNA, although that was not part of the current study. Overall, the optimal collection technique depends on a laboratory's capabilities and the case scenario. If exogenous material is readily visible, swabbing or scraping is preferable. However, if no exogenous material is visible then soaking nails may still be advantageous given that it reduces the chance of negative results.

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Introduction

According to the Uniform Crime Report (2011), a violent crime occurs in the United States every 26.2 seconds. In many of these instances, direct contact is made between a victim and perpetrator. This is particularly true of physical assault, both sexual and non-sexual. For instance, of the 750,000 aggravated assaults in the United States in 2011, approximately 25% were committed using hands, fists, or feet (Uniform Crime Report, 2011). As a victim struggles with an assailant, there is a chance of transfer of trace material between them, such as epithelial cells, fibers, hair, or blood. Given this, assault victims are routinely checked for transfer evidence. If the victim is alive, this may be performed by emergency room personnel or specially trained individuals such as sexual assault nurse examiners (SANEs), while the pathologist performing an autopsy will examine the body if the victim is deceased.

The Forensic Biology Laboratory at Michigan State University works closely with the board-certified forensic pathologists at Sparrow Hospital in Lansing, MI, who are the medical examiners for multiple counties in the state. As noted, these pathologists have been trained to collect trace evidence from cadavers when a violent act is suspected that may have involved direct contact between assailant and victim. Among the items often collected are fingernails, given that the victim could have scratched the assailant or otherwise acted so that foreign material was deposited beneath the nails. These nails are then passed along to the crime laboratory, where they may or may not be analyzed.

The methods for both collecting and processing nail evidence vary widely among practitioners. At Sparrow Forensic Pathology, nails from a hand are removed over a cloth using nail clippers, and the five nails, clippers, and cloth are placed into an envelope for transport, resulting in two envelopes per victim. Other practitioners may swab the underside of the nail or

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scrape it with a wooden applicator, particularly if the victim does not want their nails cut. If nails themselves are submitted to the crime laboratory, they can be swabbed, scraped, or placed directly into a tissue digestion buffer. Likewise, DNA isolation and analysis methods can vary. The former includes standard organic extraction or the use of a commercial kit or other more automated processes. For the latter, DNA can be analyzed based on standard autosomal STRs or Y-STRs, given that assaults often involve a male attacking a female.

The number of fingernail evidence submissions that result in useful genetic evidence is unknown; however, it is clear that this type of evidence is collected and analyzed on a regular basis in the United States. Due to this, several academic studies of fingernail evidence have been conducted, including our own published (Cline et al. 2003) and unpublished work, which acted as an introduction to the research performed in this study. Specifically, Cook and Dixon (2007), and subsequently Malsom et al. (2009) found that the presence of foreign/exogenous DNA beneath nails is quite rare, with the exception of intimate couples. An examination in our laboratory also showed that foreign DNA beneath nails is extremely rare, since STR profiles consistent with the individual from whom the nails were produced were generated, but generally few or no other alleles were present (unpublished). Other authors have reported mixed results from nails (e.g., Wiegand et al. 1993; Oz and Zamir 2000; Lederer et al. 2001; Harbison et al. 2003; Piccinini et al. 2003), even after 30 scratches (Matte et al. 2012), although in general, alleles from other than the source of the nails are relatively infrequent.

What is clear from all the nail studies cited above is that standardized laboratory procedures were used when attempting to collect and analyze exogenous DNA from nails. In other words, comparative experiments were not conducted that might lead to an optimal procedure for collecting and testing such evidence. The same holds true in crime laboratories,

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where nail evidence is generally treated the same as any other type of evidence (personal communications). A microscopic examination might be conducted to determine if there are any obvious substances on the underside of the nails. The nail or nails may then be swabbed, often using one swab for all nails (cumulative swabbing), and DNA isolated similarly to any other swab in the lab. DNA quantitation would take place, perhaps using a method that specifically estimates male DNA. Finally, STR analysis is undertaken if DNA yields are high enough. Unfortunately, the crime laboratories typically have limited resources and manpower to rigorously test each one of these steps in order to determine if they are optimal for retrieving evidence from nails.

Other potentially troubling factors may also come into play regarding fingernail evidence. As noted above, it is not unusual for nails from a victim's hand to be placed together for transportation to the crime laboratory. This could potentially result in transfer of exogenous biological material from one nail to another, or from a nail to the envelope or other material into which it was placed. The former, if prevalent, could lead to more nails appearing to contain exogenous DNA than actually did, while the latter results in the direct loss of evidence. Likewise, as noted, laboratory technicians will often swab or otherwise process nails in sets, to save both time and resources. However, this again has the potential to result in cross contamination and/or evidence loss, wherein exogenous cells from the first nail swabbed are transferred to nails swabbed subsequently. If only a nail swabbed earlier harbors foreign cells, some may be left on nails swabbed after it. Further, if nails swabbed consecutively have different exogenous materials on them, mixtures can result, which could be avoided if nails were swabbed individually. On the other hand, individual swabbing utilizes more resources and may not

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recover enough DNA for viable STR analysis, whereas swabbing nails in groups may yield higher quantities of DNA.

The above variables, inconsistencies, and concerns led us to propose the research detailed below, which was designed to address and answer several questions that exist regarding DNA-based evidence derived from fingernails. We consulted with both forensic pathologists and SANEs who regularly collect fingernail evidence, and with crime laboratory personnel who process such evidence. In the end, we performed a large variety of experiments designed to objectively and rigorously determine:

- What is the best method for collecting nail evidence so as to maximize DNA yields?
- What is the best method for collecting nail evidence so as to maximize DNA retrieval from exogenous cells?
- What is the best method for collecting nail evidence so as to minimize DNA retrieval from the nails themselves?
- What is the best method for purifying DNA from nail evidence?
- What is the best method for obtaining STR data from collected nail DNA?
- What is the best method for obtaining STR data from only exogenous material on nails?
- What is the effect of processing nails as a group (cumulative swabbing) versus individually?
- What is the source of endogenous DNA from a nail (the nail, or cells beneath the nail)?
- What is the effect of transporting nails as a group?

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- What is the influence of common adulterants (i.e., fingernail polishes) on processing nails, given the results above?

Methods

In all instances, biological samples used in this study were completely deidentified, and all collection procedures were approved by the MSU IRB. Fingernails were clipped from female volunteers, assigned a random number, and stored at -20˚C until use. Buccal swabs were collected from volunteers, which were also randomly assigned a number. A key was created to associate the buccal swab with the corresponding fingernails. Blood used in all studies was donated by a single male volunteer.

Molecular Techniques

DNA Extraction and Purification

Two DNA isolation methods were compared: an organic extraction and a commercial kit extraction. All supplies and solutions were UV irradiated for 5 min (~2.5 J/cm^2), with the exception of proteinase K. For the organic extraction, 500 µL of digestion buffer (20 mM Tris—pH 7.5; 50 mM EDTA, 0.1% SDS) and 5 µL of proteinase K (20 mg/mL) were added to the 1.5 mL microcentrifuge tubes containing the samples and incubated overnight at 55˚C. A positive control was created by adding 1 µL of male blood directly into a 1.5 mL microcentrifuge tube with the same volumes of digestion buffer and proteinase K. A reagent blank, consisting of only digestion buffer and proteinase K, was created and carried through subsequent steps.

After incubation, 500 µL of phenol was added, followed by vortexing for 15 sec and centrifuging at 14,000 rpm for 5 min. Aqueous layers were transferred to new 1.5 mL microcentrifuge tubes and 500 µL of chloroform was added. Tubes were vortexed for 15 sec and

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centrifuged at 14,000 rpm for 5 min. Aqueous layers were transferred to Amicon® Ultra-0.5 Centrifugal Filter Devices[1], 30 kDa (Millipore) and centrifuged at 14,000 x g for 10 min. Flow-through was discarded, and the filters were washed with 300 µL of TE (10 mM Tris—pH 7.5; 1 mM EDTA). The filters were centrifuged at 14,000 x g for 10 min. Filters were washed two more times, once with TE and once with low TE (10 mM Tris—pH 7.5; 0.1 mM EDTA), for a total of three washes. The filters were inverted into new Amicon® tubes and centrifuged at 1,000 x g for 3 min to collect the DNA extracts, which were stored at -20˚C until use.

A QIAamp® DNA Investigator Kit (QIAGEN) was also used for DNA isolation. All supplies and solutions were UV irradiated for 5 min (~2.5 J/cm^2), with the exception of proteinase K. The manufacturer's protocol for DNA isolation from nail clippings was used, with slight modifications. Four hundred microliters of Buffer ATL was added to completely submerge the nail, and volumes of subsequent solutions were increased proportionately. Furthermore, to minimize extraction of endogenous nail DNA, only 10 µL of proteinase K was added while DTT was not.

