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Rapid Identification of *Staphylococcus aureus* Directly from Bactec Blood Culture Broth by the BinaxNOW *S. aureus* Test

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The BinaxNOW *Staphylococcus aureus* testing showed sensitivity, specificity, and positive and negative predicative values of 97.6%, 100%, 100%, and 98.4%, respectively, for identification of *S. aureus* from Bactec blood culture broth. Importantly, the test performed equally well on aerobic and anaerobic culture broth.

Staphylococcus aureus is a major cause of bacteremia (1). Bloodstream infection is often associated with serious complications, such as endocarditis and osteomyelitis (2–4). The complication rates rise with duration of untreated bacteremia (2). Therefore, to expedite the use of appropriate antibiotic therapy and reduce morbidity, mortality, and length of hospitalization, early differentiation of *S. aureus* from coagulase-negative staphylococci (CoNS) is crucial. Various tests have been developed or adapted to rapidly identify *S. aureus* directly from positive blood culture broth, including use of fluorescence *in situ* hybridization (85 to 100% sensitivity and 100% specificity) (5, 6), PCR (93.8 to 100% sensitivity and 98.6 to 100% specificity) (7–9), the enzyme-based RAPIDEC Staph test (90.5 to 100% sensitivity and 96.6 to 100% specificity) (10–12), and the direct tube coagulase test (65 to 84.1% sensitivity and 98.7 to 100% specificity) (10, 13). However, these tests either are expensive, are labor-intensive, and/or have suboptimal sensitivity and specificity. Recently, a rapid and simple immunochromatographic BinaxNOW *Staphylococcus aureus* (BNSA) test (Alere, Scarborough, ME) was FDA approved for direct identification of *S. aureus* from BacT/Alert-positive blood culture broth. In considering this assay for off-label use in our clinical laboratory, we performed an evaluation of the BNSA test on Bactec 9240 (Becton, Dickinson BioSciences, Sparks, MD) blood culture media and also compared the performance of this BNSA test on aerobic versus anaerobic culture broth.

During our evaluation, blood culture broth from Bactec Standard 10 Aerobic/F or LYTIC/10 Anaerobic/F bottles was evaluated by the BNSA test when Gram stain suggested staphylococci (positive cocci in pairs and clusters). The test was performed according to the manufacturer's instructions. Briefly, positive blood culture broth was mixed with kit reagent A in two simple-addition, centrifugation steps. After removal of the remaining supernatant, kit reagents B and C were added and mixed with the pellet. A total of 50 μl of this resuspended pellet was added to the test card, followed by 5 drops of kit reagent D. The test card was read 10 min later. The card shows both a pink-purple control line that confirms activity of the antistaphylococcal antibody and a sample line which turns positive in the presence of *S. aureus*. The identification of the bacteria from positive blood cultures was confirmed by a Staphaurex latex agglutination test on subcultures (Remel Inc., Lenexa, KS). Discordant results were resolved by a tube coagulase test and Vitek 2 identification (BioMeriéux, Marcy l'Etoile, France).

From 2012 to 2013, 104 positive blood cultures from 104 patients were tested by BNSA test, including 41 *S. aureus* cultures (27 aerobic and 14 anaerobic bottles), 60 coagulase-negative staphylococcus (CoNS) cultures (40 aerobic and 20 anaerobic bottles), and 3 other bacterial cultures which were reported as Gram-positive cocci in pairs and clusters (1 *Micrococcus* species, 1 anaerobic Gram-positive coccus, and 1 *Gemella* species). For 41 *S. aureus* cultures, 14 (34%) were methicillin resistant (MRSA) and 27 (66%) were methicillin sensitive (MSSA). Overall, the sensitivity, specificity, positive predicative value (PPV), and negative predicative value (NPV) were 97.6%, 100%, 100%, and 98.4%, respectively. Importantly, we also showed that the BNSA test performed equally well on both aerobic and anaerobic blood culture broth. Among 17 blood culture sets evaluated in which both aerobic and anaerobic bottles flagged simultaneously and were therefore tested contemporaneously by BNSA (including 12 *S. aureus* and 5 CoNS cultures), aerobic and anaerobic broth results were identical. Furthermore, according to the manufacturer's instruction, the results are read at 10 min after adding the last reagent to the card. However, we found that all results could be read reliably at 5 min. The one false-negative test was performed on a blood culture collected from a patient already treated for several days for known *S. aureus* bacteremia. We speculate that the false-negative result may relate to antibiotic treatment-associated reduction in the level of the antigen detected by the test. Notably, our findings were similar to the performance of PCR-based methods (7–9), unpublished package insert data for BNSA on BacT/Alert bottles (98.8% sensitivity, 100% specificity), and a recent evaluation of BNSA on VersaTREK bottles (95.8% sensitivity, 99.6% specificity). Notably, several samples needed to be excluded from the last study because of pellet loss during assay preparative steps. In our study, no pellets were lost, perhaps because we removed most of the supernatant by using a 1-ml plastic transfer pipette (Samco Scientific, San Fernando, CA) and then aspirated the remaining supernatant carefully using a 200-μl-capacity micropipette.

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We believe that the BSNA assay will prove useful in clinical practice. When Gram-positive cocci in clusters are identified in positive blood cultures, doctors usually treat empirically with vancomycin to cover potential infection with MRSA. However, this approach may not be used by all doctors (14), especially when only one bottle flags positive and clinical suspicion is low (15), since CoNS is a frequent blood culture contaminant (16). In these cases, early identification of *S. aureus* directly from blood culture broth will prevent delay of therapy (14, 16). Alternatively, because of the high negative predictive value of BSNA, earlier deescalation of therapy and avoidance of unnecessary follow-up studies would be possible because of the ability to distinguish *S. aureus* from organisms such as CoNS that may be blood culture contaminants. The limitation of the BNSA assay is that it does not differentiate MRSA from MSSA. However, it may be used in combination with a rapid detection method for methicillin resistance, such as the penicillin binding protein 2a latex agglutination test (17), to establish optimal antibiotic therapy. Specifically, a change from empirical vancomycin to a more effective β-lactam agent may be instituted earlier in patients determined to have MSSA using this testing strategy (18–22). Because the BNSA test is simple, rapid (30 min), and inexpensive (approximately $10), with very high sensitivity and specificity, we believe it will serve an important role for early diagnosis of *S. aureus* bacteremia in laboratories which can neither afford nor implement expensive and/or complex all-shift molecular testing.

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