

INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Development Of The Verifast System For Multi-echelon Diagnostics Of Multiplex Agents

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Introduction: Conventional and emerging biological threat agents comprise a diverse group of bacteria, toxins and viruses that can pose a substantial threat to human health, both for civilians and military personnel. These select agents have significantly high mortality rates, and early diagnosis for targeted intervention is critical to save lives, especially for personnel engaged into a military mission. To increase accessibility to point-of-need (PON) we developed under a DTRA contract a multiplexed detection platform using a Vertical Flow paper-based Immunoassay (VFI), named VeriFAST (Vertical Integrated Flow Assay System Technology). Our VFI configuration demonstrated the functionality and advantage for multiplex assays revealing large improvement (up to 500x for some targets) in limit of detection (LOD) of Tier1 biothreats [1-3]. Further advances were also obtained using novel gold nanostars and device miniaturization [4,5]. In this project, we present our recent development of the VeriFAST system for detection of a wide range of biothreat agents.

Methods: The instrument and assay have all the key features required operation under Echelon of Care 1-2. It is designed in an ultra-compact footprint, light weight (<20lbs), rechargeable battery and DC port, 16 bits digital imaging sensor, wireless communication with smartphone (iPhone 12 Pro) that integrates into a rugged case (16.25"x12.88"x6.75") for transport and field use with user-friendly interface that supports minimal training. The digital App allows signal analysis, reporting and potential cloud-based communication. Sandwiched immunoassay with capture antibody array were printed on nanoporous nitrocellulose membranes and detection antibodies were immobilized on gold nanoparticles and/or nanostars. The antibodies were either generated in house or acquired commercially. The suitable pairs were identified by ELISA and LFIs.

Results and Conclusion: A battery-powered centrifuge has been integrated into the portable VeriFAST footprint. Serum separated from whole blood using a mini serum-separation tube has been successfully applied in the VeriFAST system. Image processing to enhance signal-to-noise ratio for their effect and impact on LOD are characterized and implemented into the mobile App. A complete workflow procedure has been developed for the PON operation. VeriFAST's capability to detect a wide range of biothreat agents (<2 hr.) will be reported for the detection of both antigens and nucleic acids for different diseases and targets, e.g., melioidosis, plague, tularemia, SEB and saxitoxin, SARS-CoV-2 virus etc.

In summary, highly sensitive and multiplexed detection of pathogens and toxins was demonstrated by VeriFAST. The system is scheduled to enter a first validation study at the Navy Research Laboratory.

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