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# Direct Detection of Methicillin Resistance in *Staphylococcus aureus* in Blood Culture Broth by Use of a Penicillin Binding Protein 2a Latex Agglutination Test[▽]

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**We studied the utility of performing a penicillin binding protein 2a latex agglutination (PBP-LA) assay directly on Bactec blood culture broth samples containing *Staphylococcus aureus* to rapidly detect methicillin resistance. The sensitivity, specificity, positive predictive value, and negative predictive value of this method were 94.1%, 97.5%, 98%, and 92.9%, respectively.**

*Staphylococcus aureus* is the leading cause of bloodstream infections, with high levels of morbidity and mortality arising from complications (7, 9, 19). Complication rates increase with the duration of bacteremia and delay in appropriate therapy (5). Therefore, the early identification of *S. aureus* and determination of its susceptibility to methicillin are crucial to optimize outcomes. Classical culture-based identification and susceptibility testing of *S. aureus* bacteria isolated from blood cultures take at least 48 h. More rapid PCR methods have been developed for identification from culture broth samples (4, 15–17); however, PCR testing is expensive and often labor-intensive. The penicillin binding protein 2a latex agglutination (PBP-LA) assay (Denka Seiken Co., Japan/Oxoid Ltd., United Kingdom) is a rapid, simple, inexpensive, and FDA-approved test for the identification of methicillin resistance in *S. aureus* bacteria from culture plates (10, 12, 21). Here, we examined whether this test could be used directly on blood culture broth to expedite diagnosis. Previously, the application of this test on simulated specimens incubated in ESP blood culture broth showed excellent specificity (100%) but poor sensitivity (18%) (3). In contrast, other studies (1, 20) that examined a small number of samples with the BacT/Alert and Bactec blood culture systems claimed excellent sensitivities (96 to 100%) but low specificities (84 to 86%). We therefore sought to examine more definitively the utility of the PBP-LA method in clinical practice by testing a large set of *S. aureus*-positive Bactec 9240 Standard/10 Aerobic/F and Lytic/10 Anaerobic/F bottles from clinical blood cultures.

In this study, positive blood cultures with Gram stain morphologies suggestive of *Staphylococcus* spp. were first tested by a direct tube coagulase (DTC) test to rapidly identify *S. aureus* (11). DTC test-positive cultures were then tested by the PBP-LA assay as follows. Briefly, 2 ml of blood culture broth was mixed with 3 or 2 ml of distilled water for aerobic and anaerobic cultures, respectively (see below for the rationale of different volumes). The tubes were centrifuged at 1,000 × *g* for 10 min, and the pellet was resuspended with 1.5 ml (aerobic culture) or 1 ml (anaerobic cultures) of 0.1 N NaOH. The suspensions were then centrifuged at 2,500 × *g* for 5 min in Microfuge tubes, and the pellet was then used as the sample for the PBP-LA assay according to the manufacturer's instructions for testing of bacterial colonies. The identification of *S. aureus* was subsequently confirmed by slide latex agglutination (Staphaurex; Remel) or tube coagulase testing of subculture colonies. Methicillin susceptibility was determined by both Vitek-2 oxacillin MIC and *mecA* gene PCR (12).

A total of 91 blood cultures positive for *S. aureus* were evaluated (51 methicillin-resistant *S. aureus* [MRSA] and 40 methicillin-susceptible *S. aureus* [MSSA] samples). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the PBP-LA test were 94.1%, 97.5%, 98%, and 92.9%, respectively, compared with *mecA* gene PCR (Table 1). In addition, the PBP-LA test showed identical performance characteristics with aerobic and anaerobic blood culture broth samples. When both aerobic and anaerobic bottles from the same set were positive for *S. aureus* ($n$ = 40; 21 MRSA and 19 MSSA samples), the PBP-LA results from each bottle were concordant.

The observed differences in sensitivity and specificity compared with those reported from studies were likely due to different sample preparation protocols (1, 3, 13, 20). For example, in a previous study showing low sensitivity with the ESP system, bacteria were collected by serum separator tubes, which may not efficiently recover the organisms (3). The lower specificities found in previous studies (1, 13, 20) were likely due to an inefficient removal of substances causing nonspecific reactivity and/or to miscalling low-level, nonspecific agglutination as a positive result, a phenomenon which we noted predominantly from some aerobic culture broths. We overcame this problem in two ways: first, we treated the aerobic culture broth with more water and NaOH, as this reduced nonspecific agglutination; second, we defined any granulation against a milky background as being nonspecific reactivity and considered only granulation against a clear background to be a true-

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TABLE 1. Overall performance of the PBP-LA test on positive blood culture broth

| PBP-LA test result | No. of samples with *mecA* PCR result | |
|---|---|---|
| | MRSA (*mecA* PCR$^+$) | MSSA (*mecA* PCR$^-$) |
| Positive | 48 | 1 |
| Negative | 3 | 39 |

positive result. Of note, Shovlin et al. (13) found that resin may potentially contribute to reduced sensitivity and specificity. Therefore, further alterations in the procedure may be necessary to optimize performance with resin-containing media or other media with substantially different formulations.

In comparison to PCR, PBP-LA in conjunction with DTC (i) leads to timely results, (ii) is technically simple, (iii) requires no expensive equipment cost, and (iv) uses inexpensive reagents. Technical and turnaround times are 2 min (2) and 2 to 4 h for DTC, respectively, and 10 to 12 and 30 min for PBP-LA, respectively. The reagent costs per test for DTC and PBP-LA are $1 and $7, respectively. In contrast, reagent costs for commercial PCR tests are usually over $30/test, and the cost of FDA-approved instrumentation is very high. Homebrew PCR methods are less expensive but highly complex. The simple PBP-LA test can be implemented on multiple shifts, while the complexity of PCR methods generally necessitates batch testing by dedicated molecular diagnostic staff, increasing turnaround times. It should be noted that the DTC assay has a sensitivity of 65 to 90% (11), reducing the sensitivity of the DTC and PBP-LA assays below that of PCR. However, the PBP-LA assay may also be combined with other rapid identification assays with higher sensitivities (96 to 100%), such as thermostable DNase (6), API Rapidec, and peptide nucleic acid-fluorescence *in situ* hybridization (FISH) (2).

Despite a lower sensitivity than that of PCR assays (14), the PBP-LA test still has utility in a number of settings. At many institutions, patients with a single set of Gram-positive cocci in clusters will not be placed on vancomycin treatment or treatment with other antibiotics if clinical suspicions are low. Furthermore, in areas with low MRSA carriage rates (8, 18), patients with Gram-positive cocci in clusters may not be placed on vancomycin treatment. For these patients, the unexpected identification of MRSA by the PBP-LA test will trigger the rapid initiation of appropriate therapy. Lastly, the identification of MRSA will lead to the earlier institution of contact precautions.

We conclude that the PBP-LA assay is a rapid, reliable, and inexpensive test for the direct detection of methicillin resistance in *S. aureus* bacteria growing in blood culture broth.

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