

TECHNICAL NOTE**CRIMINALISTICS**

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Collecting and Analyzing DNA Evidence from Fingernails: A Comparative Study^{†,‡,§}

ABSTRACT: Forensic practitioners and crime laboratories regularly collect and analyze fingernail evidence; however, the best techniques for processing such evidence have not been established. In this study, numerous aspects of fingernail evidence processing—collection of exogenous cells, transportation, purification of DNA, and STR analysis—were analyzed using fingernails harboring applied blood or epithelial cells from scratchings. Autosomal STR mixtures resulted when fingernails were soaked or swabbed, while scrapings rarely generated mixtures but exhibited allelic dropout. Y-STRs yielded single source profiles, with scrapings again showing dropout. A silica-based kit extraction recovered significantly more exogenous DNA than did organic extraction, neither of which was affected by nail polish. Swabbing nails in succession resulted in some cross-contamination from exogenous material, while transporting nails together did not, although there was loss of exogenous cells. Optimized nail processing produced complete Y-STR profiles of male volunteers from female fingernails following scratchings.

KEYWORDS: forensic science, DNA, sexual assault, fingernail evidence, scratching, Y-short tandem repeats, cumulative swabbing, DNA mixtures

According to the FBI's Uniform Crime Reports (<http://www.fbi.gov/about-us/cjis/ucr/crime-in-the-u.s/2011/crime-in-the-u.s.-2011/violent-crime/violent-crime>), there were over 80,000 forcible rapes reported in the United States in 2011. In the same year, more than 750,000 aggravated assaults occurred, a quarter of which involved the use of personal weapons such as hands, fists, or feet. Such assaults entail direct contact between a victim and assailant, at which point trace material, including fibers, hair, epithelial cells, or blood can be transferred. Owing to this, victims of physical assault are routinely checked for transfer evidence, given such evidence can be highly probative. If the victim survives, 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 inspects the body if the victim is deceased.

Scientists in the Forensic Biology Laboratory at Michigan State University regularly work with medical examiners in the

region who are trained to collect trace evidence from cadavers. Such evidence includes fingernails, given that the victim may have scratched the assailant or otherwise acted so that foreign material was deposited beneath them. 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. In contrast, local SANEs generally swab the underside of the nail or scrape it with a wooden applicator over clean paper, particularly if the victim does not want their nails cut. As is apparent, a high level of variability exists in the collection of fingernail evidence.

Once nail evidence reaches the crime laboratory, DNA isolation and analysis methods also vary. Microscopic examinations may be undertaken to see whether obvious foreign material exists (personal communications). Options for removing exogenous cells from nails include swabbing or scraping them, or placing the entire nail clipping directly into a tissue digestion buffer. Typical methods for DNA isolation include organic extraction or the use of a commercial kit or other more automated processes. Finally, DNA can be analyzed via autosomal or Y chromosome STRs, based on the presence of mixtures and the background of the case.

The amount of nail evidence submitted to crime laboratories in the United States that results in useful genetic data is unknown; however, the number of nail submissions is clearly substantial. Because of this, some academic studies of fingernail evidence have been conducted including our own published (1) and unpublished work, which acted as an introduction to the research performed here. All researchers who have examined the presence of foreign/exogenous DNA beneath fingernails have reported that it is, in general, quite rare, with the exception of intimate couples, where exogenous nail DNA is somewhat common (2,3). Studies in our laboratory confirm that foreign DNA beneath fingernails is uncommon, resulting in STR profiles

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consistent with the individual from whom the nails were produced, and generally few or no other alleles (unpublished). Other authors have reported mixed results from fingernails (4–8), even after 30 aggressive scratches (9), although in general, foreign alleles are relatively infrequent. All of these studies incorporated a single protocol to collect and analyze exogenous material from the nails; hence, there was no attempt to optimize DNA retrieval and analysis. Likewise, the crime laboratories we work with use a standard protocol for analyzing fingernail evidence, not one optimized for nails. Thus, there is a need for comparative studies to test each step in fingernail evidence processing, from exogenous DNA collection through autosomal STR and Y-STR analysis, in order to determine whether current practices are optimal for retrieving DNA evidence.

A number of other factors have the potential to influence fingernail DNA results. 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 material than actually did, while the latter results in the direct loss of evidence. Likewise, nails may be swabbed or otherwise processed 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. Further, if nails swabbed consecutively with a single swab have different exogenous materials on them, mixtures can result, which could possibly be avoided if nails are swabbed individually. On the other hand, individual swabbing not only utilizes more resources, but also may not accumulate enough DNA for successful testing.

The above variables, inconsistencies, and concerns led us to propose the research detailed below, which was designed to address and answer the many 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. A large number of experiments was designed and conducted to objectively and rigorously determine the best methods for collecting exogenous cells from fingernails, extracting and purifying DNA from those cells, and producing STR profiles. Further, the effects of transporting nails together, along with swabbing them cumulatively, were examined. In the end, an optimized procedure for processing nail evidence was developed.

