## TECHNICAL NOTE

*Theodore D. Anderson,[1] M.F.S.; James P. Ross,[1] B.S.; Rhonda K. Roby,[1] M.P.H.; Demris A. Lee,[1] M.S.F.S.; and Mitchell M. Holland,[1]Ph.D.*

# A Validation Study for the Extraction and Analysis of DNA from Human Nail Material and Its Application to Forensic Casework*

**REFERENCE:** Anderson TD, Ross JP, Roby RK, Lee DA, Holland MM. A validation study for the extraction and analysis of DNA from human nail material and its application to forensic casework. J Forensic Sci 1999;44(5):1053–1056.

**ABSTRACT:** A validation study was conducted to demonstrate that deoxyribonucleic acid (DNA) could be successfully extracted from human nail material and analyzed using short tandem repeat (STR) profiling and/or mitochondrial DNA (mtDNA) sequencing. This study involved the development of a DNA extraction protocol that includes a cleaning procedure designed to remove external contaminants (e.g., biological, chemical). This protocol was used to test human nail material that had been soaked in whole blood from a second donor and coated with gold-palladium to simulate scanning electron microscopic analysis. The results showed no indication of a mixture and were consistent with that of the nail donor. Fresh human nail material usually yielded both STR profiles and mtDNA sequence information; however, aged human nail material (~eight years old) yielded only mtDNA sequence information. Upon completion of the validation study, the extraction protocol was used for the analysis of a torn fingernail fragment recovered from the scene of a violent homicide in 1983. A partial STR profile and mtDNA sequence information indicated that the fingernail fragment was excluded as originating from the suspect and was, in fact, consistent with originating from one of the victims.

**KEYWORDS:** forensic science, DNA typing, fingernail, validation, short tandem repeat, Home Office Quadruplex I, HUMV WFA31, HUMTH01, HUMF13A01, HUMFESFPS, mitochondrial DNA, sequencing

Forensic deoxyribonucleic acid (DNA) testing is a powerful technique for human identification and/or the resolution of criminal and civil legal disputes. One powerful characteristic of DNA testing is that it may be applied to a wide variety of biological sources. Some of these sources include blood (1,2), semen (3), hair (4–6), saliva (7), bone (8–14) and teeth (15–17). In addition, human nail material has been identified as a potential source of biological material for forensic DNA testing (18,19).

The collection of trace evidence from beneath the fingernails of victims of violent crime is one part of a routine autopsy examination. However, the collection of unknown fingernail fragments from a crime scene is much less common. The forensic examination of human nail material typically includes striation pattern analysis and/or tear comparisons. In addition, the analysis of human nail material utilizing immunological assays and conventional serological markers (e.g., ABO) has been performed (20–25).

The analysis of DNA extracted from human nail material has been utilized in both clinical diagnostics and research (26,27). However, its application to forensic, evidentiary material has been limited to reverse dot-blot technology at the Human Leukocyte Antigen (HLA) DQA1 locus (18,19). Hence, a validation study was performed to demonstrate that DNA could be successfully extracted from human nail material and analyzed using short tandem repeat (STR) profiling and/or mitochondrial DNA (mtDNA) sequencing.

This study involved the development of a DNA extraction protocol that includes a cleaning procedure designed to remove biological (e.g., whole blood) and chemical (e.g., gold-palladium (Au-Pd)) contaminants. Organic DNA extractions were performed on small fragments of fresh human nail material which were contaminated by bare-handed manipulation, immersion in whole blood, Au-Pd coating, or a combination of immersion in whole blood and Au-Pd coating. In addition, DNA extractions were performed on small fragments of fresh human nail material from 21 random individuals. Finally, DNA extractions were performed on an aged human nail (~eight years old).

Upon completion of the validation study, the extraction protocol was used for the analysis of a torn fingernail fragment recovered from the scene of a violent homicide in 1983. The torn fingernail fragment was contaminated with blood at the time of recovery. Subsequently, the fingernail fragment was cleaned via sonication in sodium dodecylsulfate (SDS), coated with Au-Pd, and subjected to scanning electron microscopy (SEM) for the purpose of striation pattern analysis and tear comparison.

