B98 A Novel, Extraction-Polymerase Chain Reaction (PCR) Microdevice on a Rotation-Driven Platform: Buccal Swab to Short Tandem Repeat (STR) Product in Less Than Two Hours

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After attending this presentation, attendees will better understand the applications and capabilities of forensic microdevices, as well as the challenges of transitioning conventional DNA workflow onto the microscale.

This presentation will impact the forensic science community by describing an integrated plastic microdevice for DNA extraction and STR amplification that is capable of producing Capillary Electrophoresis (CE) -ready STR product from a buccal swab in less than two hours.

Microdevices have numerous advantages over conventional methods, including smaller reagent volumes, smaller equipment footprints, reduced contamination risk, and faster sample-to-answer time. Three unique aspects of the device presented in this work allow for successful integration. First, microfluidic flow control is achieved by exploiting centrifugal force on a simple rotational platform. This is coupled with the use of simple "passive" valves specifically positioned to physically isolate the different pre-electrophoresis chemistries. Second, enzyme-mediated DNA liberation (non-solid phase extraction) is fast (<10 minutes total) and effective as it circumvents the need for wash steps, hazardous chemicals, and solid beads, thus simplifying its incorporation into the overall microdevice architecture. Third, non-contact heating is integral to both DNA liberation and PCR amplification of STR loci. An Infrared (IR) -mediated heating system consisting of a platform, lamp, and fan is used to directly heat and cool the device, avoiding the need to transfer liquid into microtubes as required by conventional thermocyclers. During DNA liberation, the enzyme (a thermostable protease) is heated by the infrared lamp, lysing cells and exposing the nuclear DNA. During Infrared-mediated PCR (IR-PCR), thermal cycling of the sample is facilitated by the lamp and fan. Direct heating of the sample (instead of indirect heating on a heat block), small sample volume, and the effect of IR radiation on water (exciting the stretch mode) combine to dramatically reduce ramp and hold times, allowing IR-PCR with 28 cycles and a 10-minute final extension to occur in 45 minutes.

All microdevices were fabricated using laser ablation and thermal bonding of Poly(Methyl-Methacrylate) (PMMA) layers. Using this microdevice, the enzyme-mediated DNA liberation module produced DNA yields similar to or higher than those produced using the traditional (tube-based) protocol. Initial microdevice IR-PCR experiments to test the amplification module and reaction (using Phusion® Flash/SpeedSTAR™) generated near-full profiles that suffered from inter-locus peak imbalance and poor adenylation (significant –A); however, subsequent attempts using KAPA2G and *Pfu* Ultra polymerases generated full STR profiles with improved inter-locus balance and the expected adenylated product. An integrated run designed to test microfluidic control successfully generated CE-ready STR amplicons in less than two hours (<1 hour of hands-on time). Using this approach, high-quality

STR profiles were developed that were consistent with those produced using conventional DNA purification and STR amplification methods. This method is a smaller, more elegant solution than current microdevice methods and offers a cheaper, hands-free, closed-system alternative to traditional forensic methods.

Forensic Microdevice, Microfluidics, IR-PCR