

Rapid identification of *Escherichia coli* from finished water by bacteriophage amplification and lateral flow immunochromatography detection

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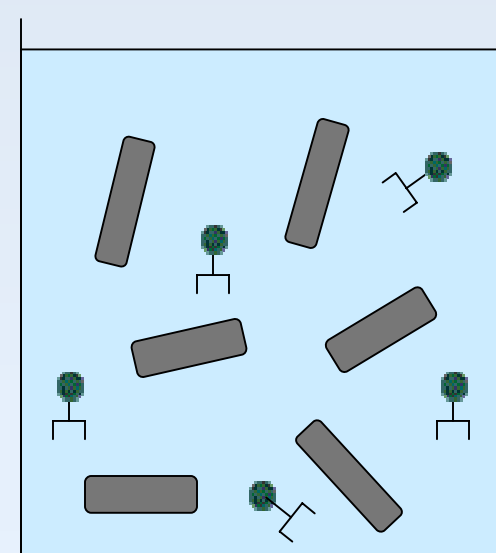
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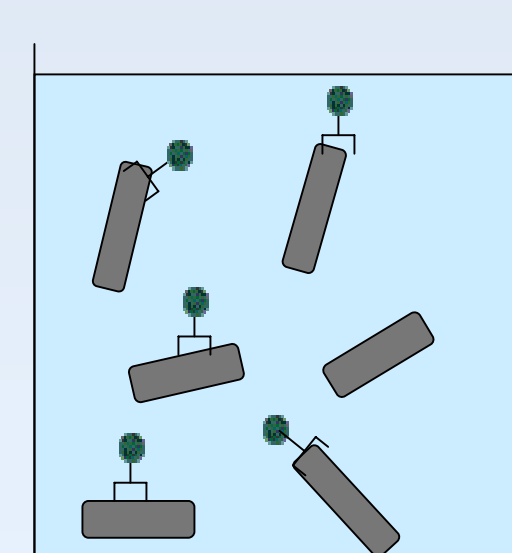
Overview

Bacteriophage amplification detection as a means of identifying bacteria is receiving renewed interest. Combined with modern methods of protein and nucleic acid detection, bacteriophage amplification provides an attractive platform for bacterial identification because of the large number of proteins and nucleic acids generated during the phage replication cycle. Whereas means of identifying bacteria by a traditional plaque assay is not rapid nor sensitive, using modern protein detection methods of mass spectrometry or lateral flow immunochromatography provide sensitive results in a less than four hours.

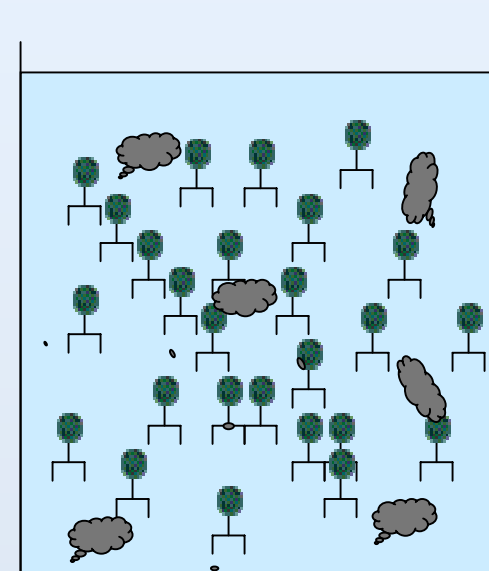
Bacteriophage Amplification



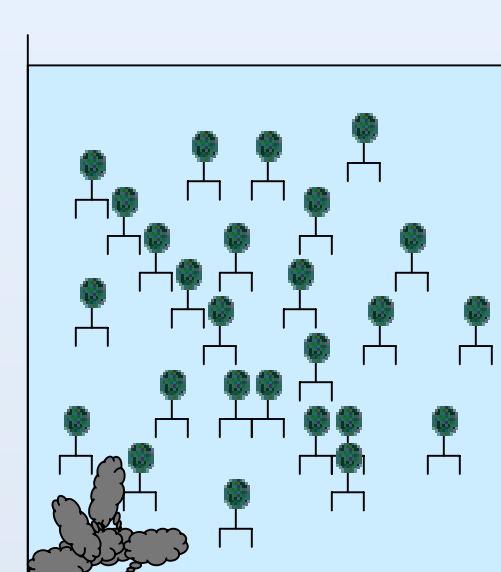
Step 1 - Bacteriophage at a concentration below the detection limit (of the chosen detector) is added to the medium containing the targeted bacterium



