METHOD COMPARISON FOR THE QUANTIFICATION OF EXTRACTED RNA: IMPLICATIONS FOR FORENSIC ANALYSIS

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The assessment of RNA recovery after extraction can prove useful in determining the course of action to follow with respect to downstream applications. A series of technologies that exploit different properties of the molecule being investigated are available for the quantification of nucleic acids.

In this study, three of these systems were evaluated for RNA quantification of extracts obtained from forensic-like body fluid stains. The Ribogreen fluorescence assay and the Nanodrop 1000 UV-VIS instrument were found to inconsistently measure the concentration of total RNA present in the sample due to the effect of phenol or other possible extraction reagent contaminants. Although most of the contaminants can be removed from the sample, the process adds a time-consuming step to the overall procedure. In addition, the Nanodrop system was found to yield inaccurate results for samples with concentrations below 3 ng/µl, which was the case with most samples tested.

Quantitative PCR is a sensitive technique that uses primers to target specific genes and estimate their level of expression. Using this technology one would be able to target human-specific mRNA instead of total RNA and to obtain a more accurate determination of the sample quantity. The GAPDH housekeeping gene was examined using two commercial kits. The first, GAPDH JOE™-TAMRA assay, targets a 232 base portion of the gene, which was concluded to be too large to quantify suspected smaller fragments. A 122 base target region assay was custom-designed and investigated along with a comparable commercial GAPDH FAM™-MGB assay. Both assays were found to be more sensitive than the previous assay. Because there were some false negatives observed with the GAPDH FAM™-MGB, the possibility of the presence of an alternate GAPDH allele in its primer-binding site was suspected and later confirmed.

Studies have suggested several housekeeping genes as potential candidates to establish the presence and quantity of human mRNA in a sample. It has been implied that GAPDH might not be the best option available since others are more actively expressed in the body fluids being examined. Present results confirm this assumption. Samples that are positive for a fluid specific marker have yielded negative GAPDH results. Therefore, five additional housekeeping genes were assessed as possible substitutes for the GAPDH gene. Results thus far suggest that the B2M marker is more sensitive, reliable and consistent than the other markers investigated for all body fluids examined.