The nails were incubated at 55˚C overnight. Tubes were centrifuged briefly and 400 µL of Buffer AL/carrier RNA solution (prepared according to QIAamp® DNA Investigator Kit manual) was added. Tubes were vortexed for 15 sec and incubated in a 70˚C water bath for 10 min, vortexing every 3 min. After briefly centrifuging, 200 µL of 100% ethanol was added. Tubes were vortexed for 15 sec, incubated at room temperature for 3 min, and briefly centrifuged. Lysates were transferred to QIAamp MinElute® columns in 2 mL tubes and centrifuged at 6000 x g for 1 min. The columns were transferred to clean 2 mL collection tubes. Five hundred

[1] These filter columns were not pretreated in any way. Subsequent research has shown they can trap large amounts of DNA. This problem can be reduced substantially through column pretreatment with a neutral nucleic acid such as yeast RNA (Foran and Doran 2014).

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microliters of Buffer AW1 was added to the columns, which were centrifuged at 6000 x g for 1 min. Columns were placed in clean 2 mL collection tubes, and 700 µL of Buffer AW2 was added. Tubes were centrifuged at 6000 x g for 1 min and transferred to clean 2 mL collection tubes. Seven hundred microliters of 100% ethanol was added, and tubes were centrifuged at 6000 x g for 1 min. Columns were transferred to clean 2 mL collection tubes, which were centrifuged at 14,000 rpm for 3 min. Columns were placed in clean 1.5 mL microcentrifuge tubes. Lids of the columns were opened and tubes were dried at room temperature for 10 min. Twenty microliters of Buffer ATE was added to the center of the membranes, lids were closed, and tubes were incubated at room temperature for 5 min. Tubes were centrifuged at 14,000 rpm for 1 min to elute DNA. Further optimization of the kit extraction's elution step examined whether the elutant, volume of elutant, or multiple elutions increased DNA recovery.

Exogenous DNA Quantification via Real-Time PCR

DNAs were quantified using a Quantifiler® Y Human Male DNA Quantification Kit (Applied Biosystems). Eight standards were created via serial dilution of the Quantifiler® Human DNA Standard according to the Quantifiler Kit User Manual. Each reaction consisted of 7.5 µL of Quantifiler® PCR Reaction Mix, 6.3 µL of Quantifiler® Y Human Male Primer Mix, and 1.2 µL of DNA extract or standard, for a total volume of 15 µL. Real-time PCR was conducted on an iCycler™ Thermal Cycler (BioRad) and fluorescence detected with an iQ™5 Multicolor Real-Time PCR Detection System (BioRad). Cycling conditions were: 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. Data were analyzed using iQ™5 Optical System Software (BioRad).

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Autosomal STR and Y-STR Analyses

DNAs were amplified using an AmpFℓSTR® Identifiler® PCR Amplification Kit and an AmpFℓSTR® Yfiler® PCR Amplification Kit (Applied Biosystems). Identifiler® and Yfiler® reactions followed the manufacturer's protocols; however, reactions were scaled down to a total volume of 10 µL. The volumes of DNA added to reactions were based on the Quantifiler® Y DNA quantification, with a target of 0.75 ng of DNA. The DNA volume was maximized at 5.5 µL due to low DNA quantities in some instances, including all scratching experiments. PCR was conducted on a 2720 Thermal Cycler (Applied Biosystems) or a GeneAmp® PCR System 2400 (Applied Biosystems). Identifiler® PCR cycling conditions were: 95˚C for 11 min, 28 cycles of 94˚C for 1 min, 59˚C for 1 min, and 72˚C for 1 min, and 60˚C for 80 min. Yfiler® PCR cycling conditions were: 95˚C for 11 min, 30 cycles of 94˚C for 1 min, 60˚C for 1 min, and 72˚C for 1 min, and 60˚C for 80 min.

All PCR products were electrophoresed on a 3500 Genetic Analyzer (Applied Biosystems) with a 50 cm 3500 Capillary Array (Applied Biosystems). One microliter of PCR product was added to 0.3 µL of GeneScan™-500 LIZ™ Size Standard (Applied Biosystems) and 9 µL of Hi-Di formamide (Applied Biosystems) on a 96-well plate. AmpFℓSTR® Identifiler® Allelic Ladder or AmpFℓSTR® Yfiler® Allelic Ladder was included as appropriate for each injection. POP-7™ (384) Performance Optimized Polymer (Applied Biosystems) was used for electrophoresis, as well as Anode Buffer 3500 Series and Cathode Buffer 3500 Series (Applied Biosystems). 3500 Series Data Collection Software, v1.1 was used to collect data from injections, and allele calls were made using GeneMapper v4.1. Instrument protocol for Identifiler® injections was: Oven Temperature: 60˚C, Run Time: 1330 sec, Run Voltage: 19.5 kV, PreRun Time: 180 sec, PreRun Voltage: 15 kV, Injection Time: 8 sec, Injection Voltage: 1.6 kV, and Data Delay: 1 sec.

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Instrument protocol for Yfiler® injections was: Oven Temperature: 60˚C, Run Time: 1800 sec, Run Voltage: 15 kV, PreRun Time: 180 sec, PreRun Voltage: 15 kV, Injection Time: 15 sec, Injection Voltage: 1.2 kV, and Data Delay: 1 sec.

Initial Scratching Experiments

Volunteers scratched one another on the underside of the forearm, using 2 pounds of force and a single scratch, which reddened but did not break the skin. The volunteer being scratched rested his or her arm on an Eat Smart Precision PRO Kitchen Scale (Health Tools LLC), the scale was zeroed, and another volunteer used his or her three center fingers to scratch the length of the forearm. Fingernails were swabbed using the double swab technique (Sweet et al. 1997), and swab heads were cut off and placed in 1.5 mL microcentrifuge tubes for organic extraction. Autosomal STR (n=71) and Y-STR (n=32) analyses were performed.

Comparison of Collection Techniques for Obtaining Exogenous DNA from Nails

Due to highly variable results from initial scratching experiments, a more consistent method of cellular deposition was implemented in order to compare exogenous DNA collection techniques. Known amounts of male blood, originating from a single individual, were placed on clipped female fingernails, resulting in similar amounts of exogenous DNA and allowing DNA recovery to be compared among the techniques.

Nail Preparation

A Kimwipe™ (Kimberly-Clark) moistened with 40 µL of sterile water was used to clean clipped female fingernails prior to use. Male blood, to which a small amount of EDTA had been added to prevent coagulation, was vortexed before 1 µL was deposited on each cleaned nail and allowed to dry for 24 h. To help obtain consistent volumes among trials, the same pipette was used for all nail samples within an experiment.

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DNA Collection Techniques

All supplies and solutions were UV irradiated for 5 min (~2.5 J/cm^2) prior to use. Three techniques were utilized to collect biological material from clipped nails spotted with blood. First, a nail was placed directly into a 1.5 mL microcentrifuge tube for digestion, termed "soaking" (n=30). Second, a Small Compressed CleanFoam® Swab (ITW Texwipe) was moistened by immersing it twice into digestion buffer and used to swab the nail, followed by a dry CleanFoam® Swab, until upon visual inspection all blood was collected. The swab heads were cut off and placed together into a 1.5 mL microcentrifuge tube (n=30). Finally, a nail was scraped with a wooden applicator (American Scientific Products) over weigh paper and the dislodged material placed into a 1.5 mL microcentrifuge tube (n=31). DNA was isolated via an organic extraction and exogenous (male) DNA quantified as described above. Autosomal STR and Y-STR analyses were conducted.

Comparison of Organic and Commercial Kit Extractions

DNA recovery was compared for the commercial kit and organic extraction (as described above), using nails harboring blood. Male blood was deposited on clipped fingernails from multiple female donors (detailed above) and processed via soaking. Organic extraction (n=15), or kit extraction (n=15) using a single 20 µL elution of Buffer ATE, was performed. Exogenous DNA was quantified, and extract volumes were taken into account when comparing total DNA recovery between methods.

The kit's elution step was further studied to determine if type of elutant (Buffer ATE vs. low TE) or volume of elutant (20, 28, 50, or 100 µL of Buffer ATE) increased DNA recovery. Further, four elutions of 20 µL were performed to determine if multiple elutions continued to

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release DNA. In the end, our standard kit extraction protocol utilized three 20 µL elutions with Buffer ATE, since minimal DNA was recovered on the fourth elution.

Scratchings Using Improved Cell Collection and DNA Extraction Protocols

Female volunteers scratched the underside of the forearm of male volunteers using the standard scratching procedure (3 scratches using the center 3 fingers at 2 pounds of force). Fingernails were cut with scissors and placed into separate 1.5 mL microcentrifuge tubes for digestion. DNA was extracted according to the standard kit extraction protocol described above. Autosomal STRs (n=8) and Y-STRs (n=19) were amplified using maximum DNA volumes, owing to low exogenous DNA recovery from scratchings.

Ancillary Tests

Transportation of Nail Evidence

Sets (n=18) of one female nail harboring male blood and two nails without blood were packaged in a coin envelope, which was sealed and transported in a backpack for 5 days. The nail with blood was processed singly, while the two nails without blood were processed together. Nails were soaked and DNA extracted via an organic extraction. Exogenous DNA was quantified and Y-STR analysis was conducted on the extracts from nails without blood.

