B5 Sonication Removal of Cellular Material From Nail Clippings and DNA Typing of Separated Components

Shelly A. Steadman, MS, Daniel J. Fahnestock, MS, Melissa D. Murphy, BS, Van T. Nguyen, MS, and Robert C. Hansen II, MS, Sedgwick County Regional Forensic Science Center, 1109 North Minneapolis, Wichita, KS*

The goals of this presentation are to present the forensic community with an efficient method for removal of foreign cellular material from fingernail clippings followed by a rapid method for washing the nail material, such that removed cellular material and the nail can be individually typed using PCR DNA technology.

This poster presentation will introduce a routine processing method for fingernail clippings submitted as evidence for DNA typing. This method allows for rapid and efficient separation of nail material from foreign cells adhering to the nail prior to DNA typing and results in little product loss from either component. This is useful for addressing a variety of forensic questions that may arise; typing of the material beneath a nail is important in cases where victims have struggled in defense, while typing of a fingernail fragment can be used to associate an individual with a crime scene.

The method described employs soaking and sonication to remove cellular components adhering to the nail. With the nail material suspended in a filter basket, cellular components removed from the nail were pelleted by centrifugation and the supernatent removed from the cell pellet. The pelleted fraction was then referred to as fraction A. Should dividing this fraction become necessary, the resulting pellet may be resuspended in a known quantity of buffer and divided into equal halves. The nail material itself is then subjected to a sonication wash, a vortex wash, and a shaking wash to remove any trace amounts of remaining foreign material that may adhere to the nail. The cleaned nail material is then referred to as fraction
B. Pellets (fraction A) and nail material (fraction B) can then separately undergo DNA extraction and typing.

Fingernail clippings from individuals were collected and treated with blood or semen originating from sources other than the fingernail donor to simulate forensic casework situations. Nails were also collected following vigorous scratching of a second individual. These nails, as well as untreated nail material, underwent the standard protocol for processing fingernail fragments. Resulting fractions were separately extracted using an organic extraction methods by Microcon® (Millipore, Corp., Bedford, MA) concentration and purification of the DNA. For nails treated with seminal material, the resulting pellets underwent a differential extraction designed to first isolate that portion of the sample containing “non-sperm” cells (referred to as the F1 portion of fraction A) and secondly to isolate DNA from all remaining cells including the sperm cells (referred to as the F2 portion of fraction A). Extracted DNA from all samples was quantified using 1% agarose yield gel analysis in conjunction with the QuantiBlot (Applied Biosystems, Foster City, CA) human DNA quantification kit. Samples were then amplified using the PowerPlex 1.1 and Amelogenin Sex Identification Systems (Promega Corp., Madison WI) and amplified products were detected by fluorescent imaging for genetic loci CSF1PO, TPOX, Amelogenin, TH01, vWA, D16S539, D7S820, D13S317, and D5S818 using the Hitachi FMBIO II Fluorescent Imaging Device (MiraiBio Inc., Alameda, CA). Following initial protocol development and validation, the processing method was applied to a forensic case in which one fingernail of unknown origin and one fingernail of known origin were submitted for PCR DNA analysis.

The DNA profiles obtained from both fractions of the untreated nail were consistent with the profile of the nail donor. This indicates that the quantity of cellular material present beneath the nail is sufficient for DNA typing. However, studies have indicated that when nails are treated with body fluids foreign to the nail donor, cellular material suitable for DNA typing was efficiently removed from the nail such that the contributors of foreign cells and the donor of the nail were easily differentiated. In cases where carry-over between fractions occurred, identification of a major contributor was possible, therefore alleviating the need for interpreting complex mixtures. This was true of both the blood-treated nail and the semen-treated nail. Clear major contributors were apparent in the nonsperm fraction of the pellet (AF1), the sperm cell fraction of the pellet (AF2), and the nail fraction (B). Profiles obtained from fractions AF1 and B were consistent with the nail donor, while the profile obtained from fraction AF2 was consistent with the seminal donor. However, in scratching studies, profiles obtained from both fractions (A and B) of the extracted nails yielded profiles consistent with the nail donor only; these findings are consistent with those previously reported by Oz and Zamir[1]. This is likely due to the limited quantity of cellular material transferred to the nail during the scratching process relative to that already present beneath the nail from the donor. With respect to forensic casework applications, the pellet fraction (A) of the nail of known origin yielded a singlesource profile consistent with that of the donor; the nail fraction (B) was not typed since the origin was known. However, a mixture of at least two individuals was obtained from the pellet fraction (A) of the nail of unknown origin, while the nail fraction (B) yielded a single source profile. The nail fraction (B) profile could not be excluded from the pellet mixture profile, allowing for interpretation of obligatory alleles from other possible contributors present within the pellet fraction (A) profile.

In conclusion, the method employed for separation of nail material from foreign components proved efficient for most samples where a sufficient quantity of cells from the foreign source were deposited on the nail. Because this method allows for the separate typing of nail and adhering cellular material, it can be applied in a standard

 ** Presenting Author*

manner regardless of the forensic question at hand.

[1]Oz, C, Zamir, A. An evaluation of the relevance of routine DNA typing of fingernail clippings for forensic casework. *J Forensic Sci* 2000;45(1):158-160.

Fingernail, PCR DNA Analysis, PowerPlex 1.1

 ** Presenting Author*