A130 Maximizing Allele Detection by Selecting Optimal Analytical Thresholds

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After attending this presentation, attendees will learn how the Analytical Threshold (AT) has a significant influence on rates of Type I (false positives) and Type II (false negatives) errors. Attendees will be introduced to Receiver Operating Characteristics (ROC) analysis as a method to compare each type of error in order to provide a method to select optimal ATs and disregard suboptimal ones.

This presentation will impact the forensic science community by providing practical guidelines on how to evaluate the signal-to-noise in order to resolve whether ATs derived from running a set of blanks can be applied during the analysis of samples amplified with low- to medium-DNA input levels and major/minor mixtures. Recommendations regarding the use of ROC will also be given.

Interpretation of DNA evidence depends upon the ability of the analyst to accurately compare the DNA profile obtained from an item of evidence and the DNA profile of a standard. This interpretation becomes progressively more difficult as the rates of Type I and II errors increase. Traditionally, ATs have been chosen to ensure the false detection of noise is minimized. However, there exists a tradeoff between the erroneous labeling of noise as alleles and the false non-detection of alleles (i.e., drop-out). In this study, the effect ATs had on both types of error was characterized. Various ATs were tested, where three relied upon the analysis of baseline signals obtained from 31 negative samples. The fourth AT was determined by utilizing the relationship between RFU signal and DNA input. The other ATs were the commonly employed 50, 150, and 200 RFU thresholds. Receiver Operating Characteristic (ROC) plots showed that although high ATs completely negated the false labeling of noise, DNA analyzed with ATs derived using analysis of the baseline signal exhibited the lowest rates of drop-out and the lowest total error rates. In another experiment, the effect small changes in ATs had on drop-out was examined. This study showed that as the AT increased from ~10-60 RFU, the number of heterozygous loci exhibiting the loss of one allele increased. Between ATs of 60 and 150 RFU, the frequency of allelic drop-out remained constant at 0.27 (+/-0.02) and began to decrease when ATs of 150 RFU or greater were utilized. In contrast, the frequency of heterozygous loci exhibiting the loss of both alleles consistently increased with AT.

Peak height ratios at each of the 7 ATs were also calculated by dividing the peak height of the smaller allele (RFU) by the peak height of the larger allele (RFU) at a locus. Those ratios were then averaged and the standard deviations from the mean were determined. The data show that as the AT decreased, the average PHR decreased, while the standard deviation remained unchanged. The average PHR at ATs of 150 RFU and an AT based on baseline analysis were 0.8 (+/-0.2) and 0.6 (+/-0.2) respectively. The drop in the mean PHR with respect to AT is suggested to be a function of the increased stochastic variation associated with the ability to detect alleles from lower quantities of DNA. This supports the proposition that forensic DNA analysis of low-template samples needs to be accompanied by guidelines appropriate for samples containing small quantities of biological material. Therefore, if a PHR threshold is used to determine the number of contributors when only two distinguishable alleles are present, this data suggests stringent PHR thresholds such as 0.7 are not optimal for samples containing <0.5ng of input DNA.

The Type I and II error rates for major:minor mixtures were also examined and showed the same general trend: lower ATs decrease the level of Type II error. However, if a significant amount of DNA from the major is present (i.e., >0.5ng), non-specific amplified product increases Type I error at low ATs which are derived from examining the baseline noise. Therefore, in order to minimize the detection error rate within the laboratory, different ATs may need to be applied to different sample types (i.e., low-template versus high-template).

Analytical Threshold, Forensic DNA, Error Rates

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