B62 A 2-D Microchip-Based Process for Volume Reduction and Purification of Total Nucleic Acids

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Upon completion of this workshop, participants will gain an understanding of a microdevice that can be used to reduce the volume of a dilute biological sample for downstream solid phase-based extraction and purification of nucleic acids on a single microdevice.

This presentation will impact the forensic community by introducing a microdevice for nucleic acid purification from the types of dilute biological evidence collected in forensic investigations, providing the potential to increase the speed of processing forensic casework samples. This work is a step toward the use of a micro-total analysis system for forensic genetic analysis.

The purpose of this research is to demonstrate the ability to reduce the volume and then purify the nucleic acids from dilute crude biological samples on a single microfluidic device.

There are numerous advantages in utilizing microfluidic devices for forensic analyses including reductions in analysis time, cost of instrumentation, and reagent consumption, as well as the ability to be combined with downstream analytical processes on a single microfabricated device.[1] In addition, there is an inherent reduction in sample consumption, important when sample is limited in forensic casework. Microchip solid phase extractions (SPE) have proven to be highly efficient and reproducible for the purification of DNA using silica-based solid phases, as demonstrated by Bienvenue, et al.[2] More recent studies demonstrate the effectiveness of the same silica-based microchip extraction method in the isolation and purification of RNA.[3] The self-contained microdevice provides an environment for RNA extraction with less opportunity for the introduction of RNases, allowing for more efficient purification of RNA by reducing sources of contamination and degradation.

Working with small amounts of sample is realized in microfluidic extractions, however, larger samples, on the order of milliliters, are often generated in casework in the collection of samples from fabrics and surfaces; these still require standard processing as they are not compatible with current microchip devices. The presented research describes a two-dimensional microchip-based method that brings together two orthogonal processes that sequentially carry out sample volume reduction and purification of nucleic acids from dilute biological samples. Volume reduction solid phase extraction (vrSPE) will be performed using a silica phase to remove impurities and concentrate the sample down to a suitable volume for a subsequent SPE on a single microdevice. A newly-developed method for DNA and RNA extraction will be used in the SPE performed after the volume reduction. The method involves the use of chitosan-coated silica beads as a solid phase, which binds and releases nucleic acids based on a pH-induced charge switch.[4] The advantage of this phase over silica is that it completely avoids the use of PCR inhibitors such as guanidine hydrochloride and 2- propanol used in typical silica-based SPE. Nucleic acids are eluted at a pH compatible with PCR buffer, allowing for future integration into a micro-total analysis system. This work will demonstrate the effectiveness of the vrSPE method coupled with chitosan-based SPE as a method for isolation and purification of nucleic acids. An integrated device design will be presented, along with preliminary studies describing the capacity and extraction efficiency of the device. Data from downstream PCR and RT-PCR analysis will be reported, and results from this work will demonstrate the first integrated microfluidic device for volume reduction and total nucleic acid purification utilizing an aqueous chitosan SPE method.

References:

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