E3 Run-Specific Limits of Detection and Quantitation for STR-Based DNA Testing

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After attending this presentation, attendees will understand a new and objective means of distinguishing between signal and noise in DNA typing electropherograms.

This presentation will impact the forensic community by demonstrating that arbitrary minimum peak height thresholds can be replaced with objective and more sensitive limits of detection that will allow more reliable information to be extracted from DNA profiling electropherograms.

STR-based DNA profiling is an exceptionally sensitive analytical technique that is often used to obtain results at the very limits of its sensitivity. The challenge of reliably distinguishing between signal and noise in such situations is one that has been rigorously addressed in numerous other analytical disciplines. However, an inability to accurately determine the height of electropherogram baselines has caused forensic DNA profiling laboratories to utilize alternative approaches. Minimum thresholds established during laboratory validation studies have become the *de facto* standard for distinguishing between reliable signal and noise/technical artifacts.

The conservative nature of these commonly employed thresholds can also arbitrarily remove from consideration legitimate signal from trace and secondary contributors to an evidentiary sample – matters of critical importance in many criminal investigations. These minimum peak height thresholds also generally fail to consider variability in the sensitivity of instruments, reagents, and the skill of human analysts involved in the DNA profiling process over the course of time.

Software made publicly available by the National Center for Biotechnology Information now provides an alternative means of establishing limits of detection and quantitation that is more consistent with those employed in other analytical disciplines. The presenters have used that software to determine the height of each data collection point for each dye along a control sample's electropherogram trace. Those values were then used to determine a limit of detection (the average amount of background noise plus three standard deviations) and a limit of quantitation (the average amount of background noise plus ten standard deviations) for each control sample. Analyses of the electropherogram data associated with the positive, negative and reagent blank controls included in 50 different capillary electrophoresis runs validates that this approach could be employed to objectively determine run-specific thresholds for use in forensic DNA casework.

A known mixed DNA profile from two unrelated individuals of an approximately 10:1 ratio was also examined using this methodology and all alleles associated with the secondary contributor were found to be at or above the run's limit of detection yet were also below the testing laboratory's validated minimum peak height threshold of 100 relative fluorescent units.

Limit of Detection, Limit of Quantitation, DNA

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