Females scratched males 3 times using their 3 center fingers at 2 pounds of force. The four sets of clipped nails (n=12) were placed in a coin envelope along with the thumb nail, which was not used for scratching. The coin envelope was sealed and transported in a backpack for 5 days. After transport, each nail was transferred to a separate 1.5 mL microcentrifuge tube. The envelope was held upright and tapped several times so that any residual material would fall to the bottom. A Sterile Cotton-Tipped Applicator (MediChoice®) was moistened with 10 µL of digestion buffer and used to swab the inside, bottom portion of the envelope. The head of the

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swab was cut and placed in a 1.5 mL microcentrifuge tube. The standard kit extraction was conducted on the nails and swabs. Exogenous DNA was quantified and Y-STR analysis was undertaken.

Cumulative Swabbing of Fingernails

Cumulative swabbing was conducted on female fingernails harboring male blood as depicted in Figure 1. Each trial (n=15) consisted of two nails with blood and two without blood. First, a Small Compressed CleanFoam® Swab was moistened by immersing it twice into digestion buffer and then used to alternately swab a nail with blood and without blood, using 8 strokes back and forth (Fig.1, 1). Second, a dry swab was used on the two nails with blood to determine if exogenous cells had been left behind by the first swab (Fig.1, 2). Third, the two nails without blood were double swabbed (Sweet et al. 1997) to determine if they had been contaminated with blood (Fig.1, 3); these two swabs were processed together. Swab heads were placed into 1.5 mL microcentrifuge tubes for organic extraction. DNAs were quantified, and Y-STR analysis was conducted on the swabs of nails without blood that showed the highest exogenous DNA yields.

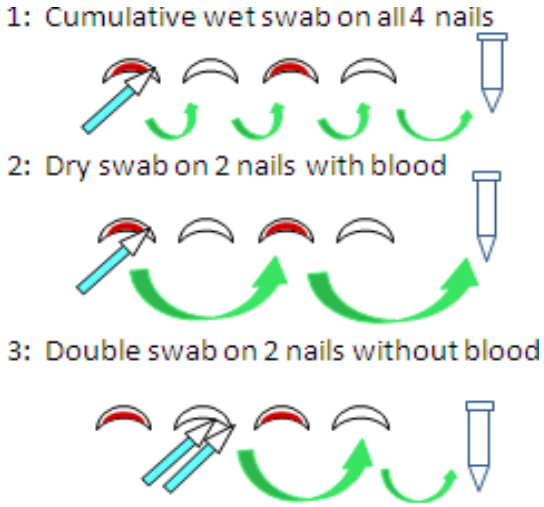

Figure 1: Diagram of Cumulative Swabbing Procedure. Refer to the text above for details.

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Effect of Nail Polish on DNA Amplification and Analysis

Female fingernails were clipped, and three different types of nail polish—Super Nails Natural Wonder (Revlon Inc.; red), Wild Shine Black Crème (Wet 'N' Wild), and Pure Ice Silver Crackle (Bari Cosmetics, Ltd.)—were painted on the top side of the nails. Polish was allowed to dry for approximately 30 min before the nails were cleaned as detailed above. One microliter of male blood was deposited on the bottom side of the nails. DNA was isolated using the standard kit extraction. PCR inhibition was assessed via real-time PCR and Y-STR analysis conducted.

Source of Endogenous DNA from Fingernails

Female fingernails were clipped and swabbed several times using a Small Compressed CleanFoam® Swab moistened by immersing it twice into digestion buffer, followed by a dry swab, in order to determine if loose cells normally exist on a nail. The two swabs were cut and placed in the same 1.5 mL microcentrifuge tube for DNA extraction. This process was then repeated. Finally, the nails themselves were placed into a 1.5 mL microcentrifuge tube. A standard organic extraction was performed, followed by DNA quantification using a Quantifiler® Human DNA Quantification Kit (Applied Biosystems). Reactions consisted of 7.5 µL of Quantifiler® PCR Reaction Mix, 6.3 µL of Quantifiler® Human Primer Mix, and 1.2 µL of nail DNA extract.

Statistical Tests

Statistical differences between DNA quantities and percent of STR profiles recovered were calculated via t-tests and ANOVA using Microsoft Excel (Microsoft Corporation). Boxplots were created using SPSS (IBM). Boxes represent the middle 50% of the data, with the heavy line indicating the median. Whiskers extend to the minimum and maximum values that are not outliers. Open circles indicate mild outliers, while extreme outliers are displayed as asterisks.

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All data were rounded to one decimal place (DNA quantities) or to the nearest percent (STR profiles) for tables presented; therefore, means listed may be slightly off due to rounding.

Results

Initial Scratching Experiments

The percent of autosomal STR and Y-STR profiles recovered from nails after scratching are shown in Tables 1 and 2, respectively. Nails generated partial autosomal STR profiles from the individual doing the scratching (avg. 30%, Table 1), while profiles from the individual being scratched were often not present or consisted of minimal alleles (avg. 2%, Table 1). In instances of females scratching males, an average of 7% of the male Y-STR profile was present; however, in most cases, no alleles were observed (Table 2).

Sample #	Profile of Scratcher	Profile of Individual Being Scratched
1	44%	0%
2	0%	0%
3	6%	0%
4	0%	0%
5	100%	0%
6	0%	0%
7	100%	0%
8	19%	0%
9	80%	0%
10	65%	5%
11	0%	0%
12	5%	0%
13	16%	0%
14	5%	0%
15	5%	5%
16	0%	0%
17	0%	0%

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18	93%	0%
19	14%	0%
20	71%	0%
21	0%	0%
22	44%	0%
23	11%	0%
24	89%	0%
25	100%	0%
26	100%	6%
27	100%	0%
28	94%	38%
29	6%	0%
30	75%	11%
31	94%	0%
32	27%	0%
33	0%	0%
34	0%	0%
35	0%	0%
36	0%	0%
37	13%	0%
38	0%	0%
39	13%	0%
40	16%	0%
41	0%	0%
42	0%	0%
43	0%	0%
44	0%	0%
45	0%	0%
46	11%	0%
47	0%	0%
48	0%	0%
49	0%	0%
50	0%	0%
51	0%	0%
52	0%	0%
53	25%	0%
54	0%	0%
55	100%	6%
56	100%	0%
57	56%	0%
58	17%	0%

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59	61%	42%
60	0%	0%
61	31%	0%
62	25%	0%
63	100%	0%
64	40%	20%
65	13%	0%
66	0%	7%
67	13%	7%
68	27%	0%
69	33%	0%
70	0%	0%
71	53%	0%

Average	30%	2%

Table 1: Percent of Autosomal STR Profiles Recovered from Initial Scratchings (1 scratch using center 3 fingers and 2 pounds of force). Percent indicates the number of alleles present that were specific to the individual out of the total number of alleles specific to the individual. Note that alleles from the scratcher were often present, whereas alleles from the individual being scratched were generally few or completely absent.

Sample #	Y-STR Profile of Male Being Scratched
1	0%
2	0%
3	6%
4	0%
5	0%
6	0%
7	0%
8	0%
9	0%
10	0%
11	0%
12	18%
13	0%
14	0%
15	0%
16	0%
17	6%
18	0%
19	6%

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20	0%
21	0%
22	18%
23	0%
24	76%
25	41%
26	29%
27	6%
28	18%
29	0%
30	0%
31	0%
32	0%

Average	7%

Table 2: Percent of Y-STR Profile Recovered from Male Being Scratched (1 scratch using center 3 fingers and 2 pounds of force). Percent indicates the percentage of a Y-STR profile recovered. No alleles were seen in a majority of cases.

Comparison of Collection Techniques for Obtaining Exogenous DNA from Nails

During these initial scratching experiments it became apparent that consistency in cellular deposition among trials was problematic, even when controlling for pounds of force applied. Further, the scratchings resulted in minimal autosomal STR data and thus did not allow for rigorous comparison of collection methods. Therefore a more objective method, in which 1 µL of male blood was placed on a nail from a female donor, was implemented. This resulted in a much more consistent amount of starting exogenous cellular material, allowing for extensive, objective comparison of the three methods that were tested to retrieve evidence from nails.

The quantities of exogenous DNA recovered using the soaking, swabbing, and scraping methods are shown individually in Table 3 and in boxplot form in Figure 2. DNA recovery varied significantly among the methods (ANOVA $p < 0.00001$). Specifically, soaking resulted in a significantly greater yield of male DNA (avg. 433.7 pg/µL) than did swabbing (avg. 275.1 pg/µL, $p = 0.007$), which in turn recovered more male DNA than did scraping (avg. 146.3 pg/µL,

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$p < 0.001$). A summary of all results comparing DNA yields and STR profiles for cell collection techniques is displayed in Table 4. Compared to the average DNA quantification of 1 µL of blood added directly to digestion buffer, soaking recovered the majority of exogenous material (96%), whereas swabbing and scraping recovered lesser amounts (61% and 33%, respectively, Table 4). These results clearly showed that soaking nails in digestion buffer was superior for exogenous DNA retrieval, thus subsequent experiments utilized the soaking method.

