EVALUATION OF APPLIED BIOSYSTEMS' RT-PCR QUANTIFICATION ASSAYS

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Molecular techniques that utilize DNA require an accurate measurement of the quantity of extracted DNA. Currently, quantitation methods include spectrophotometry, fluorometry, and hybridization assays. Not only can some of these methods be subjective, but also several lack the ability to differentiate human versus non-human DNA templates. In a forensic setting, the DNA Advisory Board requires that a laboratory have and follow a procedure for evaluating the quantity of human DNA in an extract. Applied Biosystems has developed human specific and Y-chromosome specific quantitation assays using Real-Time Polymerase Chain Reaction (RT-PCR). We hypothesized that human DNA could be objectively quantitated from various tissue sources for use in forensic work. We also hypothesized that in case of mixtures, the male component could be quantitated independently from the female fraction.

Comparisons were made between the total human specific RT-PCR quantification assay and three other quantification methods: spectrophotometry, PicoGreen® fluorescent dye, and QuantiBlot™ using genomic DNA extracted with a variety of methods and from several biological sources. Additionally, experiments were conducted to quantify low copy number samples using the human specific RT-PCR quantification assay. To determine the sensitivity of the Y-chromosome specific quantification assay, female and male DNA extracts were mixed in ratios ranging from 50:50 to 95.5:0.5 (female:male). This was followed by quantification of DNA extracted from female epithelial fractions of vaginal swabs and of fetal material with both the human specific and the Y-chromosome specific RT-PCR quantification assays to determine if male DNA carry-over could be detected.

Results show that DNA quantitations using ABI's human specific and Y-chromosome specific RT-PCR quantification assays are more sensitive and specific than current methods. Additionally, low levels of male DNA can be detected in suspected mixture samples. We conclude that this assay will prove valuable in quantitating human DNA and male DNA for forensic work. This approach could prevent repeating downstream applications due to excess or minimal DNA inputs and also provide an estimate of the extent of mixed samples.