B50 Examining Candidate DNA Quantitation Standards With Real-Time Quantitative PCR Assays

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After attending this presentation, attendees will have information regarding the evaluation of commercial and in house real-Time PCR assays for the development of a human DNA reference material.

This presentation will impact the forensic community and/or humanity by demonstrating how the application of qPCR methods for the estimation of human genomic DNA is increasing. The reproducibility and ability to automate qPCR assays is an attractive characteristic for use in a forensic laboratory is of importance to understand the effects different qPCR assays and their standards have on the intended result: the ability to obtain quality signal from a human identity typing kit.

Numerous real-time quantitative PCR (qPCR) methods have been developed in the last several years for use with forensic DNA samples. Ten different qPCR methods were used to evaluate DNA samples distributed in the NIST Interlaboratory DNA Quantitation Study 2004 (QS04). The target DNA concentrations of the QS04 samples were from 1.5 ng/µL to 50 pg/µL. About one-fifth of all QS04 results were from qPCR methods. These data show differences among the qPCR methods, both with regard to precision and bias. It is unclear from these data whether the observed differences are inherent to the methods or reflect differences in the stan- dards used in their calibration.

The evaluation of several qPCR methods using six different human DNA calibration materials will be presented. All of the qPCR methods are either commercially available or have been published recently. Three of the calibration materials are commercially available; three are derived from in- house purified single-donor blood samples. This study is being used to direct development of candidate Standard Reference Material 2372, Human Genomic DNA Quantitation Standard.

A total of 5 methods for quantifying human genomic DNA were examined. The assays were run on the ABI 7500 instrument platform. All of the 5 methods were run in duplicate using six different human DNA cal- ibration materials. Three of the human DNA standards were commercially obtained and three were prepared in house and quantified by a UV mea- surement (wavelength = 260 nm). The genomic DNA standards were examined by serially diluting a range of 10 ng down to 0.41 ng. The pre- scribed analysis thresholds and baseline values were applied and linear standard curves for the DNA standards were generated. Cross comparisons of the DNA standards and assays were made.

Standard curves for all six DNA standards were generated for the 5 assays. This allowed a comparison between different genomic DNA stan- dards as well as the 5 qPCR assays. Variation in the relative amounts of DNA in a “standard” is illustrated by way of a practical example; running an autosomal STR test.

The application of qPCR methods for the estimation of human genomic DNA is increasing. The reproducibility and ability to automate qPCR assays is an attractive characteristic for use in a forensic laboratory. It is of importance to understand the effects different qPCR assays and their standards have on the intended result: the ability to obtain quality signal from a human identity typing kit.

References:

1. Kline, M.C., Duewer, D.L., Redman, J.W., Butler, J. (2005) Results from the NIST 2004 DNA Quantitation Study *J Forensic Sci.* 50: 570-578.

Quantitative PCR, DNA Quantification, Real-Time PCR

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