Methods

All biological samples used in this study were completely de-identified, and procedures for their collection and use were approved by the MSU Institutional Review Board. Nails and buccal swabs were collected from volunteers, which were associated through randomly assigned numbers. Blood was drawn from a single male donor and used throughout all studies.

DNA Isolation and Purification Techniques

Supplies and solutions used in DNA isolation and purification were UV irradiated for 5 min ($\sim 2.5 \text{ J/cm}^2$) prior to use. For organic extractions, 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 1.5-mL microcentrifuge tubes,

containing the samples. A reagent blank was created by adding the same reagents to a 1.5-mL microcentrifuge tube. Tubes were incubated at 55°C overnight. Five hundred microliters of phenol was added, and tubes were vortexed for 15 sec and centrifuged at 14,000 rpm ($21,000 \times g$) 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 centrifuged at 14,000 rpm for 5 min. Aqueous layers were transferred to 30 kDa Amicon® Ultra-0.5 centrifugal filter devices (Millipore, Billerica, MA) and centrifuged at $14,000 \times g$ 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 $14,000 \times g$ for 10 min. Filters were washed two more times, once with TE and then with low TE (10 mM Tris—pH 7.5; 0.1 mM EDTA). The filters were inverted into new Amicon® tubes and centrifuged at $1000 \times g$ for 3 min to collect the DNA extracts in a final volume of 23 μL , which were stored at -20°C until use.

A QIAamp® DNA Investigator Kit (QIAGEN, Hilden, Germany) was also used for DNA purification. Four hundred microliters of Buffer ATL and 10 μL of the kit's proteinase K were added to completely submerge the nails. Digests were incubated at 55°C overnight. Four hundred microliters of Buffer AL and 1 μL of carrier RNA were added as per the kit's manual. DNA was eluted from the column using 20 μL of Buffer ATE. Further, repeated elutions were tested by transferring columns to clean microcentrifuge tubes and adding 20 μL of Buffer ATE. This was performed three additional times, yielding four separate eluates, and DNA recovery was quantified from each.

Quantification of Exogenous DNA

Exogenous (male) DNA was quantified using a Quantifiler® Y Human Male DNA Quantification Kit (Applied Biosystems, Foster City, CA). Eight standards were created via serial dilution of the Quantifiler® Human DNA Standard according to the Quantifiler® Kit user manual. Reactions 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 . Quantitative PCR was conducted on an iCycler™ Thermal Cycler (Bio-Rad, Hercules, CA) and fluorescence detected with an iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad). Cycling conditions were per the Quantifiler® Kit manual. Data were analyzed using the iQ™5 Optical System Software (Bio-Rad), and inhibition was assessed via internal PCR control (IPC) curves. Differences in final DNA volumes using the two extraction methods were taken into account for DNA quantification results.

Autosomal and Y-STR Analyses

STR analysis was conducted using an AmpFISTR® Identifier® PCR Amplification Kit or an AmpFISTR® Yfiler® PCR Amplification Kit (Applied Biosystems). Reaction volumes were scaled to 10 μL , a modification that was previously validated in our laboratory (data not shown). The volume of DNA added was based on Quantifiler® Y quantification, with a target of 0.75 ng. In some instances, including all scratching experiments (detailed below), the input DNA volume was maximized due to low DNA quantities.

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). AmpFISTR® Identifiler® Allelic Ladder or AmpFISTR® Yfiler® Allelic Ladder was included as appropriate for each injection. POP-7™ (384) Performance Optimized Polymer (Applied Biosystems) was used for electrophoresis, and allele calls were made using GeneMapper v4.1. Injection protocols were 1.2 kV/15 sec for Yfiler® and 1.6 kV/8 sec for Identifiler®.

Comparison of Collection Techniques for Obtaining Exogenous Cells from Nails

Studies evaluating exogenous cell collection techniques were based on organic extraction, using the protocol detailed above. Three cell collection techniques were compared through deposition of 1 μL of male blood (exogenous cells) on the underside of clipped female nails, which was allowed to dry for 24 h. First, a nail was placed directly into a 1.5-mL microcentrifuge tube, termed “soaking” ($n = 30$). Second, double swabbing (10) was performed using a Small Compressed CleanFoam® swab (ITW Texwipe, Kernersville, NC) moistened with digestion buffer to swab a nail, followed by a dry swab, until visually all blood was removed. The paired swab heads were placed together into a 1.5-mL microcentrifuge tube ($n = 30$). Third, a nail was scraped with a wood applicator (American Scientific Products, Charlotte, NC) over weigh paper, and the dislodged material was placed into a 1.5-mL microcentrifuge tube ($n = 31$). Finally, a positive control was created by adding 1 μL of male blood directly into a 1.5-mL microcentrifuge tube. DNA was isolated from each, exogenous DNA was quantified, and autosomal or Y-STRs were amplified.

