B149 Stability and Recovery of mRNA in Biological Stains

Mindy E. Setzer, BS, Jane S. Juusola, BS, and Jack Ballantyne, PhD, University of Central Florida, PO Box 2367, Orlando, FL 32816-2367*

The attend will learn about the persistence of mRNA in biological stains.

This presentation will impact the forensic community by demonstrating mRNA is useful in determining the type of biological stain present and persists under various environmental factors.

In theory, RNA expression patterns, including the presence and relative abundance of particular RNA species, provide cell and tissue specific information that could be of use to forensic scientists. An mRNA based approach could allow the facile identification of the tissue components present in a body fluid stain and conceivably could supplant the battery of serological and biochemical tests currently employed in the forensic serology laboratory. Some of the potential advantages include greater test specificity, and the ability to perform simultaneous analysis using a common assay format for the presence of all body fluids of forensic interest.

Previously we have reported that it is possible to isolate total RNA of sufficient quality and quantity from biological stains to enable subsequent detection of particular mRNA species using the RT-PCR technique. In the extraction method that we employ, total RNA is isolated from biological stains by extraction with guanidine isothiocyanatephenol:chloroform and precipitated with isopropanol. The extracted total RNA is then treated with DNase I, at which time the extract can be quantitated, when desired, using a sensitive fluorescence assay based upon the binding of the unsymmetrical cyanine dye RiboGreen. The RNA is reverse-transcribed using random decamers as the first strand primer and then the cDNA is amplified using gene-specific primers. We have already identified numerous candidate body fluid specific genes for blood, saliva, semen, and vaginal secretions using a combination of literature and public database searches.

To address concerns over stability of RNA in forensic samples, we have conducted an in-depth study on the persistence of RNA in biological stains. Stains were prepared from blood, saliva, semen, and vaginal secretions and exposed to a range of environmental conditions so that affects of different light sources (UV, fluorescent, natural), temperature (room temperature, refrigeration, freezer), and environment (outside covered, outside exposed) could be assessed. Initially, quantitation experiments were performed using biological samples of various sizes (whole 50 ul stain, ¾ stain, ½ stain, ¼ stain, and $^1/_8$ stain) in order to determine how much RNA can be recovered from the different sized stains. We also performed RT-PCR with these stains using housekeeping and tissue-specific gene candidates to determine sensitivity of the different primer sets. The results from these experiments were then applied to the analysis of the stability samples which were collected at specific time intervals (1 day, 3 days, 7 days, 1 month, 3 months, 6 months, 1 year, >1 year). The extent of RNA degradation within each type of body fluid stain was determined using quantitation of total RNA and reverse-transcription polymerase chain reaction with at least one highabundance and one low-abundance gene. DNA was also extracted from the stability samples and quantitated to determine how the stability of RNA compares to the stability of DNA in biological stains. The results of these studies will be presented in detail.

RNA, Body Fluid Stain Identification, RNA Stability

 ** Presenting Author*