PRELIMINARY RESULTS FROM THE 2001 NIST MIXED-STAIN STUDY #3: DNA QUANTITATION AND IDENTIFICATION OF MINOR CONTRIBUTOR (S)

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The National Institute of Standards and Technology (NIST) has provided the forensic community with a number of challenging and educational inter-laboratory measurement exercises. NIST Mixed Stain Studies #1 and #2 explored the performance of 1997- and 1999-era multiplexed Short Tandem Repeat (STR) systems for samples containing DNA from more than one source. While demonstrating that these systems reliably amplify multiple-source DNA given an appropriate amount of DNA in the reaction mixture, partial or complete minor component amplification failures were observed when the amount of DNA in a sample was determined inaccurately.

Designed to directly explore the relationships between DNA quantitation and sample typing without the complications of sample extraction, sample shipment for the Mixed Stain Study #3 (MSS3) began in January 2001. The seven MSS3 samples are Tris/EDTA buffer DNA extracts in screw-capped vials with DNA concentrations ranging from ≈1.0 to ≈10 ng/μL. One sample (designated "R") is a single source "control material" to be amplified and analyzed each time one of the other six mixture samples is amplified or analyzed. Five of the six mixture samples ("S" to "W") are two-component male and female donor mixtures. The remaining mixture ("X") contains DNA from one female and two male donors. The mixtures were designed with ratios of the components ranging from ≈3:1 to ≈10:1.

MMS3 participants were asked to quantitatively determine the total concentration of DNA in each of the samples and amplify an "appropriate volume" with the STR multiplex(es) of their choice. The following results were requested: DNA concentration of each sample in ng/μL, sample volume amplified, alleles present/detected in the samples, peak height/peak area (optical density) data (and appropriate hard copy from all analyses), the potential type(s) of the minor contributor(s), and an estimate of each mixture ratio.

As of the end of June 2001, more than 80 laboratories have received MSS3 samples and more than 20 have returned results. The median of the estimates of total DNA concentration agree fairly well with the design values but the estimates vary by more than a factor of 10. To date, the most common typing failure is again minor component alleles not seen or not called. The vast majority of these incomplete typings occur with the extreme mixture ratio samples when inappropriately small volumes of the sample are amplified. Interestingly, most participants accurately call the alleles in the three-component mixture while (and, paradoxically, perhaps because of) under-estimating its total DNA concentration.