B215 The Proper Use of Standard Reference Material 2372 (SRM 2372) Human DNA Quantitation Standard for the Calibration of Commercial Quantitative Polymerase Chain Reaction (qPCR) Kit DNA Standards

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After attending this presentation, attendees will understand why the National Institute of Standards and Technology (NIST) produces SRM 2372 Human DNA Quantitation Standard and how that standard should be used within an individual laboratory to calibrate internal commercial qPCR kit DNA standards.

This presentation will impact the forensic science community by bringing attention to the differences in commercial qPCR kits based on the lot of the DNA standard and the quantitation chemistry employed. The proper use of SRM 2372 will also be presented.

The NIST SRM 2372 Human DNA Quantitation Standard was produced to support the need for a human-specific DNA quantitation standard in forensic casework as a calibrant for commercially produced DNA standards. SRM 2372 is intended primarily for use in the value assignment of human genomic DNA forensic quantitation kit materials. SRM 2372 consists of three materials, one single-source male, one multi-source female, and one multi-source male/female mixture, all solubilized in TE-4 buffer.[1] The application of SRM 2372 is for calibration of commercial qPCR kit standards to aid in proper quantification of unknown DNA samples within a forensic workflow.

Commercial Short Tandem Repeat (STR) assays used by the forensic human identity community require tight control of the amount of sample DNA amplified in the Polymerase Chain Reaction (PCR). This requires the ability to reproducibly measure the concentration of human DNA in a casework sample extract prior to input in the PCR reaction. Approximately 500pg-1,000pg of input DNA will provide a balanced and an interpretable STR profile. Commercially available qPCR kits routinely are relied upon to determine the concentration of casework extract within forensic laboratories; however, assays employed rely upon commercial DNA standards for relative quantitation estimates.

Data shown will demonstrate the need for an SRM for the calibration of commercially produced qPCR DNA standards. This is due to an observed ~50% variation in measured DNA concentration between multiple lots of a commercially available DNA standard. This variation may lead a laboratory to possibly overestimate or underestimate the concentration of an unknown sample that could lead to an incorrect dilution within the PCR workflow. In addition, the variation in assigned concentration across several qPCR chemistries will be shown. The use of SRM 2372 is intended to enable the comparison of DNA concentration measurements over time, production lots, and within an individual laboratory. Additionally, manufacturers can use SRM 2372 to validate the values assigned to their own commercial qPCR kit DNA standards. Individual forensic laboratories should use SRM 2372 to validate the concentration of DNA qPCR quantitation kit standards and to verify the assigned concentration of an in-house or commercial DNA standard prior to use. This recalibration will result in decreased variability between varying lots of qPCR kit DNA standards. A specific example of how to properly use SRM 2372 to recalibrate a commercial qPCR kit DNA standard will be presented.

Reference(s):

1. Kline M.C., Duewer D.L., Travis J.C., Smith M.V., Redman J.W., Vallone P.M., Decker A.E., Butler J.M. (2009) Production and certification of NIST Standard Reference Material 2372 Human DNA Quantitation Standard. *Anal. Bioanal. Chem.* 394: 1183-1192.

SRM 2372, qPCR, Standard

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