### Materials and Methods

Fresh human nail material (i.e., fingernail, toenail) was obtained from 21 random individuals. Aged (~eight years old) human nail

[1] Armed Forces DNA Identification Laboratory, Department of Defense DNA Registry, Office of the Armed Forces Medical Examiner, Armed Forces Institute of Pathology, Washington, DC.

* The opinions and assertions expressed herein are solely those of the authors and are not to be construed as official or as the views of the United States Department of Defense or the United States Department of the Army.

Received 20 Oct. 1998; and in revised form 11 Jan. 1999; accepted 19 Jan. 1999.

material was obtained from one individual. Individual nail fragments (~5.0 mg) obtained from two random individuals were contaminated by bare-handed manipulation, immersion in whole blood, Au-Pd coating, or a combination of immersion in whole blood and Au-Pd coating. The Au-Pd coating was performed to simulate the analysis of the nail material using SEM. Each sample of the contaminated nail material was cleaned by one of several procedures. These procedures included scraping, vortexing, sonication, immersion in water, ethanol, acetone, SDS, and/or bleach, double-extraction, or a combination of these procedures. The following procedure was used to clean individual nail fragments (~2.5 mg) from the 21 fresh nails as well as the aged nail: 1a) If the nail material is coated with Au-Pd, then vortex for 2 min in 10% commercial bleach. Remove bleach. 1b) If the nail material is coated with nail polish, then vortex for 2 min in acetone. Remove acetone. 1c) Vortex the nail material for 2 min in sterile, deionized water. Remove water. Repeat as necessary to clean surface of nail material (at least two times). 2) Sonicate the nail material for 10 min in 10% SDS. Remove SDS. 3) Vortex the nail material for 2 min in 10% commercial bleach. Remove bleach. 4) Rinse the nail material thoroughly with sterile, deionized water. Remove water. Repeat three times. The nail material was then cut into fragments and boiled in sterile, deionized water for 5 min. The DNA was organically extracted from the nail material by the modification of a previously published protocol (18). Proteinase K (20 mg/mL) and dithiothreitol (1M) were added to the extraction buffer (10 mM Tris, pH 8.0, 100 mM NaCl, 50 mM EDTA, pH 8.0, 0.5% SDS) at regular intervals until the nail material was completely dissolved. The DNA extracts were purified and concentrated using Amicon Centricon-100® concentrators.

Selected DNA extracts were quantified using the Perkin-Elmer QuantiBlot™ Human DNA Quantitation Kit. In addition, selected DNA extracts were analyzed using forensic, polymerase chain reaction (PCR)-based DNA testing systems (e.g., STR profiling, mtDNA sequencing). Amplification reactions were carried out in a Perkin-Elmer Thermal Cycler Model 9600. The STR profiling system used was the Home Office Quadruplex I (HUMVWFA31, HUMTH01, HUMF13A01, and HUMFESFPS). The mtDNA region that was sequenced includes positions 16024 to 16238 (28). Mitochondrial DNA amplification products were visualized on 2% agarose gels using ethidium bromide and purified and concentrated using Amicon Centricon-100 concentrators. Sequencing reactions were performed using the Perkin-Elmer Applied Biosystems Division PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit. For both STR analysis and mtDNA sequencing, electrophoresis was performed using 6% polyacrylamide gels. The detection system used was the Perkin-Elmer Applied Biosystems Division DNA Sequencer Model 373.

Several of the cleaning procedures were considered unsuccessful based upon the detection of mixtures in the resultant STR profiles. However, all cleaning methods resulted in a single, correct mtDNA sequence. This observation inspired an investigation into the amount of contaminating DNA necessary to result in the detection of a mixed mtDNA sequence. Various volumes (i.e., 1 μL, 5 μL, and 10 μL) of contaminating whole blood were added to the organic DNA extraction reactions of small fragments (~2.5 mg) of human nail material from two random individuals. The DNA extraction procedure was performed as previously described.