The effects of cumulatively swabbing a set of nails were also examined, using the strategy depicted in Fig. 1. Each trial ($n = 15$) consisted of two female nails with male blood and two without. First, a CleanFoam® swab was dipped twice into digestion buffer and alternately used for eight strokes back and forth on a nail with blood, a nail without blood, another nail with blood, and another nail without blood. Second, a dry swab was used on the two nails with blood, to determine whether exogenous cells had been left behind. Third, the two nails without blood were double swabbed as above, to determine whether they

had been contaminated with blood; these two swabs were processed together. Swab heads were cut off and placed in a 1.5-mL microcentrifuge tube for organic extraction. Exogenous DNA was quantified and Y-STR analysis was performed.

Transportation of Fingernails Harboring Blood

The effect of transporting fingernails was examined using sets ($n = 18$) of one female nail harboring male blood and two female nails without blood packaged in coin envelopes, which were 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 purified via organic extraction as described above. Exogenous DNA was quantified, and Y-STR analysis was conducted. Results were compared to those of non-transported soaked nails to assess any loss of exogenous DNA during transportation.

Organic and Silica-Based Kit Extractions

The organic extraction was compared to the silica-based kit extraction (both described above) to assess relative DNA recovery. One microliter of male blood was deposited on 30 female fingernails. Half were processed with each extraction method using the soaking technique, and exogenous (male) DNA was quantified.

Inhibitory effects of nail polish on DNA recovery and analysis were examined using both extraction methods. Nail polish (Super Nails Natural Wonder [Revlon Inc.; red, New York City, NY], Wild Shine Black Crème [Wet N Wild, Orlando, FL], or Pure Ice Silver Crackle [Bari Cosmetics, Ltd., Vails Gate, NY]) was applied to the top of the nail and allowed to dry for 30 min. Blood was added and allowed to dry for 24 h. Nails were processed using organic ($n = 4$) and commercial kit extractions ($n = 9$) as detailed above. PCR inhibition was assessed via IPC curves, and Y-STRs were amplified.

STR Profiles from Scratchings Using Optimized Procedures

After determining the collection and extraction methods that produced the highest exogenous DNA yields, a scratching study was performed to better mimic forensic casework. Male volunteers rested their forearm, palm side up, on an Eat Smart Precision PRO Kitchen Scale (Health Tools LLC, Wyckoff, NJ). The scale was zeroed, and a female volunteer scratched the length of the forearm three times using her center three fingers and two pounds of force, which was enough to redden the skin but not draw blood. Fingernails were cut with scissors and processed using the commercial kit as detailed above, followed by three elutions with 20 μL of Buffer ATE. Autosomal ($n = 8$) and Y-STRs ($n = 19$) were amplified using the maximum volume of input DNA.

The effect of transporting nails following scratching was then examined. Four female volunteers scratched male volunteers as described above. Each set of three nails was placed in a coin envelope along with a thumb nail that was not used for scratching. Envelopes were sealed and transported in a backpack for 5 days. Each nail was placed in a separate 1.5-mL microcentrifuge tube. Envelopes were held upright and tapped several times, so that any residual material would fall to the bottom. Sterile Cotton-Tipped Applicator swabs (MediChoice®, Mechanicsville, VA) were moistened with 10 μL of digestion buffer and used to swab the inside, bottom portion of the envelopes. The kit-based extraction, using three 20 μL elutions, was conducted on the

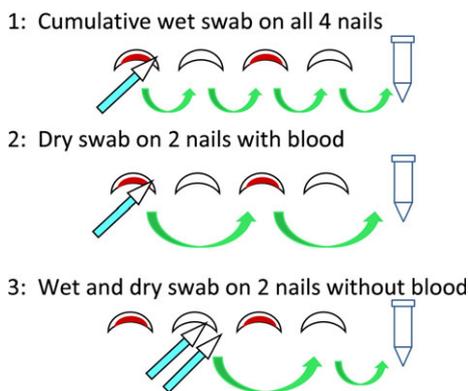


FIG. 1—Illustration of the cumulative swabbing procedure. (1) Swab, moistened with digestion buffer, was used on all four nails, alternating between a nail with blood and a nail without blood. (2) Nails with blood were re-swabbed using a dry swab. (3) Nails without blood were re-swabbed utilizing the double swab technique: a moistened swab followed by a dry swab.

transported nails and swabs. Y-STR analysis was performed as detailed above.

Statistical Tests

Statistical difference in DNA yields or STR profiles (t-test and ANOVA) were assessed using Excel (Microsoft Corporation, Everett, WA). Boxplots were produced in SPSS (IBM, Armonk, NY), wherein the box represents the middle 50% of the data, with the dark line indicating the median. Each “whisker” extends to the minimum or maximum values obtained, excluding outliers.

