DEVELOPMENTAL EVALUATION OF A COMBINATORIAL REVERSE TRANSCRIPTION-QPCR MULTIPLEX FOR FORENSIC BODY FLUID IDENTIFICATION

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Molecular methods for forensic body fluid identification (BFID), such as proteomics, microRNA (miRNA) analysis, or microbial classification, have been extensively researched in theforensic community due to flaws in current serological techniques. Recognition of advantages and disadvantages of each method have led to the proposition that combining molecular markers would increase discrimination efficiency of multiple body fluids from a single assay. While microbial taxonomic classification for BFID is successful in body fluids with high abundances of bacteria, such as saliva, feces, and vaginal/menstrual secretions, miRNA analysis shows promise in body fluids with less bacterial content, such as venous blood and seminal fluid. Since miRNAs co-extract with DNA, a separate RNA extraction is not necessary, eliminating a major hurdle to casework implementation. This study aims to synergize on the benefits of both miRNAs and microbial DNA to characterize six body fluids from a single DNA extract through RT-qPCR analysis and statistical modeling. The qPCR multiplex would allow for simultaneous BFID from standard casework DNA extracts at the quantification step of the current forensic DNA workflow.

Primers and hydrolysis probes targeting the 16S gene in three microbial species (*Lactobacillus crispatus* for vaginal/menstrual secretions, *Bacteroides uniformis* for feces, and *Streptococcus salivarius* for saliva) were designed and optimized. Likewise, primers and probes were designed and optimized for miRNA targets let-7g, miR-200b-3p, and miR-891a, which have shown differential expression patterns specific to blood and semen in previous research when normalized to let-7g. The validated 16S triplex was tested for body fluid specificity in 30 donors of blood, semen, saliva, feces, vaginal fluid, and menstrual secretions. A stepwise analysis of raw cycle threshold values was developed to identify vaginal/menstrual secretions, feces, and saliva, which correctly classified 96.7% of samples, where only six samples failed due to being outliers (3 standard deviations greater than the mean). Meanwhile, the reverse transcription and qPCR reaction conditions were optimized for the normalization marker (let-7g) and are currently being optimized for miR-891a and miR-200b-3p. Body fluid specificity of the miRNA markers will be assessed in DNA extracts of the same 30 donors, and the data will be incorporated into the analysis method to discriminate between blood and semen, thus predicting all six body fluids in the final assay. Additionally, DNA was extracted from ten body fluid mixtures most likely to be found at crime scenes and will be evaluated once the final assay is validated. In summary, this project demonstrates the ability to utilize an integrated molecular approach for body fluid identification that could be easily implemented into forensic DNA laboratories. Future work may include expanding the population size to generate more data to include in the model and incorporating discriminatory molecular markers for other forensically relevant body fluids.