A71 Lessons Learned in the Application of the NIST DNA Quantification SRM 2372 to Quantitative Real-Time PCR Analysis

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The goal of this presentation is to assist attendees who are attempting to improve their DNA quantification analysis.

This presentation will impact the forensic science community by sharing information that will provide assistance to crimes labs to assess the accuracy of their protocols and the precision of their qRT-PCR instruments when determining the amount of target DNA to amplify for STR analysis of casework material.

One of the issues encountered in our laboratory system when qRT- PCR (Applied Biosystem Quantifiler™) analysis was implemented to replace the old hybridization dot/slot blot quantification procedures (Quantiblot™) was that the target amount of DNA amplified to obtain equivalent STR peak heights on the genetic analyzer changed substantially. The gap between the DNA concentration measured by qRT- PCR and other procedures such as UV absorbance could be substantial depending on the lot of qRT-PCR kits used during internal validation. Another issue was the lot to lot variability in concentration of the DNA standard (Standard A) provided in the qRT-PCR kit. Consistency in calibration of the target DNA concentration measured by the qRT-PCR to the amplified STR peak heights detected could be maintained from lot to lot by monitoring and adjusting the concentration of the standard. The availability of regression analysis parameters in qRT-PCR assays such as y intercept, slope and r^2 from the standard curve were welcome features; however, there were no internal calibrator controls provided with the kits like there were in the old hybridization based protocol.

The goal of this study was to improve DNA quantification and make the Applied Biosystem Quantifiler™ DNA qRT-PCR analysis traceable to the NIST SRM 2372 quantification standard. Neilson et al (FSI- Genetics, 226-230, #2, 2008) did a comparison of 5 different DNA quantification methods and came to the conclusion that the accuracy of the Quantifiler™ Human DNA Quantification kit could be improved by switching the DNA standard from the Raji cell line DNA provided in the kit to genomic DNA (G147A obtained from Promega). As part of the NIST SRM 2372 traceability implementation it was decided to do an evaluation of the Quantifiler™ Human DNA kit using both the DNA standard provided in the kit and the G1471 genomic DNA from Promega as an alternative standard. It was also decided to test a commercially prepared human genomic DNA from buffy coats (Roche) as a qRT-PCR calibrator control at two different concentrations. Work started using the Applied Biosystems 7000 SDS qRT-PCR instrument but the SRM 2372 did not give the expected results. Consequently the study continued with different plate configurations and a comparison analysis performed on the newer Applied Biosystems 7500 SDS qRT-PCR instrument.

The results of the study supported the following:

1) A material modification should be made to improve the Quantifiler™ Human DNA kit by changing the DNA standard and by adding a calibrator control. The G1471 genomic DNA as a DNA standard was found to provide a DNA concentration estimate of buffy coat pooled DNA concentrations that was very

close to the DNA concentration obtained from UV absorbance by the manufacturer. The calibration of target DNA amplified to the expected STR peak heights detected by the genetic analyzer is closer to that historically used for the old hybridization slot blot method.

2) There is an optimum plate configuration for the 7000 SDS qRT-PCR instrument for using the SRM 2372. In general SRM 2372 component A works better in columns 5 and 6 while components C and B work well in columns 1 through 4.

3) There is better precision for DNA quantitation when using the 7500 rather than the 7000 SDS qRT-PCR instrument. By loading a 96 well optical plate of uniform DNA concentration with Quantifiler Human and plotting the CT versus plate position it was ascertained that the 7500 has much more consistent readings across the plate then the 7000 SDS qRT-PCR instrument.

qRT-PCR, NIST SRM 2372, DNA quantification

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