B20 Microchip-Based Volume Reduction and Sample Concentration of Crude Sample Digests for Micro-Solid Phase DNA Extraction

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After attending this presentation, attendees will learn of lysates for DNA extraction on microdevices.

This presentation will impact the forensic community and/or humanity by reducing the large volume crude sample lysates for DNA extraction on microdevices.

This research presentation describes the development of a microchip- based method for reduction of large-sample volumes and sample pre-con- centration for downstream micro solid phase extraction (SPE). This research would enable a more facile transition from currently existing forensic protocols for DNA extraction to microchip-based methods, enabling faster, high-efficiency recovery of DNA.

As the forensic community continues to explore new technologies and analysis techniques to improve/supplant current methods, microdevices have an increasing appeal as an alternative platform to costly time- and reagent-consuming analyses. Microdevices are now readily utilized to carry out PCR amplification and DNA separations with reduced volumes/analysis times, and the application/testing of these devices for forensic genetic analysis is now underway. In addition, sample preparatory steps, such as DNA extraction, have also been miniaturized, again with a concomitant reduction in sample size, reagents consumed, and time. However, current sample preparation methodologies for forensic DNA analysis often require the creation of a cell lysate or a cell suspension before the extraction procedure is performed which can involve the solubilization of material in 0.5 mL or more of solution. Indeed, larger volumes of solution (0.5 to >1 mL) are often required to effectively elute nuclear DNA from samples with large surface areas or samples such as fabric cuttings, cotton swabs, and other materials, due to their bulky, absorbent nature. In addition, it has been demonstrated that larger extraction volumes can enhance DNA yields from contaminated sources, diluting potential PCR inhibitors prior to DNA extraction and increasing the likelihood of recov- ering intact, amplifiable DNA. However, many microchip platforms, limited by the volumes that they can accept, cannot adequately accom- modate volumes of this size without adding extensively to the time frame for extraction and/or the cost, while increasing the potential susceptibility of the system to contamination. As a result, new methods are required to provide high efficiency, high purity extractions (free from PCR inhibitors) that can both recover and concentrate small amounts of DNA from complex and potentially contaminated mixtures for forensic analysis.

The research presented here describes a microchip-based method being developed for reduction of large sample volumes and sample pre- concentration for downstream micro solid phase extraction (SPE). This method, designed as a crude extraction prior to a more stringent SPE will simultaneously reduce sample volume, removing some contaminants and inhibitors, while providing a high-concentration, small volume eluate for subsequent purification. A device designed to accomplish this volume reduction solid phase extraction (vrSPE) in less than 30 minutes is pre- sented. A method for vrSPE is described, with details on the translation from the macro- to micro-scale. In addition, preliminary studies defining the capacity and extraction efficiencies of these devices are reported. Also described:

1) the use of the device to recover small amounts of DNA from volumes typically encountered in forensic analysis (0.5 to 1 mL) with successful STR amplification,

2) elution profiles showing recovery of sample with a 10-fold reduction in volume (and 10-fold increase in sample concentration),

3) a method and device integrating vrSPE with previously established µSPE methods for complete purification of DNA.

These results represent the successful transitioning of macro-scale volumes required for recovery of DNA from commonly encountered samples down to micro-scale systems for rapid sample purification.

DNA Extraction, Microchip, Volume Reduction

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