

Rapid Direct Detection of Bacteria from Positive Blood Cultures by Bacteriophage-Enabled Immunoassay

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ABSTRACT*

Background:

Current antibiotic therapy approaches for suspected bacteremic and septic patients regularly take a conservative approach treating broadly. There is a need to identify (or rule out) the specific pathogens known to be important markers of severity of sepsis and patient outcome. A number of methods have recently been described for the direct detection of bacteria from blood culture samples. Detection of bacteria has previously been demonstrated using bacteriophages that bind to target cell receptors. Upon infection, input phage direct production of thousands of progeny per cell, culminating in a release of phage into the sample. Here we apply this phenomenon for rapid detection of

Staphylococcus aureus and *Escherichia coli*, two principal bloodborne pathogens strongly linked to patient outcome.

Materials and Methods:

Phage were sourced from clinical samples and existing collections. Phage were then isolated from lawns of *S. aureus* and *E. coli* on agar plates. Standard plaque assay methods were employed to define host ranges and burst characteristics of candidate phage. Lateral flow immunoassays (LFI), comprised of anti-phage polyclonal antibody were then developed for each target to detect the phage amplification signal. Amplification and performance studies were carried out by spiking known *E. coli* strains at 100 CFU/mL into BD Bactec blood culture bottles and incubated until positive. One hundred microliters of blood culture was removed and added to a phage/nutrient broth and were incubated at 37°C for 2 hours.

Results and Conclusion:

On 100 *E. coli* and 78 non-*E. coli* Gram Negative strains, the assay demonstrated 95% sensitivity and 95% weighted specificity direct from culture. *E. coli* detection in 2 hours by LFI in blood culture positives produced consistent results. 78 *Staphylococcus aureus* (28 MRSA) clinical strains and 48 CNS strains tested with the *S. aureus* assay, and by the indicator BioAssay method, it is shown to be specific to *S. aureus* with greater than 87.5% sensitivity and 95.3% weighted specificity.

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.

METHODS:

Bacteria

Bacteria were sourced from the ATCC (Manassas, VA), NCTC (Birmingham, UK), and JMI Laboratories (Iowa City, 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.

Phage Purification/ Antibody Development

Phage particles for antibody preparation were filtered through .2 µm 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 Azure Institute (San Diego, CA).

RESULTS:

Detection of *E. coli* using Coliphage Amplification

E. coli was chosen to represent a model system for using bacteriophage to identify bacteria from cultured samples. This bacteria represents a commonly isolated pathological specimen and coliphage represent some of the most well understood bacteriophage. Sources of coliphage are readily available from a number of sources:

Wastewater Fly traps Environmental samples Collections

RESULTS (Cont'd):

Results of Phage Screening

Coliphage were screened by the indicator BioAssay method on as many as 100 *E. coli* isolates to determine sensitivity and specificity characteristics. These are summarized to the right in Table 1. Each coliphage demonstrated a unique host range pattern, infecting different strains of *E. coli* differently. Table 1 outlines the sensitivity of three candidate coliphage. Likewise, specificity of the examined phages varied from the very specific to the broad. Table 2 outlines the gross specificity and weighted specificity of these three candidates. Weighted percentiles were calculated using data from the published literature to assess the clinical applicability of the method.

Phage Cocktailing

Interested to increase the host range of assay bacteriophage to more clinically acceptable levels, we explored cocktailing candidate phage. Cocktailing is a practice first described in the phage therapy literature to expand phage coverage for a particular target. We analyzed the host range data by each strain of *S. aureus* and *E. coli* tested and found that while some phage did well with certain capsular types, they lacked coverage in others, and that the converse was true. Keeping to a minimal cocktails (pairs and triples only), we revisited the existing data to look at theoretical phage cocktail performance values. Here we took only the union of strains which both phage were individual tested against, and quantitated their gross and weighted sensitivity and specificity values.

