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Evaluation of the BinaxNOW *Staphylococcus aureus* Test for Rapid Identification of Gram-Positive Cocci from VersaTREK Blood Culture Bottles

Neelam Dhiman,[a]* Tamara L. Trienski,[b] Linda P. DiPersio,[a] Joseph R. DiPersio[a]

Department of Pathology and Laboratory Medicine, Summa Health System, Akron, Ohio, USA[a]; Department of Pharmacy, Summa Health System, Akron, Ohio, USA[b]

The ability of the rapid BinaxNOW *Staphylococcus aureus* (BNSA) immunochromatographic test (Alere Scarborough, Inc., ME) to accurately differentiate *S. aureus* from coagulase-negative staphylococci (CoNS) and other Gram-positive cocci (GPC) directly from VersaTREK blood culture bottles was evaluated. A total of 319 positive patient blood culture bottles with GPC seen in clusters with Gram staining were tested using the BNSA test and a direct tube coagulase test (DTCT). The BNSA test was accurate for the detection and differentiation of *S. aureus* from CoNS and other GPC within 30 min from the time of blood culture positivity and demonstrated a test sensitivity and specificity of 95.8% and 99.6%, respectively. BNSA test results were faxed to the antimicrobial stewardship pharmacist by noon each day in order to evaluate empirical antimicrobial therapy and facilitate more rapid changes or modifications if necessary. Same-day reporting of BNSA test results in conjunction with an antimicrobial stewardship program was more impactful in improving treatment for inpatients with documented *S. aureus* bacteremia than in reducing empirical vancomycin use in inpatients with CoNS during the first 24 h following reporting.

Staphylococci are the most commonly recovered organisms from blood culture specimens and represent approximately 35% of total patient isolates at our hospital system (1). Approximately 75% of staphylococcal isolates are identified as coagulase-negative *Staphylococcus* species (CoNS) or other Gram-positive cocci (GPC), most of which represent contamination during blood collection. When laboratories first report the presence of GPC seen in clusters (GPCCs) with Gram staining from blood culture bottles, it is important to differentiate as rapidly as possible between *Staphylococcus aureus* and other GPCCs that may not be clinically significant. Many patients are started on empirical therapy to cover *S. aureus*, including methicillin-resistant *S. aureus* (MRSA), until final identification and antimicrobial susceptibilities are reported 18 to 48 h later. A number of laboratory testing procedures have been used to rapidly differentiate CoNS from *S. aureus* soon after Gram stain reading. These include nonmolecular methods as well as newer molecular methods that can also determine methicillin susceptibility (2–6). Our laboratory has used a direct tube coagulase test (DTCT) for many years to rapidly identify *S. aureus* from blood culture bottles. Positive DTCT results are reported to appropriate medical personnel, but negative DTCT results are not reported because a low but significant number of *S. aureus* isolates do not turn the DTCT positive within 4 h. This has been demonstrated in our validation studies as well as in published reports (2, 3).

The BinaxNOW *Staphylococcus aureus* (BNSA) test (Alere Scarborough, Inc., Scarborough, ME) is a new *in vitro* immunochromatographic membrane assay that uses polyclonal antibodies to qualitatively detect an *S. aureus*-specific protein directly in samples taken from positive blood culture bottles with GPCCs. The test currently has FDA approval for use only with BacT/Alert standard aerobic and anaerobic blood culture media (bioMérieux, Inc., Durham, NC), and Alere has no immediate plans to expand approval to other instrument or media types. A multicenter clinical study of 325 blood culture samples containing GPCCs demonstrated a sensitivity of 98.8% compared to standard laboratory methods (BinaxNOW *Staphylococcus aureus* test package insert; Alere Scarborough, Inc., Scarborough, ME). Only 1 of 85 *S. aureus* isolates was misidentified. The purpose of our study was to evaluate the ability of the BNSA test to accurately differentiate *S. aureus* from CoNS and other GPCCs growing in VersaTREK blood culture aerobic/anaerobic media and to compare the performance of the BNSA test to that of our in-house validated DTCT method. The study also evaluated the immediate (same day) clinical impact on antimicrobial prescribing that rapid BNSA reporting had when used in conjunction with an antimicrobial stewardship program.

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MATERIALS AND METHODS

Organism identification. Routine blood cultures were performed using the VersaTREK instrument (Trek Diagnostic Systems, Ltd., Cleveland, OH) following the manufacturer's guidelines. Positive blood cultures were removed from the instrument, and Gram staining was performed. Only bottles with GPCCs were included in the study. Blood-broth samples were removed by syringe to perform the BNSA and DTCT procedures as described below. A total of 319 patient blood culture samples collected between October 2011 and April 2013 were included in the study. *S. aureus* isolates subcultured from positive blood culture bottles were confirmed using a standard latex agglutination method (Staphaurex; Remel, Inc., Lenexa, KS). Discordant results were resolved using the standard

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Address correspondence to Joseph R. DiPersio, dipersij@summahealth.org.
* Present address: Neelam Dhiman, Medfusion, Lewisville, Texas, USA.

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TABLE 1 Performance of the BNSA test

Results for *Staphylococcus aureus*[a] (no. of isolates)	BNSA test results (no. of isolates)[b]	
	Positive	Negative
Positive (96)	92	4
Negative (223)	1	222

[a] Confirmed by latex agglutination.
[b] Sensitivity, 95.8%; specificity, 99.6%.

tube coagulase test and/or API Staph biochemical identification strips (API Analytab Products, Plainview, NY).

