B111 Development of a Microfluidic Differential Extraction Module and Refinement of Infrared (IR) -Mediated Short Tandem Repeat (STR) Amplification for a Rotation-Driven Microdevice

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After attending this presentation, attendees will better understand the forensic applications of microfluidic platforms, micro Total Analysis Systems (μTAS), and recent advancements toward integrating a differential DNA extraction module for sexual assault samples.

This presentation will impact the forensic science community by describing a differential extraction module on a rotation-driven microdevice platform for sexual assault casework samples as well as refinement of IR-mediated STR amplification occurring on the device. If implemented, an automated device based on this microchip could remove the human variability often seen with manual differential extractions, speed the workflow for sexual assault samples, and result in high-quality data with fewer STR mixtures needing lengthy interpretation.

Microdevices offer several potential advantages over conventional methods for quick determination of STR profiles, including smaller reagent and sample volumes, less manual intervention, and decreased risk of contamination — all leading to decreased sample-to-answer time. IR-Polymerase Chain Reaction (PCR) and enzyme-driven DNA preparation have improved the ability of scientists to integrate microdevice modules that represent all aspects of the traditional forensic DNA workflow. While these technologies have the potential to significantly impact the way forensic science DNA laboratories function, extensive modification of these technologies is needed before microdevices can be implemented for processing casework samples. Commercially-available microdevice-based instruments (i.e., Remote Access Program for Interactive Diagnostics (RAPID)) require extensive external pumps and actuators for microfluidic control and are designed only for the processing of single-source reference (known) buccal swab samples. Unfortunately, forensic casework samples, ~52% of which involve testing of sexual assault evidence and which require a differential cell lysis process, are not currently amenable to microchip-based processing. Manual differential lysis is a time-consuming DNA purification process that often still results in mixtures, which require a significant amount of interpretation effort. As the media draws more attention to the number of rape kits left untested each year, it will become imperative that crime laboratories find ways to process more of these samples more quickly. To this end, the first goal of this project was the incorporation of a differential extraction module onto the aforementioned microdevice.

In this project, sperm cells were separated from non-sperm cells via an antibody-labeled, bead-based capture mechanism. Initially, chip architecture was redesigned to include an antibody capture chamber that allows for dual valving and microfluidic movement into side-by-side sperm cell and non-sperm cell DNA liberation chambers. This design allows for antibody-coated microbeads to selectively capture sperm cells, which would prevent movement through a burst valve designed to capture non-sperm cells. Next, two sperm-specific antibodies (SP-10 and SPAG8) and one male-specific antibody (MEA-1) were tested off-chip for binding efficacy using flow cytometry. To assure that other contributing female and epithelial cells were not binding to the sperm antibodies, two antibodies specific for epithelial cells were also used. Based on binding specificity and efficiency results from the flow cytometry data, the best sperm-specific antibody was selected for further on-chip testing.

The second objective of this work was to explore STR reaction chemistry alterations in an effort to improve STR profile issues often seen with IR-mediated amplification. Existing microdevice platforms that utilize rapid small volume IR-PCR and polymerase combinations often lead to the presence of significant non-adenylated (-A) products and an overall "ski-slope" effect that leaves alleles in the larger-sized loci either very low or undetectable by capillary electrophoresis. In previous studies, a commercially available primer set was used along with Phusion® Flash master mix and SpeedSTAR™ HS polymerases in a reduced volume reaction for IR-mediated on-chip amplification. In this study, AmpliTaq Gold® polymerase and AmpFℓSTR® Identifiler® Plus chemistry were evaluated, along with adjustments to the original combination of Phusion® Flash and SpeedSTAR™ HS, other enzyme combinations (AmpliTaq Gold® Fast and KAPA2G Fast), and longer final extension times. When used alone, AmpliTaq Gold® yielded negative Identifiler® Plus results; however,

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using it in combination with the Phusion® Flash-SpeedSTAR™ HS combination and a longer final extension (180s) resulted in a nearly complete profile (29 of 30 alleles called) with more than 40% of alleles (12 of 29) showing greater adenylated product. Additionally, none of the STR loci showed *only* -A peaks. Taken together, these improvements represent large strides toward the development of a sexual assault microdevice.

Microdevice, Differential Extraction, IR-PCR

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