Sample #	Exogenous DNA Quantification for Soaked Nails	Exogenous DNA Quantification for Swabbed Nails	Exogenous DNA Quantification for Scraped Nails
1	211.0	327.0	290.0
2	356.0	316.0	35.2
3	855.0	223.0	156.0
4	167.0	203.0	25.8
5	18.7	325.0	34.2
6	56.0	269.0	49.7
7	232.0	153.0	10.1
8	199.0	673.5	167.0
9	772.0	300.0	83.8
10	751.0	351.0	224.0
11	188.0	140.0	17.6
12	371.5	159.0	18.9
13	83.7	169.5	17.0
14	376.0	184.5	38.5
15	351.0	376.5	293.5
16	433.0	462.0	294.0
17	776.5	189.0	375.0
18	484.5	314.5	0.0
19	797.0	88.1	143.0
20	273.5	328.5	139.0
21	292.5	257.5	349.5
22	563.0	234.0	521.0
23	346.5	202.0	0.0
24	963.0	189.0	145.0
25	429.5	226.0	171.5

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26	825.5	324.0	203.0
27	665.5	252.0	26.1
28	409.5	478.5	538.5
29	294.5	94.9	3.2
30	469.5	442.5	15.3
31	NA	NA	150.5
Average	433.7	275.1	146.3

Table 3: Comparison of Exogenous DNA Yields Based on Cell Collection Techniques. DNA quantities are reported as pg/µL. Soaking resulted in the highest exogenous DNA yield, followed by swabbing. Scraping nails recovered the least amount of exogenous DNA.

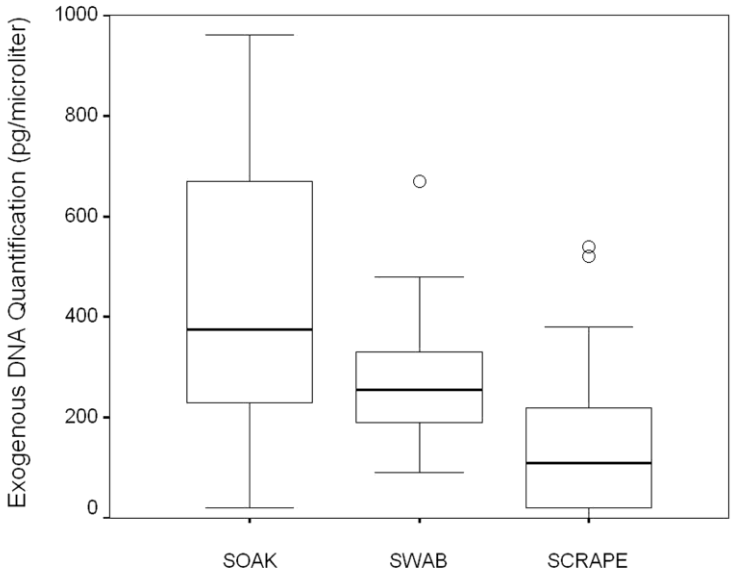

Figure 2: Boxplot of Exogenous (Male) DNA Quantities from each Collection Technique. Y axis is DNA quantity in pg/µL. Note that the soaking method recovered the most exogenous DNA, while scraping recovered the least.

	Soak	Swab	Scrape
Exogenous DNA Quantification	433.7, n=30	275.1, n=30	146.3, n=31
Percent Recovery of Exogenous DNA	96%	61%	33%
Percent of Exogenous Autosomal STR Profile	99%, n=29	99%, n=28	80%, n=27
Percent of Exogenous Y-STR Profile	100%, n=16	100%, n=16	90%, n=16

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Table 4: Summary of Exogenous (Male) DNA Yields and Percent STR Profiles Obtained by Collection Method. Average DNA quantities are reported as pg/µL. Percent recovery was determined by DNA quantification of each method relative to the quantification of the blood controls. Percentages of profiles are reported as the average percent of exogenous DNA-specific autosomal STR alleles or Y-STR alleles recovered by each collection method.

When these same DNAs underwent STR analysis (Tables 5 – 7; summarized in Table 4), several trends emerged. First, autosomal STR profiles of nails soaked or swabbed generally contained most or all possible male alleles (Tables 5 and 6, respectively). However, both methods resulted in mixtures of the nail donor and the exogenous cell donor. In most instances, soaking the nail produced a major profile from the nail (e.g. Fig. 3a) or similar DNA contributions from endogenous/exogenous cells. In some cases, an exogenous major profile was present. In contrast, swabbing consistently resulted in a major profile from exogenous material (e.g. Fig. 3b), which was often mixed with endogenous alleles. Autosomal STR alleles from scrapings were typically only from exogenous cells (e.g. Fig. 3c), and significantly fewer exogenous alleles were recovered than when using the other two techniques ($p < 0.05$ for both). About half of scrapings recovered no endogenous-specific alleles, while one or more endogenous-specific alleles were present in the other half.

Soak Sample #	Exogenous-Specific Alleles Observed	Exogenous-Specific Alleles Possible	Percent of Exogenous Autosomal STR Profile Recovered
1	19	19	100%
2	19	19	100%
3	16	19	84%
4	18	19	95%
5	20	21	95%
6	21	21	100%
7	21	21	100%
8	20	21	95%
9	21	21	100%
10	21	21	100%
11	18	19	95%
12	19	19	100%

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13	21	21	100%
14	21	21	100%
15	21	21	100%
16	21	21	100%
17	19	19	100%
18	19	19	100%
19	19	19	100%
20	21	21	100%
21	21	21	100%
22	21	21	100%
23	21	21	100%
24	21	21	100%
25	21	21	100%
26	21	21	100%
27	21	21	100%
28	21	21	100%
29	21	21	100%

Average	99%

Table 5: Exogenous Autosomal STR Profiles from Soaked Nails Harboring Blood. Soaking nails recovered the entire exogenous profile in most instances.

Swab Sample #	Exogenous-Specific Alleles Observed	Exogenous-Specific Alleles Possible	Percent of Exogenous Autosomal STR Profile Recovered
1	19	19	100%
2	19	19	100%
3	17	19	89%
4	21	21	100%
5	21	21	100%
6	21	21	100%
7	19	21	90%
8	21	21	100%
9	21	21	100%
10	21	21	100%
11	19	19	100%
12	19	19	100%
13	21	21	100%
14	21	21	100%
15	17	17	100%
16	17	17	100%

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17	21	21	100%
18	21	21	100%
19	21	21	100%
20	19	19	100%
22	21	21	100%
23	21	21	100%
24	21	21	100%
25	21	21	100%
26	21	21	100%
27	21	21	100%
28	21	21	100%
29	21	21	100%

Average	99%

Table 6: Exogenous Autosomal STR Profiles from Swabbed Nails Harboring Blood. Swabbing nails recovered the entire exogenous profile in most instances.

Scrape Sample #	Exogenous-Specific Alleles Observed	Exogenous-Specific Alleles Possible	Percent of Exogenous Autosomal STR Profile Recovered
1	18	19	95%
2	16	19	84%
4	20	21	95%
5	21	21	100%
6	8	21	38%
7	2	21	10%
8	21	21	100%
9	18	21	86%
10	20	21	95%
11	14	21	67%
12	0	21	0%
13	17	21	81%
14	17	21	81%
15	17	17	100%
16	17	17	100%
17	17	17	100%
18	5	21	24%
19	21	21	100%
20	21	21	100%
21	19	19	100%
22	19	19	100%
23	0	21	0%

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24	21	21	100%
25	21	21	100%
26	21	21	100%
27	21	21	100%
28	21	21	100%

Average	80%

Table 7: Exogenous Autosomal STR Profiles from Scraped Nails Harboring Blood. Half of the nail scrapings generated complete exogenous profiles, but allelic dropout was often observed.

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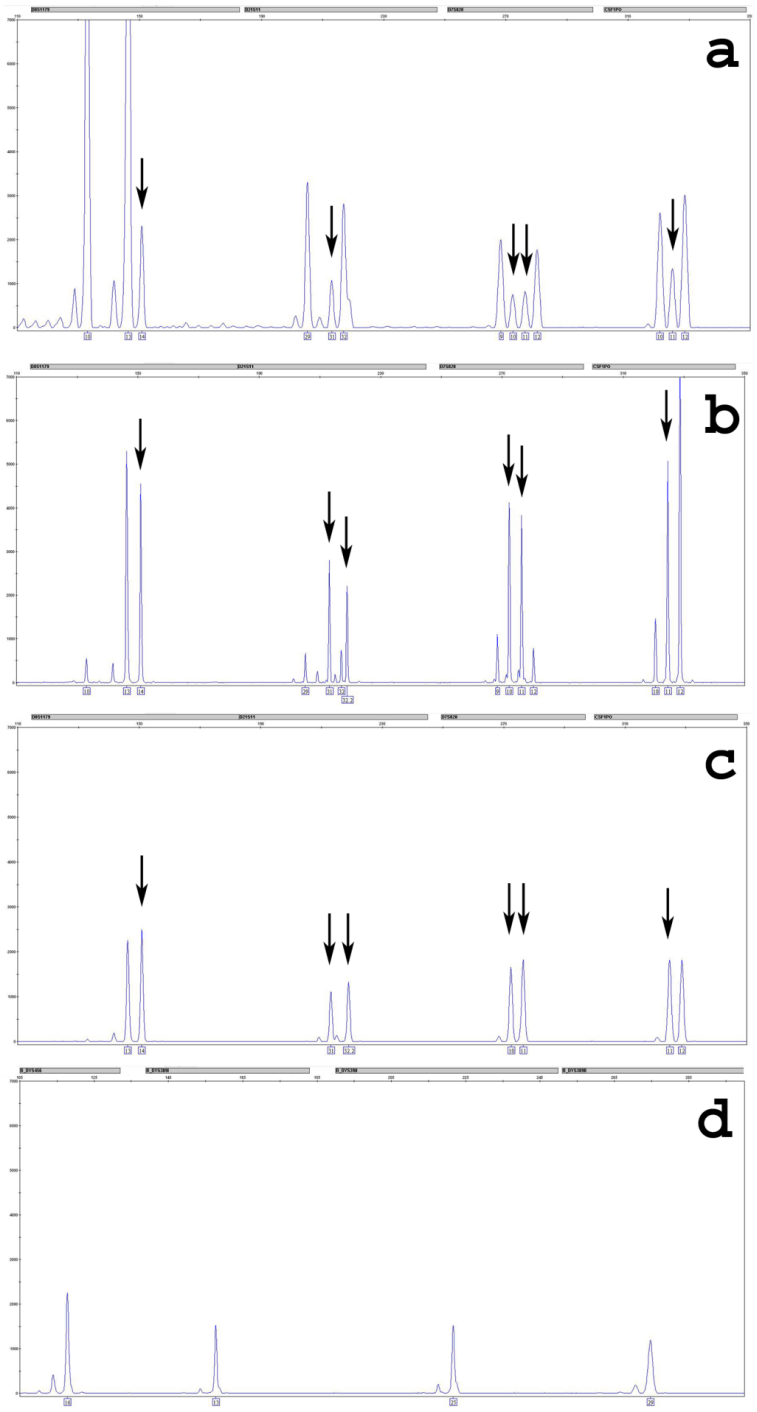

