A174 Recertification of Standard Reference Material 2372 – the Human DNA Quantitation Standard: The What, the Why, and the How

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After attending this presentation, attendees will understand why the National Institute of Standards and Technology (NIST) has recertified the standard reference material 2372 (SRM 2372) human DNA quantitation standard, and how that recertification has been performed to achieve traceability to SI units.

This presentation will impact the forensic science community by assuring the understanding of procedures and policies followed by NIST to assure the quality and fitness-for-purpose of the standard reference materials that the community uses to assure the quality of their measurements and to demonstrate that quality to their accrediting bodies.

SRM 2372 is intended primarily for use in the value assignment of human genomic deoxyribonucleic acid (DNA) forensic quantitation 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. SRM 2372 was originally certified for spectroscopic traceability in units of decadic attenuance, D_{10}. The D_{10} scale is a measure of absorbance and is traceable to the unit 1. The certified values of all three SRM 2372 components had D_{10} values of approximately one. The conventional conversion factor for aqueous solutions of double stranded DNA (dsDNA) is: 1.0 D_{10} at 260nm = 50ng/µL DNA. Data on the performance of the SRM 2372 components with three qPCR methods is supplied at http://www.cstl.nist.gov/biotech/strbase/srm2372.htm.

The genomic DNA in all three of the SRM 2372 components was prepared to be dsDNA; however, five years after production these dsDNA solutions transformed into mixtures of dsDNA and single stranded DNA (ssDNA). The conventional conversion factor for aqueous solutions of ssDNA is: 1.0 D_{10} at 260 nm = 40ng/µL DNA. Therefore, this partial conformational change increased D_{10} at 260nm but did not change the DNA mass concentrations or the behavior of the materials in qPCR assays. However, recall that the SRM 2372 components were certified for their spectroscopic properties and not for the conventional conversion to mass concentration. Sales of SRM 2372 were therefore suspended as soon as the spectroscopic instability was recognized.

After determining that the solution volumes of the SRM 2372 components were unchanged and there had been no appreciable degradation of the DNA, it was decided to recertify the remaining units for the spectroscopic properties of ssDNA. Since the conversion between dsDNA and ssDNA was incomplete and somewhat variable among units, methods for users of these materials to force complete conversion to ssDNA are required. Two methods have been developed: heat treatment at 98°C and strand disassociation by sodium hydroxide (NaOH). The recertified values incorporate the variability of this conversion and, as such, the uncertainty intervals for the new values are larger than the original ones. The re-certified spectroscopic values remain traceable to the unit 1.

In addition, the SRM 2372 components are now certified for “copy number,” that is, the total number of genome-equivalents present in the solution based on results from numerous digital PCR assays. qPCR assays associated with chromosomes 1, 4, 5, 6, 9, and 14 were developed in-house to be able to assess possible copy number variability issues associated with the three SRM components. Once the qPCR assays were optimized using an AB7500, they were then transposed onto the digital PCR platforms for analysis of copy number. Historically, component C of SRM 2372 has shown variability in some commercial qPCR assays. Developing qPCR assays that cover areas of six different chromosomes was done to try and understand the variability associated with these past results.

SRM 2372 components have also been checked for their performance with commercially available qPCR assays that may be of interest to the forensic DNA community.

DNA Quantitation, Digital PCR, Decadic Attenuance

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