INVESTIGATION OF REPRODUCIBILITY AND ERROR ASSOCIATED WITH REAL TIME QUANTITATIVE PCR METHODS

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The number of tools available for processing DNA samples continues to increase providing opportunities to produce more definitive results from difficult samples. One of the most important tools is the ability to use quantitative PCR (qPCR) for the estimation of the amount of DNA obtained from a sample. With the advent of multiplex systems which allow measurement of both total human and total male DNA the quantitation result can be used to direct the first steps in the analysis.

Quantifiler® Duo DNA Quantification Kit by ABI and other qPCR multiplex systems provide information by incorporating amplification of both a human specific and a male specific DNA sequence. However to make maximum use of the data a complete understanding of how variability impacts results is essential. Sources of variation need to be experimentally isolated for effective measurement.

To measure the inherent variation of the reaction mix/instrument combination, a single master mix was made with DNA at each concentration tested. This master mix/DNA sample combination was made up in an amount large enough for analysis of four identical reactions. A second set of four reactions was tested by pipetting the sample DNA individually into each well. Comparison of the variation in the CT values for each sample set provides information about the inherent minimum deviation of the assay. The effect of the measured error in CT on the calculated DNA concentration and ultimately on the STR amplification set up is discussed and illustrated. Accurate estimation of the error associated with DNA concentration requires consideration of the logarithmic relationship between CT and concentration as well as the errors associated with the slope and Y-intercept of the standard curve.