

A Novel Phage Technology for the Detection of *S. aureus* and Differentiation of MSSA and MRSA in Positive Blood Culture Bottles

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REVISED ABSTRACT

Background: *Staphylococcus aureus* is a major cause of bloodstream infections. Ineffective MRSA therapy increases adverse outcomes and length of hospital stay. Performance of a novel, lytic bacteriophage prototype assay (LBP; MicroPhage, CO) for the rapid identification of *S. aureus* and differentiation of MSSA/MRSA directly from positive blood culture bottles was investigated by three separate clinical microbiology laboratories. Amplification of a mixture of phages specific for *S. aureus* is used to indicate presence of the organism. MRSA is differentiated by phage amplification in the presence of cefoxitin.

Methods: Positive blood culture bottles from the BACTEC and the BacT/Alert were assayed. A 10 μ L aliquot was inoculated into each of two devices, the first for identification of *S. aureus* while the second, which also contained cefoxitin, was used for MRSA determination. Bacteriophage amplification was detected after a 5 h incubation, using a lateral flow device containing phage-specific antibodies. Identification and MRSA determination were run in parallel with serially interpreted results; antibiotic results only apply to *S. aureus*. Each site compared the LBP results to their standard bacterial culture identification (ID) and susceptibility testing (AST) procedures.

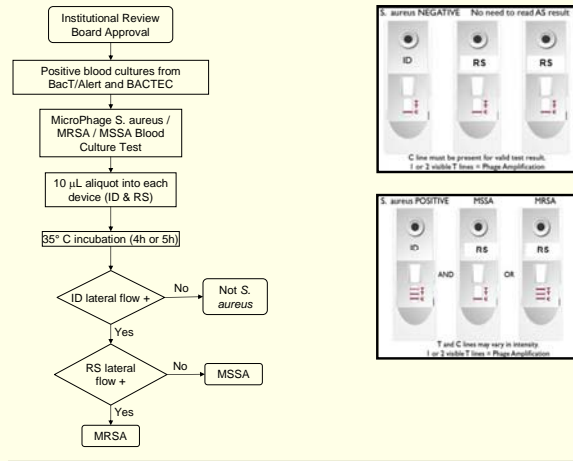
Results: A total of 728 samples were tested. Overall agreement between the LBP and standard ID and AST was 96% (685/711). Testing included 162 *S. aureus* (99 MRSA), 291 CoNS, 94 other gram positive and 164 gram negative organisms. Performance characteristics for *S. aureus* detection were: sensitivity, 90.1% (146/162); specificity, 100% (549/549), PPV, 100% (146/146); NPV, 97.2% (549/565). For MRSA detection, the sensitivity, specificity, PPV and NPV were 84.8% (84/99); 99.0% (606/612), 93.3% (84/90) and 97.6% (606/621), respectively.

Conclusion: Given the high PPV of the prototypic LBP *S. aureus* test, a result of *S. aureus* need not be verified. However a negative result should be considered presumptive. For MRSA differentiation, the prototype is less sensitive than conventional methods.

INTRODUCTION

As a significant cause of blood stream infections *Staphylococcus aureus* requires rapid and accurate identification, including determination of methicillin resistance. Current bench methods rapidly identify *Staphylococcus* to the species level but are not capable of determining resistance. Complex molecular assays are available to provide species identification as well as the detection of resistance (*mecA*). Molecular tests however are frequently quite expensive, require special expertise and therefore may need to be batched, minimizing their utility as rapid tests. Assays that can be set up in real time, are easy to use and relatively inexpensive are still needed. One such prototypic assay is a phenotypic test using a proprietary amplification technology utilizing lytic bacteriophage. Samples are mixed with bacteriophage specific to *S. aureus*, if the organism is present the phage infects it and multiplies, eventually lysing the cells and repeating the amplification cycle. After incubation (35° C) bacteriophage amplification is detected using lateral flow devices containing phage specific antibodies. Determination of cefoxitin resistance is achieved by using a second sampling device (RS) that also includes the presence of cefoxitin in the media, preventing growth of susceptible organisms. Three separate clinical microbiology laboratories evaluated a novel lytic bacteriophage prototype assay for the rapid identification of *S. aureus* and determination of methicillin resistance.

