B52 Application of the CFS-HumRT Quantitive PCR Assay With Non-Probative Forensic Casework Sample

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The goal of this presentation is to demonstrate an efficient method of quantifying amplifiable human DNA for use in forensic science.

The Centre of Forensic Sciences' (CFS) laboratory is in the process of completing developmental validation of a rapid and automated RealTime Quantitative PCR assay for detection of human DNA utilizing the ABD 7900HT SDS platform. This assay utilizes CFS-HumRT, a TaqMan®-MGB sequence specific probe developed at the CFS. The probe has a fluorescent reporter dye (VIC) attached at the 5' end and a quencher dye attached to the 3' end. When the probe is intact, the reporter dye emission is quenched. During PCR, the probe anneals specifically between the forward and reverse primers. When the probe is cleaved by the 5' nuclease activity of the DNA polymerase, the reporter dye is separated from the quencher and a sequence-specific signal is generated. With each additional PCR cycle more reporter molecules are cleaved and thus more fluorescence is generated. At some point during the PCR, sufficient target has been amplified and a significant change in fluorescence is detected. This point is referred to as the threshold cycle (CT) and at any given cycle during the geometric phase of the PCR, is proportional to the log of the starting amount of nucleic acid.

Developmental validation of the CFS-HumRT has followed TWGDAM guidelines and has demonstrated the following: a) analysis of over 550 human DNA samples, including 100 samples from each of our Caucasian, Black, South Asian, and South East Asian databases indicates the assay performs similarly for all racial classifications tested, b) analysis of numerous non-human samples indicates the probe to be highly specific, only cross reacting with some primate species, c) the assay yields accurate results for human/non-human mixtures even when the latter is in excess by 1000-fold, d) repeated analysis of DNA standards have shown the assay to be highly sensitive capable of detecting as little as 6pg of template DNA and reliably quantifying from 25ng down to 25pg of DNA, e) replicate amplifications of known samples have shown the technique yields consistent results with standard deviations of less than ±0.15, f) the assay can identify human DNA extracted from a variety of body fluids/tissues including blood, saliva, vaginal epithelial, seminal fluid, and hair, and g) when operating within the dynamic range of the system and using known high quality DNA samples, the technique yields comparable quantification results to the current QuantiBlot™ assay with the added benefit of automation.

During this presentation the utilization of the CFS-HumRT assay with non-probative forensic casework samples will be highlighted. Specific issues that will be addressed are as follows:

- The effects of sample quality and purity on PCR efficiency;
- The interpretation of assay results;
- The impact of DNA quantification using the CFS-HumRT assay on subsequent STR typing using ABI AMPFISTR Profiler Plus™ amplification kits; and
- The development of laboratory specific protocols for the deployment of a fully automated Real-Time Quantitative PCR assay for detection of human DNA.

DNA Quantitation, Real-Time PCR, CFS-HumRT

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