## Results

The organic extraction of DNA from fresh human nail material (~2.5 mg) often yielded a sufficient quantity and quality of human DNA to allow successful forensic, PCR-based DNA testing (i.e., STR profiling, mtDNA sequencing) to be performed. Mitochondrial DNA sequence information was obtained from all specimens tested. Those specimens, which were not tested using mtDNA sequencing, yielded complete STR profiles. This suggests that all specimens would have yielded mtDNA sequence information. Complete STR profiles were obtained from approximately 76% of the individuals sampled. No STR results were obtained from the remaining individuals sampled (Table 1).

The selected cleaning procedure, used to clean individual nail fragments from the 21 fresh nails as well as the aged nail, sufficiently removed: 1) biological contaminants so as to eliminate all detectable indications of a mixture, and 2) chemical contaminants so as to eliminate inhibitory effects upon the extraction, amplification, or sequencing reactions. In fact, it was demonstrated that to produce a mixture in the mtDNA sequence information obtained from a DNA extraction of human nail material (~2.5 mg) it is required that approximately five microliters of whole blood from a second donor be added to the nail extraction reaction (Fig. 1). However, the addition of a single microliter of whole blood from a second donor to the DNA extraction reaction of human nail material (~2.5 mg) will produce a mixture in the resultant STR profile.

The organic DNA extraction of aged (~eight years old) human nail material (~2.5 mg) yielded a sufficient quantity and quality of DNA to allow successful mtDNA sequencing to be performed. Short tandem repeat analysis did not produce a profile for the aged nail material; however, fresh nail material from the same individual also failed to provide a STR profile. Therefore, no meaningful conclusions could be drawn about the effectiveness of STR profiling with respect to aged nail material.

### *Application to Forensic Casework*

On 23 September 1983, three men and two women were abducted during a robbery at a Kentucky Fried Chicken restaurant in

TABLE 1—*Specifications and results for the DNA analysis of nail material.*

| Individual | ~Nail Mass (mg) | ~DNA Yield (pg/μl) | mtDNA Result | STR Result |
|---|---|---|---|---|
| 1 | 2.0 | <30 | NP | POS |
| 2 | 2.5 | <30 | NP | POS |
| 3 | 3.0 | 35 | POS | NEG |
| 4 | 3.5 | 35 | POS | POS |
| 5 | 3.8 | <30 | NP | POS |
| 6 | 3.7 | <30 | POS | POS |
| 7 | 1.6 | 35 | NP | POS |
| 8 | 2.5 | 35 | NP | POS |
| 9 | 3.2 | 35 | NP | POS |
| 10 | 2.7 | <30 | POS | POS |
| 11 | 3.3 | <30 | POS | NEG |
| 12 | 2.6 | <30 | POS | NEG |
| 13 | 2.6 | 35 | POS | NEG |
| 14 | 1.5 | <30 | POS | POS |
| 15 | 2.0 | <30 | NP | POS |
| 16 | 2.5 | <30 | POS | POS |
| 17 | 2.5 | <30 | NP | POS |
| 18 | 3.3 | 185 | NP | POS |
| 19* | 2.8 | <30 | POS | NEG |
| 20 | 2.5 | <30 | POS | POS |
| 21 | 3.0 | 300 | POS | POS |
| Average | 2.7 | | | |

*= This individual donated the aged (~eight year old) nail material.
NP = Testing not performed.

