## A96 Extraction and Identification of Lifestyle Markers During Mitochondrial DNA Testing of Human Hair

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After attending this presentation, attendees will learn a procedure for the chemical analysis of human hair for lifestyle markers using the discarded fractions from a protocol for mitochondrial DNA sequencing.

This presentation will impact the forensic science community by showing how the fields of microscopic, chemical, and genetic analysis of hair can interact and be used to determine lifestyle markers of questioned hair samples. This presentation will demonstrate how the gaps between the fields of forensic hair analysis can be bridged by adopting the theme of "One Biologist's Trash is Another Chemist's Treasure."

Forensic hair analysis can be divided into three main areas: microscopic exams, chemical analysis, and genetic analysis. Each of these areas is focused on its own particular questions and analytes, and as such, these areas have developed independently of each other with their own literature, procedures, and practitioners.

The research presented here bridges the gaps between the areas of forensic hair analysis by adopting a theme of "One biologist's trash is another chemist's treasure." Several methods by which discarded fractions from a typical protocol for mitochondrial DNA (mtDNA) sequencing can be subjected to chemical analysis and the results provide information about the lifestyle of the subject, including cosmetic modifications to the hair, use of tobacco, and demographic information such as age and gender. It is hypothesized that any small organic compounds incorporated into the hair (e.g., hair dye components, nicotine/cotinine, integral lipids) will be released and partition into the organic layer during liquid-liquid extractions.

In practice, forensic science laboratories recommend that appropriate hairs should be selected for nuclear DNA (nDNA) analysis during the microscopic examination of the hair. In order to be suitable for nDNA, the hair root must be present. Hairs removed while actively growing (anagen phase) are more likely to yield partial or full DNA profiles. Hairs naturally shed when the hair follicle is dormant (telogen phase) are less likely to yield DNA profiles. Given that many cases when the root of the hair is absent or nDNA is otherwise unavailable (such as in telogen hairs), mitochondrial DNA (mtDNA) analysis can be utilized.

The first phase of a multi-phase project is presented here. Each phase involves three main developmental steps: validation, scale down, and extrapolation. Using literature methods for analytes of interest, the methods have been recreated and shown to be reliable using known standards. The published methods have been scaled down to the level of a typical mtDNA analysis (2cm length of hair) and the methods were extended to yield successful results when explicitly applied to the discarded rinses, washes, and organic layers of a mtDNA protocol.

The first phase of this project is concerned with the discarded fraction from the first step of an mtDNA hair analysis protocol. It is hypothesized that in addition to any residues of mounting media, neutral surface components will be extracted in the xylene wash. This includes surface lipids such a free fatty acids, fatty esters, squalene, and cholesterol. Residues of hair care products such as conditioners and hair gels are also extracted.

The validation step of this project was conducted by creating a standard mixture of surface lipids and relevant components of hair care products in xylene. Large volume injection gas chromatography/mass spectrometry (GC/MS) was used to achieve baseline resolution of all components. Linearity and limits of detection for each standard were determined.

The scale down step was accomplished by ultra-sonicating milligram quantities of hair in xylene and analyzing the extract. Microgram quantities were then analyzed which correspond to single hair fragments whose length is on the order of centimeters.

The first phase of this analysis was extrapolated by ultra-sonicating and analyzing xylene extracts of 2cm hair segments. The xylene extract was analyzed by GC/MS using large volume injection in a programmed temperature vaporizer (PTV).

This procedure will open up new possibilities for information that can be obtained from a hair sample, to include factors such as age (youth versus adult), tobacco use, and hair dyes. These methods could be applied during a typical extraction protocol for mitochondrial DNA and would result in an unknown sample being more fully characterized, while a comparison of a questioned and known sample would be more probative.

**Forensic Hair Analysis, Lifestyle Biomarkers, Mitochondrial DNA Sequencing of Hair**

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