B60 NIST Mixed Stain Study #3: Does DNA Concentration Measurement Accuracy Matter?

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The goal of this research project is to present to the forensic community findings from the NIST Mixed Stain Study #3 linking DNA concentrations ([DNA]) measurement accuracy to among-sample STR multiplex analytical signal variability.

Short-tandem repeat (STR) multiplex assays are now the dominant forensic DNA human identification technology. While multi-step and chemically complex, current commercial STR multiplex assays provide results that are robust to typical among-laboratory differences in sample preparation, polymerase chain reaction (PCR) equipment and protocols, and separation and visualization systems. The National Institute of Standards and Technology (NIST) has coordinated a series of interlaboratory examinations of multiplexed STR systems. In addition to documenting the evolution of STR assays within the forensic community, these studies search for latent analytical difficulties by challenging analysts and assay systems with designedly difficult samples, presented in atypical contexts, and described with minimal instructions. No problem intrinsic to properly performed STR multiplex analyses has been encountered. In the 1999 Mixed Stain Study #2 (MSS2) [*J Forensic Sci* 2001;46(5):1199-1210], linkages between certain STR measurement anomalies and inaccurate DNA quantitation were observed. The 2001 Mixed Stain Study #3 (MSS3) was designed to further explore the performance of high-plexity STR systems and to resolve the DNA quantitation issues raised in the earlier interlaboratory challenges.

Participation in MSS3 was open to all human identity laboratories utilizing multiplex STR systems of five or more loci. Seventy-four institutions returned partial or complete results for the study.

Samples consisted of one control (labeled “R”) and six study samples (labeled “S” to “X”). Control R was a single-source material, S to W were two-source materials, and X was a three-source material. With the exception of samples T and V, no source was used in the preparation of more than one material. Samples T and V were prepared from the same two sources to have identical total DNA concentrations, but with reciprocal 5:1 and 1:5 female:male source composition ratios. The MSS3 consisted of two major activities: (1) quantifying the DNA (as ng/lL) in the control and study samples and (2) analyzing all of the samples using one or more STR multiplex. From the first activity, participants were asked to report the [DNA] in each sample and to specify the quantification protocol used. From the second, participants were asked to report the volume of each sample used in each PCR amplification, to report the type and intensity of all observed alleles in each sample, and to assign where possible alleles to major and minor contributor sources. Participants were requested to analyze the control sample as the first and last sample in every set of analyses performed and to report the intensity of all alleles observed in each analysis. Participants were also requested to provide hardcopy of all gel image or electropherogram results. No sample handling, analysis, data analysis, nor result reporting procedures or formats were specified. All results were required to be submitted to NIST no later than 10-Oct-2001.

The consensus medians of the [DNA] agree very well with the design values. The among-participant variability in measuring [DNA] can be estimated from the average interquartile range of the individual distributions. This robust estimate of the among-participant [DNA] standard deviation, expressed as a multiplicative factor, is 1.6x. Since the similarly defined estimate of among-participant [DNA] variation in MSS2 was 1.8x, the [DNA] measurement comparability among the forensic community appears to have improved from 1999 to 2001.

The average signal per ng DNA amplified by one participant is not predictive of the signal observed by other participants. However, the average signal per ng DNA amplified of one sample does predict the signals for the other samples analyzed within a laboratory. The absolute efficiencies of the over-all STR multiplex measurement process (including the amplification, injection, separation, and detection subprocesses) are fairly variable among participants, even when the processes are nominally identical. However, the relative efficiency of each participant’s measurement process is quite stable, at least over the days-to-months required by the MSS3 study.

Interlaboratory Study, Measurement Accuracy, STR Multiplex

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