Step 2 - The phage is allowed time to infect the targeted bacterium

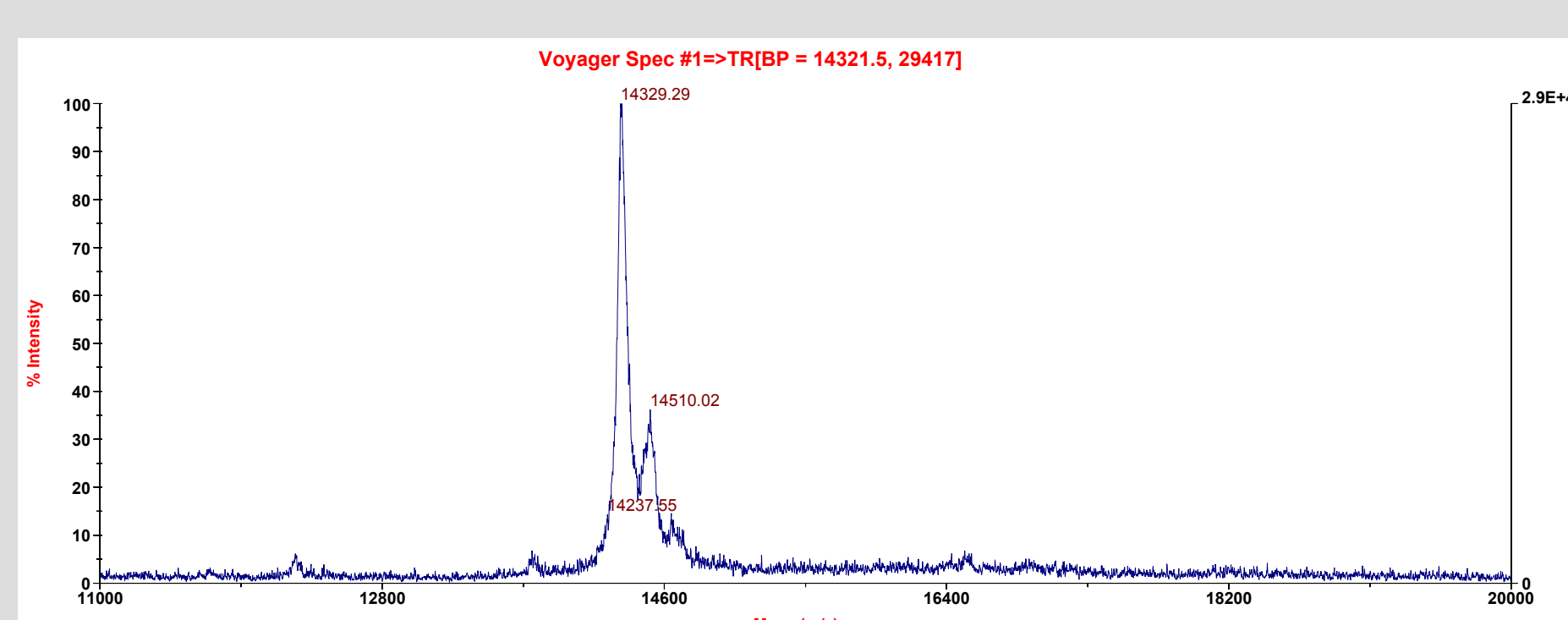


Step 3 - The bacteriophage replicates and lyses the bacteria, releasing the progeny phage into solution, thereby driving up the concentration to detectable levels

