A86 Quantitation of Total and Male Chromosomal DNA Using Multiplex PCR and Capillary Electrophoresis

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After attending this presentation, attendees will have discussed alternative available methods for DNA quantitation using existing instrumentation in the DNA laboratory. Understand the capabilities and limitations of capillary electrophoresis for quantitative measurements. Introduce validation steps for use of Genetic Analyzers for quantitative applications.

This presentation will impact the forensic community by demonstrating the DNA quantation method provides a comparable alternative to qPCR for quantitating total human and male DNA in a sample. With modest validation of an ABI310 for quantitative use, the assay provides comparable sensitivity and reliability to existing DNA quantitation methods for a fraction of the cost, and with available instrumentation currently in the lab.

The utility of a DNA quantitation assay (Q-TAT) incorporating amplification of the amelogenin gene on the X and Y chromosomes was recently reported (Allen and Fuller 2006). The assay was shown to be comparable to the Quantiblot assay in terms of sensitivity and reliability although it was less sensitive and had a lower dynamic range than qPCR. In this study, the Q-TAT assay has been modified to incorporate additional PCR targets into a multiplex consisting of primers for the amelogenin locus (on both the X and Y chromosomes), the SRY gene (on the Y chromosome), and the luciferase gene from the sea pansy (*Renilla sp*) which was included in the PCR reaction as a template to detect the presence of PCR inhibitors. The enhanced assay (Q-TAT 1.1) was evaluated for accuracy in quantitation of total human and total male DNA, as well as for the detection of known PCR inhibitors. Results showed that the Q-TAT 1.1 assay is reliable and effective at providing accurate estimates of total human DNA. Moreover, in male:female mixtures consisting of as low as 3% male DNA, Q-TAT 1.1 was able to provide quantitation estimates of male DNA suitable for deciding between autosomal STR or Y-STR analyses as the method of choice for DNA typing. Finally, the inhibition control system incorporated into Q- TAT 1.1 is a very sensitive indicator of PCR inhibition caused by EDTA, hemin, indigo dye, and humic acid.

DNA Quantitation, Capillary Electrophoresis, PCR Multiplex

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