Results

Comparison of Collection Techniques for Obtaining Exogenous Cells from Nails

Deposition of a constant amount of blood on clipped nails allowed for an objective comparison of the three methods for retrieving exogenous cells, which could not be attained via scratching. Exogenous DNA recoveries from the soaking, swabbing, and scraping methods are shown in Fig. 2, and data summarized in Table 1. Results varied significantly among the methods (ANOVA $p < 0.00001$), with soaking resulting in a

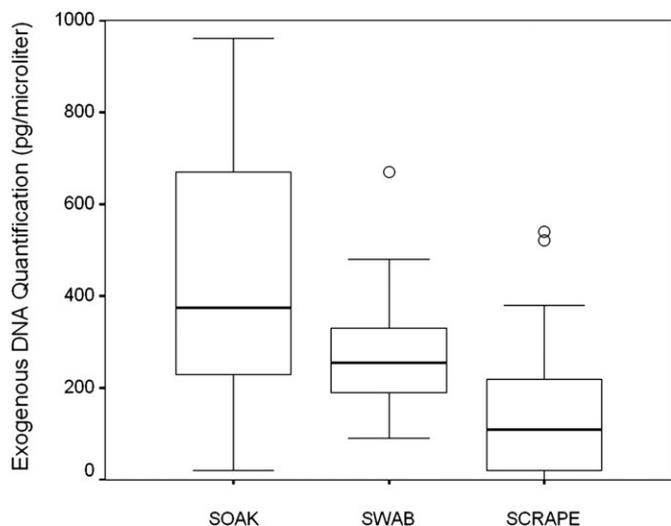


FIG. 2—Boxplots comparing exogenous DNA yields from blood on nails using each cell collection method. The x axis indicates the collection method while the y axis is the DNA quantity in pg/μL. Mild outliers are indicated by an open circle. Significantly different amounts of DNA were recovered among the techniques.

TABLE 1—Comparison of exogenous DNA yields and percent of STR profiles obtained by collection method.

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

significantly greater yield of exogenous DNA (avg. 433.7 pg/μL) than swabbing (avg. 275.1 pg/μL, $p = 0.007$), which in turn recovered more exogenous DNA than did scraping (avg. 146.3 pg/μL, $p < 0.001$). In comparison with the controls of 1 μL of blood added directly to a digestion (avg. 449.7 pg/μL), soaking averaged 96% exogenous DNA recovery, while swabbing and scraping averaged 61% and 33%, respectively. Because soaking nails in digestion buffer was superior for retrieval of exogenous cells, subsequent testing utilized the soaking method.

When these same samples underwent autosomal STR analysis, several trends emerged. First, profiles of soaked or swabbed nails generally contained all possible exogenous alleles. However, both methods usually resulted in mixtures of exogenous and nail DNA. In the majority of instances, soaking produced a major profile from the nail (e.g., Fig. 3a) or roughly equal nail/exogenous DNA contributions. The major profile was periodically from the exogenous cells (about 1/3 of cases), which in one instance was single source. In contrast, swabbing produced major profiles from the exogenous cells (Fig. 3b), one of which was single source. Autosomal STR profiles of scraped nails generally contained only alleles from exogenous material (Fig. 3c), although there was significantly more dropout than when using the other two techniques ($p < 0.05$). Soaked, swabbed, and scraped fingernails that previously produced autosomal STR mixtures generated complete single source profiles when Y-STR analysis was performed (e.g., Fig. 3d), although scrapings displayed some allelic dropout.

Cumulatively swabbing nails with and without blood using a single swab recovered an average of 560.5 pg/μL of exogenous DNA (Fig. 4). Dry swabbing of the two nails harboring blood recovered an average of 68.1 pg/μL of exogenous DNA that the first swab left behind. Swabbing the two nails without blood following cumulative swabbing showed that contamination and cell loss from the nails with blood occurred, resulting in 59.7 pg/μL of DNA. Further, complete Y-STR profiles consistent with the blood were generated from the clean nails after cumulative swabbing.

Transportation of Fingernails Harboring Blood

Cross-contamination between nails with and without blood was not a substantial problem during transport, as evidenced by the low exogenous DNA quantity recovered from nails without blood (avg. 21.5 pg/μL, Fig. 5). Y-STR amplification of the five nails without blood that generated the highest exogenous DNA yields resulted in no profiles consistent with the blood. Two of these “clean” nails, from the same female donor, produced identical, unidentified Y-STR profiles. Two from another female donor produced a different Y-STR profile inconsistent with the blood, while the fifth did not generate alleles. Hence, the bulk of the exogenous DNA recovered from the nails without blood seems to have originated from other individuals or materials the volunteers had contact with. However, in spite of no obvious cross-contamination during transport, a significant amount of exogenous material on nails was apparently lost through the process, as the average exogenous DNA yield was 270.3 pg/μL, whereas soaked nails not undergoing transport (from Table 1) had an average yield of 433.7 pg/μL ($p < 0.05$).

