

EVALUATION AND COMPARISON OF QUANTIFICATION METHODS FOR HUMAN RNA OVER A 104 CONCENTRATION RANGE

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Recent research has reported the use of molecular methods in identifying stains from human tissue that would replace standard serological typing. The RT-PCR methods utilize specific tissue-based expression markers, which characterize the source of the biological stains. An essential first step in the analysis involves accurately quantifying the extracted RNA in order 1) to determine the appropriate amount of extract to use in subsequent analyses and 2) to conserve the recovered RNA by minimizing the amount used in the analyses. Thus, we evaluated three quantification techniques for measuring human RNA: UV absorbance, micro-capillary electrophoresis (MCE), and fluorescence-based quantification. Total human uterus RNA was measured in the 500–0.05 ng· μ l⁻¹ range via a ND-1000 spectrophotometer (UV), Agilent RNA 6000 Nano and Pico kits (MCE), and Quant-iT RiboGreen RNA Assay kit (fluorescence). The precision and accuracy of each method was assessed and compared with an independently derived RNA concentration that was obtained using inductively coupled plasma-optical emission spectroscopy (ICP-OES). Robustness, cost, operator time and skill, and conservation of evidence were also considered in the evaluation. Results indicate an appropriate concentration range for each RNA quantification technique to optimize accuracy and precision. The ND-1000 spectrophotometer accurately and precisely quantifies small volumes (1–1.5 μ l) in the 500–5 ng· μ l⁻¹ range. The Quant-iT RiboGreen assay also exhibits high precision in the 1–0.05 ng· μ l⁻¹ range, but the assay consumes 100 μ l of diluted or undiluted sample and the reagents are more costly. The Agilent kits exhibit the lowest precision compared with the ND-1000 and Quant-iT RiboGreen assay in the 500–0.05 ng· μ l⁻¹ range. However, the Agilent kits can quantify with only 1 μ l of sample and determine the integrity of the RNA sample, a useful feature when determining the success of extracting RNA from a forensic sample.