Testing in Blood Culture

Blood culture bottles were spiked with 10ml human donor blood or 10 ml bacterial medium. Bacteria were selected based on their previous results by the BioAssay indicator method. Table 4 outlines three such example strains. One ml of medium containing approximately 100 cfu/ml of bacteria were added to represent a clinical with 10 cfu/ml. The bottle was incubated in a BacTec (BD Diagnostics) until alarmed. Positive samples were then removed and tested as described on both the LFI and BioAssay indicator. All results remained consistent in testing up to 12 hours post alarm.

E. coli Model Lessons

In first developing the *E. coli* assay several lessons were learned that were applied to the development of the *S. aureus* assay. First, Bacteriophage are a tool that can be used to identify bacteria on a species level. Second, Single phage detection is unlikely to cover all strains necessary for a clinical product. Third, mixtures of phage (cocktails) can be employed to improve coverage. Finally, bacteria in blood culture grow to levels detectable by phage amplification in 2 hours.

Detecting *S. aureus* by Phage Amplification

Following work on *E. coli*, work expanded to demonstrate similar results on *S. aureus*, a bacteria with many clinical implications.

Figure 1. Coliphage BioAssay indicator photos. (A) shows the control on a host strain, (B) shows replication in a test strain, and (C) shows no replication in a test strain.



Table 1. Coliphage candidate preliminary host range performance.

Coliphage	Sens
155	79% (79/100)
Fly-002	74% (74/100)
MP-498	58% (58/100)

Table 2. Coliphage candidate preliminary specificity performance.

Challenge Genus / Phage	155	Fly-002	MP-498
<i>Acinetobacter</i>	0/3	0/3	1/3
<i>Aeromonas</i>	0/3	0/3	0/3
<i>Bacteroides</i>	0/1	0/1	0/1
<i>Citrobacter</i>	0/4	0/4	0/4
<i>Enterobacter</i>	1/14	1/14	0/13
<i>Escherichia (non-coli)</i>	1/7	0/7	0/7
<i>Klebsiella</i>	2/10	1/10	0/7
<i>Pasteurella</i>	1/2	1/2	1/2
<i>Proteus</i>	0/9	0/9	0/1
<i>Pseudomonas</i>	0/10	0/10	1/10
<i>Salmonella</i>	0/3	0/3	0/3
<i>Serratia</i>	1/6	0/6	0/6
<i>Shigella</i>	3/5	3/5	0/5
<i>Yersinia</i>	0/1	0/1	0/1
Overall score	8 / 78	6 / 78	3 / 78
Gross Specificity			
Weighted Spec.			

Table 3. One 3-phage cocktail performance data.

Phage Cocktails	155 + Fly-002 + MP-498
<i>E. coli</i> infectibility:	95% (95/100)
Non- <i>E. coli</i> Specificity: (Gross - Weighted)	88.5% - 95% (9/79)

Table 4. Selected testing in Blood Cultures.

Bacterial Strain	Indicator BioAssay	LFI
<i>E. coli</i> Nap IV	+	+
<i>E. coli</i> 7625	+	+
<i>E. coli</i> 14400	-	-

