A195 Minimum Distinguishable Signals and Limits of Detection: Applications of Uncertainty in Analytical Measurement for Determination of an RFU Threshold for Forensic DNA Analysis

Joli Bregu, BS, Danielle Conklin, MS, Elisse R. Coronado, MS, Robin W. Cotton, PhD, and Catherine M. Grgicak, PhD, Boston University School of Medicine, Biomedical Forensic Sciences, 72 East Concord Street, Room R806B, Boston, MA 02118*

After attending this presentation, attendees will be exposed to a number of analytical techniques that were used to determine the minimum distinguishable signal (MDS), the limit of detection (LOD), and the sensitivity of signals/alleles generated using STR typing.

This presentation will impact the forensic science community by illustrating methods commonly used to calculate and elucidate the minimum distinguishable signals, limits of detection, sensitivity, and signal to noise ratios. The assessment will allow the determination of an relative fluorescent unit (RFU) threshold that will represent a value which analytically distinguishes noise from "true allele" signal.

The amplification and subsequent fragment analysis of DNA recovered from crime scenes remains one of the most sensitive and powerful techniques available for the purposes of human identity testing.

A number of technologies, methodologies, and chemistries have been introduced permitting amplification (and subsequent analysis and interpretation) of samples with low DNA concentrations. Such technologies include, but are not limited to, post-PCR clean-up, modifications to the cycle number and/or thermal cycling parameters, increased injection times, and optimized STR amplification kits/chemistries; however, as these advancements become more frequently implemented, it is necessary to determine their limitations. As such, it is imperative to determine the minimum target DNA concentration that can be detected (at a known confidence level) using these technologies.

The minimum mass of DNA that can be detected with confidence is known as the limit of detection. This amount not only concerns the signal of the target, but also the magnitude of the analytical signal in relation to the levels of fluctuation in the blank signal. Therefore it is dependent upon the laboratory's ability to calculate and elucidate the minimum distinguishable signals, limits of detection, sensitivity, and signal to noise ratios.

In this study a number of analytical techniques were used to determine: (1) the minimum distinguishable signal (MDS); (2) the limit of detection (LOD); and, (3) the sensitivity of signals/alleles generated using AB's AmpFlSTRTM Identifiler® kit.

The MDS was experimentally determined by running 33 blanks (formamide + LIZ600) using a 2, 5, and 10 s injection on a 3130 Genetic Analyzer (Applied Biosystem) using the manufacturer's recommended protocol. This is accomplished by using the following definition

$$MDS = \overline{S_{bl}} + 3SD_{bl}$$

where MDS is the minimum distinguishable signal, *Sbl* the average of the blank signal, and *SDbl* is the standard deviation of the blank signals. The MDS therefore represents the RFU value at which one cannot statistically distinguish between "true" signal and baseline noise of the instrument. However, since this only represents the expected MDS of the instrument - which is comprised of the noise from the electronics, detector, etc - it does not represent the MDS obtained from amplified blanks. Therefore the MDS for 86 amplified negatives run over a period of approximately one year were analyzed and it showed that the MDS was significantly larger than those calculated from the 33 run blanks (Table 1.). It was also observed that the MDS did not increase between injection times when calculated using the run blanks, but did significantly increase between 5 and 10 s injections when determined by utilizing the amplified negatives. This suggests a characteristic MDS is better calculated from a series of amplified negatives run over a period of time using representative injection times.

Lastly, determination of the sensitivity (i.e., slope) and y-intercept which are computed using linear regression (R2 >0.994) of a calibration curve - which plots signal (RFU) versus target (0.0625 – 1 ng) - shows the y-intercept is less than the MDS, thereby suggesting: (1) the MDS should experimentally be determined using amplification blanks; and, (2) the limit of detection for the AmpFlSTRTM Identifiler® kit using these experimental conditions and defined by

$$LOD = \frac{3SD_{bl}}{m}$$

where LOD is the limit of detection, *m* is the slope, and is the standard deviation of the blank signals, is 42, 17, and 9 pg for 2, 5, and 10 s injections respectively.

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Table 1. Minimum Distinguishable Signals for 2, 5, and 10 second injections calculated with run blanks and amplification blanks.

	MDS (RFU)					
Color	2s		5s		10s	
	Run Blanks	Amplification Blanks	Run Blanks	Amplification Blanks	Run Blanks	Amplification Blanks
Blue	6	13	7	11	9	15
Green	9	15	9	15	10	19
Red	14	34	17	28	14	54
Yellow	15	17	14	18	14	25
Overall	11	23	12	22	12	35

Further analysis to determine the signal:noise, signal:amplification target and the LOD of a weighted regression were also analyzed and compared. Results show that as the signal increased, so did the level of amplified noise. These noise peaks were not consequences of bleed-thru, -A, positive/negative stutter, etc, but were amplified noise/artifacts. Analysis of the signal:noise and target:signal:noise shows that when analyzing data, the signal of the “major” peak(s) must be taken into consideration.

DNA Analysis, RFU Threshold, Minimum Distinguishable Signal

 ** Presenting Author*