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Field-Forward Diagnostics

# Development of a Vertical Flow Paper-Based Immunoassay (VFI) for Multiplexing Detection of Tier I Bio-threat Agents

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**Introduction**: More than seventy biological agents and toxins have been determined to pose severe threats to both human and animal health. Exposure to these agents often occurs in austere settings such as battlefields and rural areas where resources are limited. Therefore, it is imperative to develop point-of-care diagnostic tools that are sensitive, cost-effective and simple-to-use that are amenable to multiplexing. Our team has developed and characterized a vertical flow paper-based immunoassay (VFI) microfiltration device that performs multiplexed detection of Tier I bio-threats. The platform is based on microbial antigen capture that generates colorimetric signals for direct visualization in less than 10 min.

**Method:** *Burkholderia pseudomallei* (Tier I agent) is the causative agent of melioidosis, a devastating bacterial infection. A sandwich immunoassay was constructed to detect the *B. pseudomallei* capsular polysaccharide (CPS) in the vertical flow format. A CPS-specific monoclonal antibody (mAb 4C4) was immobilized on a nitrocellulose membrane (pore size <1µm) and served as the capture antibody. A micro-dispenser was used to spot mAb 4C4 in an array format on the nitrocellulose. Gold nanoparticles (GNP) linked with mAb 4C4 served as the detection antibody, which produces colorimetric signals following binding to CPS. The VFI was run by pre-mixing detection antibody with buffer solution spiked with CPS then the sample was passed through the membrane vertically with a syringe pump. After a washing and a drying step, the membrane was scanned with a standard tabletop scanner and analyzed using an automated imaging analysis software.

To characterize the VFI system, a design of experiment (DOE) screening analysis was created in JMP Pro 13. Seven VFI parameters were studied, including six continuous factors - flow rate, assay time, GNP amount, premixing time, buffer pH, buffer ionic strength, and one categorical factor - membrane type.

**Results & Conclusion:** Traditional paper-based lateral flow assays may have limited sensitivity and multiplexing capabilities due to the small sample volume and the need of large membrane pore size (>10 µm), which is inefficient for target capture. VFI provides a good solution to these problems by implementing active fluid pumping.

According to the DOE results, flow rate and assay time were the two most important factors affecting the average signal intensity, followed by membrane type, pH, and premixing time. There was a 2-factor interaction between flow rate and assay time, indicating that the sample volume per unit area might be the key to further improving VFI's sensitivity. As for the signal variation, GNP amount and membrane type were the dominating factors, followed by flow rate. The screening design identified key factors that will be studied for further VFI optimization.

Under these optimum experimental conditions, the current VFI's limit-of-detection (LOD) for the CPS assay is 4 pg/mL (10 times lower than our previous lateral flow device). We also demonstrated multiplexing detection of CPS and PGA (a biomarker for *B. anthracis*, the causative agent of anthrax). Next, we will characterize the VFI system for detection of a variety of biothreats, validate the multiplexing capabilities and improve the performance through miniaturization.

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