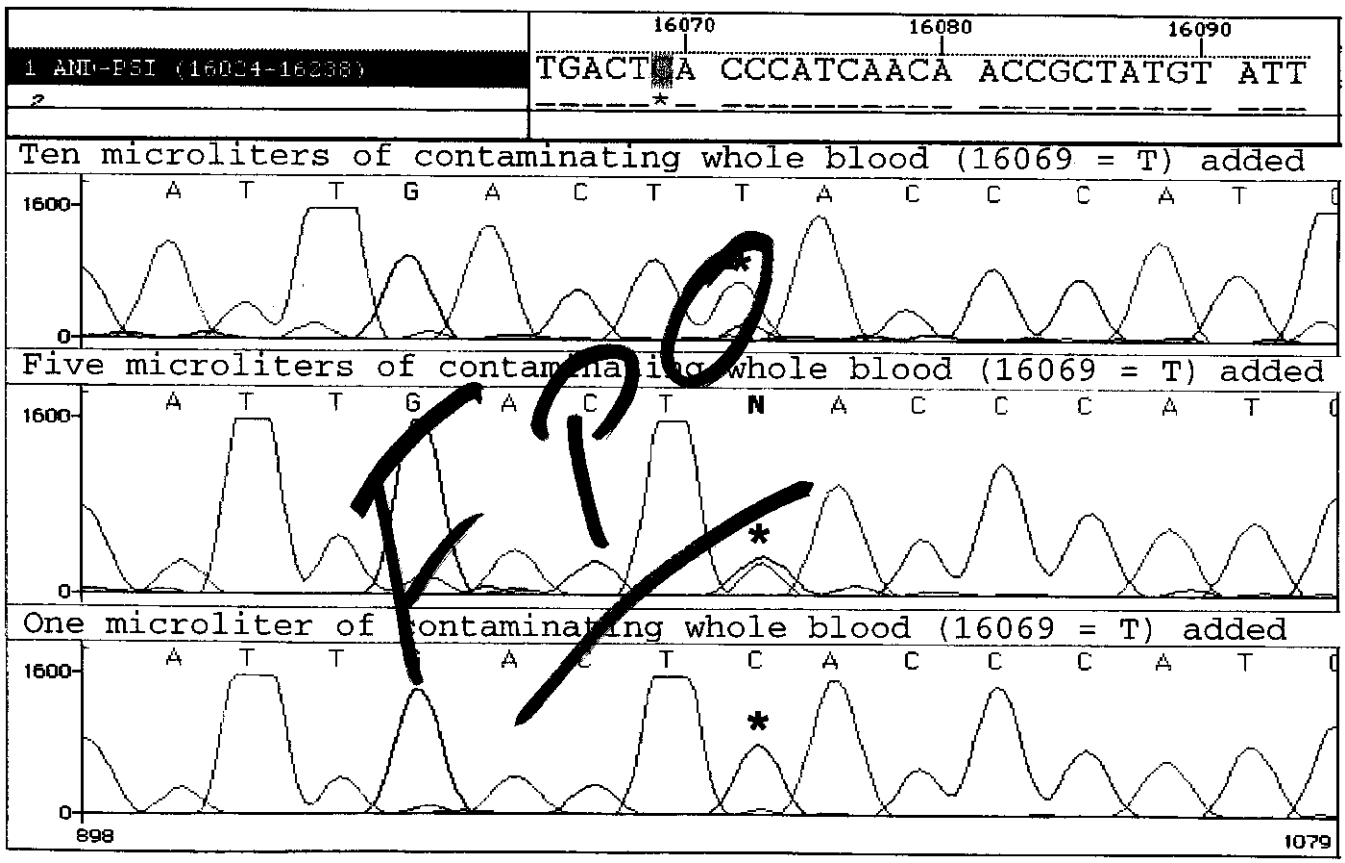


FIG. 1—*Mitochondrial DNA sequence data generated from the DNA extracted from human nail material. Each extract has been contaminated with a certain volume (10 μL, 5 μL, or 1 μL) of whole blood from a second donor. The nail donor possesses a cytosine (C) at the indicated position. The blood donor possesses a thymine (T) at the indicated position.*

Kilgore, Texas. The next day, five bodies were discovered in a Rusk County oil field. Each of the deceased had been shot in the back of the head. While autopsies were being performed, a torn fingernail fragment was recovered from the belt loop of one of the victims. The fingernail fragment was determined to be from an individual having blood type O.

Within several days, a suspect was apprehended and questioned concerning the murders. The suspect had a torn fingernail and type O blood. Striation pattern comparisons were performed between the fingernail fragment and a fingernail cutting taken from the suspect. Two experts concluded that the striation patterns from the two fingernail samples matched one another. However, subsequent striation pattern analysis, performed by a third expert, indicated that a match did not exist between the fingernail fragment recovered during autopsy and the fingernail cutting taken from the suspect (29).

