Comparison of Two Techniques for Human Identification from Hair: Mitochondrial DNA Sequencing and Supercritical Fluid Extraction-Gas Chromatography/Mass Spectrometry

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Human hair is becoming more common as a source material for human identification analysis, especially in criminal investigations. In the past, hair samples have been characterized by microscopic examination, which provides limited discriminatory power. More recently, both nuclear DNA and mitochondrial DNA (mtDNA) genetic analyses have been used. Successful nuclear DNA analysis depends to a great extent on starting with sufficient root material, whereas, mtDNA has been detected in both the root and the shaft and due to its high copy number is more likely to be successfully amplified from limited samples.

In this study, hairs from the same individuals were examined by two different techniques – on-line supercritical fluid extraction-gas chromatography/mass spectrometry (SFE-GC/MS) and mitochondrial DNA (mtDNA) sequencing. SFE-GC/MS determines the organic components that are extractable from the surface of the hair, including naturally-deposited compounds from sebaceous and sweat glands, and artificially-deposited species from conditioners, other hair treatments, and environmental exposures. The hypothesis is that a unique chromatographic spectra, displaying a series of peaks representing the various extracted components, would be generated for each individual. Mitochondrial DNA sequencing of the human hypervariable regions HV1 and HV2 has shown unique sequences from non-maternally related individuals.

Advantages of the SFE-GC/MS are: 1. Small sample sizes – 30 to 900 µg; technique can be performed on a single hair; 2. Can be conducted using the shaft of the hair; does not require the root; 3. Added sensitivity of the on-line system (50 to 100x the conventional extraction procedure); 4. Ability to distinguish between siblings as well as mothers and their children; 5. Non-destructive technique (i.e., hair is still intact at end of procedure); 6. Rapid results – three hours /hair sample from receipt of hair to spectra (24 samples would take 72 hours); and 7. GC/MS instrumentation should be available in a well-equipped forensic laboratory. Advantages of the mtDNA sequencing procedure are: 1. Small sample size – single hair or single hair root; 2. Can be conducted on either the hair root or hair shaft; 3. Can confirm identity by comparison to maternal lineage; 4. Multiple samples (24 to 36 are done routinely) can be analyzed at same time; 5. Time to final results: 48 to 72 hours; and 6. The necessary instruments are becoming more common in forensic laboratories.

Disadvantages of the SFE-GC/MS system are: 1. Small differences observed in the spectra from hair from the same individual suggest an inhomogeneity in the deposits coating the hair surface; 2. Spectra currently analyzed by personal observation; and 3. Instrumentation is expensive -$70 to 80 K. Disadvantages of the mtDNA sequencing procedure are; 1. Different hairs from same individuals may differ by one base pair due to heteroplasmy; 2. Can not distinguish mothers from their children or siblings from each other; and 3. Instrumentation (especially if one wishes to do automated sequencing) can be expensive - $65 to 160 K.

Results of the comparison of these two techniques on human hair samples indicate that SFE-GC/MS could distinguish between individuals and mtDNA sequence analysis was able to distinguish non-maternally related individuals. DNA from