Figure 2. Negative (control) and Positive LFIs

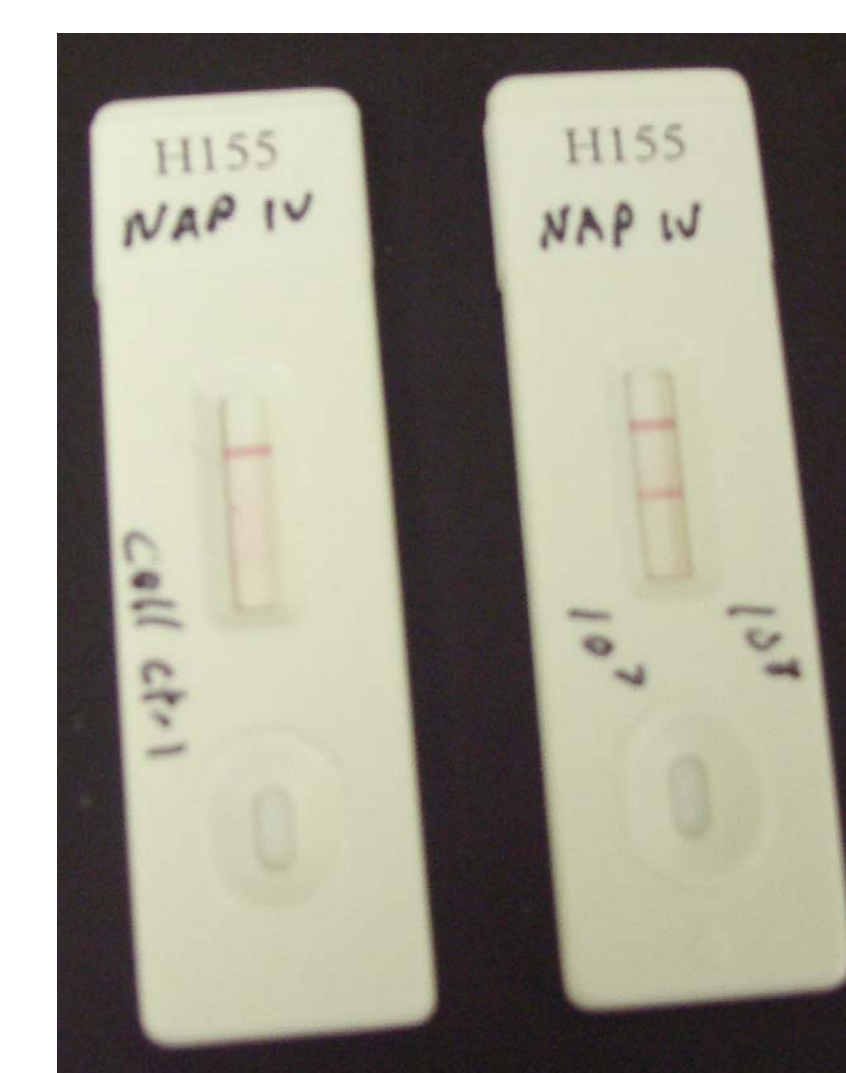


Table 5. *S. aureus* candidate preliminary host range performance.

<i>S. aureus</i> phage	Sensitivity
MP-21	79.5% (62/78)
MP-80	74.4% (58/78)
MP-110	60% (24/40)

RESULTS (Cont'd):

Results

S. aureus phage were screened by the indicator BioAssay method on as many as 78 *S. aureus* isolates (28 MRSA, 30 with unknown MIC profile) to determine sensitivity and specificity characteristics. These are summarized to the right in Table 5. Again, each phage demonstrated a unique host range pattern, infecting different strains. Table 6 outlines the sensitivity of three candidate *S. aureus* phage. Table 7 outlines the gross and weighted specificity of these three candidates. Weighted percentiles here were calculated using composite data from the published literature to assess the clinical applicability of the method. CNS species were only broken down to *S. epidermidis* (75%), *S. hominis* (8%), *S. haemolyticus* (8%), *S. warneri* (4%), *S. cohnii* (1%), *S. intermedius* (1%), *S. saprophyticus* (1%), *S. simulans* (1%), and Other CNS (1%).

Phage Cocktailing

Reviewing the data on these three *S. aureus* phage, it is apparent that M-21 and MP-110 have complimentary host ranges which could be exploited in a single assay.

Table 6. *S. aureus* phage candidate preliminary specificity performance.

Challenge Genus / Phage	MP-21	MP-80	MP-110
<i>S. epidermidis</i>	1/31	0/31	0/31
<i>S. haemolyticus</i>	0/5	4/5	0/5
<i>S. hominis</i>	4/9	1/9	0/9
<i>S. warneri</i>	1/3	1/3	0/3
<i>S. cohnii</i>	0/3	0/3	0/3
4 Other CNS	0/1	1/1	0/4
Overall score	10/52	7/52	0/59
Gross	80.8 %	86.5 %	100%
Weighted Spec.	87.2 %	92.7 %	100 %

Table 3. One 2-phage cocktail performance data.

