TECHNICAL NOTE

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An Evaluation of the Relevance of Routine DNA Typing of Fingernail Clippings for Forensic Casework

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ABSTRACT: DNA extracted from fingernail clippings of victims in forensic cases is a possible source of DNA from the perpetrator in cases where victims struggled or defended themselves. The source of this DNA on a victim's fingernails could possibly originate from contact with the suspect's blood, saliva, semen or scratched skin. In this technical note we evaluate the relevance of routine DNA typing of fingernail clippings in the forensic biology laboratory when, in real casework, normally only small quantities of nail material is sent. This was carried out by extracting DNA from fingernail clippings from a number of volunteers, before and after aggressively scratching other volunteers. No blood was drawn from the scratching, but skin flakes were observed under the nails before cutting and subsequent DNA typing. The DNA extracted was then typed using the STR systems: HUMTHO1, HUMTPOX and HUMCSF1PO (CTT triplex) and the system of D1S80. These profiles were compared with profiles achieved by similar typing of buccal swabs as a reference from each volunteer. In this study, the profile detected from each volunteer's clippings was the same before and after scratching, and matched the profile of the corresponding volunteer as defined by typing each volunteer's reference buccal swab. Fingernail clippings that are sent to our lab in actual casework are usually so small that additional treatment by swabbing or removing debris from below the clipping is not possible. For this reason, in this simulation the entire clippings were used for DNA extraction, to maximize the possibility of finding an additional profile.

In conclusion, the findings from this study show that although the profiles obtained when typing fingernail clippings are those of the donors themselves, we suggest that typing of fingernail clippings should be carried out in forensic cases only when relevant. We would suggest that fingernail clippings not be *routinely* sent to the biology laboratory as items of evidence to be tested.

KEYWORDS: forensic science, fingernail clippings, DNA typing, polymerase chain reaction, contamination, D1S80, CSF1PO, THO1, TPOX, short tandem repeats

Fingernail clippings or torn nail fragments from victims in assault cases, principally sexual assault cases, are occasionally sent to forensic laboratories as a possible source of DNA originating from a suspect (1). This DNA could possibly originate from sources other than the skin scratched from the suspect. During an attack, the victim's hands and fingernails could possibly come in contact with a perpetrator's blood, or semen or saliva, which may leave traces on the victim's fingernails. It has been reported that a foreign profile can occasionally be obtained from debris scraped from underneath nails, but nail clippings, and not material from underneath nails, are usually sent to forensic laboratories (2). The clippings most often sent to our lab in actual casework are of such a small quantity that in order not to lose any foreign material, no preliminary examination is carried out to try and identify specific sources of DNA. These clippings provide an ample source of DNA originating from the victim (or donor of the nails). In order to identify the presence of a secondary source of DNA originating from a possible suspect in the mass of a victim's DNA, PCR amplification of the nail clippings would be the preferred method (3). It has been suggested that clippings provide such a minimal contribution of evidentiary value in an investigation (4) that they no longer need to be sent as items of evidence. Before accepting this notion we decided to investigate the relevance of routine DNA typing of fingernail clippings as received in actual casework, in order to possibly recover a suspect's DNA profile. In addition, we realize that a realistic concern within the forensic science community is the prevention of false positives when using PCR (5). In addition to the possibility of identifying a suspect's DNA from nail clippings, we wanted to address the unwanted possibility of retrieving nonrelevant opportunist profiles, different than the victim's profile, if routine nail examination was to be undertaken in the laboratory. In this paper the authors present results of DNA typing of PCR amplification products from fingernail clippings from volunteers before and after skin scraping of other volunteers.

Materials and Methods

Four to five nail clippings were collected from each volunteer. Fingernail clippings varied in length and hardness from volunteer to volunteer. A buccal swab from each volunteer was also collected as a reference control for comparison with the amplification products generated from the nail clippings of the corresponding donor. In order to simulate a forensic situation, the same volunteers were paired and one volunteer was then requested to aggressively scratch the arm of the other volunteer, only to the point of superficial abrasion. The scratching did not result in the drawing of blood, but skin par-

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ticles were observed under the nails before clipping. These fingernails were then cut and collected in preparation for DNA typing.

Sample Treatment

The nail clippings were treated as all usual items of evidence received at the laboratory. No swabbing of nails or debris scraping was done, inasmuch as the samples we receive at the lab are usually small and threadlike and do not present surface areas for these procedures. DNA was extracted from the entire fingernail clippings using the phenol/chloroform extraction method (6). In addition, two sets of nail clippings (from paired volunteers B and D) had DNA extracted using the Chelex method (7). Sample DNA recoveries were estimated to be between 5 and 15 ng/μL as determined using a 1% agarose minigel containing ethidium bromide.

DNA Typing Methods

Four DNA loci were typed from the respective DNA extracts. Approximately 0.5 to 5 ng of DNA per sample were taken for amplification. PCR amplification was carried out simultaneously for three STR loci—CSF, TPOX and THO1, (CTT triplex), according to the Promega Manual (8), with a modification of 16 μg BSA being added to the amplification mixture. The fourth locus to be typed was D1S80, (using approximately 10 ng DNA per sample), which was amplified as described by Sajantila et al. (9), with a modification in this reaction of 3.2 μg BSA being added to the amplification mixture. Quantitation of PCR products was done before separation on polyacrylamide gels. The D1S80 PCR products were separated on a 0.4 mm thick, 6% polyacrylamide gel. The triplex products were separated on 4% denaturing polyacrylamide gel. Products were visualized using the silver-staining method (10).

