A MICROCHIP MODULE FOR DIFFERENTIAL EXTRACTION OF SEXUAL ASSAULT SAMPLES AND IMPROVED IR-MEDIATED STR AMPLIFICATION FOR A ROTATION-DRIVEN MICRODEVICE

Kemper Gibson B.S.[1], Jordan O. Cox, M.S.[1], Kimberly Jackson M.S.[2], James P. Landers Ph.D.[2], Tracey Dawson Cruz, Ph.D[1].

[1]Virginia Commonwealth University, Department of Forensic Science, 1015 Floyd Avenue, Room 2011 Box 843079 Richmond VA 23284
[2]University of Virginia, Department of Chemistry, McCormick Road, P.O. Box 400319, Charlottesville, VA 22904

IR-PCR and enzyme-driven DNA preparation have improved scientists ability to integrate microdevice modules that represent most aspects of the traditional forensic DNA workflow. Unfortunately, extensive modifications will be needed before microdevices can be implemented for processing casework samples. This is especially true for sexual assault evidence which currently requires a time-consuming manual differential cell lysis process, which often still leads to tedious mixture interpretation efforts. Automatable, more precise differential procedures are sorely needed so labs can process more samples faster and reduce associated backlogs. To this end, the first goal of this project was the incorporation of a differential extraction module onto a previously developed rotation-driven microdevice. In this module, sperm cells were separated from non-sperm cells via an antibody-labeled, bead-based capture mechanism designed to selectively capture sperm cells, and prevent their movement through a burst valve designed to capture unbound non-sperm cells. Initially, chip architecture was redesigned to include an antibody capture chamber with dual valving for microfluidic movement into side-by-side sperm cell and non-sperm cell DNA liberation chambers. Next, two sperm-specific (SP-10, SPAG8) and one male-specific (MEA-1) antibodies were tested off-chip for binding efficacy using flow cytometry; based on this data, the best sperm-specific antibody was selected for further on-chip testing. The second goal was to explore STR reaction alterations to improve STR profile issues often seen with on-chip IR-PCR. The existing microdevice utilizes rapid small volume IR-PCR and polymerase combinations (Phusion Flash/SpeedSTAR™ HS) that often lead to significant –A product and a "ski-slope" effect. As such, AmpliTaq Gold® polymerase and AmpFlSTR® Identifiler® Plus chemistry were evaluated, along with adjustments to the Phusion Flash/SpeedSTAR™ HS ratio, 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 when used with the Phusion Flash/SpeedSTAR™ HS and longer final extension (180s) a near full profile resulted (29/30 alleles) with over 40% of alleles showing improved –A. Taken together, these modifications represent large strides towards the development of a sexual assault microdevice, which could ultimately remove the human variability often seen with manual differentials, speed the workflow for sexual assault samples, and produce high quality data with fewer mixtures.