H133 Normalization of Polymerase Chain Reaction (PCR) -Based Quantification Using 9947A Human Standard DNA

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Learning Overview: After attending this presentation, attendees will better understand the use of 9947A human standard DNA as a normalization control for comparing data from multiple quantitative PCR (qPCR) plates.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by creating a method that increases the ability to compare DNA concentrations collected via qPCR on different days or when standards are used toward the end of the recommended storage life.

This study evaluated the effectiveness of using purchased 9947A human standard DNA as a normalization control for Plexor® HY and Quantifiler® Trio quantification kits. Currently, there is no normalization control included in qPCR kits for plate-to-plate comparisons when data is collected on different days or across multiple plates. Differences in quant values when comparing qPCR data collected in different runs may arise for a range of reasons, including differences in the dilution accuracy of the standards, pipetting accuracy of the amplification mix, or because the standard dilutions are near the protocol-specified storage limit and may be degraded. To assess some of these variations, a comparative analysis of quantification standards over time was performed using purified 9947A human standard DNA as a normalization control.

Quantification standards from Plexor® HY and Quantifiler® Trio kits were made and assessed at time points up to 14 days. Each set of standards was plated with purified DNA samples and 9947A human standard DNA using the QIAgility® automated robot. The observed quant value for 9947A can then be used in relation to its known concentration to normalize the sample concentration and allow for a more accurate comparison of quant values. Preliminary data shows that aged standards artificially increase the DNA concentration of plated samples. However, when using 9947A as a normalization control, data can be corrected to real concentration values. The effect of this normalization strategy will be discussed for samples across a wide range of quant values.

Based on the trends of qPCR standards degrading as they approach the recommended storage life and the inherent variables present in the quantification process, a normalization technique is necessary to compare data across multiple plates or from multiple days. This research has shown that artificially increased or skewed DNA concentrations can be corrected to their true value by using purified human standard DNA with a known concentration as a normalization control. Results from this study show this normalization method is useful for longitudinal research studies looking at the effect of modifications to sampling, storage, or extraction procedures.

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DNA Quantification, Normalization, qPCR