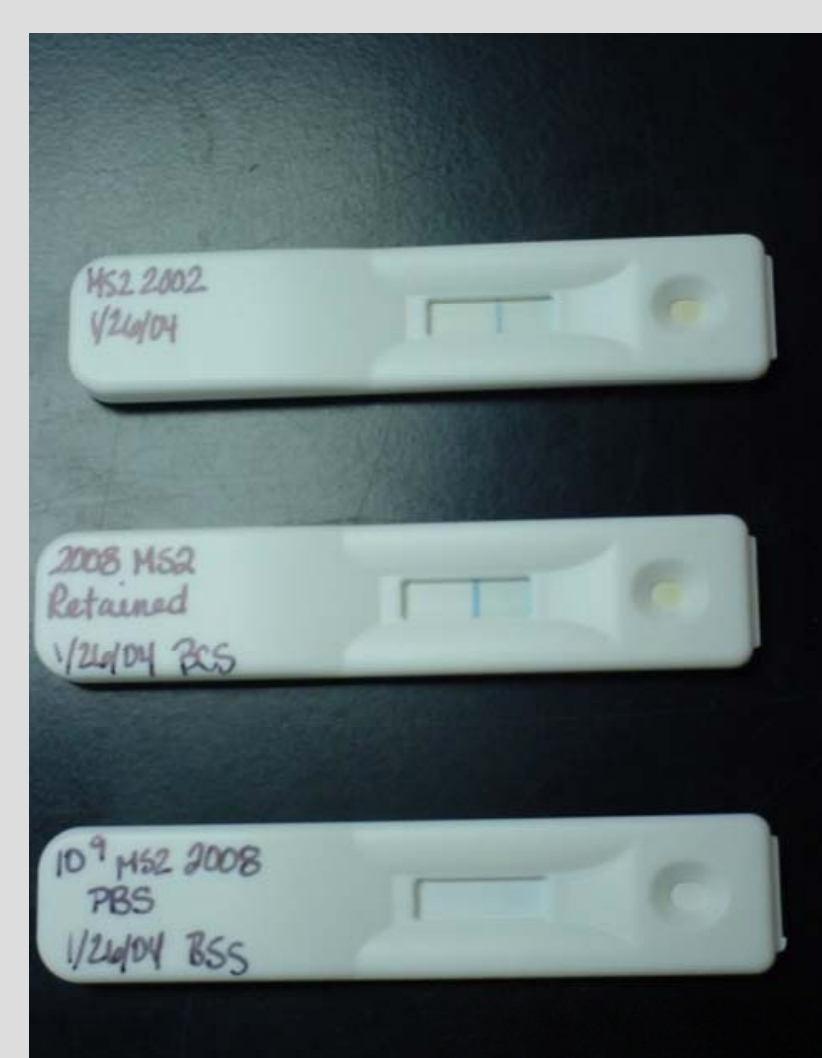


Step 4 - The sample can be analyzed by numerous detectors for phage amplification.

