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BODY FLUID IDENTIFICATION: MICROCHIP-BASED SOLID PHASE EXTRACTION OF NUCLEIC ACIDS FROM COMPLEX MATRICES

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Microfluidic technology has the potential to greatly impact the forensic community with the significant advantages it provides over many conventional analyses. Microchips are an appealing alternative to time-consuming, costly conventional analyses because of the reduction in sample and reagent volume, time of analysis, and overall cost inherent with their use. Microdevices also provide a platform for integration of multiple analytical processing steps on a single device. DNA extraction and purification, PCR amplification and microchip electrophoresis have been demonstrated on a microdevice with the associated reduction in time and reagent volume.[1] The potential to process sample volumes on the order of microliters makes the microchip an attractive device for forensic analyses where sample is often limited.

Traditionally, DNA purification involves organic extraction, consisting of several incubation and centrifugation steps which greatly extend the analysis time, sample handling, and amount of reagents consumed. Solid phase extraction (SPE) has been shown to be a viable alternative to organic extractions, and is now routinely used in forensic analysis. Microchip SPE, using silica beads packed in a microchannel, has been used to purify DNA from biological samples, providing efficient and reproducible extraction of PCR-ready DNA in a reasonably small volume.[2] Although efforts have been expended to develop a microchip-based SPE method for DNA purification, significantly less effort has been directed towards the isolation and purification of RNA. With recent advancements in the identification of body fluids using mRNA expression analysis[3] the ability to obtain information from RNA transcripts that DNA cannot provide becomes important. Thus, development of a robust system for the purification of RNA from biological samples is a necessity. With RNA being sensitive to degradation and contamination, microchip-based SPE provides the advantage of purification in a self-contained environment. Highly efficient extractions provide co-purified RNA and DNA free from PCR inhibitors and nucleases, which after digestion of DNA, allows for body fluid identification from the mRNA.

The presented research demonstrates the use of a microchip SPE method for purification of nucleic acids from biological samples of forensic interest. The microdevice design will be presented, along with preliminary studies describing the capacity and extraction efficiency of the device for co-purification of RNA and DNA. Elution profiles will detail the recovery of RNA in elution fractions along with results from conventional PCR of the extracted DNA and RT-PCR analysis on RNA in each fraction

after degradation of the DNA. Purification of RNA from biological samples will focus on mRNA expression analysis for body fluid identification.

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