



A99 Evaluation of Concordance, Sensitivity, STR Amplification Input, and a True Zero Value for the Qiagen Investigator™ Quantiplex Kit

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After attending this presentation, attendees will understand the novel aspects of a new DNA quantitation kit, Qiagen's Investigator Quantiplex kit, and learn about the outcome of an internal validation as well as how effectively the kit was used as a predictive screening tool for STR amplification success in a data set with low yield DNA samples.

This presentation will impact the forensic science community by informing it of the evaluation of a novel quantitation kit in a forensic DNA research setting and its ability to predict STR amplification success with a set of low yield DNA samples, thus aiding laboratories who may be interested in implementing a new quantitation kit.

Qiagen's Investigator™ Quantiplex kit, a total human DNA quantitation kit, has a 200 base pair Internal Control, a fast cycling time relative to current forensically relevant quantitation kits utilized, and a novel scorpion molecule containing a primer, probe, fluorophore, and quencher covalently linked for an optimized interaction. In this study, the Investigator™ Quantiplex kit was compared to the Quantifiler™ Human kit for concordance, sensitivity, optimal DNA input for STR amplification reactions, and determination of a true zero value, a value under which no useful STR data is consistently obtained, thus reliably predicting STR failure. Full and half volume reactions were also evaluated with the Investigator™ Quantiplex kit. For this study, buccal swabs were extracted using the Qiagen QIAamp® DNA Blood Mini Kit, quantified with Quantifiler™ Human or Investigator™ Quantiplex kits, and amplified with the ABI AmpF/STR® Identifiler kit using a previously validated reduced volume reaction. Amplified samples were separated on the 3100Avant Genetic Analyzer with a default ten second injection and standard STR analysis parameters. Data were analyzed with GeneMapper® ID v.3.2 using an analytical threshold of 50 RFU. On average, Investigator™ Quantiplex quantitated samples 0.558ng/μl lower than Quantifiler™ Human, but standard deviations were large. When compared to previous studies conducted in the laboratory, Investigator™ Quantiplex is slightly more sensitive than Quantifiler™ Human, and preliminary data in this study suggests Investigator™ Quantiplex may be more robust to inhibitors. Four samples known to contain an inhibitor were undetected using the Quantifiler™ Human kit but were detected by the Investigator™ Quantiplex kit and exhibited quantitation values ranging from 0.043ng/μl to 0.461ng/μl. With the Investigator™ Quantiplex kit, the half volume reaction was found to be analogous to the full volume reaction based on consistency of data and the range of quantitation values detected. Optimal DNA input into reduced volume AmpF/STR® Identifiler amplification reactions was determined to be 2.0ng, as this was the only input with which average heterozygote peak heights were obtained within the desired 1000 – 1500 RFU range. Samples with 0.25ng input DNA or greater resulted in average peak heights above 200 RFU, a typical stochastic threshold, but substantial standard deviations were observed. Samples with DNA inputs of 0.05ng and 0.10ng exhibited average peak heights above the analytical threshold but below 200 RFU. Allelic dropout was first observed with samples under 0.5ng total DNA input and was more extensive with samples under 0.25ng total input. For the samples with a 0.25ng total DNA input, all allelic dropout was attributed to the same sample. No true zero value was identified in this study, but samples undetected with the Investigator™ Quantiplex were consistently unlikely to result in enough useful data for statistical calculations or upload into CODIS (two alleles and 0.82 complete loci above the analytical threshold on average). Of the undetected samples in the true zero study, 66% yielded no allele calls and 78% yielded only a single allele or none. The Investigator™ Quantiplex kit displays a number of advantages, including time and cost savings, high sensitivity, and potential robustness to inhibitors. Future work should include completion of a reproducibility study with the half versus full volume reactions, and testing of more low level and inhibited samples.

Investigator Quantiplex, STR Success, DNA Quantitation