B1 The Development and Initial Evaluation of a MicroRNA (miRNA) System for Forensically Relevant Body Fluids Using Capillary Electrophoresis

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After attending this presentation, attendees will better understand the utility of miRNAs for Body Fluid Identification (BFID) in a forensic setting. Recently, the development of molecular-based assays has shown some improvement over conventional biological screening methods through increased body fluid specificity, sensitivity, the identification of a greater number of body fluids, and less consumption of the evidentiary sample.

This presentation will impact the forensic science community by demonstrating a possible confirmatory method of BFID for venous blood, menstrual blood, semen, vaginal material, and saliva using miRNAs that utilizes similar techniques and instrumentation already in place in crime laboratories.

BFID can be of great importance during the course of an investigation and in the courtroom. Determining the body fluid origin of a stain may provide probative information about the events that took place during the commission of a crime. Current methods for BFID, such as chemical tests, microscopy, enzymatic activity, and immunological tests, do not offer the level of specificity the forensic field requires. In recent years, miRNAs have been suggested as a viable biomarker for BFID and have shown considerable body fluid specificity. MiRNAs are small, typically 18-25 nucleotides in length, which makes them ideal for analyzing highly degraded forensic samples. In addition, miRNA interrogation allows for the co-analysis of DNA for individualization and miRNA for BFID from a single sample.

A common strategy for miRNA profiling systems is to analyze relative expression (delta Cycle Threshold (ΔCT)) values of various miRNAs compared to an endogenous reference gene using Real-Time quantitative Polymerase Chain Reaction (RT-qPCR); however, most instrumentation for qPCR can only detect up to five different fluorescent dyes, which would limit the amount of markers that can be amplified simultaneously. Additional reactions would be required to analyze multiple markers, which increases sample consumption, the risk of contamination, cost of reagents, and time of analysis. To address these issues, a previously reported linear primer system was expanded in order to incorporate additional miRNA markers by forming a comprehensive four-dye multiplex system using capillary electrophoresis.

In this study, a new ten-marker system for BFID was designed to differentiate between venous blood (miR-451 and miR-142-3), menstrual blood (miR-144 and miR-412), semen (miR-891 and miR-10), vaginal material (miR-124 and miR-617), and saliva (miR-205 and miR-658). Each marker was tested in singleplex reactions to assess marker viability in the multiplex as well as evaluate cross-reactivity. It was observed that the vaginal markers interacted during reverse transcription and were removed from the multiplex at this time. All other primers amplified the correct miRNA targets. Five samples each of venous blood, menstrual blood, semen, vaginal material, and saliva were evaluated with the multiplex. Although further work is needed, the miRNA system was able to generate an STR profile (from the DNA extract) and distinguish between venous blood, menstrual blood, and semen (RNA extract) from a single sample.

microRNA, Body Fluid Identification, Capillary Electrophoresis