DEVELOPMENT OF AN INNOVATIVE APPROACH TO HUMAN DNA QUANTIFICATION ANALYSIS

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Recently introduced Quantifiler™ Trio human DNA quantification kits are more sensitive, resistant to inhibitors, include a more robust DNA standard, and provide sample quality and quantity information. The result of these changes is that the laboratory can more accurately predict downstream STR results including deciding whether to continue with STR analysis, which STR kit to use, and how much DNA to add to the STR reaction. Although improved and optimized, accurate quantification results still depend heavily on the standard curve generation and metrics. To further increase efficiency and decrease variability a flexible Virtual Standard Curve (VSC) feature was developed within the HID Real-Time PCR Analysis Software v1.3. VSC functionality allows the user to input standard curve quality metrics for each quantification target and analyze or re-analyze data with the user-defined values. Ultimately, this feature reduces quantification variation by negating the variation introduced during the creation and addition of the standard curve dilution series. This approach also increases productivity by allowing for the addition of more samples to each plate and saves analyst time. Three sources of variation were assessed, including multiple users, lots of kits, and QuantStudio™ 5 Systems. The use of optimized VSC protocols demonstrated how to decrease quantification variability while increasing efficiency. In addition, the impact of quantification values determined using a VSC versus in-plate standards on downstream GlobalFiler™ kit STR results was evaluated. This study demonstrates a VSC can be utilized in a forensic DNA laboratory to improve accuracy and increase efficiency while not impacting STR results.