Body fluid identification (BFID) can provide crucial information during the course of a forensic investigation. Recently, the development of molecular-based assays has shown 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. In addition, these methods can also facilitate the co-analysis of DNA for individualization, and RNA for BFID purposes. In recent years, microRNAs have shown considerable body fluid specificity, and their small size (18-22 nucleotides) make them ideal for analyzing highly degraded forensic samples.

In this study, we designed a new 8-marker system for BFID to differentiate between venous blood (miR-451 and miR-142-3), menstrual blood (miR-141-3 and miR-412), semen (miR-891 and miR-10), and saliva (miR-205 and miR-658) using a capillary electrophoresis approach. This panel expands on a previously reported linear primer system in order to incorporate additional miRNA markers by forming a comprehensive four-dye multiplex system.

Each marker was tested in singleplex reactions to assess primer viability and evaluate cross-reactivity. The saliva primers were not specific to the miRNA targets and were removed from the multiplex. The singleplex reactions also revealed a marker proposed for the identification of menstrual blood (miR-141-3) was found to cross-react with semen, which has not been previously described in literature. All other primers amplified the miRNA targets. 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 from a single sample.