REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Rapid Electrochemical Immunoassays For Low-cost, Multiplex Detection Of Food Pathogens Using Capillary Driven Microfluidic Devices

Michael Wiederoder US Army DEVCOM Soldier Center **Jason Boes** Colorado State University **Cody Carrell** Colorado State University **Shannon McGraw-Manza** US Army DEVCOM Soldier Center **Charles Henry** Colorado State University

Foodborne illness costs Soldiers an average of 3 work days per year resulting in $900 million in lost productivity. Current techniques to detect foodborne pathogens such as molecular diagnostics and culture counting require long wait times (8-48 hrs), centralized labs, complex instruments, and trained personnel. Here we describe the development of capillary microfluidic devices for rapid (1-2 hrs), low-cost (~$2-10 per test) electrochemical detection of pathogenic bacteria.

Utilizing an enzymatic assay coupled with low-cost electrochemical detection via re-usable thermoplastic electrodes, we are proposing a system suitable for use in a resource limited environment. Prior work demonstrated detection of Salmonella sp. at 640 CFU/mL within 2 hrs in milk using an electrochemical immunoassay. This consisted of incubating antibody functionalized magnetic beads with the sample followed by magnetic extraction and concentration. Then the remaining solution was incubated with a biotinylated secondary antibody and a streptavidin tagged electrically active enzyme. Finally, the mixture was added to a microfluidic device containing the thermoplastic electrode and an electrically active substrate for analysis with a portable potentiostat.

To build on this work we are investigating methods to improve sample treatment and multiplexing detection of pathogenic bacteria. Sample pre-treatment uses capillary-driven immunomagnetic filtration to concentrate and extract target bacteria instead of a test tube and manual pipetting. The captured antibody functionalized magnetic microbeads then provide a platform for an enzymatic sandwich assay. The electrochemical substrates remain physically separated throughout the process, allowing simultaneous detection of multiple bacteria. The microfluidic-driven nature of the device facilitates rapid turnaround, allowing it to run at a point-of-need timescale, and provides a user-friendly experience to enable use by an untrained end user. The development methods are amenable to diverse targets of interest (bacteria, toxin, virus, protozoa/cyst) and sample types (food, environmental samples, and clinical samples) by adapting the antibodies or recognition elements.

Approved for Public Release: PAO # PR2022_28016