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ASSESSMENT OF TWO METHODS FOR QUANTITATING RNA EXTRACTED FROM HUMAN BODY FLUIDS

Michael L. Lin[1], Rachel A. Bartholomew[1], Rhonda Craig[2], and Kerri A. Dugan[3]
[1]Oak Ridge Institute for Science and Education Visiting Scientist, Counterterrorism and Forensic Science Research Unit, FBI Laboratory, Quantico, Virginia
[2]DNA Analysis Unit I, FBI Laboratory, Quantico, Virginia
[3]Counterterrorism and Forensic Science Research Unit, FBI Laboratory, Quantico, Virginia

A protocol has been developed to analyze messenger RNA content from biological samples (Juusola and Ballantyne, 2005). Methods such as this one will allow identification of specific fluid and tissue types such as blood and semen based on expression patterns of cell-type specific messenger RNA rather than traditional serological procedures. Before these assays can be implemented, accurate quantitation of RNA extracted from a sample is needed for successful downstream analysis by reverse transcription and PCR (RT-PCR). For the low amounts of RNA expected, the Quant-iT™ RiboGreen® RNA Kit (Invitrogen Corporation, Carlsbad, CA) and the 2100 Bioanalyzer (Agilent Technologies, Inc., Palo Alto, CA) can be used to quantitate RNA. Both systems were evaluated for reproducibility and sensitivity of determining RNA concentration. The RiboGreen assay produced consistent values at final RNA concentrations from 0 to 50 ng/ml with coefficient of variation (CV) values from 1.35% to 6.06%. The 2100 Bioanalyzer with the RNA 6000 Pico and Nano Assay Kits was tested with RNA concentrations from 50 pg/ml to 500 ng/ml. The Bioanalyzer values tended to be less consistent than the RiboGreen assay with CV values ranging from 3.99% to 49.88%. Both methods have been used to quantitate RNA before RT-PCR, and additional testing will be performed to determine the suitability of these methods to casework.