B2 Optimization of the Extraction of Total Ribonucleic Acid (RNA) From Semen

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The goal of this presentation is to optimize the extraction of total ribonucleic acid (RNA) from body fluids for reverse transcription polymerase chain reaction (RT-PCR) and subsequent determination of source attribution.

This presentation will impact the forensic community and/or humanity by demonstrating how messenger RNA expression patterns can provide cell and tissue specific information that can be used to positively identify a tissue or body fluid source. Subsequent amplification of mRNA via RT-PCR using tissue specific genes can be to identify the tissue/body of origin.

Although DNA technology can be used to identify (i.e., type) a suspect, the source of DNA itself (blood, semen, *etc.*) cannot always be definitively identified. Therefore there is a need for the development of tissue or body-fluid specific protocols to identify forensically relevant body fluid or tissue samples. The development and use of RNA technology may complement existing DNA technology where the source of the stain (i.e., body fluid or tissue type) can be identified by examining the expression profile of body fluid or tissue-specific genes. Each cell type has a distinctive pattern of messenger RNA (mRNA) expression. Messenger RNA expression patterns provide cell and tissue specific information that can be used to positively identify a tissue or body fluid source. Subsequent amplification of mRNA via RT-PCR using tissue specific genes can be to identify the tissue/body of origin.

The RNA extraction protocol was streamlined for ease of use in the forensic laboratory. The traditional RNA extraction protocol is lengthy, produces toxic bi-products (i.e., phenol), and requires a skilled user. To be validated and used in casework, stringent quality control measures must be performed on each batch of new reagents, decreasing sample throughput. The use of a RNA extraction kit has several benefits: (i) increased sample throughput, (ii) lack of toxic/hazardous chemical bi-products, (iii) ease of training, and (iv) "built in" quality control measures.

The extraction of RNA from liquid body fluids was optimized using cryopreserved human semen. RNA was extracted from 0.5-500ul of semen using the RNAeasy Micro kit (Qiagen, Valencia, CA). The Agilent 2100 Bioanalyzer was used to determine RNA quantity and quality, including RNA Integrity Number (RIN) analysis. RNA samples (0.5pg to 50ng) were reversed transcribed using the Sensiscript Reverse Transcription kit (Qiagen, Valencia, CA). PCR products were amplified with semen-specific Protamine-1 primers. The subsequent PCR products were analyzed using the Agilent 2100 Bioanalyzer.

The amount of total RNA extracted from semen samples ranged from 9pg/ul to 20ng/ul. RNA quality was variable, with RINs ranging from 1-8.6 (scale 1-10). RNA samples having a RIN of 1 lacked 18S and 28S ribosomal peaks, but still contained quantifiable RNA. Samples with a RIN of 8.6 contained intact 18S and 28S ribosomal RNA bands. RNA samples (degraded and non-degraded) were efficiently reverse transcribed and amplified to produce a semen specific RNA product. A semen-specific Protamine-1 RT-PCR product was obtained from as little as 5pg (RIN=7.4) or 15-25 pg (RIN=1).

The amplification of a semen specific product was possible with degraded RNA, suggesting that RNA can be used for cellular source attribution even when there is a question regarding the overall integrity of the sample. The potential benefits of an RNA based approach for body fluid stain or tissue characterization include: (i) the ability to perform parallel tests for numerous markers of a single body fluid in a single assay format, (ii) the ability to perform parallel tests for different body fluids in a single assay format, (iii) a definitive identification of body fluids for which presently no specific test exists, and (iv) the ability to automate the process. The use of RNA technology could supersede current protocols for forensic body fluid identification.

Serology, Semen, RNA Analysis

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