Phage Cocktails	MP-21 + MP-110
<i>S. aureus</i> infectibility:	87.5% (42/48)
Non- <i>S. aureus</i> Specificity: (Gross - Weighted)	89.6% - 95.3% (7/67)

Figure 4. Major components of a lytic bacteriophage.

BACTERIOPHAGE STRUCTURE

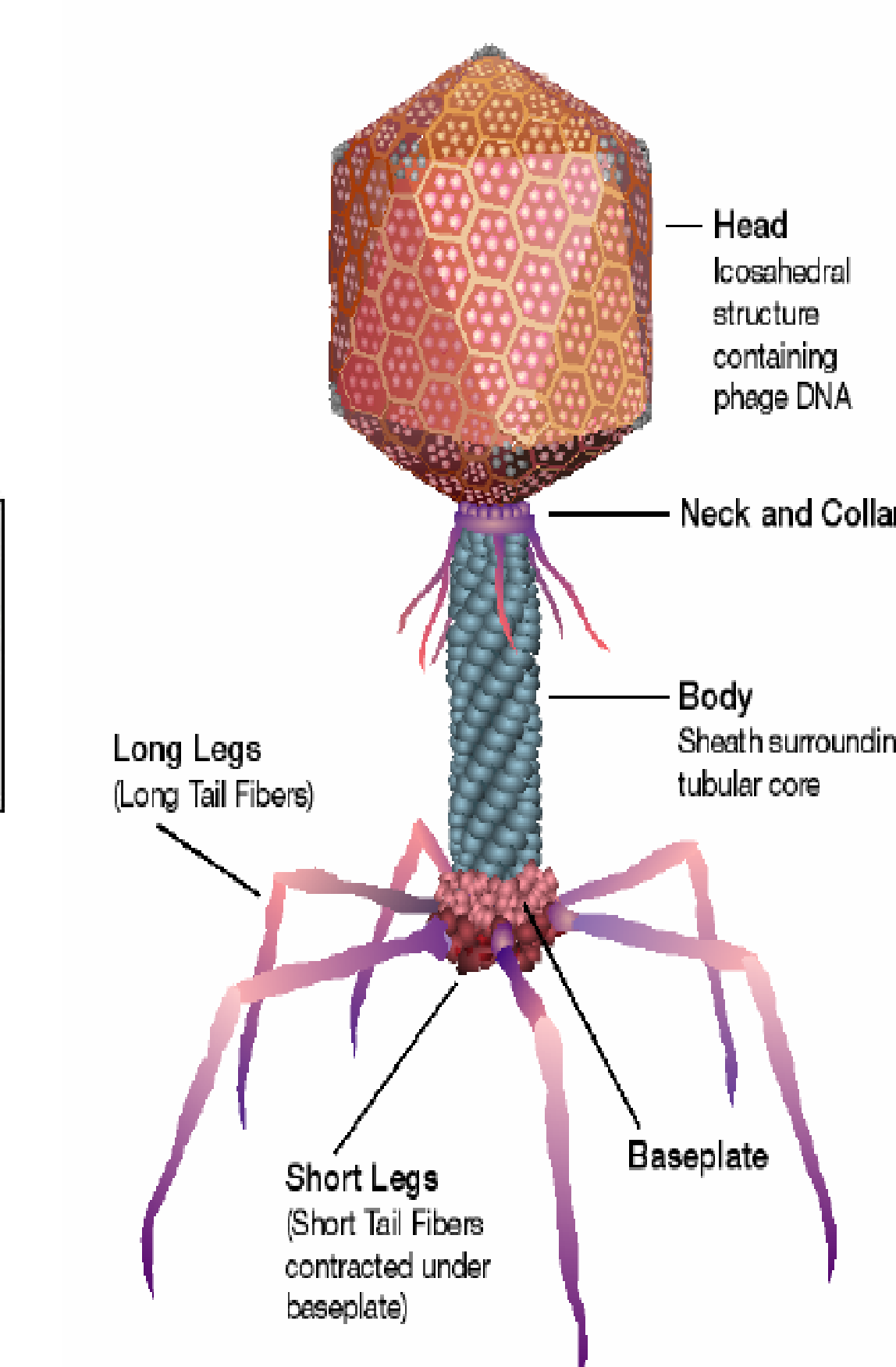
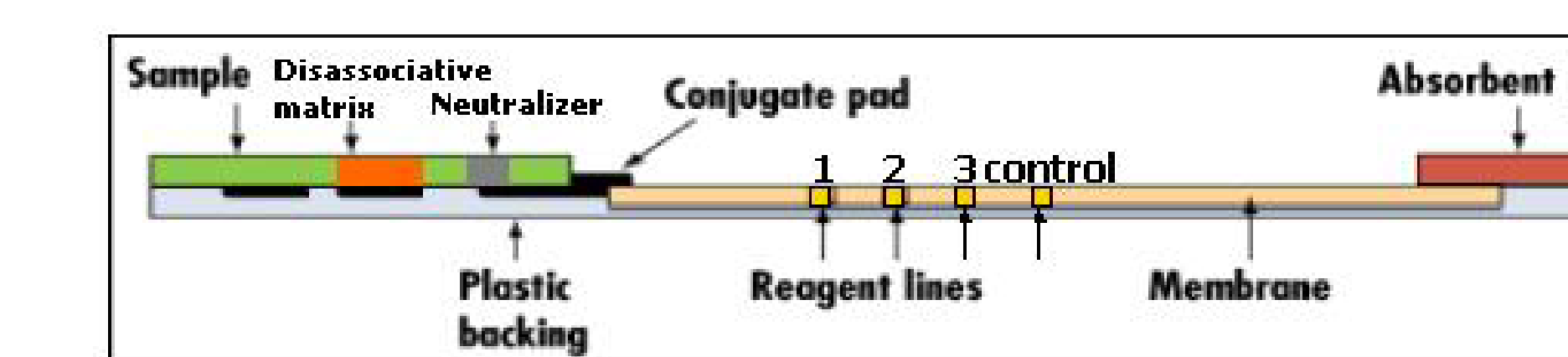