MATERIALS AND METHODS



RESULTS

- 728 samples collected (711 samples analyzed)
 - 17 excluded from analysis (16 greater than 24 hours from alarm & 1 pediatric bottle)
 - Blood culture bottles analyzed (387 aerobic, 285 anaerobic, 39 charcoal)
 - Overall agreement 96% (685/711) at 5 hours, 91% (648/710) at 4 hours
- 711 Organisms isolated (162 *S. aureus* (99 MRSA), 291 CoNS, 94 other gram positive, including yeast, 164 gram negative)

CONCLUSIONS

- S. aureus* identification**
- Prototype has a high PPV (100%) at 5 hours and does not need to be verified
 - No cross reaction seen when tested against non-target organisms
 - Negative result for *S. aureus* should be considered presumptive (NPV 97.2%)
- MRSA differentiation**
- Current prototype is less sensitive than conventional methods

Although a 4 hour incubation was incorporated to assess feasibility, the five hour bacteriophage amplification is necessary to achieve acceptable sensitivity

TABLE 1A. Comparison of *Staphylococcus aureus* culture identification to lytic bacteriophage prototype

LBP	Culture			95% CI			
	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
4h +	102	0	102	63.0% (0.56-0.70)	100% (0.99-1.00)	100% (0.96-1.00)	90.1% (0.88-0.93)
4h -	60	548	608				
Total	162	548	710*				
5h +	146	0	146	90.1% (0.84-0.94)	100% (0.99-1.00)	100% (0.98-1.00)	97.2% (0.96-0.99)
5h -	16	549	565				
Total	162	549	711				

* One coagulase negative *Staphylococcus* sample excluded at 4h time point due to repeat lateral flow failures

TABLE 1B. Comparison of methicillin sensitive *Staphylococcus aureus* (MSSA) culture identification to lytic bacteriophage prototype

LBP	Culture			95% CI			
	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
4h +	35	0	35	55.5% (0.43-0.68)	100% (0.99-1.00)	100% (0.90-1.00)	95.9% (0.94-0.97)
4h -	28	647	675				
Total	63	647	710*				
5h +	52	4	56	82.5% (0.73-0.92)	99.4% (0.99-0.99)	92.9% (0.86-1.00)	98.3% (0.97-0.99)
5h -	11	644	675				
Total	63	648	711				

* One coagulase negative *Staphylococcus* sample excluded at 4h time point due to repeat lateral flow failures

TABLE 1C. Comparison of methicillin resistant *Staphylococcus aureus* (MRSA) culture identification to lytic bacteriophage prototype

LBP	Culture			95% CI			
	Positive	Negative	Total	Sensitivity	Specificity	PPV	NPV
4h +	65	2	67	65.7% (0.56-0.75)	99.7% (0.99-1.00)	97.0% (0.93-1.00)	94.7% (0.93-0.96)
4h -	34	609	643				
Total	99	611	710*				
5h +	84	6	90	84.8% (0.78-0.92)	99.0% (0.98-1.00)	93.3% (0.88-0.98)	97.6% (0.96-0.98)
5h -	15	606	621				
Total	99	612	711				

* One coagulase negative *Staphylococcus* sample excluded at 4h time point due to repeat lateral flow failures

TABLE 2. Comparison of culture result to prototype discrepant result

Culture	Prototype	4 hours	5 hours	Error type
MRSA	Not <i>S. aureus</i>	34	11	False negative
MRSA	MSSA	0	4	Very major error
MSSA	Not <i>S. aureus</i>	26	5	False negative
MSSA	MRSA	2	6	Major error

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