EVALUATION AND COMPARISON OF THE HID REAL-TIME PCR VIRTUAL STANDARD CURVE TO COMMONQUANTITATION TECHNIQUES

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Quantitative PCR (qPCR) plays a critical role in the field of forensic biology to determine the amount of “amplifiable” human specific DNA. An assay-specific dilution of standards is the most prevalent technique utilized to determine the quantity of DNA in a forensic sample. This research evaluates and compares two alternative methods in determining the quantity of DNA that does not require the preparation of a standard curve for each quantitation run and aims to minimize run-to-run variation and reduce costs and analyst time. These two methods are the generation of an in-house external standard curve that can be implemented into a workflow or LIMS systems and using the virtual standard curve function included in the HID Real-Time PCR software by ThermoFisher with the Quantifiler™ Trio quantification kit. Various standard curvedilution sets were prepared and analyzed to evaluate different variables include lot numbers, curve preparations by different analysts, and between instrument calibrations. Samples were quantified in duplicate, and a linear regression was determined utilizing the of the average of all runs to calculate the slope and y-intercept per variable and quantitation target (Small, Large, or Y) to generate a virtual standard curve in the new HID Real-Time PCR Analysis Software v1.3. It was determined that the external standard curve method and virtual standard curve method ledto identical results, which allows laboratories with different instrumentation to choose the method that fits best into their current workflow. Results showed there was no significance between instrument calibration and no difference between kit lots when comparing the assay specific curve to the virtual/external curve methods. For the virtual standard curve, there was no significant difference to the assay specific method. There were significant differences between pipetting from different analysts when looking at the different standards prepared. A recommendation from this research regarding the use of these techniques is to have as many analysts as possible pipetting. If more than one variable is introduced throughout the process, a new virtual standard curve needs to be generated. This study demonstrates the feasibility of the implementation of the virtual standard curve function into a case working laboratory workflow and that a laboratory can benefit using these methods.