Organic and Silica-Based Kit Extractions

Quantitative PCR demonstrated that the QIAamp[®] DNA Investigator Kit, using a single elution, recovered a significantly

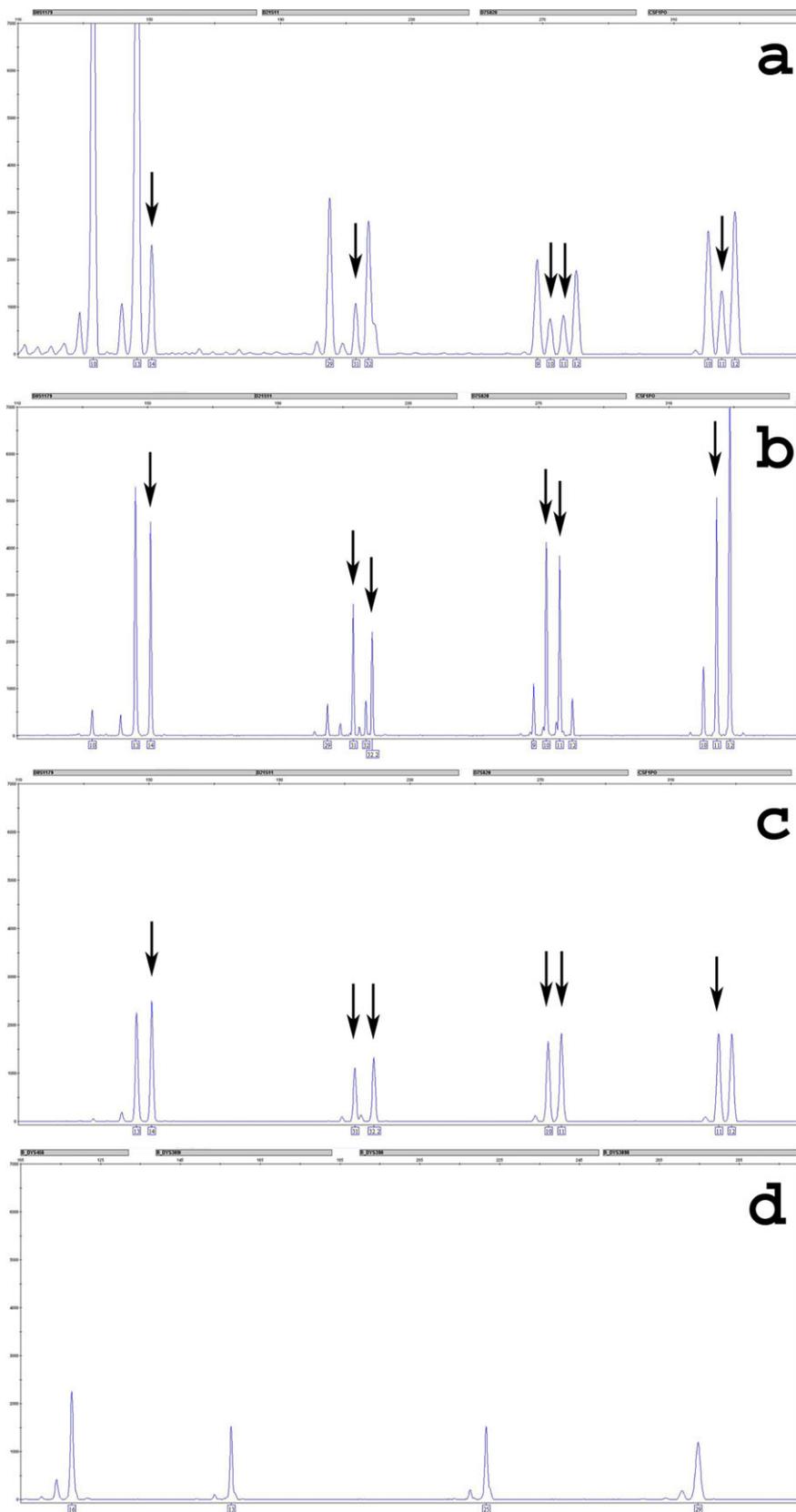


FIG. 3—Exemplary electropherograms of autosomal STR profiles from a soaked (a), swabbed (b), or scraped (c) nail harboring blood. Arrows indicate alleles specific to the exogenous material. Y-STR analysis of a swabbed nail is shown in panel d. The y axis of all electropherograms is scaled to 7000 RFU. Autosomal STR loci shown are D8S1179, D21S11, D7S820, and CSF1PO (blue channel for Identifiler®). Y-STR loci shown are DYS456, DYS389I, DYS390, and DYS389II (blue channel for Yfiler®). Allele calls are indicated in boxes below the peaks. Note that both soaking and swabbing resulted in STR mixtures, with soaking typically producing a major profile from nail DNA, and swabbing a major profile from exogenous material. Scraping recovered fewer nail alleles than the other methods, although there was dropout of exogenous DNA alleles. All DNAs resulted in single source profiles when Y-STR analysis was performed.

