B8 The Detection of MicroRNAs (miRNAs) in DNA Extraction Methods Commonly Used for Forensic Casework

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After attending this presentation, attendees will understand how miRNAs function and why they can be of significant value in forensic analyses. Recent forensic literature has focused on molecular markers for forensic body fluid identification, including miRNAs, messenger RNAs, methylation, and proteomics. Each has been proposed as a supplement to or replacement of the current serological methods. The miRNA and RNA work for forensic body fluid identification thus far has been performed using RNA extraction of samples, which requires an additional step in the evidence analysis process. Not only is analyst time lost in the secondary extraction process, but it could also consume additional sample, which is problematic when the sample is of limited size. Although two reports have shown that miRNAs can potentially be detected within silica column-based DNA extracts, only one type of silica column isolation method was tested and other extraction methodologies have not been explored for miRNA co-isolation and detection. Attendees will understand the importance and limitations of miRNA detection using common forensic DNA extraction methods and be encouraged to promote the necessary research for implementing miRNAs into forensic casework.

This presentation will impact the forensic science community by illustrating how forensic research on miRNAs continues to build evidence for their utility in forensic casework.

MiRNAs are small non-coding RNAs that regulate gene expression by binding to messenger RNA in the cytosol to prevent further translation. Their short length of 18-22 nucleotides, cellular function, and resistance to degradation allow for easy detection in highly degraded samples, as is often the case in forensic casework samples. This study was conducted to explore detection of miRNAs in a variety of DNA extraction methods. Liquid donations of semen, blood, and saliva were collected from three individuals, and 100µL was aliquoted onto cotton swabs. Seven of the most common DNA extraction methods used by forensic laboratories were performed on all samples, with a total RNA isolation method for each sample as a control. A portion of all DNA and RNA extracts were DNase-treated to ensure that the data was a reflection of miRNA detection rather than genomic DNA contamination. MiRNA presence was evaluated using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR) analysis of miRNAs let-7g and let-7i because research has shown that they have relatively similar expression levels across multiple body fluids and between individuals. DNase treatment of extracts had little effect on miRNA expression levels between untreated and DNase-treated samples. Subsequent work revealed that multiple silica columns as well as other non-silica-based DNA isolation methods yielded detectable miRNA levels consistent with those found in the RNA extracts of the same samples. Based on these data, miRNAs are present at detectable levels in DNA extracts when using common DNA extraction methods. Co-extracting miRNAs with DNA would be beneficial for forensic investigations since it could provide probative information about an evidence sample without a separate RNA isolation, thus consuming less sample and reducing the amount of hands-on time for biological evidence analysis.

microRNAs, DNA Extraction, RT-qPCR