



A123 Validation of the Quantifiler® Duo DNA Quantification Kit

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After attending this presentation, attendees will acquire information about the Quantifiler® Duo DNA Quantification Kit from Applied Biosystems.

This presentation will impact the forensic community by assisting other labs in deciding if integrating the Quantifiler® Duo DNA Quantification kit will enable the laboratory to quantify the amount of total DNA and male DNA in a sample.

This presentation will provide more information or understanding about the Quantifiler® Duo DNA Quantification Kit from Applied Biosystems. This kit utilizes the enhanced multiplexing technology of the Applied Biosystems 7500 Real-Time PCR System and the Sequence Detection Software v.1.2.3 in order to simultaneously detect human and male DNA in samples that are commonly encountered in forensic laboratories. To better understand both the attributes and limits of the Quantifiler® Duo DNA Quantification Kit, a series of validation experiments were carried out to test precision, reproducibility, sensitivity and mixture interpretation, for its possible casework implementation at the Westchester County Forensic Laboratory.

The precision of the Quantifiler® Duo DNA Quantification Kit was determined through examining the cycle threshold number for the dilutions of the DNA standard provided with the kit. The quantitation values reported for the dilutions of the National Institute of Standards and Technology (NIST) standards were used to establish the kit's ability to provide reproducible results intra- and inter-plate. The NIST male quantitation standard was serially diluted to approximately 1.44pg/μL to test the kit's sensitivity. Mixtures were prepared from known male and female samples. Ratios of male to female DNA were made to assess the detection capabilities of the male component relative to increasing amounts of female component. Additionally, fourteen mock casework samples were extracted using a differential extraction procedure and quantitated using the Quantifiler® Duo kit.

Further investigation was done to see if results obtained using the Quantifiler® Duo DNA Quantification Kit had any direct effect on the subsequent DNA profiles detected. This was done by amplifying the sensitivity, mixture, and mock samples with the AmpfSTR® PCR Amplification Kits and subsequently placing them on the Applied Biosystems 3130 Genetic Analyzer and analyzing them using GeneMapper v3.2.

Quantifiler® Duo DNA Quantification Kit was found to be both precise and reproducible. The kit was sensitive to DNA concentrations of approximately 11.5pg/μL. It detected the male component down to a male to female mixture of 1:200. The kit functioned well with the mock casework intimate samples that were differentially extracted. The reported DNA quantities of the sperm fraction were consistent in terms of both the total human DNA and the total male detected (total DNA ~ male DNA). In many of the epithelial fractions, both male and female contributors were indicated.

Overall, the Quantifiler® Duo DNA Quantification Kit enables the laboratory to quantify the amount of total DNA and male DNA in a sample. Furthermore, results obtained can be useful in enhancing the profile of a male contributor in a mixed sample when it previously could not be detected using the current standard human DNA quantitation method. However, it should be noted that there are some limits to the kit's sensitivity regarding the upper and lower limits of detection. The results obtained from this internal validation will hopefully help other labs in the forensic community decide if integrating the Quantifiler® Duo DNA Quantification Kit enables the laboratory to quantify the amount of total DNA and male DNA in a sample. Furthermore, results obtained in their laboratory would be beneficial.

Quantifiler Duo, DNA Quantification, 7500 Real-Time PCR System