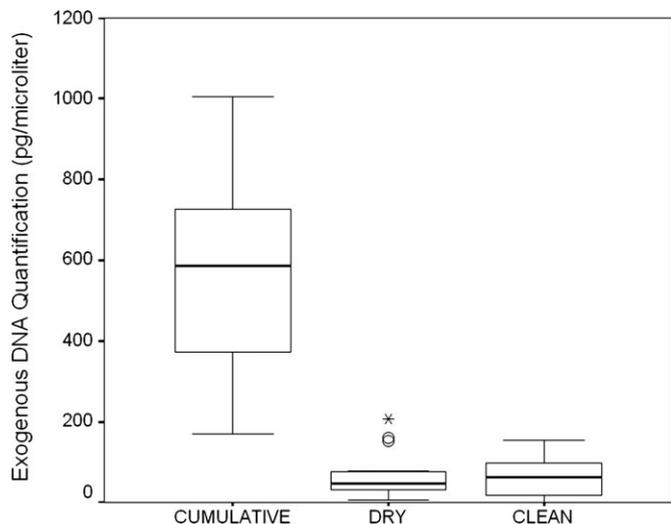


FIG. 4—Boxplots comparing exogenous DNA yields from cumulatively swabbed nails, the first and third of which harbored blood (Fig. 1). The x axis indicates swabbing procedure (cumulative swab, dry swab on nails with blood, and double swab on 'clean' nails without blood). The y axis is the exogenous DNA quantity in pg/μL. Mild outliers are indicated by an open circle, while extreme outliers are shown by an asterisk. The cumulative swab recovered the majority of exogenous DNA, while a second dry swab recovered exogenous cells that were left behind. The cumulative swabbing resulted in some cross-contamination.

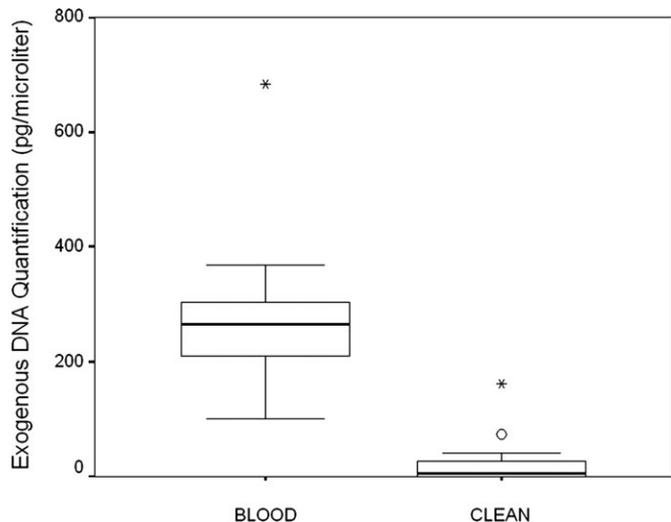


FIG. 5—Boxplots comparing exogenous DNA yields from nails with or without blood after transporting them together and using the soaking technique. The x axis indicates type of nail (harboring blood, or 'clean') while the y axis is the DNA quantity in pg/μL. Mild outliers are indicated by an open circle, while extreme outliers are shown by an asterisk. Cross-contamination apparently did not occur during transport; however, a significant amount of DNA was lost compared to nails that did not undergo transport (Fig. 2).

greater amount of exogenous DNA than did organic extraction (avg. 13.8 ng and 10.2 ng, respectively, $p < 0.05$). Testing of the kit's column elution step showed that DNA was successfully recovered from up to four 20 μL elutions. The first, second, and third elutions yielded an average of 414.9 pg/μL, 337.7 pg/μL, and 225.0 pg/μL of DNA, respectively, while the DNA quantity from the fourth elution was negligible. Subsequent experiments utilized three 20 μL elutions.

The three brands of nail polish tested did not prove detrimental to PCR amplification nor STR analysis, following either