Figure 3: Autosomal STR and Y-STR Analysis of Nails Harboring Blood. Autosomal STR profiles from a soaked (a), swabbed (b), and scraped (c) nail are depicted. Arrows indicate alleles specific to the exogenous cells; large peaks in (b) and (c) without arrows are shared alleles. Note that soaking often resulted in a major profile from endogenous cells, whereas swabbing had an exogenous major profile. Both soaking and swabbing resulted in mixtures. In contrast, scraping often produced a single source exogenous profile; however, allelic dropout was seen. (d) Y-STR

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profile of the same swabbed nail in (b), which produced a single source profile attributed to the exogenous cells.

All DNAs from soaked or swabbed nails that contained autosomal STR mixtures produced single source profiles from exogenous cells when Y-STRs were amplified, with no allelic dropout (Table 8). This is exemplified in Figure 3d, which is the same swabbed sample as Figure 3b that showed an autosomal STR mixture. Scraped nails again had Y allelic dropout in some instances.

Sample #	Exogenous Y-STR Profile (Soak)	Sample #	Exogenous Y-STR Profile (Swab)	Sample #	Exogenous Y-STR Profile (Scrape)
1	100%	1	100%	1	100%
2	100%	2	100%	2	100%
3	100%	3	100%	4	100%
4	100%	4	100%	5	100%
5	100%	5	100%	6	82%
6	100%	6	100%	7	0%
7	100%	7	100%	8	100%
8	100%	8	100%	9	82%
9	100%	9	100%	10	100%
10	100%	10	100%	15	100%
11	100%	11	100%	16	100%
12	100%	12	100%	17	100%
13	100%	13	100%	22	100%
14	100%	14	100%	24	100%
18	100%	24	100%	28	100%
24	100%	26	100%	29	71%
Average	100%		100%		90%

Table 8: Percent of Exogenous Y-STR Profile Recovered from Nails Harboring Blood for Each Cell Collection Technique. Soaked and swabbed nails produced complete Y-STR profiles of exogenous cells, while scraped nails experienced allelic dropout in some cases.

Comparison of Organic and Commercial Kit Extractions

Exogenous DNA quantifications for organic and kit extractions are shown individually in Table 9 and in boxplot form in Figure 4. Real-Time PCR results indicated that the Qiagen DNA

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Investigator Kit recovered a significantly greater amount of exogenous DNA based on a single column elution than did organic extraction (avg. 13.8 ng and 10.2 ng respectively; $p < 0.05$, Table 9), however, as noted above, this was only the case if the Amicon® columns used in organic extractions were untreated, while organic extractions produced slightly higher DNA yields if columns were pretreated as described by Doran and Foran (2014). Optimization of the kit elution step showed that DNA was successfully recovered from up to four elutions (data not shown); however, the DNA quantity was negligible in the fourth. The volume of buffer used to elute the DNA did not have a substantial effect on DNA yields, nor did replacing the kit elution buffer with low TE (data not shown), thus a final protocol of three elutions using 20 µL of Buffer ATE was utilized for subsequent experiments.

Sample #	Exogenous DNA Quantification (pg/µL)	Total DNA Recovered (ng)
Organic		
1	524.0	12.1
2	389.5	9.0
3	358.5	8.2
4	276.0	6.3
5	343.5	7.9
6	566.5	13.0
7	317.0	7.3
8	299.5	6.9
9	607.0	14.0
10	525.5	12.1
11	426.5	9.8
12	722.5	16.6
13	419.5	9.6
14	324.5	7.5
15	560.0	12.9
Average		10.2

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Kit		
1	359.0	7.9
2	420.0	9.2
3	457.5	10.1
4	511.5	11.3
5	719.0	15.8
6	698.0	15.4
7	684.0	15.0
8	1120.0	24.6
9	403.0	8.9
10	1110.0	24.4
11	573.5	12.6
12	740.5	16.3
13	331.5	7.3
14	741.0	16.3
15	519.0	11.4

Average		13.8

Table 9: Comparison of Exogenous DNA Recovery for Organic and Commercial Kit Extraction. Volume of the DNA extracts was considered when determining total DNA (ng) recovered using each extraction method. The kit extraction recovered a significantly greater amount of exogenous DNA from nails harboring blood than organic extraction (13.8 vs. 10.2, $p < 0.05$), though see footnote 1 above.

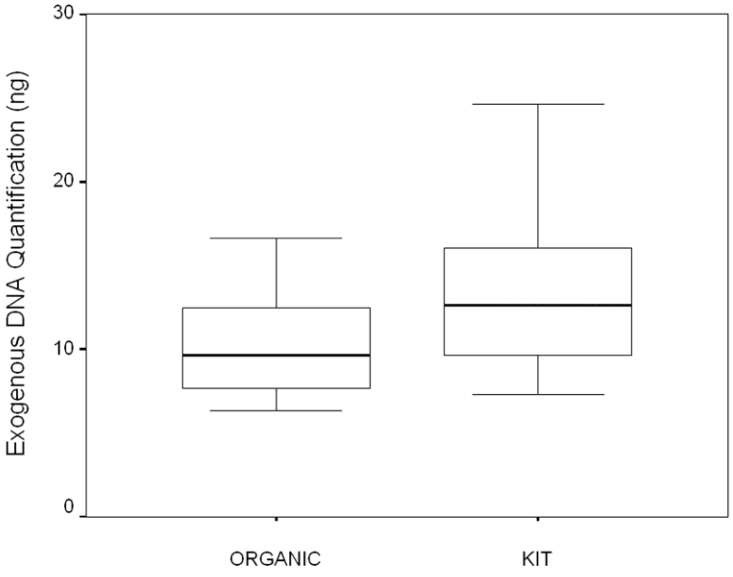

Figure 4: Boxplot of Exogenous (Male) DNA Yields from Organic and Kit Extractions. Y axis is DNA quantity in ng. Note that the kit extraction recovered a greater amount of exogenous DNA than organic extraction, though see footnote 1 above.

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Scratchings Using Improved Cell Collection and DNA Extraction Protocols

The standard scratching procedure (3 scratches using the center 3 fingers at 2 pounds of force) and the optimized cell collection (soaking) and DNA extraction protocols, along with the maximum amount of input DNA, resulted in strong autosomal STR profiles of the female nail donor (e.g. Fig. 5a). Male alleles, though often present, were substantially weaker (e.g. Fig. 5a, indicated by arrows), and in some instances were not detected. In contrast, Y-STR analysis (Table 10) produced several full profiles (e.g. Fig. 5b). Overall, 69% of Y-STR loci had alleles matching the known male profile. One nail had a Y-STR profile that clearly originated from a different, unknown male that was not consistent with any laboratory personnel (not represented in Table 10).

