

# Rapid Identification of Bacteria by Bacteriophage-Enhanced Immunoassay

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## ABSTRACT:

### Background:

Identification of bacteria from clinical samples often requires more than 48 hours, limiting the clinical utility of automated diagnostic platforms. Molecular-based tests are emerging on the market for more rapid bacterial identification for use in clinical samples such as blood culture and nasal swabs. These newer methods though do not address the resource restrictions facing today's clinical microbiology laboratory where adequately trained medical technologists are in short supply. Likewise, applications to resource-poor settings outside of the United States require technologies that are inexpensive and easy to use, in addition to meeting high clinical standards.

In bacteriophage amplification, input phage direct production of thousands of progeny per infected target bacterium, culminating in a release of phage into the sample that can be detected as a surrogate marker. This phenomenon is classically observed through plaque formations on a bacterial lawn. Here we apply this to an immunoassay detector for rapid and easy to use detection of *Escherichia coli* as a bacterial model to test the validity of this identification platform.

### Materials and Methods:

Bacteriophage were sourced from clinical samples and existing collections. They were then isolated from lawns of *E. coli* on agar plates. Standard plaque assay methods were employed to define host ranges and burst characteristics of candidate phage. A lateral flow immunoassay (LFI), comprised of anti-phage polyclonal antibody, was then developed to detect the phage amplification signal to a single lead phage candidate to conduct these studies. Ninety-two (92) clinical strains of *E. coli* and Gram Negative non-*E. coli* were used to define the new method's sensitivity and specificity. To test assay sensitivity, forty-eight (48) *E. coli* strains at approximately  $1 \times 10^6$  CFU/mL were spiked with  $5 \times 10^6$  pfu of bacteriophage and incubated at 37°C for 2 hours. 100  $\mu$ L of incubated sample was transferred to the LFI detector, where it was read against positive and negative controls at 10 minutes. Forty-four (44) clinical strains of Gram Negative non-*E. coli* were tested in the same manner to define assay specificity. Both performance tests were compared to overnight plaque assays to define method agreement.

### Results and Conclusion:

Of the 48 *E. coli* strains that were standard plaque assay positive for bacteriophage amplification, 47 were also positive by the lateral flow immunoassay method at 2 hours for agreement and sensitivity of 98%. Non-*E. coli* strain testing demonstrated 100% agreement between the two methods. The specificity of the test phage showed 89% specificity to the Gram Negative organisms. Weighted to blood culture clinical prevalence, the specificity rate appears to exceed 95%.

Rapid (2 hours) and easy to use, bacteriophage-enabled immunoassay, shown here with lateral flow immunoassay appears to be a valid method for rapid bacterial identification. This work supports further development efforts to describe direct identification from clinical matrices, as well as to describe rapid identification with additional bacteria for which there are well described bacteriophage, such as *M. tuberculosis*.

## INTRODUCTION:

With currently available technology, it takes 1 to 3 days to accurately ID bacteria and determine antibiotic susceptibility. This is too slow to assist physicians in formulating initial treatment plans. Consequently, physicians prophylactically prescribe broad-spectrum antibiotics to treat most clinical conditions. Even when infections are bacterial, research shows that targeted, narrow spectrum antibiotic therapy results in better patient outcomes than does broad-spectrum antibiotic therapy. For life threatening conditions such as sepsis, this difference can be critical. The long-term public health consequences of indiscriminate antibiotic usage are also serious because it accelerates the growth of antibiotic resistance and shortens the useful lifetime of important antibiotics. Providing accurate and timely diagnostic data to physicians about bacterial infections will reduce health care costs and save lives.

## MATERIALS and METHODS:

### Bacteria

Bacteria were sourced from ATCC (VA), NCTC (Birmingham, UK), and JMI Labs (IA).

### Indicator BioAssay

Bacteria were grown to log phase are added to serially diluted phage preparations and plated in soft agar. Plates were examined for plaque formation the following day and phage titer was determined based on plaque formation. An increase in plaque formation relative to a control is indicative of phage replication.

## MATERIALS and METHODS:

### Phage Purification/ Antibody Development

Phage particles for antibody preparation were filtered through 0.2  $\mu$ m cellulose acetate, PEG precipitated, and purified by size exclusion chromatography. Rabbit polyclonal antibodies were adsorbed against whole heat-killed cells and cell-free extracts of *E. coli* to generate necessary specificity in the antibody. Adsorbed antiserum was purified using Protein A affinity chromatography.