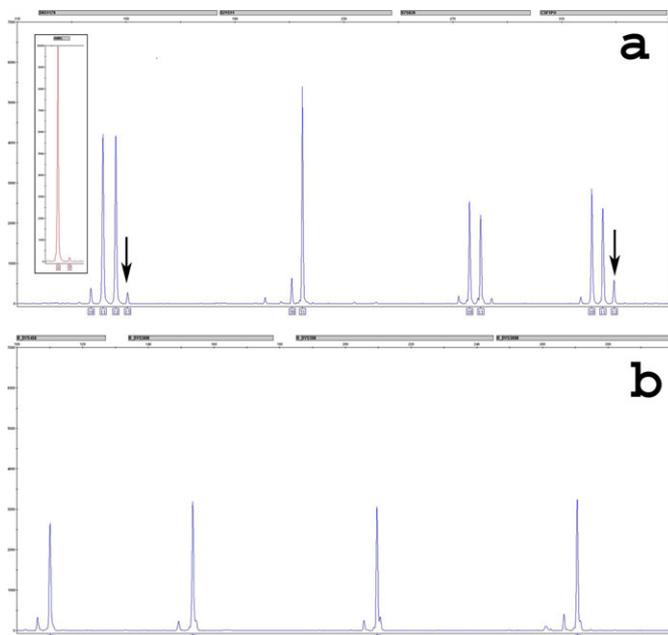


FIG. 6—Exemplary electropherograms from nails following scratching using the optimized procedures. (a) Autosomal STR electropherogram of a soaked nail. Arrows indicate alleles specific to the exogenous material. (b) Y-STR electropherogram of the same soaked nail in panel a. The y axis of both electropherograms is scaled to 7000 RFU. Autosomal STR loci shown are D8S1179, D21S11, D7S820, and CSF1PO (blue channel for Identifiler®). Amelogenin is shown in the insert on the left side; note the very small Y peak. Y-STR loci shown are DYS456, DYS389I, DYS390, and DYS389II (blue channel for Yfiler®). Allele calls are indicated in boxes below the peaks. The male autosomal STR alleles are present, albeit at much lower peak heights compared to the nail alleles, while Y-STR analysis resulted in a single source profile of the male being scratched.

organic or kit-based extraction. The IPC curves showed no indication of inhibition from nail polish, and Y-STR analysis resulted in complete profiles consistent with exogenous cells, with the exception of one nail extract that lacked a single allele (data not shown).

STR Profiles from Scratchings Using Optimized Procedures

Scratchings (three scratches using the center three fingers at two pounds of force) in conjunction with nail soaking, kit extraction, and the maximum amount of input DNA, resulted in strong autosomal STR profiles of the nail donor, while alleles from the exogenous cells, though often present, were substantially weaker and in some instances were not detectable (e.g., Fig. 6a). In contrast, Y-STR analysis produced several full profiles (e.g., Fig. 6b). Overall, 69% of Y-STR loci had alleles consistent with the known exogenous profile. One nail had a Y-STR profile that clearly originated from a different, unknown male (not represented in the 69% average).

No cross-contamination was found between clean nails and those used for scratching following co-transportation. However, Y-STR profiles from transported nails used for scratching produced significantly fewer alleles (25%) than did nails processed immediately following scratching (69%, $p < 0.001$). Further, only one transported nail generated a full Y-STR profile, while several had no alleles. Swabbing the inside of the envelopes used to transport the nails yielded no quantifiable exogenous DNA nor Y-STR alleles consistent with the scratched individual (data not shown).

Discussion

The goal of the research presented here was to test and compare methods for the collection and analysis of DNA evidence from fingernails. Our communications with medical examiners, SANEs, and crime laboratory personnel led to several questions about retrieving such evidence following assault, and how it is most effectively processed in the crime laboratory. It quickly became apparent that the collection of nail evidence is not standardized and essentially has not been optimized. Neither MEs nor SANEs knew whether their practice for nail evidence collection was useful, much less optimal, nor had they ever received feedback on such evidence from crime laboratories. Similarly, crime laboratories often use protocols on nails developed for other types of evidence, such as swabbing them, that may or may not be ideal.

Owing to this, we performed a detailed and systematic analysis of exogenous cell isolation from nails following scratching, DNA purification from the isolates, and profiling of that DNA, all based on methods familiar to crime laboratories. In order to quantitatively compare exogenous cell retrieval methods, a consistent amount of cells needed to be deposited onto nails. To accomplish this, male blood was placed on female nails, not because it mimics all assaults (although the vast majority of sexual assaults involve a male perpetrator and female victim), but because it allowed a simple, direct assay—Y chromosome quantification—of how much exogenous DNA was being recovered.

Once this procedure was developed, comparisons of cutting and soaking nails directly in digestion buffer, swabbing the blood from nails, or scraping the blood from them commenced. Results showed that all techniques retrieved cells/DNA, but at varying levels. Soaking nails resulted in significantly more exogenous DNA, which makes sense, as all exogenous material was subjected to cell lysis. In contrast, swabbing or scraping nails likely leaves exogenous material behind, and did so in these experiments, most notably for scrapings, where cells have to be scraped onto a surface (in our case weigh paper) and then transferred to a tube, which could easily result in cell loss.

In addition to recovering exogenous DNA, the three cell retrieval methods also recovered DNA from the nail itself, generating mixtures. Soaking nails harboring blood generally resulted in situations wherein the nail produced the dominant profile, yet the exogenous profile was readily discernible. At the opposite extreme, scraping the blood from the nail resulted in the fewest nail alleles, however, there was far more dropout in the exogenous profiles. Between these was swabbing, which produced both nail and blood alleles, although the exogenous material gave the major profile, which was almost always complete. In all instances, Y-STR testing produced clean single source profiles, but again scraping suffered from some allelic dropout. Given all of this, swabbing may be the best method overall, although soaking could be advantageous if there is little or no visible evidence that exogenous material is present, while scraping could be advantageous when exogenous material is readily observable (though see scratching results below).