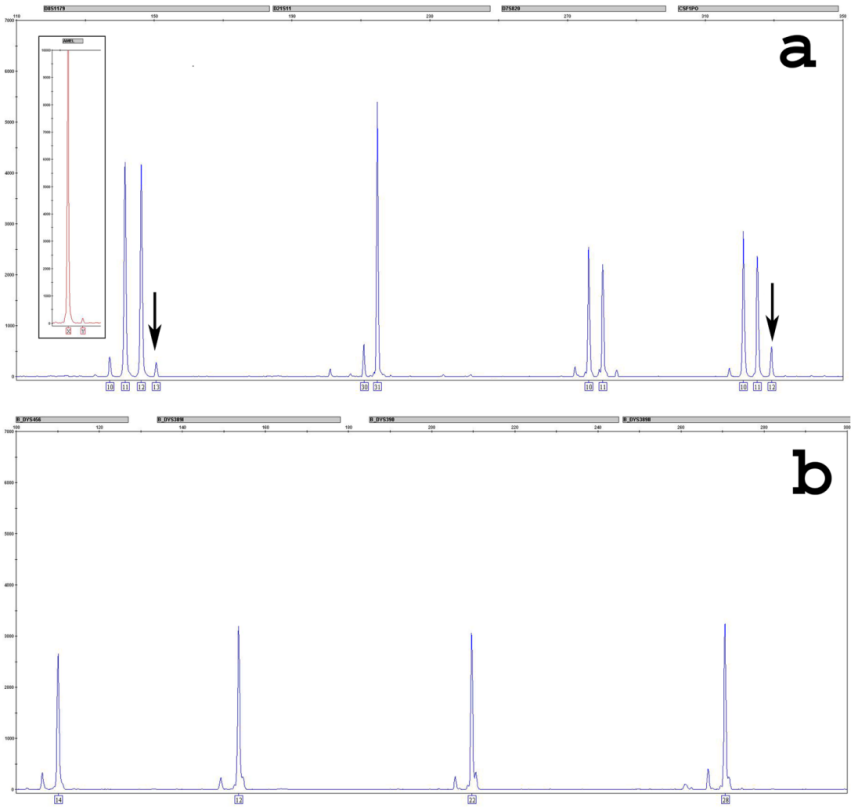

Figure 5: Autosomal STR and Y-STR Profiles from Nails Following Scratching Using Optimized Procedures. (a) Autosomal STR profile from a soaked nail after scratching. Arrows

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indicate exogenous-specific autosomal STR alleles. Note the endogenous major profile and that exogenous alleles are present but with much lower peak heights. (b) Y-STR profile from the same soaked nail in (a).

Sample #	Percent of Exogenous Y-STR Profile
1	100%
2	65%
3	100%
4	35%
5	100%
6	100%
7	65%
8	88%
9	76%
10	100%
11	53%
12	82%
13	24%
14	12%
15	41%
16	65%
17	71%
18	94%
19	0%

Average	69%

Table 10: Percent of Y-STR Profile Recovered from the Scratched Individual. Y-STR analysis of nails after scratching produced both partial and full profiles consistent with the scratched individual, with an average of 69% of the profile recovered.

Ancillary Tests

Transportation of Nail Evidence

Exogenous DNA quantities from nails harboring blood following transport are shown in full in Table 11 and in boxplot form in Figure 6. Low levels of exogenous DNA were recovered from nails without blood (avg. 21.5 pg/µL, Table 11). Y-STR profiling of the five nails without blood that generated the highest exogenous DNA yields showed that none were consistent with the blood profile (Table 12). Specifically, two of the ‘clean’ nails from one female donor

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produced identical, unidentified Y-STR profiles. Two other nails from a second female donor produced a different unidentified Y-STR profile. Neither profile was consistent with laboratory personnel, indicating that the male DNA was present on the female nails prior to the deposition of blood. Still, a significant amount of exogenous DNA was lost from the nails during transport, as the average exogenous DNA yield was 270.3 pg/µL (Table 11), whereas soaked nails not undergoing transport had an average yield of 433.7 pg/µL (from Table 3, $p < 0.05$).

Nail with Blood	Exogenous DNA Quantification	Nails without Blood	Exogenous DNA Quantification
1	229.5	1	26.4
2	324.5	2	11.0
3	222.5	3	10.1
4	683.0	4	4.0
5	150.5	5	29.9
6	355.0	6	162.5
7	304.5	7	6.1
8	263.0	8	18.5
9	271.0	9	73.8
10	296.5	10	41.7
11	252.5	11	0.0
12	211.0	12	3.5
13	101.0	13	0.0
14	269.0	14	0.0
15	133.0	15	0.0
16	138.0	16	0.0
17	292.0	17	0.0
18	368.0	18	0.0
Average	270.3		21.5

Table 11: Exogenous DNA Quantification of Transported Nails with and without Blood. DNA yields are in pg/µL. Nails with blood had substantially more exogenous DNA, and several of the 'clean' nails had no or very little exogenous DNA. However, some 'clean' nails had notably higher exogenous DNA levels, although this did not originate from the blood (see Table 12). There was a significant loss of exogenous DNA from the nails with blood when compared to nails not undergoing transport (Table 3).

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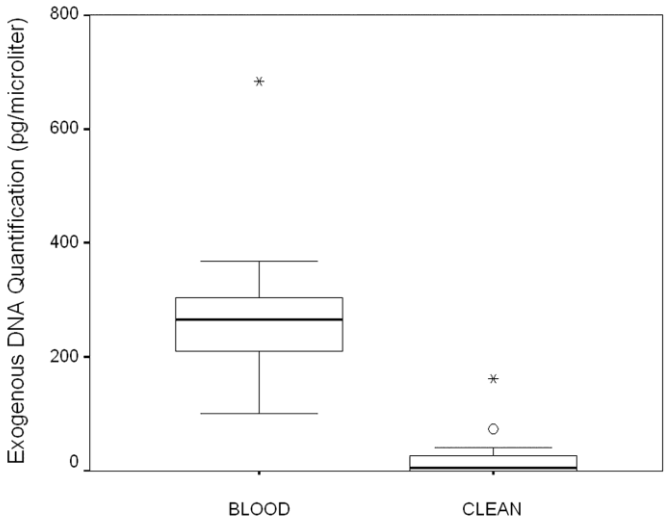

Figure 6: Boxplot of Exogenous (Male) DNA Quantities from Nails Harboring Blood Following Transport. Y axis is DNA quantity in pg/µL. Note that nails with blood had a greater yield of exogenous DNA, and several of the 'clean' nails had little to no exogenous DNA present.

Locus	5	6	9	10	1	Male Blood
DYS456	17	17	15	15, 17	.	16
DYS389I	13	13	12	12, 13	.	13
DYS390	23	23	22	22	.	25
DYS389II	29	29	.	25, 28, 29	.	29
DYS458	18	18	15	15, 16	.	15
DYS19	14	14	14	14	.	15
DYS385	11, 14	11, 14	13, 15	13, 15	.	11, 14
DYS393	13	13	13	13	.	13
DYS391	10	10	10	10	.	10
DYS439	12	12	11	11	.	10
DYS635	23	23	20	20, 24	.	23
DYS392	13	13	11	11	.	11
GATA H4	12	12	11	11	.	13
DYS437	15	15	16	15, 16	.	14
DYS438	12	12	10	10	.	11
DYS448	19	19	20	20	.	20

Table 12: Y-STR Profiles of 'Clean' Transported Nails with Highest Male DNA Yields. (.) denotes no alleles present. Samples 5 and 6 originated from the same female nail donor. Samples 9 and 10 originated from a different female donor. Note that Y-STR profiles for samples 5, 6, 9, and 10 do not match the male blood, indicating that cross contamination did not occur. The source of the Y-STR profiles is unknown. Sample 1 did not generate Y-STR alleles.

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Similar results were obtained from transported nails following scratching (Table 13), wherein no cross contamination was found between co-transported nails used and not used for scratching. However, DNA yields from transported nails were low (Table 13), and only one produced a full Y-STR profile, while several had no alleles (Table 14). Further, exogenous DNA was lost during transport, as Y-STR profiles of nails used for scratching contained only 25% of the possible alleles (Table 14), which was significantly less than those that were not transported (from Table 10, $p < 0.001$). Swabbings of the inside of the envelope used to transport the nails produced no quantifiable male DNA (Table 13) nor Y-STR alleles (data not shown).

Scratched Nail	Exogenous DNA Quantification	Unscratched Nail/Envelope	Exogenous DNA Quantification
1a	0.0	1d	0.0
1b	0.0	2d	0.0
1c	0.0	3d	0.0
2a	0.0	4d	0.0
2b	0.0	1 envelope	0.0
2c	0.0	2 envelope	0.0
3a	22.5	3 envelope	0.0
3b	9.2	4 envelope	0.0
3c	0.0		
4a	4.1		
4b	64.4		
4c	2.5		
Average	9.3		0.0

Table 13: Exogenous DNA Quantification of Transported Nails Post-Scratching and Envelopes used for Transportation. Numbers 1 – 4 indicate different females performing the scratching. Letters a – c represent an individual nail used for scratching, while d represents the thumbnail not involved in scratching. DNA yields are in pg/µL. Note that cross contamination did not occur, as thumbnails resulted in no exogenous DNA quantification. Nails used for scratching resulted in low exogenous DNA quantities after transport (avg. 9.3 pg/µL), while the swabs of the envelopes recovered no exogenous DNA.

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Sample #	Percent of Exogenous Y-STR Profile
1a	0%
1b	6%
1c	0%
2a	59%
2b	6%
2c	0%
3a	82%
3b	0%
3c	0%
4a	41%
4b	100%
4c	0%

Average	25%

Table 14: Percent of Exogenous Y-STR Profile Recovered from Post-Scratching Transported Nails. Note that several nails generated no alleles. Compared to post-scratching nails that were not transported, fewer Y-STR alleles were present after nails were transported (25% vs. 69%, Table 10, $p < 0.001$).