The case was not prosecuted until 1995. Before a grand jury, the suspect was implicated, in part, by the results of ABO typing and nuclear DNA testing (AmpliType® HLA DQα typing) which failed to exclude him as the donor of the fingernail fragment. Subsequently, the torn fingernail fragment and appropriate reference material from the suspect and the five victims were sent to the Armed Forces DNA Identification Laboratory for additional DNA testing. Nuclear DNA testing (partial STR profile at HUMVWFA31, HUMTH01, and HUMF13A01) and mitochondrial DNA sequencing (~610 base pairs) excluded the suspect as the source of the fingernail fragment. No alleles were detected at HUMFESFPS. A single allele was detected at HUMVWFA31. A single allele was reported at HUMTH01 and HUMF13A01. These single alleles were accompanied by a second allele that was below the signal intensity threshold required for reporting. Furthermore, this testing indicated that the fingernail fragment was consistent with having originated from one of the victims (Table 2). The alleles at HUMTH01 and HUMF13A01 that could not be reported were con-

TABLE 2—*Results of DNA analyses performed on a forensic case involving evidentiary human nail material.*

| STR Locus/mtDNA Sequence Position | Suspect Profile | Fingernail Profile | Victim Profile |
|---|---|---|---|
| HUMVWFA31 | 18,19 | 18,18 | 18,18 |
| HUMTH01 | 6,9 | 8,< | 6,8 |
| HUMF13A01 | 5,7 | 4,< | 4,5 |
| HUMFESFPS | 10,11 | NR | 11,12 |
| 16129 | N | A | A |
| 16162 | G | A | A |
| 16189 | C | T | T |
| 16223 | C | T | T |
| 16304 | T | C | C |
| 73 | G | G | G |
| 151 | T | C | C |
| 199 | T | C | C |
| 250 | T | C | C |
| 263 | G | G | G |
| 315.1 | C | C | C |

NR = No Result.
< = Inconclusive result (allele was below signal intensity threshold for reporting).

sistent with the second alleles reported for the victim. In 1996, all charges against the suspect were dropped.

*Acknowledgments*

The authors wish to thank the American Registry of Pathology. The authors also wish to thank Victor W. Weedn and James J. Canik for their administrative support. In addition, the authors wish to thank Jennifer S. Holland, Jeanne M. Willard, Suzanne M. Barritt, and Pamela G. Freeman for their technical assistance. Finally, the authors wish to thank George T. Duncan, Thomas J. Parsons, and John H. Ryan for their review of this manuscript.

