VALIDATION OF QUANTIFILER QUANTITATION KITS USING THE ABI 7000 AND ABI PRISM 3100 GENETIC ANALYZER

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The Quantifiler Quantitation Kits, and the ABI 7000, give the forensic community a new alternative for the quantitation of DNA samples. The system utilizes Real Time PCR to measure the amount of amplifiable DNA in a sample. Quantifiler kits are available in both Human and Y(Male). The human kit measures total human DNA, while the Y(Male) kit measures only male DNA. The Y kit, specifically, is an obvious benefit to the forensic community.

In evaluating Quantifiler, a number of studies were completed to determine if the Quantifiler system would be more advantageous than the current quantitation method. Comparisons between Quantifiler, Quantiblot and AluQuant were performed. Additionally, the cost per sample was calculated for each system. The quantitation results of all three systems were used to determine which system generated the highest quality DNA profile as determined via amplification. Initial evaluations demonstrated that Quantifiler was superior to the current method, and therefore, the decision was made to move forward with the validation of Quantifiler and the ABI 7000. Validation studies for Quantifiler included sensitivity, accuracy, precision, matrix, robustness, mixture and species specificity analyses.

Both Quantifiler kits, Human and Y(Male), were able to detect DNA from a variety of biological samples and substrates, regardless of the extraction method. The Y(Male) kit did not cross-react with female DNA and, with the exception of gorilla and one dog, Quantifiler did not detect non-human DNA. The gorilla and dog samples did not amplify with Profiler Plus. Further studies suggested that dog DNA did not react with Quantifiler and that these results may have been due to human contamination of the sample, possibly during collection.

Both kits were shown to be precise via multiple quantifications of the same sample demonstrating similar results. However, there were inconsistencies when comparing total Human to Y DNA in a single sample. That is, the ratio did not remain constant in an individual or sample type. Based on the mixture precision studies, the Y(Male) kit did appear to have slightly more variability than the Human kit.

Determining the ratio of total Human to Y DNA proved very informative when analyzing mixtures. It was determined that for amplification of 0.5 ng of target DNA, the Human and Y ratio needed to be 5:1 or less.

When compared to AluQuant and Quantiblot, Quantifiler demonstrated the lowest standard deviation for peak heights across all nine loci of Profiler Plus, suggesting that out of the three methods, Quantifiler most accurately quantifies DNA. Quantifiler also demonstrated greater sensitivity than the current method. Samples yielding zero quantitation results with Quantifiler did not contain amplifiable DNA. Additionally, samples quantified at approximately 5 pg/µl did not produce profiles in Profiler Plus. Therefore, any sample quantified at 0.005 ng/µl or less need not be amplified for STR analysis. This allows blanks and samples with no or non-amplifiable amounts of DNA to be dropped/removed from the process, thus making Quantifiler cost effective. It also allows for low level samples (below the detection limit of our current STR technology) to be saved for possible future testing or Y-STR analysis.