B69 Microchip-Based Solid Phase Purification of RNA

Joan M. Bienvenue, MS, Kristin Hagan, BS, Jerome P Ferrance, PhD, and James P. Landers, PhD, University of Virginia, Department of Chemistry, McCormick Road, Charlottesville, VA 22904*

The goal of this research project is the purification of RNA from crude samples using microfluidic devices.

This presentation will impact the forensic community and/or humanity by presenting research which represents the first demonstration of RNA purification and total nucleic acid isolation in a microfluidic device, which may positively impact the ability to identify body fluids for forensic analysis.

As microfluidic technology continues to evolve for both forensic and clinical applications, microdevices have increasing appeal as an alternative platform to costly, time- and reagent-consuming analyses. PCR amplification and DNA separations are now accomplished with reduced volumes/analysis times,[1] and the application/testing of these devices in forensic laboratories is now underway. In addition, sample preparatory steps, such as DNA extraction and purification, have also been miniaturized, again with a concomitant reduction in sample size, reagents consumed, and time.[2] Glass microdevices packed with solid phases such as silica beads, sol-gel immobilized silica beads, or sol-gels alone have demonstrated utility as potentially low-cost alternatives for highly efficient and reproducible extraction of DNA.[3] Micro solid phase extraction (μSPE) provides the benefits of reproducibility and high extraction efficiency, while also yielding highly purified, PCR-ready DNA in reasonably small volumes. While much attention has been focused on the development of microfluidic systems for DNA purification, little research effort has been directed towards the development of robust systems for RNA isolation and with the recent development of mRNA expression analysis systems for body fluid identification by other research groups,[4] the ability to effectively purify RNA from crude samples becomes a necessity. Isolation and purification of RNA, without compromising the integrity of the DNA present in samples, however, has proven challenging. Current methodologies often require organic extractions with time- and reagent- consuming incubation and centrifugation steps not to mention multiple points where contamination with nucleases is a possibility. Thus, closed microfluidic systems capable of rapid, sensitive, total nucleic acid isolation become a logical and potentially important solution. Silica- based SPE methods are not only effective for DNA purification, but also RNA purification and in self-contained microdevices provide a tremendous advantage for forensic analysis by inherently removing many potential sources of contamination and degradation of nucleic acids by nucleases. As a result, these new methods can provide high efficiency, high purity extractions (free from PCR inhibitors and nucleases) resulting in both the recovery and concentration of small masses of DNA and RNA from complex and potentially contaminated mixtures for a complete forensic genetic analysis.

The research presented here describes a microchip-based method being developed for the purification of total nucleic acids from biological samples. This method, designed to co-purify DNA and RNA from multiple biological sources, utilizes a previously described silica- based solid phase extraction for the isolation of nucleic acids. A device designed to accomplish this purification is presented along with a method for simultaneous recovery of DNA and RNA fractions in purified and concentrated form. Using commercially-available nucleic acid quantitation kits, DNA and RNA are co-purified and quantified from biological samples. In addition, preliminary studies describing the capacity and extraction efficiencies with these devices are reported. Elution profiles detailing the recovery of RNA and DNA from biological samples are also presented, along with the results of downstream processing of these purified nucleic acids via PCR and RT-PCR. The advantages of microfluidic systems for nucleic acid purification will be highlighted, with focus on the results presented, which represent the first demonstration of RNA purification and total nucleic acid isolation in a microfluidic device.

References:

1 Easley, CJ, Karlinsey, JM, Landers, JP. 2006. “On-chip pressure injection for integration of infrared-mediated DNA amplification with electrophoretic separation”. (Journal Cover) Lab Chip. May;6(5):601-610.

2 Easley, CJ, Karlinsey, JM, Bienvenue, J.M., Legendre, L.A., Roper, M.G., Feldman, SH, Hughes, MA, Hewlett, EL, Merkel, TJ, Ferrance, J.P. and Landers, J.P. “A Fully-Integrated Microfluidic Genetic Analysis System with Sample in-Answer out Capability.” PNAS (in revision).

3 Bienvenue, JM, Duncalf N, Marchiarullo, D, Ferrance, JP, and Landers, JP. 2006. **“**Microscale Cell Lysis and DNA Extraction of Sperm Cells for Forensic Analysis,” J. Forensic Sciences.” J. Forensic Sciences. 2(51): 266-73.

4 Juusola, J, Ballantyne, J. 2005. “Multiplex mRNA Profiling for the Identification of Body Fluids.” For. Science International. 152: 1-12.

RNA, Extraction, Microchip

 ** Presenting Author*