Detectors



Mass Spectrometry

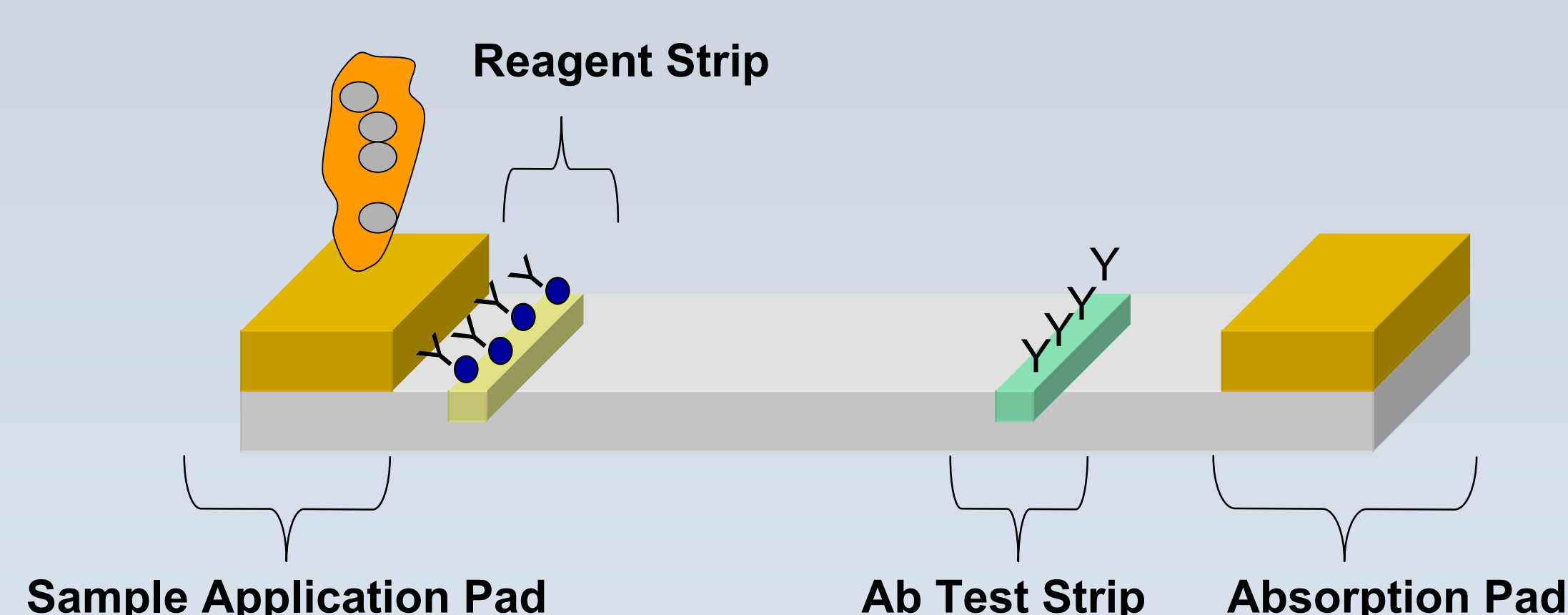


Lateral Flow

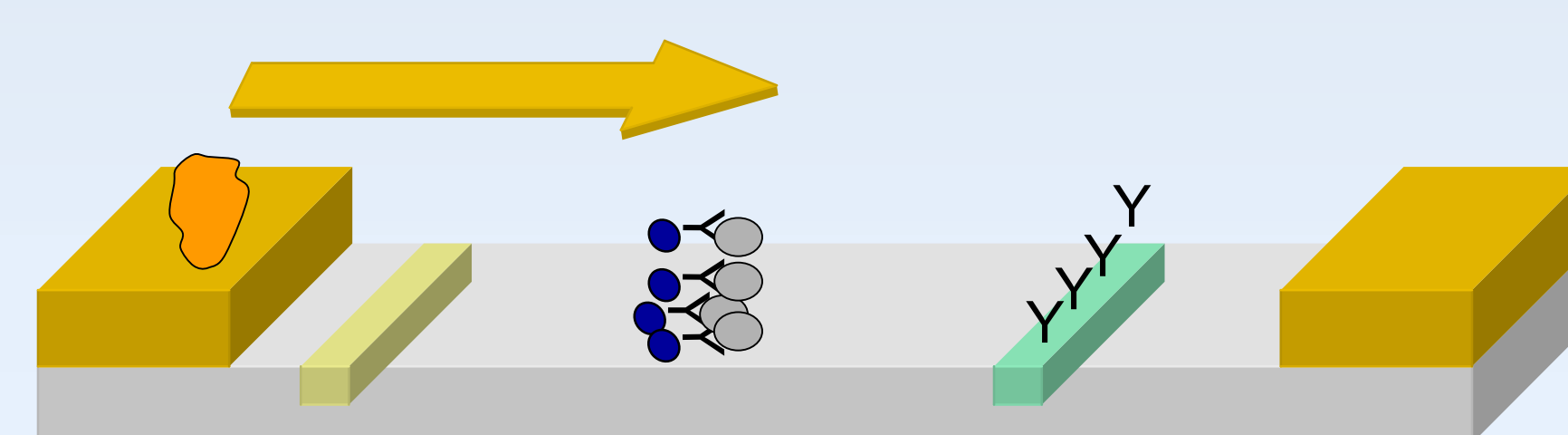


Optical Immunoassay

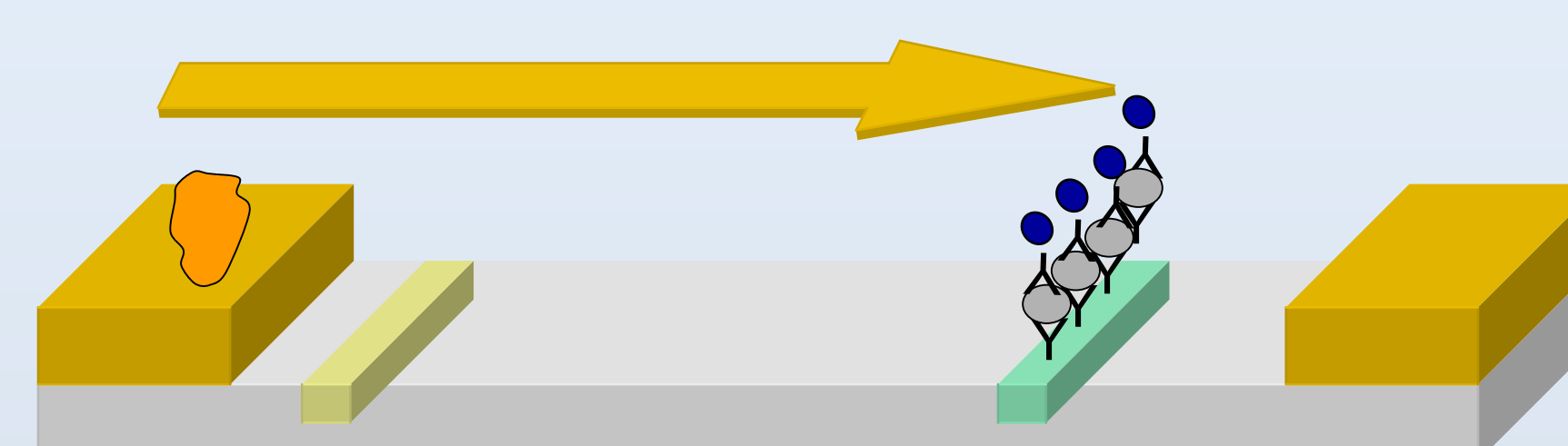
Lateral Flow Immunochromatography



Step 1 - A sample containing the amplified bacteriophage is added to the sample application pad



Step 2 - As the sample flows over the reagent pad, an antibody conjugated to a blue bead attaches to the phage, and continues to flow toward the absorbent pad



Step 3 - As the sample flows over the test strip, the immobilized antibody captures the phage-blue bead complex, forming a blue line.

E. coli samples obtained from Hach Company (Loveland, CO) were grown overnight in a nutrient rich broth to a final concentration of approximately 1×10^9 cells/ml. A portion of the *E. coli* cells were diluted in a 10 fold dilution series, while another portion of the cells were treated with stock chlorine bleach to induce stress so that the final concentration of chlorine in the solutions was 0.5 mg/L, then similarly diluted.

E. coli suspensions were added to MS2 coliphage solutions so that no detectable signal could be seen on the LFI strips immediately after addition of the bacteria. The MS2-*E. coli* mixtures were allowed to incubate for 3 hours, after which aliquots of the samples were applied to the LFI strips for detection of phage amplification. Final *E. coli* concentrations were determined by the Hach mColi-Blue assay.

Results