Results and Discussion

The results shown in Table 1 represent the data for the triplex CTT and D1S80 typing from the fingernail clippings of the volunteers (single and paired) and the corresponding buccal swabs as references.

As can be seen from the table, the DNA profiles retrieved from the nail clippings of the various volunteers matched the profiles typed from the buccal swabs of the same volunteers. DNA profiles from fingernails of volunteers who had scratched other volunteers did not provide a profile differing from their own, as determined by the typing of the reference. No multiple profiles were noticed as might be expected if a sufficient quantity of biological material had collected under the fingernails when the scratching took place. The fingernails treated by Chelex extraction and subsequent PCR amplification also failed to provide a profile differing from the donor of the nails.

In our actual casework experience, only small quantities of fingernail material are received for DNA comparison. Our study employed similar quantities of fingernail material for DNA typing. This may or may not be a cultural or regional characteristic which subsequently influences results obtained when searching for a foreign profile belonging to a suspect. It was also noted that no nonspecific DNA profiles appeared after PCR amplification of the nail clippings and the subsequent silver staining of these products. A recently published article suggests that DNA can so easily be transferred that even a casual handshake can provide a profile originating from the person whose hand was shaken (11). If this were the case, we would have expected that fingernails, which so easily come in contact with many objects touched by other people, would have provided what we referred to as "opportunist profiles." No special precautions were undertaken (i.e., hand washing, nail cleaning) before the fingernails were collected and treated. Not only did we fail to see impressions of random DNA, we did not observe the profile of the person who had purposely been scratched. This suggests that although there is an ever-present fear of contamination when using the PCR method, especially with small samples, a reliable result can be achieved if sufficient precautions are undertaken during the process (5).

In conclusion, from the data presented in this work, we see that it is safe to assume that if normal precautions to prevent contamination during PCR typing (as is excepted within the forensic community) are carried out, a reliable result can be expected. In addition, we saw that the profiles received from all fingernail typing matched that of the donor, and therefore we feel that the routine typing of fingernail clippings does not contribute essential information in forensic casework. An additional contribution from this study might also be to suggest that in cases where blood or saliva are not available, fingernail clippings could provide a reference sample for DNA typing.

TABLE 1—*PCR typing results of volunteer's fingernail clippings before and after scratching, and reference samples.*

Sample	D1S80	THO1	TPOX	CSF
	VOLUNTEERS—FINGERNAIL CLIPPINGS			
A	28,28	6,9	8,9	9,10
B	24,24	6,10	11,11	10,12
C	18,25	8,9	8,11	11,11
D	21,24	6,9.3	8,9	11,11
E	Fingernails	not typed		
F	18,22	7,9.3	11,12	11,12
	REFERENCES—BUCCAL SWABS			
A	28,28	6,9	8,9	9,10
B	24,24	6,10	11,11	10,12
C	18,25	8,9	8,11	11,11
D	21,24	6,9.3	8,9	11,11
E	24,24	8,9	8,12	11,12
F	18,22	7,9.3	11,12	11,12
	*PAIRED VOLUNTEERS—FINGERNAIL CLIPPINGS			
A/B	28,28	6,9	8,9	9,10
F/D	18,22	7,9.3	11,12	11,12
B/D	24,24	6,10	11,11	10,12
C/B	18,25	8,9	8,11	11,11
D/B	21,24	6,9.3	8,9	11,11
B/E	24,24	6,10	11,11	10,12

* The first letter designates who performed the scratching in the pair.

References

1. Keating SM, Allard JE. What's in a name?—Medical samples and scientific evidence in sexual assaults. Med Sci Law 1994 Jul;34(3): 187–201.
2. Wiegand P, Bajanowski T, Brinkmann B. DNA typing of debris from fingernails. Int J Legal Med 1993;106(2):81–3.
3. Tahir MA, Watson N. Typing of DNA HLA-DQ alpha alleles extracted from human nail material using polymerase chain reaction. J Forensic Sci 1995 Jul;40(4):634–6.
4. Hochmeister MD, Eisenberg AJ, Budowle B, Binda S, Serra A, Whelan M, et al. The development of a new sexual assault kit for the optimization of collection, handling, and storage of physical and biological evidence. Proceedings from the First European Symposium on Human Identification: Promega Corporation 1996;21–6.
5. Kwok S, Higuchi R. Avoiding false positives with PCR. Nature 1989;339:237.
6. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989; Section 9.16.

7. Walsh PS, Metzger DA, Higuchi R. Chelex © 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 1991;10:506.
8. Promega Technical Manual Gene Print TM STR Systems. Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711-5399 Revised 5/98.
9. Sajantila A, Budowle B, Strom M, Johnsson V, Lukka M, Peltomen M, et al. PCR amplification of alleles at the D1S80 locus: Comparison of a Finnish and a North American Caucasian population sample, and forensic casework evaluation. Am J Genetics 1992;50:816–25.
10. Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen, RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Genet 1991;48:137–44.
11. Oorschot RA, Jones MK. DNA fingerprints from fingerprints. Nature 1997;387:767.

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