B99 Evaluation of the ABI Quantifiler Human DNA Quantification Kit: Optimization of Input DNA for STR Analysis by CE and Determination of a True Zero Value

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After attending this presentation, attendees will retain suggestions for re-validation of CE analysis methods when implementing a new DNA quantitation method. Additionally, attendees will learn useful information about the use of real-time quantitation methods as screening tools for DNA analysis.

This presentation will impact the forensic community and/or humanity by displaying the importance of completing a thorough internal validation prior to implementing new quantitation technologies into the DNA analysis process.

Current methods for the analysis of forensic biological samples commonly require polymerase chain reaction (PCR) amplification of short tandem repeat (STR) loci for human identification using commercial multiplex and megaplex STR kits. Each kit has been optimized by the manufacturer to amplify a narrow range of input DNA, typically 0.50 – 2.50ng. Requiring a narrow range of template DNA necessitates the need for human DNA quantitation methods that are sensitive, precise, and accurate in order to ensure optimal amplification of the STR loci. Recent comparisons of commonly used quantitation methods have shown differences in their sensitivity, accuracy, and/or precision; differences such as these can impact the ability of a laboratory to detect alleles after STR amplification. Therefore, it is important that labs refine their methods and re-optimize downstream procedures in the DNA analysis process as advancements in technologies are made and as new quantitation procedures are implemented. Specifically, failure to revalidate electrophoretic conditions after implementation of a new quantitation method could impact the end result – a successful DNA profile. In this study, the Quantifiler™ Human DNA Quantitation Kit used in conjunction with the ABI Prism® 7000 Sequence Detection System was evaluated to 1) determine the appropriate amount of DNA to be amplified to give optimal heterozygote peak heights during analysis via capillary electrophoresis, and 2) determine if a true zero value exists for this quantitation method – a value below which no detectable STR profile would be observed. Three DNA extraction methods commonly used in forensic casework (organic, the DNA IQ™ System, and the QIAamp® Mini DNA Kit) were used to determine if extraction method had any further influence on heterozygote peak height. All samples were amplified with the AmpF*l*STR® Profiler Plus™ PCR Amplification Kit, and PCR products of STR loci were separated and detected on the ABI Prism® 3100-*Avant* Genetic Analyzer. Profiles and heterozygote peak heights were observed using GeneMapper™ ID, version 3.2.

For each of the extraction methods, a strong correlation ($r > 0.80$) between input DNA and heterozygote peak height was observed, however DNA extracted with the DNA IQ™ System consistently resulted in higher peak heights. In addition, the data show increased variability in average peak heights at inputs of >1.500 ng. Based on the data collected, it is recommended that the input DNA for multiplex STR amplification be increased to 1.500 ng of extracted DNA. At this input level, if the volume of PCR product for CE analysis is maintained (1.2 ìl) along with a 10sec electrokinetic injection, average heterozygote peak heights near 1500rfu should be obtained. In the true zero value study, 96 low-level DNA samples (ranging from “undetected” to 0.225 ng/ìl) were amplified using the AmpF*l*STR® Profiler Plus™ kit. It should be noted that typable loci were obtained from samples that were “undetected” by Quantifiler™, including a complete profile obtained from one “undetected” sample. However, 73% of the time, samples that were “undetected” by the Quantifiler™ kit were truly undetectable, resulting in no typable STR alleles. Although several of the “undetected” samples did display partial profiles, these samples generally did not yield enough typable loci to provide useful information for identification purposes. Given these observations, an absolute true zero value cannot be defined for Quantifiler™, but these data show that it may be a useful screening tool for predicting the success of a downstream STR amplification.

DNA Quantitation, Quantifiler, Capillary Electrophoresis

 ** Presenting Author*