A168 Alternative Methods for Human Identification: DNA Base Composition Profiling by Electrospray Ionization Time- of-Flight Mass Spectrometry

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After attending this presentation, attendees will be familiar with the methods used for forensic identification of mitochondrial DNA (mtDNA) by electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) and the capabilities of the Plex-ID instrument designed for this purpose. The research findings can guide forensic experts in practical aspects of adopting mass spectrometry as a technique for use in human identification by analysis of the mtDNA control region.

This presentation will impact the forensics science community by delineating the performance capabilities of mass spectrometry-based mitochondrial DNA base composition profiling. A full understanding of the capabilities of ESI-TOF MS instrumentation is critical to the adoption of the technology in the forensic sciences so that cost and labor advantages can be gained without exceeding expectations of performance in routine casework.

Mass spectrometry base composition profiling represents an alternative technology for forensic identification of biological material via mtDNA with improvements over the current method of DNA sequencing through reductions in cost, labor, and time required to generate a result. Mass spectrometry-based detection of DNA is not hindered by length heteroplasmy or mixed contributor samples, both of which are challenging to analyze by DNA sequencing.

In order to investigate the suitability of the Plex-ID ESI-TOF MS for use in forensic identification, experiments have been performed at NIST to assess the operational characteristics of the system when used for base composition profiling of mtDNA. Research aimed to assess the function of the Plex-ID ESI-TOF MS in the areas of: (1) concordance with Sanger sequencing data; (2) limits of detection; (3) monitoring for contamination; and, (4) ability to detect mixtures. Concordance studies evaluated results from the Plex-ID against Sanger sequencing data for DNA templates originating from four major population segments in the United States: Caucasian, African American, Hispanic American, and Asian American. PCR sensitivity was assessed through serial dilution of three unique DNA templates. Sensitivity was monitored over time to ascertain whether there may be changes in sensitivity levels of the PCR reagents or the instrument. Contamination was assessed through an experiment designed to evaluate multiple potential sources of contaminants. The ability to detect and successfully identify mixtures of DNA templates was gauged by mixing two templates together at ratios of 99:1, 19:1, 9:1, 3:1, 1:1, 1:3, 1:9, 1:19, and 1:99 and co-amplifying the templates for subsequent detection on the mass spectrometer.

Concordance study results from 669 samples identified four samples which did not generate full profiles of 24 amplicons, with a single amplicon failing to be detected. This yields a concordance rate of 99.4% (665/669) when using the criteria that a full profile is necessary for registration with the instrument database. When comparing the number of successfully measured amplicon masses, the concordance rate is 99.9% (16,052/16,056). Importantly, the discordances were limited to incomplete profiles rather than incorrect measurements. The limit of detection of template DNA was found to be well below the manufacturer's suggested minimum quantity of 200pg per sample, divided into eight multiplex PCR reactions. Full mtDNA profiles were generated over the range of 8 to 40pg of nuclear DNA template. Sensitivity levels remained within acceptable limits with slight fluctuations during four months of monitoring. Samples were variable in their mtDNA copy number, as expected. To monitor for contamination, 18 experiments were run over the course of eight months. No contamination was detected. Mixtures could be consistently detected when the two components were present at 3:1, 1:1, or 1:3 ratios. However, complete profiles for both mixture components could not reliably be generated due to limitations in the instrument's ability to resolve two masses that are within 11 Daltons of each other.

Savings in cost and labor inputs, combined with high levels of sensitivity and accuracy make, the ESI-TOF mass spectrometry technique well suited to the forensic human identification laboratory.

A full understanding of the capabilities of ESI-TOF MS instrumentation is critical to the adoption of the technology in the forensic sciences so that cost and labor advantages can be gained without exceeding expectations of performance in routine casework. This presentation will impact the forensic science community by delineating the performance capabilities of mass spectrometry-based mitochondrial DNA base composition profiling.

Mass Spectrometry, Base Composition, Mitochondrial DNA

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