B34 Quantitative and Qualitative Assessment of Total Human and Human Male DNA in Forensic Type Biological Samples Using a Multiplexed Real-Time PCR System

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After attending this presentation, attendees will learn about a methodology for simultaneous quantitation of human DNA and human male DNA in forensic biological samples in single PCR reaction using real-time PCR technology and its utility in the assessment of the quality of extracted DNA.

This presentation will impact the forensic science community by demonstrating a real time assay for simultaneous quantitation of human male and total human DNA in biological samples.

In order to select the appropriate STR analysis methodology and obtain optimal STR analysis results for forensic type samples, it is desirable to determine the relative quantities of male and female DNA and detect the presence of PCR inhibitors at an early stage in the analysis. This assessment is necessary because the forensic samples often contain mixtures of DNA from male and female contributors and are exposed to environmental insults. A multiplex TaqMan® assay has been developed to:

1. Quantitate total human DNA and human male DNA simultaneously
2. Determine the ratio of human male and female DNA
3. Detect PCR inhibitors
4. Allow selection of appropriate STR amplification kit
5. Predict success with downstream STR amplification

The multiplex assay is designed for the 7500 Real Time PCR System using the ribonuclease P RNA component H1 (RPPH1, VIC® labeled probe) human target and the sex determining region Y (SRY, FAM™ labeled probe) male-specific target. A synthetic oligonucleotide sequence was co-amplified as an internal PCR control (IPC, NED™ labeled probe). A validation study of the multiplex assay was performed according to the Revised Validation Guidelines by Scientific Working Group on DNA Analysis Methods (SWGDAM;http://www.fbi.gov/hq/lab/fsc/backissu/july2004/standards/2004_03_standards02.htm#perfcheck). Briefly, standard curves for both human (RPPH1) and human male (SRY) specific targets were generated using human male genomic DNA. The RPPH1 and SRY assays demonstrated human specificity with minimal cross-reactivity to DNA from other species. Reproducible DNA concentrations were obtained within a dynamic range of 0.023 to 50 ng/µl. In addition, the multiplex assay was highly sensitive to human male DNA in the presence of high amounts of female DNA, detecting as little as 25 pg/µl of human male DNA in the presence of a thousand-fold excess of human female DNA (25 ng/µl). The ability of the assay to predict inhibition of PCR was demonstrated by a shift of the Internal Positive Control (IPC) Ct values in the presence of increasing quantities of hematin and humic acid, common inhibitors of PCR. Experiments that were performed to demonstrate the correlation between the quantification results using the multiplex assay and the strength of STR profiles generated using the AmpFℓSTR® Identifiler®, Yfiler® and MiniFiler™ PCR Amplification Kits will also be discussed. The multiplex assay provides guidance for the selection of the appropriate STR amplification kit to obtain interpretable STR profiles. This approach will reduce the number of samples that need re-processing thereby decreasing the turn around time in a forensic laboratory.

DNA Quantitation, Real Time PCR, DNA Analysis

 ** Presenting Author*