Cumulative Swabbing of Fingernails

Exogenous DNA quantities from cumulative swabbing nails with and without blood are shown in Table 15 and in boxplot form in Figure 7. The cumulative swab produced the most DNA (560.5 pg/µL), although contamination of the nails without blood did occur (59.7 pg/µL). Further, complete Y-STR profiles consistent with the blood were generated from the clean nails with the highest exogenous DNA yields (data not shown), clearly demonstrating that cumulative swabbing transfers biological material/DNA between the objects being swabbed. Finally, the second swab (dry swab on the two nails with blood) recovered an average of 68.1 pg/µL of exogenous DNA that the first swab left behind.

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Swab 1	Exogenous DNA Quantification	Swab 2	Exogenous DNA Quantification	Swab 3	Exogenous DNA Quantification
1	687.0	1	32.5	1	102.5
2	920.0	2	17.9	2	103.9
3	669.0	3	160.3	3	25.5
4	886.5	4	31.9	4	68.8
5	585.5	5	151.5	5	61.9
6	288.5	6	50.1	6	155.0
7	1,005.0	7	206.0	7	98.3
8	183.5	8	5.0	8	7.1
9	169.5	9	65.1	9	15.2
10	457.5	10	37.7	10	0.0
11	198.0	11	78.3	11	6.8
12	766.5	12	47.1	12	98.5
13	480.0	13	39.2	13	36.3
14	460.0	14	72.8	14	95.8
15	650.5	15	26.4	15	19.7
Average	560.5		68.1		59.7

Table 15: Exogenous DNA Quantification of Cumulatively Swabbed Nails. DNA yields are in pg/µL. Swab 1 was a moistened swab used on all four nails. Swab 2 was a dry swabbing of the two nails with blood. Swab 3 was a wet and dry swab of the nails without blood. A majority of the exogenous DNA was recovered by the first cumulative swab; however, the second dry swab of the nails with blood did recover DNA that was left behind. Some cross contamination occurred during cumulative swabbing.

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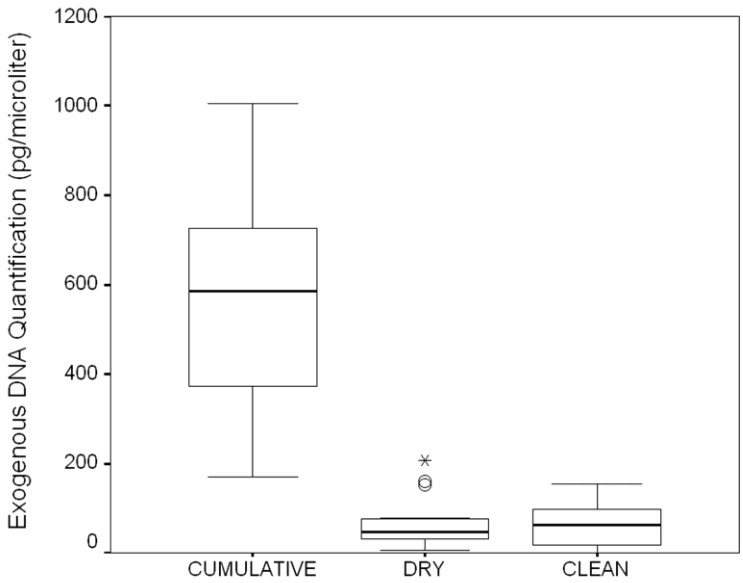

Figure 7: Boxplot of Exogenous DNA Quantities from Cumulative Swabbing. Y axis is DNA quantity in pg/µL. "Cumulative" indicates the moistened swab used on all 4 nails. "Dry" indicates a second, dry swab used on the 2 nails harboring blood. "Clean" indicates the double swab of nails without blood. Note that while the cumulative swab recovered a majority of exogenous material, it left behind exogenous material on the nails with blood, and deposited exogenous material on the nails without blood.

Effect of Nail Polish on DNA Amplification and Analysis

The three brands of nail polish tested did not prove detrimental to PCR and STR analysis using either an organic or kit-based extraction. The internal PCR control curves did not indicate inhibition when nail polish was present. Y-STR analysis of nails with polish resulted in complete profiles consistent with the blood, with the exception of one nail extract, which lacked a single Y-STR allele (data not shown).

Source of Endogenous DNA from Fingernails

An initial swabbing of untreated fingernails recovered an average of 1.9 ng/µL of human DNA, while the second swab produced 0.4 ng/µL. Subsequent soaking of the nail produced much higher DNA recovery (11.3 ng/µL).

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Discussion

The goal of the research presented here was to examine and subsequently enhance the collection and analysis of DNA evidence from fingernails. Our regular communications with medical examiners and SANEs led to several questions about retrieving such evidence following assault, and how it is processed at the crime laboratory. It quickly became apparent that a variety of methods are implemented to collect nail evidence, and little effort has been made to optimize collection methods for downstream analysis. The medical examiners/forensic pathologists in our region clip the nails of one hand with standard nail clippers over a small sheet, and package the nails, along with the sheet and clippers, into a single envelope that is included with any other evidence submitted to the crime laboratory. In contrast, one of these medical examiners noted that a morgue in which he had previously worked possessed a single pair of nail clippers when he arrived, which were used on all cases. A local SANE reported that she usually scrapes nails following assault because many of her patients do not want to have their, often highly decorated, nails cut. At neither level did the practitioner know if their practice for nail evidence collection was useful, much less optimal, for crime laboratory purposes, nor had they ever received feedback on such evidence.

Our discussion with crime laboratory personnel indicated that nail evidence is often received, but unless foreign material such as blood is visualized, nails are not usually processed. When they are, the foreign material is typically swabbed according to a laboratory's SOP, and DNA is isolated and analyzed as would be any other swab. Unless there are very large amounts of foreign material, the predominant DNA results are from the nail itself; however, mixtures are not uncommon. And as mentioned, there has been little communication between the DNA analysts and those collecting nail evidence regarding best practices.

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Based on this, we performed a detailed and thorough analysis of exogenous cell isolation from nails, DNA purification from the isolates, and testing of that DNA, all using methods routinely employed by crime laboratories. Our first goal was to examine isolation of exogenous cells from nails following scratching. At the behest of NIJ, we sought to better standardize scratching so that similar quantities of cells might be deposited on nails as different volunteers scratched. After speaking with biomechanical engineers, it was decided that the simplest and best option would be to have volunteers scratch with a reasonably constant amount of force by having a volunteer lay their forearm palm side up on a scale, zero the scale, and have a second volunteer press the middle three nails of a hand into the arm until the scale read 2 pounds. The volunteer then dragged their nails along the arm once, keeping the scale at approximately 2 pounds, which reddened but did not break the skin. Because this task was often not successful on the first attempt, we had volunteers practice 2 pound scratching by placing a computer mouse pad on the scale that roughly mimicked the arm in give and texture. In the end, this procedure was easy to conduct and helped remove some of the variability inherent in scratching.

In spite of this more standardized scratching regimen, preliminary experiments examining methods for exogenous cell recovery from nails were ambiguous, largely because consistent numbers of cells were not deposited through scratching. This meant that replicate nails gave differing results, making it difficult to establish an optimal method for retrieving exogenous cells/DNA. Owing to this, a different strategy was developed, in which one microliter of male blood was placed on the underside of a female nail. This allowed a large number of nails to be tested that harbored very similar amounts of exogenous DNA, along with a simple method for distinguishing DNA from the nail (female) from exogenous material (male).

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Comparisons of soaking the nail directly in digestion buffer, swabbing the blood from the nail, or scraping the blood from the nail, showed that all retrieved cells/DNA, but at varying levels. Soaking nails resulted in the most exogenous DNA, which makes sense, as all exogenous material was subjected to cell lysis. In contrast, swabbing or scraping nails will almost necessarily leave exogenous material behind. Swabbing was used to remove all blood that could be detected visually, including using a second, dry swab to collect any residual material, which our tests showed did exist. This technique also collected a good portion of the exogenous material, although it was significantly less that soaking. The lowest amount of exogenous DNA recovered was through scraping, most likely because not only did it not remove all exogenous material from a nail, but it also involved transfer of that material first to a medium to collect it (in this case weigh paper), and then to a tube for processing. This multi-step process is bound to result in cell loss.

Likewise, the three techniques resulted in different yields of cells from the nail itself, or from epithelial cells from the person who donated the nail. We tested this via swabbing nails two times and then soaking them, and found that many loose cells were recovered from the first swabbing, fewer from the second swabbing, and that the most DNA originated from the nail itself following soaking. This means that soaking an evidentiary nail in digestion buffer will release a large amount of DNA from it, which has obvious implications regarding mixtures with exogenous material. We found exactly that, wherein soaking female nails harboring male blood recovered the most exogenous DNA, but it also generated the most nail DNA, and hence had the largest drawback when autosomal STRs were analyzed. Indeed, most Identifiler® assays of soaked nails harboring blood produced major profiles of the nail donor, while the exogenous material had much lower peak heights. However, in some soakings the exogenous material

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produced the major profile. On the other hand, swabbing nails generated major profiles of exogenous DNA and minor profiles from the nail donor. Scraping resulted in mainly single source profiles of exogenous DNA; however, allelic dropout was prevalent, and endogenous alleles were still occasionally present. Autosomal STR mixtures resulting from soaking or swabbing were remedied when Y-STRs were assayed, and soaking resulted in the most complete male profiles. In contrast, Y-STR allelic dropout was more prevalent when nails were scraped.