Figure 3. Major components of a lateral flow immunoassay (LFI).



CONCLUSIONS:

As previously demonstrated in environmental and food samples (ref), the phage amplification method is shown here to be effective in additional samples and with an additional bacteria / host pairings. Although the data is still preliminary, adopting phage amplification to immunoassay detection appears to result in clinically responsible results.

Host range and cross reactivity of bacteriophage vary a good deal, however it has been demonstrated that developing an assay with highly specific bacteriophage is achievable. Further work applying this technology to clinical samples is needed to better understand rates of target and non-target infection as well as any competing events resulting from phage cocktails in this format.

This work supports further development by MicroPhage to develop an assay using the more user-friendly detector (LFI) and supports viability to expand the technology's menu to other clinically relevant bacteria.

OPPORTUNITIES:

Rapid direct detection of bacteria from clinical samples has the potential to influence antibiotic therapy choices and improve clinical outcomes. Immunoassays have become a staple in the point of care environment, screening bacteria from *H. pylori* to *S. agalactiae*. Direct detection of bacteria by immunoassays have shown to have deficits due to matrix complications, poor sensitivity, and non-specific binding. By combining incorporating phage amplification into immunoassays, it appears that an expanded menu can be accomplished in complex matrices such as blood. In addition to work on blood culture positive bacteria detection, the method holds promise for applications in wound care management, aiding respiratory diagnosis, and reducing repeat urinary tract infections with targeted treatment. The method also holds promise for additional bacterial targets, such as *S. pneumoniae*, *Enterococcus* sp., and *Pseudomonas* sp., all with well-described lytic phage candidates available for development.

* Abstract has been revised from the original.