## B41 Integration of Microchip Electrophoresis and a Validated Messenger RNA (mRNA) Panel as a Novel Approach to Forensic Body Fluid Identification

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**Learning Overview:** After attending this presentation, attendees will better understand a simple method for microchip electrophoretic separations, the use of a novel mRNA panel for differentiation body fluids, and how the integration of these two scientific advances applies directly to forensic body fluid identification. This information will present a novel alternative to current methods used in forensic laboratories and literature.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing a simple, yet specific, alternative method for body fluid identification that can be easily integrated into the forensic workflow, is automated to minimize user intervention, and can produce genetic profiles from mixture samples that are comparable to those generated from commercial genetic analyzers.

This research aims to develop a microfluidic approach to forensic body fluid Identification (bfID) using an mRNA panel coupled with electrophoretic separation on a centrifugally driven microfluidic device. The Institute of Environmental Science and Research (ESR) has developed a method for detection and differentiation of body fluids in an unknown sample. The approach is based on amplifying mRNA targets specific to individual body fluids, then electrophoretically separating the amplicons. Traditionally, a commercial genetic analyzer is used for separation of fragments by capillary electrophoresis. However, electrophoretic separation can instead be carried out on a microfluidic device to decrease required assay time, cost, and user intervention relative to use of a commercial instrument.

Genetic material (mRNA) was extracted from various samples containing either a single fluid (blood, semen, saliva, vaginal fluid, or menstrual blood) or a mixture, amplified, then separated by size using either a commercial genetic analyzer (3500*xl* or 3130*xl)*, or a novel microfluidic device was developed for separation of the mRNA targets. Sample injection time and channel temperature for the microfluidic approach were optimized for improved DNA fragment resolution with on-disc microfluidic electrophoresis. To determine the optimal sample injection time for an on-disc electrophoresis, different electrokinetic sample injection times prior to separation were evaluated to determine the minimal amount of time needed to produce acceptable peak heights (above 1,000 Relative Fluorescence Units [RFU]). This study also evaluated whether heating of the separation domain/polymer was necessary for sufficient peak resolution and separation, with the temperature adjusted via an embedded Peltier thermoelectric device. Analysis of the resulting electropherograms indicated that heating of the separation channel did not have a large impact on the resolution or separation of the peaks in the electropherograms. To determine detection sensitivity of the on-disc platform, two single-source samples for each body fluid were diluted to various extents (1:2, 1:6, 1:10, or 1:14) prior to injection and electrophoresis. This demonstrated an on-disc platform sensitivity that was comparable to current bfID techniques. The fluorescence signal from all peaks was detectable through 2X and 4X dilution for the majority of the body fluids, although, some drop-out was observed.

In conclusion, this study has provided evidence for an on-disc microfluidic separation of a novel mRNA body fluid panel as an alternative to current bfID techniques that demonstrates detection of individualized targets for all five body fluids, detection of all peaks for mixture fluid samples, and rapid separation times (~8 minutes). This on-disc separation provides genetic profiles that are comparable to “gold standard” genetic analyzers used in forensic laboratories, based on peak heights and ratios. In addition, the on-disc platform was able to consistently separate all peaks in the mixture samples and show detection of highly diluted amplified samples. The next steps will be to optimize amplification of the mRNA targets on-disc and integrate the amplification and separation domains to allow for full automation after lysis of a sample for a “sample in-answer out” device.

**Microchip Separation, Body Fluid Identification, Mixture Analysis**



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