B81 The Developmental Validation of a MicroRNA (miRNA) Panel for Forensic Body Fluid Identification

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Learning Overview: After attending this presentation, attendees will understand how microRNAs (miRNAs) can be of significant value for body fluid identification in forensic casework. Attendees will be apprised of the markers that can distinguish different body fluids and understand why miRNAs may be a better molecular-based method for the identification of body fluids rather than the use of current serological tests, which are based on enzymatic activity and are often prone to false positives.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing how forensic research on miRNAs continues to build evidence for their utility as forensic molecular markers.

MiRNAs are small non-coding RNAs 18-25 nucleotides in length that have been identified and evaluated as potential markers for the identification of forensically relevant body fluids. There is significant interest in the use of miRNAs for forensic casework because of their short length and high resistance to degradation. They have also been shown to co-extract and be detectable in DNA extracts, which could make the use of miRNAs a more streamlined and easily implementable molecular body fluid identification method than other described methods.

Candidate miRNAs were identified through high-throughput sequencing of the miRnome and quantitative Polymerase Chain Reaction (qPCR) panel analyses of differential expression patterns in venous and menstrual blood, vaginal secretions, saliva, feces, urine, perspiration, and semen. Identified candidate miRNAs were further validated using population sample sets from each biological fluid, ultimately identifying nine miRNAs to identify seven biological fluids. This panel of nine miRNAs includes a pair of endogenous reference markers that provide normalization of miRNA expression without evaluation of the RNA or known input quantity. This panel uses expression detected using reverse-transcription quantitative PCR (RT-qPCR) to identify and differentiate feces, urine, peripheral blood, menstrual secretions, vaginal secretions, semen, and saliva. Identification of the biological fluids was found to be reliable across population samples of mixed ages, ethnicities, and gender (where appropriate).

Panel performance for body fluid identification of miRNAs in DNA extractions was assessed and compared to paired RNA extracts and found to provide body fluid identification in 7 of the 9 body fluid samples tested. Detection in compromised samples, limit of detection, and species specificity was evaluated according to developmental validation guidelines and miRNA body fluid ID was successful despite compromising conditions and low input. However, most of the miRNAs in the panel are not human-specific.

In conclusion, the 9-miRNA panel has been shown to provide robust, accurate identification of 7 biological fluids, and continues to show potential for implementation into forensic casework.

Body Fluid Identification, microRNA, qPCR