<i>E. coli</i> concentration (cfu / ml)	Chlorine Stressed	LFI Result
5×10^8	No	+
5×10^6	No	+
5×10^4	No	+
7×10^7	Yes	+
7×10^5	Yes	+
7×10^3	Yes	+
Positive Control	No	+
Negative Control	No	-
<i>E. coli</i> Control	No	-

Experimental

An anti-MS2 coliphage polyclonal antibody for construction of the lateral flow immunochromatography (LFI) strips was purchased from Tetracore, Inc. (Gaithersburg, MD), and the LFI strips were generated in-house. MS2 coliphage stock solutions maintained on site were propagated in *E. coli* on a soft agar base, and recovered by scraping the soft agar off of the surface of the petri dish and into a tryptic soy broth solution followed by centrifugation and filtration. Phage were quantified by the traditional plaque assay, and then diluted for determination of the detection limit on the LFI strips. All LFI assays were conducted by placing 100 microliters of sample onto the LFI sample application site, and allowing the flow to migrate to the absorption pad. Reading of the LFI strips for results was conducted 30 minutes after sample application, and positive results were determined by comparison with a positive and negative control.

Conclusions

Bacteriophage amplification coupled with LFI is:

- A rapid and sensitive method for *E. coli* detection
- A viable platform for detection of stressed *E. coli*
- A potential platform for other phage / host detection methods
- A potential platform for multiple pathogen detection
- A simple methodology that a minimally trained technician can perform

