USING SRM 2372 HUMAN DNA QUANTITATION STANDARD IN QPCR ASSAYS: ARE THERE DIFFERENCES BETWEEN ASSAYS?

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Modern highly-multiplexed short tandem repeat (STR) assays used by the forensic human-identity community require tight control of the initial amount of sample DNA amplified in the polymerase chain reaction (PCR) process. This in turn requires the ability to reproducibly measure the concentration of human DNA, [DNA], in a sample extract. Quantitative PCR (qPCR) techniques can assay the number of intact stretches of DNA of specified nucleotide sequence in an extremely small sample; however, these assays must be calibrated with DNA extracts of well characterized and stable composition. Because of the variability in the targeted sequences of various qPCR assays found in the population of samples assayed some variability in the quantitation results are sometimes seen. We have been studying these differences between qPCR assays and will detail some of our findings.

Standard Reference Material (SRM) 2372 Human DNA Quantitation Standard was prepared to serve as a way to assign a [DNA] to qPCR "kit" calibrants. Interlaboratory data from 32 laboratories (6 different qPCR assays) as well as five qPCR methods performed at NIST were used in evaluating the suitability of the materials used to prepare SRM 2372. Additionally we have evaluated two newer qPCR kits with SRM 2372 components. The data from these studies as well as detailed instructions for the proper use of SRM 2372 are presented.