

## **Evaluation of mRNA Markers for the Identification of Five Human Body Fluids.**

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Messenger RNA (mRNA) profiling has been proposed as an alternative method to serology for body fluid stain identification. Current serological techniques can conclusively determine the presence of a blood or semen stain and presumptive identification of saliva stains is possible. However, forensic identification of several other body fluids is also of interest. In sexual assault cases for example, the identification of vaginal secretions and menstrual blood stains would be useful.

Several body fluid specific markers have been suggested for the identification of body fluid stains of interest to the forensic community. Among these, twelve were selected for this study: semen (PRM1, PRM2, and TGM4), saliva (HTN3 and STATH), blood (ALAS2 and SPTB), menstrual blood (MMP7, MMP10 and MMP11), and vaginal secretions (KRT16 and MUC4). These markers have been optimized and assessed for use in the identification of body fluid stains.

A preliminary assessment of their ability to specifically amplify the gene of interest and their potential to cross-react with other body fluid stains narrowed these markers to seven candidate genes. These seven markers were further assessed for cross-reactivity with other body fluid stains and their presence in individual donors. The ALAS2 blood specific marker positively identified blood stains from four different donors. No cross-reactivity was seen between these blood stains and the other six body fluid markers selected. Menstrual blood and vaginal secretion stains were examined together. Samples were taken once during the donor's period and again 3 and 7 days upon completion of menstruation. The menstrual blood marker MMP7 was seen in both menstrual blood stains and vaginal secretion stains, although its expression decreased as the time after menstruation increased. The vaginal secretion marker MUC4 was strongly expressed in both menstrual blood and vaginal secretion stains. Additionally, the expression of the ALAS2 blood specific marker could be seen in both menstrual blood and vaginal secretion stains. The cross-reactivity seen in the menstrual blood and vaginal secretion stains varied as the menstrual cycle progressed.

Individual differences in gene expression level and marker cross-reactivity were observed in the individual donors tested. Additional information about the donor can be gleaned from the mRNA profiles generated. Despite the presence of cross-reactivity in some of the body fluid stains, the body fluids examined have distinct gene expression profiles, allowing for body fluid identification based on mRNA profiling. The more robust body fluid specific markers will be further assessed for their stability under environmental conditions.