B1 Investigating the Use of MicroRNAs (miRNAs) for the Identification of Forensically Relevant Body Fluids

Kelsie R. Weir, BA, 9124 E Mill Creek Road, Troy, IL 62294; and Claire Glynn, PhD, Henry C. Lee College of Forensic Science, University of New Haven, West Haven, CT 06516*

After attending this presentation, attendees will gain insight into the field of miRNA analysis and its potential application to forensic science, in particular for body fluid identification. This presentation includes extensive research to identify the optimal method of miRNA extraction through to the validation of particular miRNAs previously suggested as markers for particular body fluids.

This presentation will impact the forensic science community by introducing the potential of this novel method for the confirmatory molecular identification of forensically relevant body fluids. This presentation will provide a valuable contribution to the growing field of miRNA analysis for body fluid identification.

MiRNAs are small, non-coding single-stranded RNA molecules, typically 19-25 nucleotides in length. Previously, they were assumed to have no function and were referred to as "junk DNA;" however, they are now known to play crucial roles in many biological processes and have been shown to be highly robust under chemical and physical conditions. Furthermore, through extensive research in the biomedical field, they have also been shown to have high tissue specificity, which infers great advantages to their role in the forensic science field.[1] Due to these qualities, it is proposed that miRNAs could be ideal for the identification of forensically relevant body fluids. The goal of this research was to investigate the ability to extract miRNAs from body fluids using multiple methods, and then to validate miRNAs previously identified to show specificity for particular body fluids using Relative Quantitative Polymerase Chain Reaction (RQ-PCR), which has been shown to be the gold standard for observation of miRNA expression.[2]

Following informed consent, five body fluids were collected from ten volunteers (n=50), including venous blood, menstrual blood, semen, saliva, and vaginal secretions. Each sample was extracted using three different methods including: mirVana™ miRNA isolation kit, miRNeasy® mini kit, and a modified mirVana™ method with Trizol. The manufacturer's instructions of each kit were followed throughout. The quality and quantity of extracted miRNA was determined using an Eon™ spectrophotometer which measures the full spectrum (220nm-750nm) for accurate measurement of concentrations (A260) and protein contamination (ratio A260/280). The concentration of each extracted sample was recorded in ng/µl and the quality assessed by analysis of the 260/280 ratio. RQ-PCR was performed using a 9700 Thermal Cycler and 7900HT Real Time PCR System, targeting six miRNAs of interest, namely miR-451, miR-412, miR-891a, miR-205, and miR-124a, with miR-16 used as the endogenous control. Data analysis was performed using Sequence Detection System (SDS) software and Mintab® 16.0.

The results of this study show that quantifiable miRNA was extracted from each sample; however, remarkable variation was observed in the yields obtained depending on the methods used for each body fluid. Overall, the results suggest the miRNeasy® mini kit provided consistently higher yields throughout all the body fluids, with the exception of saliva, where the modified mirVana™ method proved to be superior. Each miRNA of interest was detectable in the relevant body fluid with significant dysregulation observed across the body fluids for each miRNA.

This study has identified the optimal method for extraction of miRNAs from body fluids and further validates a selection of miRNAs previously suggested as potential biomarkers. This research highlights the overwhelming potential of miRNAs as novel molecular markers for the confirmatory identification of forensically relevant body fluids.

Reference(s):

1. An J., Shin K., Yang W. (2012). Bodily Fluid Identification in Forensics. *BMB Reports*, 545-553.
2. Wang Z., Luo H., Pan X., Liao M., Hou Y. (2011). A model for data analysis of microRNA expression in forensic body fluid identification. *Forensic Science International: Genetics,* 419-423.

mircoRNA, Body Fluids, Identification