## A59 Pathogen Detection Using a Unique Liquid Array Technology

*Amanda Battaglia, MS*, 249 Hillman Avenue, Staten Island, NY 10314; Andrew J. Schweighardt, MA, 108 Sandy Hollow Road, Northport, NY 11768; and Margaret M. Wallace, PhD, John Jay College of Criminal Justice, Department of Sciences, Room 4510, 445 West 59th Street, New York, NY 10019*

After attending this presentation, attendees will have knowledge on a novel, bead-based liquid array hybridization system and its capabilities in detecting low concentrations of pathogens.

This presentation will impact the forensic science community by demonstrating that detection of bacterial DNA with a new technology that incorporates the established capabilities of flow cytometry, microspheres, lasers, and digital signal processing is rapid and efficient. This proof of concept study demonstrates how instruments combining these technologies in a unique way can be used to successfully identify the source of a DNA sequence and is not an endorsement of the vendors supplying the components of the system.

Since the anthrax scare in 2001, the threat of modern biological related terrorism has become a reality. Bio-terrorist attacks are difficult to prevent and patients cannot be treated correctly without a proper diagnosis. This dilemma requires that there be a rapid means to identify pathogens in the environment and in a patient in order to respond with the proper vaccinations and care more readily. Techniques that can perform several assays on a single sample have become a necessity. Multiplexing DNA-based technologies are ideal for pathogen detection, specifically because they can provide rapid, positive identification of biological weapons. In this research, selected sequences of PCR amplified microorganisms' genomes were identified with a novel technology that combines bead-based liquid array hybridization with flow cytometry.

This unique system consecutively analyzes up to 96 samples per run with up to 100 probes per sample. Liquid kinetics allows for three- dimensional exposure and thus a multitude of collisions can occur between the polystyrene microspheres and the sample. If there is complementarity between the oligonucleotide probe affixed to the bead and a DNA sequence in the sample, a positive identification is reported.

Part of the 23S ribosomal RNA gene, *rrl*, was successfully amplified in four microorganisms: *Bacillus cereus*, *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*. A biotin tag was incorporated at the 5' end of one the strands of each PCR product. This strand included the reverse complement of the 20-base-pair probe attached to a microsphere set, which was designed as a probe for the particular microorganism. One probe was created for each DNA target sequence, producing four bead types to combine and utilize in a multiplexed assay. The instrument classifies the microspheres by using a red diode laser (635 nm) to detect the fluorescence emitted by the internal dyes of the microspheres and a green diode laser (532 nm) to detect and quantify the target DNA by measuring the intensity of the fluorescence emitted by the reporter.

PCR products were positively identified by specially designed, multi-analyte profiling beads, which are spectrally addressed. After unknown samples and a microsphere mixture were combined, hybridization was detected by adding the reporter fluorophore streptavidin-R-phycoerythrin to the reaction. In all cases, the fluorescent response was greatest for the bead set homologous to the target DNA present. The assay was highly specific and no false positives were observed. However, a few reactions resulted in false negatives, as the fluorescent intensity was sometimes less than the minimum value for a positive identification (>2 times background fluorescence). In attempts to rectify some cross-hybridization between *E. coli* and *S. enterica*, hybridization temperature and salt concentration were adjusted, yet the problem persisted.

A sensitivity test performed on decreasing concentrations of PCR amplicons showed that a very low amount of DNA could be detected and that the instrument response was directly proportional to the input concentration. The lower limit of detection was determined to be 0.5 ng for *B. cereus* and *E. coli* and 2 ng for *S. enterica*. The lower limit of detection for *S. aureus* could not be determined, as the instrument response was still very high for samples at concentrations as low as 0.25 ng.

Future studies will include analyses of pathogens that are more closely related. Additional markers will be included to increase the specificity of the assay and positively distinguish between species that have very similar nucleotide sequences. Sensitivity and mixture studies will be performed on these additional microorganisms and probes to design a robust multiplexed assay for pathogen detection.

**Multiplexed Detection, Pathogens, Bioterrorism**

 ** Presenting Author*