## A130 Integration of RNA Purification and Reverse Transcription Amplification (RT-PCR) in a Single Microfluidic Device for Biowarfare Agent and Pathogen Detection

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After attending this presentation, attendees will have learned the advantages of utilizing a microdevice for RNA purification and amplification for application to various biological samples associated with biowarfare providing a completely closed environment reducing contamination and sample degradation.

This presentation will impact the forensic science community by demonstrating the use of a microfluidic device capable of both RNA purification and reverse-transcription amplification (RT-PCR). The integration of these sample processing steps provides the next step towards a micro total analysis system (μTAS) which would reduce overall analysis time and provide imperative information to the forensic and defense communities in a timely manner.

The identification of genes encoded for by RNA is a technique commonly used within the defense community for detection of biowarfare agents, such as pandemic diseases like influenza A. In order to detect the presence of biowarfare agents in a biological sample, the RNA must first be purified from cellular and extracellular material and then undergo RT-PCR amplification of a specific region of the genome to identify the threat at hand. These processes can be completed conventionally using solid phase extraction (SPE) and RT-PCR amplification performed on a thermal cycler, however, they often require multiple sample transfer steps, exposing highly unstable samples to potential contamination or degradation as well as exposing the individual processing the sample to the infectious agent. Conventional extraction and amplification methods also require lengthy analysis times. In contrast, the use of a microfluidic purification and amplification system would benefit the defense community by providing a closed environment to reduce contamination of the sample and exposure of the individual processing the sample to the infectious disease. A microfluidic system for RNA analysis also provides reduced sample consumption, reagent consumption, and analysis time, and provides portability for on-site analysis while allowing for seamless integration of multiple sample processing steps.[1]

The implementation of SPE on a microfluidic device for the purification of RNA has been well characterized, and shown to be reproducible and widely applicable to multiple sample types using both a silica-based chaotropic method and a completely aqueous method using an alternative solid phase, chitosan.[2,3] This aqueous methodology involving the reversible binding of DNA to chitosan-coated silica based upon a buffer pH change was first demonstrated Cao et al.[4] The chitosan phase, also employed by Hagan et al.[3], was later found to perform superior to silica for RNA purification. The use of this phase allows for nucleic acid purification to be completed in a completely aqueous environment, eliminating the need for reagents commonly used in chaotropic extraction methods involving a silica solid phase that can inhibit downstream PCR analysis such as guanidine hydrochloride or isopropyl alcohol. Using completely aqueous buffers also allows elution of RNA in PCR buffer, ensuring compatibility with downstream PCR analysis. Additionally, the low-molecular weight chitosan used has been proven a RNase inhibitor, providing an ideal environment for extraction of RNA.[5] The implementation of PCR on microfluidic devices has also been well established using small sample volumes (nL).[6] One example of PCR on a microfluidic device involves the use of infrared (IR)-mediated heating, which results in increased speed of temperature transitions and, therefore, a decrease in analysis time. This was used by Legendre et al.[7] for the integration of SPE with PCR for DNA analysis, however, the integration of SPE and RT-PCR has not before been demonstrated.

This work provides the first demonstration of a microfluidic system for purification of RNA integrated with RT-PCR utilizing IR-mediated heating for more rapid thermal cycling. The use of an integrated microdevice provides a completely closed environment and decreases sample handling steps, with less opportunity for the introduction of contaminants and RNases and exposure of the individual processing the sample to an infectious disease. The decreased analysis time achieved with this method would assist the forensic and defense communities by providing information faster in situations where response time may be critical as well as provide vital information to first-responders. An integrated device design will be shown in addition to characterization of the SPE domain using purified human RNA as a simulant for cells infected with influenza A virus. Method development employing separate microdevices for SPE and RT-PCR will be demonstrated. This will entail determining the optimal SPE elution fraction containing the maximum mass of purified RNA for RT-PCR. The reduction from a two-step RT- PCR method to a one-step method on a microfluidic device will also be shown. Lastly, integration of SPE and RT-PCR on a single microfluidic device will be demonstrated.

**References:**

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**Solid Phase Extraction, RT-PCR, RNA**

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