### LFI Development

Lateral flow strips were prepared using purified antibody by ACON Laboratories (CA).

### Indicator BioAssay vs. Lateral Flow Experiments

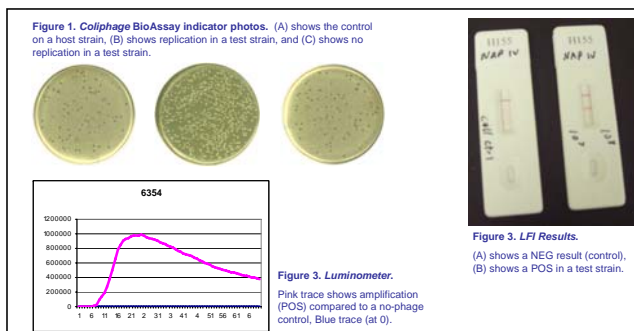
All bacteria were grown up to an OD of 0.3 to 0.5 at 630 nm wavelength. 50  $\mu$ L of bacteria were inoculated into 1 ml TSB broth supplemented with 10 mM MgCl<sub>2</sub> and  $5 \times 10^6$  pfu/ml bacteriophage (HER 155). The broth was left to incubate shaking at 37°C for 2 hours. 70  $\mu$ L was removed and applied to Lateral Flow detector well and read at 10 min. The remainder was centrifuged at 14,000 rpm for 5 minutes to pellet bacteria. The supernatant was diluted 1:10<sup>6</sup> and 100  $\mu$ L was plated with *E. coli* NAP IV (bacteriophage host bacteria strain) and top agar. Plates were read the following morning (at approximately 18 hours). A plate count of 10x greater than the input bacteriophage (control) was counted as a positive amplification.

### Lateral Flow Immunoassay vs. Luminometer

The bacteria were grown up as above. 1 ml TSB was inoculated with 10 mM MgCl<sub>2</sub> and  $5 \times 10^6$  pfu/ml bacteriophage and 100  $\mu$ L was transferred into a luminometer plate, luciferase was added (per mfr instructions) and placed into the luminometer for approx. 18 hours. The remaining contents were incubated and read on LFI as above.

### Interpretation of Results

Indicator BioAssay: Figure 1 shows the interpretation criteria for plaque assay control, POS, and NEG plates. Luminometer: Figure 2 Shows the luminometer traces for POS (pink line, with NEG control). NEG produces no trace. Lateral Flow Immunoassay: Figure 3 shows NEG (1 line, control only) and POS (2 lines, control and test) results.



## RESULTS:

Indicator BioAssay results (18 h) compared to Lateral Flow Immunoassay (2h):

	Indicator POS	Indicator NEG
Lateral Flow POS	47	7
Lateral Flow NEG	1	32
	<b>Sensitivity: 98%</b>	<b>Specificity: 82%</b>

Luminometer results (18 h) compared to Lateral Flow Immunoassay (2h):

	Luminometer POS	Luminometer NEG
Lateral Flow POS	53	2
Lateral Flow NEG	0	43
	<b>Sensitivity: 100%</b>	<b>Specificity: 96%</b>

Lateral Flow Immunoassay (2h) compared to strain identity:

	3 <sup>rd</sup> Party Identity <i>E. coli</i>	3 <sup>rd</sup> Party Identity Gram Negative Bacilli
Lateral Flow POS	47	5
Lateral Flow NEG	5	36
	<b>Sensitivity: 90%</b>	<b>Specificity: 88%</b>

## CONCLUSIONS:

- Bacteriophage amplification coupled with immunoassay detection produces similar results to traditional plaque assay (Indicator Bioassay) in 2 hours compared to 18 hours on these strains of *E. coli* and Gram Negative bacilli.
- When compared, Lateral Flow immunoassay detection produces nearly identical results compared to luminometer tracings for bacteriophage amplification for sensitivity and specificity on these strains of *E. coli* and other Gram Negative bacilli.

## OPPORTUNITIES:

- Rapid direct detection of bacteria from clinical samples has the potential to influence antibiotic therapy choices and improve clinical outcomes.
- Bacteriophage amplification-enabled immunoassay holds promise for additional bacterial targets, such as *S. aureus*, *S. pneumoniae*, *Enterococcus sp.*, and *Pseudomonas sp.*, where lytic phage exist, but no direct immunoassays exist on the market.
- This platform hold promise for microbiology detection areas where resources are scarce and/or need for a more rapid result would be beneficial.