Lateral Flow Immunoassay Development for Multiplex Detection of Waterborne Pathogens Utilizing Surface Enhanced Raman Scattering (SERS) Reporter-Encapsulated Nanoparticles

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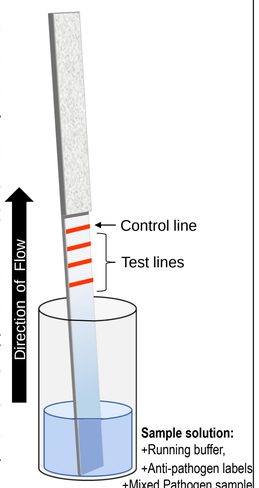
INTRODUCTION

Rapid detection of waterborne bacterial pathogens implicated in infectious diseases such as cholera, dysentery, shigellosis and typhoid fever is of great importance to public health. Timing is the limiting factor for pathogen detection as traditional diagnostic assays require a minimum of 24 hours as well as extensive resources and expertise. Our **objective** is to develop a paper-based lateral flow immunoassay (LFA) platform for the detection of multiple waterborne pathogens. Multiple labeling strategies will be compared, including SERS-based and fluorescence-based labels.

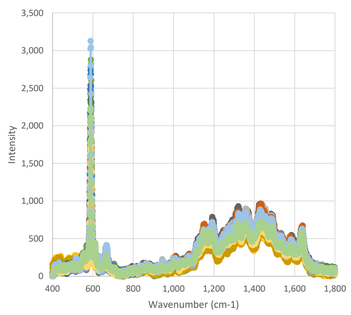
METHODS

Antibodies raised against *Salmonella typhi, Escherichia coli O157:H7*, and *Shigella dysenteriae* were sourced commercially (MyBioSource, Thermo). The capture antibodies were dispensed onto nitrocellulose membranes using a precision line-dispensing system (BIODOT) at a concentration of 1 mg/mL and rate of 1 uL/cm, then subsequently dried in a forced-air oven at 40^{O}C for 30 mins. The detector antibodies were purified and covalently conjugated to two different nanoparticle-based labels for comparison: 1) 60nm gold nanospheres bearing a high density of encapsulated Raman-active reporter molecules (Nanopartz), and 2) 200nm europium chelate (carboxy-terminated) fluorescent nanoparticles (BangsLabs). The membrane materials were assembled (with 3mm overlay), laminated onto an adhesive backing, and cut into individual 4mm wide strips using an automatic guillotine cutting machine (Arista Bio). *S. typhi, E. coli*, and *S. dysenteriae* were grown in tryptic soy broth. Functional testing of the LFA was performed by dipping the test strip into a 50uL sample solution, (as shown in the schematic), permitting the sample to migrate along the strip and interact with capture antibodies downstream. The test strips were visually inspected to discern positive and negative samples. Signal quantitation was performed using a Leelu LFA reader (LUMOS) as well as a DXR2 Raman microscope in order to measure relative signal intensities, as well as to determine the limit of detection.

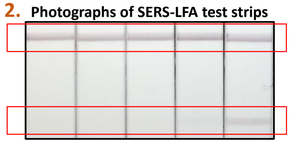


RESULTS (SERS-LFA)

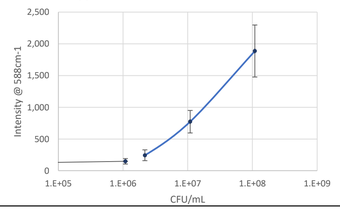
1. Raman Spectral Mapping at Test Line



2. Photographs of SERS-LFA test strips



3. *S. typhi* Calibration Curve



4. Data

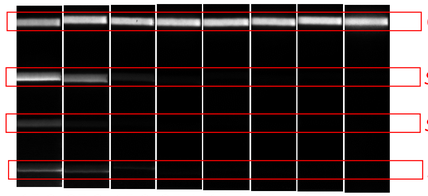
CFU/mL	TL AVG Intensity @588cm-1	Standard Deviation	Signal-to-Noise
109000000	1888	411	35
10900000	774	177	14
2180000	243	85	4
1090000	148	42	3
0	52	15	1

5. Limit of Detection (LOD) = $\text{signal}_{blank} + 3\sigma_{blank}$
LOD = 97 AU = **495,000 CFU/mL**

(1) Example Raman spectra (n=30) acquired through a 10x objective lens focused on the Test Line (TL) region of an LFA strip, using a 785nm excitation laser operating at 10mW with 1s of exposure time. **(2-4)** A limit-of-detection study for *S. typhi* demonstrated good linear correlation across the ODs measured, as shown in the photographs, calibration curves, and data. **(5)** The CFU/mL LOD was estimated based on a least squares regression line.

RESULTS (Europium-LFA)

6. Europium-based LFA multiplex limit-of-detection study



O.D. of Mixed Bacteria: 1E-2, 3E-3, 1E-3, 3E-4, 1E-4, 3E-5, 1E-5, 0000

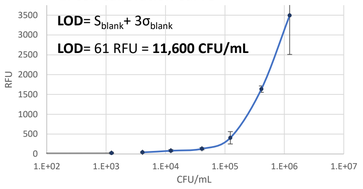
(6) Photographs of Eu-Latex LFA strips taken with a portable camera-based fluorescence reader. Photographs were taken 15 min after each strip was dipped into a mixture of three bacteria of a different optical density (0.01, 0.003, 0.001, 0.0003, 0.0001, 0.00003, 0.00001, negative).

10. Pathogen cross-reactivity study

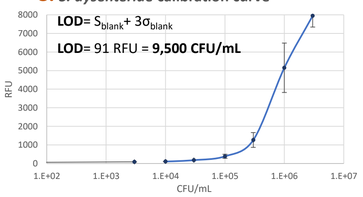
E. coli O157:H7	+	-	-
S. typhi	-	+	-
S. dysenteriae	-	-	+

(10) LFA strips were tested individually for cross-reactivity between the three pathogens. The photographs clearly show negligible cross-reactivity.

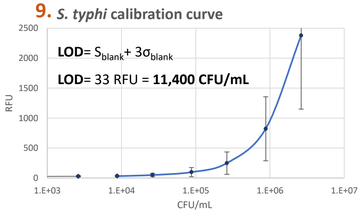
7. *E. coli* calibration curve



8. *S. dysenteriae* calibration curve



9. *S. typhi* calibration curve



(7-9) The calibration curves and calculated LOD are shown for each representative pathogen. The CFU/mL LOD was estimated based on a least squares regression line. The multiplex limit-of-detection study demonstrated good linear correlation across all ODs tested.

CONCLUSIONS

Our experiments demonstrated the ability to rapidly identify and discern between multiple high-risk pathogens present in water samples using a single test strip and 50uL of total combined sample volume. Additionally, the method demonstrated remarkable specificity, negligible cross-reactivity, and a high degree of sensitivity for all three pathogens. The LOD for *S. dysenteriae, S. typhi,* and *E. coli* O157:H7 were estimated to be approximately 9500, 11400, and 11600 CFU/mL, respectively. Furthermore, we expect the LOD can be improved by at least an order of magnitude by leveraging a modular or field-deployable sample concentrator. This method clearly demonstrates the utility of a multiplex LFA for the rapid detection of waterborne pathogens. We believe our approach has strong potential to be leveraged as a field-deployable method for rapid on-site pathogen identification and may also serve as a valuable tool for additional field monitoring applications, to include biothreat and bioterrorism detection.