Another aspect of exogenous cell collection from nail evidence examined was testing nails individually or as a group. Clearly, the former requires more time and reagents; however, it is important to understand whether there are drawbacks to cumulatively processing nails, and if so, how substantial they are. In the current study, while not detected visually, cells were readily transferred from a nail with exogenous material to one without during cumulative swabbing, resulting in full Y-STR profiles from “clean” nails. This has the potential to be 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 full Y-STR profiles of unknown origin, which indicates foreign DNA was already present on the volunteers' nails. 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 viable profile that might otherwise not be obtained. Clearly, there are pros and cons to both strategies (discussed below).

The methods for purifying DNA from nail evidence also played a key role in optimizing its analysis. The commercial kit resulted in significantly higher exogenous DNA yields than did organic extraction, and additional DNA was recovered using multiple elutions. Knowing this is critical if minimal amounts of DNA exist in the first place, and it led to a final protocol of three elutions using 20 μ L of elutant, which differs from the manufacturer's protocol. In the end, because we knew that our scrapings were likely to result in very low levels of exogenous DNA, the overall procedure settled upon was: (i) soak the nail in kit lysis buffer, (ii) purify the DNA using the standard kit reagents and carrier RNA, (iii) elute the DNA three times using 20 μ L of elutant, and (iv) assay the maximum volume of input DNA possible for STR analysis.

After cell collection and DNA extraction procedures were established, they were tested on nails following scratching. A standardized method was used, wherein volunteers scratched with two pounds of force using three nails, which did redden volunteers' skin temporarily, although it did not come close to breaking the skin or leaving any trace after an hour or so. It seems likely that in a violent struggle substantially more force would be applied during scratching, and recovering exogenous cells/DNA would be that much more successful. Autosomal STR analysis of nails after scratching produced strong nail profiles, along with exogenous alleles, albeit at much lower peak heights. Y-STR analysis resulted in a higher number of exogenous alleles than autosomal STR analysis, including several complete 17 locus profiles. These results are far better than previous studies of fingernail evidence (2–8), including one where volunteers were scratched extremely hard as many as 30 times, even drawing blood (9). Again, this accentuates how important it is to optimize all facets of nail DNA evidence collection and analysis instead of simply applying standard operating procedures to this unique form of evidence.

It was also important to assess how other variables affected exogenous DNA yields and analyses. For instance, nail polish did not appear to have any effect on STR results regardless of the DNA purification method used (organic or kit), although we did not test a large variety of polish brands or formulations. Nail evidence transportation was examined, given that in most cases such evidence will be collected by one entity (e.g., SANEs or pathologists) and processed by a different one (crime laboratory personnel). Our tests showed that contaminated and clean evidence transported together did not result in transfer of detectable amounts of exogenous material from the former to the latter. On the other hand, these experiments indicated that large amounts of exogenous material were lost from evidentiary nails following transport, which is certainly troublesome. Blood applied to nails, which was allowed to dry and presumably adhere, was retained at much higher levels than were cells following the more realis-

tic scratchings, which showed a significant loss of DNA based on the number of Y-STR alleles called. The lost exogenous material was not readily recoverable through swabbing the envelope used for transport, thus this represents an important consideration for nail evidence processing.

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, this begins with the individuals who collect it. The ideal situation seems to be to clip nails, however, if a living victim does not want their nails clipped or nails are too short for clipping, double swabbing is most useful, followed by scraping. The best packaging of clipped nails would be in a container where dislodged exogenous material would not be lost and is in a form that is easily manipulated in the crime laboratory. A simple solution might be to transport nails 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, helping to eliminate loss of exogenous material. Of course, it would be important to assure that the evidence is dry so that DNA degradation does not occur prior to processing, or at least that the air volume is large enough so the evidence could dry. It seems it would be relatively straightforward to develop such methods, although we did not pursue them in the current study.

Once nail evidence reaches the crime laboratory, several trade-offs 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 most likely scratched the assailant. However, when such 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, processing nails together increases the chances of mixtures, and at a minimum elimination samples from any intimate partners should be obtained. Conversely, using a single swab on multiple nails may bring the number of exogenous cells to a level where DNA testing is successful, and it certainly saves time and resources. In our scratching experiments using the optimized protocol, enough cells were obtained from single nails for development of Y-STR profiles, despite relatively gentle scratching, indicating that if processed correctly it may not be necessary to cumulatively swab. However, partial profiles were also obtained in these experiments, which might have been improved upon using cumulative swabbing. Finally, the type of STR testing to be performed must be considered. If a male attacks a female and there is little or no exogenous material on the victim's nails, maximizing DNA recovery through soaking and performing Y-STR testing can result in the most probative data. On the

other hand, if a different assault scenarios exist, if there is a large amount of exogenous material present, or if only autosomal STRs can be tested, then a method that releases less nail DNA, such as swabbing or scraping, is beneficial. All of these factors deserve consideration as nail evidence is collected and processed.

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