## References

1. Jung JM, Comey CT, Baer DB, Budowle B. Extraction strategy for obtaining DNA from bloodstains for PCR amplification and typing of the HLA-DQα gene. Int J Leg Med 1991;104–45.
2. Kanter E, Baird M, Shaler R, Balazs I. Analysis of restriction fragment length polymorphisms in deoxyribonucleic acid (DNA) recovered from dried blood stains. J Forensic Sci 1986;31–403.
3. Giusti A, Baird M, Pasquale M, Balazs I, Glassberg J. Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm. J Forensic Sci 1986;31–409.
4. Higuchi R, von Beroldingen CH, Sensabaugh GF, Erlich HA. DNA typing from single hairs. Nature 1988;332:543–6.
5. Uchihi R, Tamaki K, Kojima T, Yamamoto T, Katsumata Y. Deoxyribonucleic acid (DNA) typing of human leukocyte antigen (HLA)-DQA1 from single hairs in Japanese. J Forensic Sci 1992;37:853–9.
6. Wilson MR, Polanskey D, Butler J, DiZinno JA, Repogle J, Budowle B. Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. Biotechniques 1995;18:662–9.
7. Walsh DJ, Corey AC, Cotton RW, Forman L, Herrin GL, Word CJ, et al. Isolation of deoxyribonucleic acid (DNA) from saliva and forensic science samples containing saliva. J Forensic Sci 1992;37:387–95.
8. Fisher DL, Holland MM, Mitchell L, Sledzik PS, Wilcox AW, Wadhams M, et al. Extraction, evaluation, and amplification of DNA from decalcified and undecalcified United States Civil War bone. J Forensic Sci 1993;38: 60–8.
9. Hochmeister MN, Budowle B, Borer UV, Eggmann U, Comey CT, Dirnhofer R. Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. J Forensic Sci 1991;36:1649–61.
10. Holland MM, Fisher DL, Mitchell LG, Rodriguez WC, Canik JJ, Merril CR, et al. Mitochondrial DNA sequence analysis of human skeletal remains: Identification of remains from the Vietnam War. J Forensic Sci 1993;38:542–53.
11. Lee HC, Pagliaro EM, Berka KM, Folk NL, Anderson DT, Ruano G, et al. Genetic markers in human bone: I. Deoxyribonucleic acid (DNA) analysis. J Forensic Sci 1991;36:320–30.
12. Lee HC, Pagliaro EM, Gaensslen RE, Berka KM, Keith TP, Keith GN, et al. DNA analysis in human bone tissue: RFLP typing. J Forensic Sci Soc 1991; 31:209–12.
13. Lee HC, Ruano G, Pagliaro EM, Berka KM, Gaensslen RE. DNA analysis in human bone and other specimens of forensic interest: PCR typing and testing. J Forensic Sci Soc 1991;31:21–6.
14. Parsons TJ, Weedn VW. Preservation and recovery of DNA in postmortem specimens and trace samples. Forensic taphonomy: the postmortem fate of human remains. CRC Press 1997.
15. Potsch L, Meyer U, Rothschild S, Schneider PM, Rittner C. Application of DNA techniques for identification using human dental pulp as a source of DNA. Int J Leg Med 1992;105:139–43.
16. Schwartz TR, Schwartz EA, Mieszerski L, McNally L, Kobilinsky L. Characterization of deoxyribonucleic acid (DNA) obtained from teeth subjected to various environmental conditions. J Forensic Sci 1991;36:979–90.
17. Smith BC, Fisher DL, Weedn VW, Warnock GR, Holland MM. A systematic approach to the sampling of dental DNA. J Forensic Sci 1993;38: 1194–209.
18. Tahir MA, Watson N. Typing of DNA HLA-DQα alleles extracted from human nail material using polymerase chain reaction. J Forensic Sci 1995; 40(4):634–6.
19. Potsch L, Penzes L, Prager-Eberle M, Rittner C. HLA DQα typing of human fingernails. Advances in forensic haemogenetics 4 Springer-Verlag 1992.
20. Yada S, Tsugawa N, Ohya I, Mori M. ABO subtypes and the testing of hair and nail. Acta Criminologiae et Medicinae Legalis Japonica 1969;35 (2):51–4.
21. Outteridge RA. Determination of the ABO group from fingernails. Medicine, Science, and the Law 1963;3:275–6.
22. Yada S, Okane M, Sano Y. A simple method for blood-grouping fingernails. Acta Criminologiae et Medicinae Legalis Japonica 1966;32:96–8.
23. Garg RK. Determination of ABO(H) blood group specific substances from fingernails. Am J Forensic Med and Path 1983;4(2):143–4.
24. Sehajpal PK, Sharma VK. Determination of A and B group antigens in human finger and toe nails. Archiv Fur Kriminologie 1982;169(3–4): 117–9.
25. Garg RK. Determination of ABO(H) blood group substances from finger and toe nails. Zeitschrift fur Rechtsmedizin 1983;91:17–9.
26. Kaneshige T, Takagi K, Nakamura S, Hirasawa T, Sada M, Uchida K. Genetic analysis using fingernail DNA. Nuc Acid Res 1992;20(20):5489–90.
27. Uchida K, Wang L, Yahagi Y, Tokunaga K, Tadokoro K, Juji T. Utility of fingernail DNA for evaluation of chimerism after bone marrow transplantation and for diagnostic testing for transfusion associated graft-versus-host disease. Blood 1996;87(9):4015–6.
28. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et. al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–65.
29. Starrs E. Fingernail matching has met its match—DNA comes to the fore—again. Scientific Sleuthing Review 1998;22(1):1–4.

Additional information and reprint requests:
Theodore D. Anderson
DoD DNA Registry, AFDIL
AFIP Annex
1413 Research Blvd, Bldg 101
Rockville, MD 20850