Once soaking nails was identified as the optimal technique for obtaining exogenous DNA, we compared two standard methods for DNA purification based on it: organic extraction and a commercial kit (Qiagen). In these experiments, the kit resulted in significantly higher exogenous DNA yields than did the organic extraction, and thus it too was incorporated into our final protocol, however as noted above, subsequent experiments in which Amicon® columns were pretreated with yeast RNA showed that DNA was being trapped on untreated columns, and that DNA yields from organic extractions were as high or higher as the kit following column pretreatment. Testing different variables for eluting the DNA from the kit column indicated that three elutions using a reduced volume of kit elution buffer (20 µL/elution) recovered the most DNA, which was also incorporated into our protocol. In the end, the procedure that resulted in the most data from male blood spread on female fingernails was 1) soak the nail in kit lysis buffer, 2) purify the DNA using the standard kit reagents and its carrier RNA, 3) elute the DNA three times using 20 µL of elutant, and 4) assay the maximum amount of DNA possible (in our case 5.55 µL) for Y-STRs.

Using this protocol, we returned to scratchings of volunteers. The three center nails of females were used under a standard amount of force for three scratches along male forearms. In our preliminary studies autosomal STR testing resulted in partial nail profiles and minimal

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exogenous alleles, and cleaner profiles when testing Y-STRs, but still with few alleles. Once the optimized protocol was utilized, autosomal STR analysis produced strong endogenous profiles. These results were not surprising, given that large quantities of DNA were recovered from the nails themselves using the soaking method (more discussion of this follows). Exogenous autosomal alleles were also often called, albeit at much lower peak heights. Still, this represents a marked improvement from our original assays. However, the greatest difference between preliminary and optimized testing was seen using Y-STR assays, where the latter resulted in 69% of loci producing callable peaks, including complete 17 locus Y-STR profiles in about a quarter of cases. It should be noted that 2 pounds distributed among 3 fingers that became our standard protocol is not particularly high: it did redden volunteers' skin temporarily, but did not come close to breaking the skin. It seems likely that in a violent struggle substantially more force would be applied during scratching, and recovering exogenous DNA would be that much more successful.

Nail polish did not appear to have any effect on STR results regardless of the DNA purification methods used (organic or kit), although we did not test a large variety of polish brands or formulations. The IPC curves indicated there was no inhibition during PCR, and full profiles were obtained from the nail samples, with the exception of one that had one missing allele.

We also examined nail evidence transportation, given that in most cases such evidence will be collected by one entity (e.g., SANEs or MEs), and processed by a different one (crime laboratory personnel). Our test showed that contaminated and clean evidence transported together, which often occurs in such cases, did not result in transfer of detectable amounts of biological material from the former to the latter. On the other hand, all of our tests indicated that

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large amounts of exogenous material was lost from evidentiary nails following transport, which is certainly troublesome. Both blood applied to nails, which was allowed to dry and presumably adhere, as well as the more realistic scratching, showed significant loss of exogenous material. Attempts to recover exogenous cells lost after scratching from the envelope used for transport were unsuccessful. In this regard, it may be preferable to transport nail evidence in a container from which it would be easier to save material lost from nails. For example, nails might be transported in a clear microcentrifuge tube or similar, where the nail could be inspected microscopically *in situ*, and to which a digestion buffer could be added directly if desired. Obviously if such a container is used the nail evidence should be allowed to dry prior to packaging in order to prevent DNA degradation, but using a small closed container would help to preserve evidence.

Another aspect of nail evidence examined was processing nails individually or as a group. Naturally, the former requires more time and reagents; however, it is important to know if there are drawbacks to processing nails together, and if so, how substantial they are. Through our conversations with crime laboratory personnel, we found that a cumulative swabbing technique is often utilized, where a single swab is passed over multiple nails. This has two potential drawbacks: cross contamination of the nails, and relatedly, loss of exogenous material as it is collected from one nail and then deposited on nails swabbed subsequently. In our experiments, cells, while not detected visually, were readily transferred from a nail with exogenous material to one without, resulting in full Y-STR profiles from a ‘clean’ nail. Clearly this is problematic, particularly if a limited number of exogenous cells exist on only one nail that happens to be swabbed prior to others. Further, swabbing multiple nails with a single swab increases the chance of mixtures. During our testing we encountered instances of strong 17 locus Y-STR profiles

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inconsistent with the individual who was scratched. If such a nail were swabbed along with nails that harbored an assailant's cells, a mixture could easily result, or the assailant's DNA might be overwhelmed by more prevalent non-assailant material. However, if small numbers of cells existed on multiple nails, collecting most of them on a single swab could result in a successful profile that might otherwise not be obtained. In scratching experiments using the final protocol we obtained enough cells from single nails for Y-STR profiles despite relatively gentle scratching, indicating that, if processed correctly, it may not be necessary to cumulatively swab. On the other hand, we also obtained partial profiles that might have been improved using cumulative swabbing.

In the end, the goal of research such as that described here is to pass on objective, high quality, and useful information to practitioners. For fingernail evidence, that starts with those who collect that evidence, including SANEs and forensic pathologists. All three techniques—soaking, swabbing, and scraping—resulted in viable autosomal and Y-STR results. The ideal situation seems to be to clip the nails and package them as noted above, so that the maximum amount of nail material is obtained, in a form that allows crime laboratories flexibility in how to process the nails. If the victim is deceased, then the nails should be clipped and packaged accordingly. However, for living victims who do not want their nails clipped, thorough swabbing is most useful, followed by scraping. For pathologists, there seems to be no advantage to swabbing or scraping nails when they can always be clipped. The exception would be if nails are so short that clipping them produces almost nothing, thus a swabbing is more productive. It should also be noted that our tests showed that following a wet swabbing with a dry swabbing results in higher DNA yields, therefore if swabbing is utilized, a double swabbing technique is advantageous. Finally, since exogenous cells are lost during transport, packaging dry nails in a

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microcentrifuge tube or similar is advised. It may also be worthwhile to package nails individually, which would allow crime laboratory personnel more leeway in how to deal with nail evidence when it reaches them.

Once nail evidence arrives at the crime laboratory several tradeoffs must be considered. First is the ease and efficiency of processing the nails, weighed against the probative value of the results that might be obtained. When an assault victim survives and can provide information about if and which nail evidence might be most useful, it could be advantageous to focus on specifics, such as a hand or finger that scratched the assailant. However, when this information is unavailable, all nails may need to be tested, particularly if foreign material is not visually evident. The question then becomes: should nails be processed individually or in groups? As noted above, cumulatively swabbing nails necessarily increases the chances of mixtures. Given that we obtained full, unidentifiable Y-STR profiles from some female nails, mixtures are a real possibility, and an elimination sample from any intimate partners may need to be obtained if cumulative swabbing is used. Further, if a set of nails is cumulatively swabbed, exogenous cells from one nail can be deposited on the next nail, resulting in loss of evidence and possible misinformation. Alternatively, using a single swab on multiple nails may bring the number of exogenous cells to level where DNA testing is successful, and it certainly saves time and resources.

Regardless of if nails are processed individually or as a group, soaking them in digestion buffer will result in the most DNA, followed by swabbing and then scraping. However, soaking nails increases both exogenous and endogenous DNA yields, particularly the latter, which is disadvantageous when utilizing autosomal STRs, but can readily be overcome through Y-STR analysis, if the attacker is male and victim female. Decreasing the length of incubation in

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digestion buffer may also minimize the amount of nail DNA extracted, although this was not examined in the current study. At the opposite end is scraping, which produces far fewer nail alleles, but can result in dropout of exogenous alleles. Thus the preferred method in a crime laboratory should be based on the nature of the assault and laboratory capabilities. If the laboratory is limited to only autosomal STR analyses, or if exogenous material is readily visible, soaking nails is not advised, as it is likely that endogenous alleles will overshadow alleles from exogenous material. In contrast, if the assault was male-on-female, minimal exogenous material is visible, and Y-STR testing is available, the much higher quantities of DNA recovered via soaking a nail make it preferable, therefore it should be used in spite of the increase in endogenous DNA.

Overall, the goals of this research proposal were met. The strengths and weaknesses of the current strategies for processing fingernail evidence were examined, in an objective, quantitative manner. Important variables regarding nail evidence collection, DNA isolation, DNA purification, and DNA analysis were identified, and then procedures were developed to strengthen each. Pros and cons of different methods for collecting fingernail evidence were determined. Similarly, multiple methods for obtaining the most probative DNA, and successfully analyzing it, were examined. In the end, the success of this experimental strategy is best exemplified by comparing our early results using standard testing of nails involved in scratchings to those obtained using the optimized procedures. The former produced little or no data, while the latter produced worthwhile data in almost all cases. As proposed, this research culminated in multiple worthwhile recommendations for forensic practitioners who regularly collect and analyze fingernail evidence.

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