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Accuracy of *S. aureus* Identification by the MicroPhage MRSA/MSSA Blood Culture Test for Bactec

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Background: *Staphylococcal aureus* bacteremia has high morbidity and mortality, and appropriate treatment can be delayed due to the 2-3 days needed for microbiological testing. The MicroPhage MRSA/ MSSA Blood Culture Test reports results within 5 hours of positive blood culture detection. The test is based on bacteriophage amplification, detected by lateral flow immunoassay. As most blood culture positives are actually non-pathogenic contaminants, the ability of the test to reliably distinguish *S. aureus* from other bacteria is critical. Here we report species cross-reactivity results against a panel of yeasts, Gram-negatives and Gram-positives, especially coagulase-negative *Staphylococci* (CoNS).

Methods: A panel of 54 strains was tested, comprising 3 *Candida*, 8 Gram-negative, and 43 Gram-positive strains. Micro-organisms were spiked into 'SA' and 'Plus N' Bactec blood culture bottles, charged with blood drawn from volunteers, at a concentration of approximately 10 cfu/ml. The blood cultures were grown in a Bactec 9050, and samples were withdrawn after detection and inoculated into the MicroPhage MRSA/MSSA Blood Culture Test, which was run according to manufacturer's instructions. Phage amplification was monitored by plating for plaque forming unit on susceptible host as well as by lateral flow immunoassay device (Detector). Tests outcome on detectors were read by a panel of blinded readers.

Results: False positive calls were made 0/9 for *Candida*, 2/34 (6%) for Gram-negative strains, and 12/184 (6.5%) for non-*S. aureus* Gram-positive strains. The two Gram-negative false-positive results came from a *Proteus vulgaris* strain and could not be repeated on multiple tests. The Gram-positive false-positive calls came from two *S. epidermidis* strains known to cross react with the phage cocktail in the test and are due to weak but detectable phage amplification.

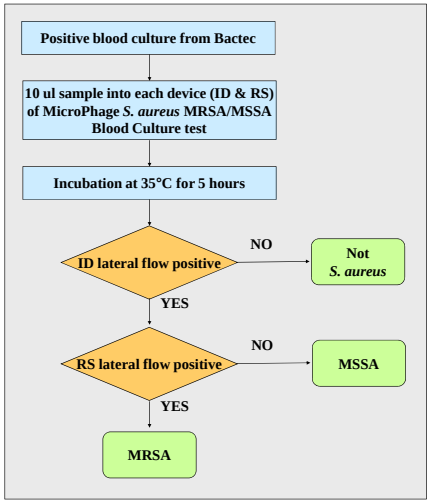

Fig. 1. The MicroPhage test. Positive blood culture sample is mixed with bacteriophage in culture broth. The mixture is incubated, allowing the bacteriophage to amplify if the target species is present and growth condition is permissive. Phage amplification is detected by anti-phage antibodies on a lateral-flow immunoassay device.

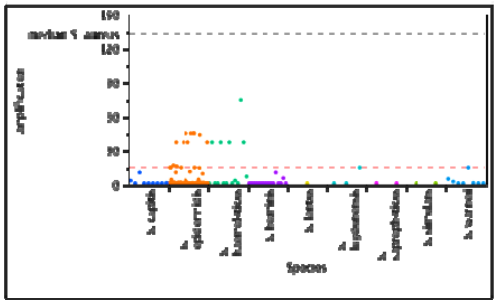

Fig. 2. Cross-reactivity from CoNS isolates. Phage amplification by CoNS isolates under permissive condition is shown by colored dots. Red dashed line is the level of amplification likely to be called positive by the test detector. Black dashed line denotes median amplification by *S. aureus* strains in the same experiment.

	Strain input		Test outcome		
	Genus	species	MRSA	MSSA	Non-SA
Gram negative	*Escherichia*	*coli*	0	0	6
Gram negative	*Klebsiella*	*pneumoniae*	0	0	9
Gram negative	*Proteus*	*mirabilis*	0	0	3
Gram negative	*Proteus*	*vulgaris*	0	2	7
Gram negative	*Pseudomonas*	*aeruginosa*	0	0	9
Gram positive	*Enterococcus*	*faecalis*	0	0	13
Gram positive	*Enterococcus*	*faecium*	0	0	9
Gram positive	*Enterococcus*	*faecium VRE*	0	0	7
Gram positive	*Micrococcus*	*luteus*	0	0	6
Gram positive	*Staphylococcus*	*capitis*	0	0	6
Gram positive	*Staphylococcus*	*epidermidis*	9	3	63
Gram positive	*Staphylococcus*	*haemolyticus*	0	0	16
Gram positive	*Staphylococcus*	*hominis*	0	0	10
Gram positive	*Staphylococcus*	*lentus*	0	0	3
Gram positive	*Staphylococcus*	*lugdunensis*	0	0	3
Gram positive	*Staphylococcus*	*saprophyticus*	0	0	3
Gram positive	*Staphylococcus*	*simulans*	0	0	3
Gram positive	*Staphylococcus*	*warneri*	0	0	7
Gram positive	*Streptococcus*	*mitis*	0	0	3
Gram positive	*Streptococcus*	*mutans*	0	0	7
Gram positive	*Streptococcus*	*pyogenes*	0	0	3
Gram positive	*Streptococcus*	*sp.*	0	0	10
Yeast	*Candida*	*albicans*	0	0	3
Yeast	*Candida*	*glabrata*	0	0	3
Yeast	*Candida*	*parapsilosis*	0	0	3

Table 1. Cross-reactivity by genus and species. A set of Gram negative, Gram positive and yeast strains were assayed in the MicroPhage Test. Out of 229 total tests, 215 were correctly called non-*S. aureus*. Two of the false positives were from Gram negatives while 12 were from non-*aureus* Gram positives.

Conclusions: Based on assays done with a set of non-*aureus* strains, the MicroPhage MRSA/MSSA Blood Culture Test exhibit little or no cross-reactivity to yeasts and Gram-negative strains. This is likely due to high species specificity of the phages used in the test.

Cross-reactivity by Gram-positive strains is relatively high in the present study because the study panel was weighted with known cross-reactive strains. The 2 cross-reacting *S. epidermidis* strains are the most reactive of 153 CoNS clinical isolates tested. Extrapolated out to expected clinical prevalence, no more than 3% cross-reactivity is expected among non-*S. aureus* Gram-positive blood cultures.