BinaxNOW *Staphylococcus aureus* test. Testing was performed according to the manufacturer's product insert (BinaxNOW *Staphylococcus aureus* test package insert; Alere Scarborough, Inc., Scarborough, ME). Blood culture bottles were gently mixed, and a 1-ml aliquot was removed to a conical tube followed by addition of 1 ml of reagent A (lysing agent). The tube was vortexed to mix and centrifuged for 5 min at 1,500 × *g* using an angle-head rotor. The supernatant was aspirated and discarded without disturbing the pellet. One milliliter of reagent A was again added and the pellet resuspended, and this was followed by repeat centrifugation. The aspirated supernatant was discarded and 5 drops of reagent B (neutralizing agent) added to the pellet. The pellet was vortexed to resuspend and 25 μl of reagent C (bacterial membrane-lysing agent) added, and this was followed by vortexing. Fifty microliters of this sample was added to the middle of the white sample pad located on the upper right-hand side of the test card. Four drops of reagent D (clearing solution) were added to the white wash pad located on the top left-hand side of the device, and 1 drop was added to the top of the white pad on the upper right-hand side of the test card. The card was closed, sealed, and left at room temperature for 10 min, at which time it was read. A blue control line turns to pink-purple to indicate a negative sample. A blue control line and a second sample line turn to pink-purple to indicate a positive test for *S. aureus*. If the blue control line does not change color, the test is invalid.

Direct tube coagulase test. The DTCT was performed by adding 0.2 ml of blood-broth sample to 0.5 ml of EDTA rabbit plasma in a tube. The tube was placed in a 36°C heating block and examined hourly up to 4 h. Any clot formation was considered positive for *S. aureus*. If the test was negative at 4 h, the tube was incubated overnight at room temperature and read the next morning. Both the BNSA and DTCT procedures were performed on the same positive blood culture bottle. In general, the first bottle to turn positive was used. If both bottles of the set were positive at the time of testing, then the aerobic bottle was used.

Antimicrobial stewardship review. Results of the first 160 patient blood culture samples tested using the BNSA method were not reported for clinical use but were used to establish test sensitivity/specificity and clinical utility. Subsequent BNSA test results were entered into the laboratory computer and all inpatient results were faxed to the antimicrobial stewardship pharmacist for review of appropriateness of empirical antibiotic therapy. Patients identified as potentially needing interventions, such as changes in therapy or de-escalation of therapy, were discussed on formal afternoon antimicrobial stewardship rounds with an infectious diseases physician. At the end of the study, a review of actions taken on 60 inpatient BNSA test results collected between September 2012 and April 2013 (30 patients with *S. aureus* and 30 patients with CoNS) was undertaken to evaluate the immediate (same-day) clinical impact of this testing procedure. This project was approved as a "quality improvement analysis" by the institutional review board at our institution (IRB).

RESULTS

A total of 319 patient blood culture samples with GPCCs were included in the final analysis. Three patient blood culture samples that gave false-negative BNSA results for confirmed *S. aureus* isolates were excluded from analysis because they did not yield a visible pellet (pellet loss) during specimen processing. A fourth patient sample yielding no visible pellet was excluded before final BNSA testing. The association between pellet loss and false-negative results was noted early in the study and led to a decision not to continue with BNSA testing if a pellet was not observed. The manufacturer intends to address this issue in a future package insert update. Reasons for pellet loss with rare *S. aureus* isolates are unclear at this time.

TABLE 2 Performance of the direct tube coagulase test on 319 blood culture specimens

Organisms (*n*)	DTCT time to positivity	No. (%) of isolates positive or negative for DTCT	
		Positive	Negative
S. aureus (96)	1 h	30 (31.3)	
	2 h	35 (36.5)	
	3 h	9 (9.4)	
	4 h	8 (8.3)	
	Overnight	8 (8.3)	6 (6.3)
Not *S. aureus* (223)	1 h to overnight	0	223

The performances of the rapid BNSA test and the DTCT are shown in Tables 1 and 2, respectively. BNSA test sensitivity and specificity were 95.8% and 99.6%, and DTCT sensitivity and specificity were 93.8% and 100%, respectively. With the use of the DTCT, 65 of 96 (67.7%) *S. aureus* isolates were identified by 2 h, and 85.4% of the isolates were identified by 4 h. No false-positive DTCT results were observed.

The BNSA test successfully identified *S. aureus* from two specimens that contained additional organisms (one contained *Acinetobacter baumannii* and *S. epidermidis* and another contained *Klebsiella pneumoniae* and CoNS). Two other mixed specimens with *S. aureus* gave false-negative BNSA test results (one contained a CoNS and the other contained mixed morphotypes of *S. aureus*). One false-positive BNSA test was seen from a blood specimen containing a presumptive *S. lugdunensis* isolate (69% likelihood on the API Staph test). The breakdown of non-*S. aureus* isolates recovered from blood cultures is shown in Table 3. Ninety-two percent of these specimens contained CoNS either in pure culture or mixed with other CoNS morphotypes. Additional GPC isolated included *Micrococcus* spp., *Enterococcus* spp., *Aerococcus* spp., and *Streptococcus* spp.

Previous reports evaluating the impact of rapid methods that differentiate *S. aureus* from other GPCCs have documented de-

TABLE 3 Non-*Staphylococcus aureus* blood culture isolates

Organism(s)	No. (%) of isolates
CoNS	204 (91.5)
CoNS and *Aerococcus* spp.	1 (0.4)
Micrococcus spp.	14 (6.3)
Streptococcus group C	2 (0.9)
Enterococcus spp.	1 (0.4)
Streptococcus (alpha-hemolytic)	1 (0.4)
Total	223

creased costs associated with more rapid de-escalation of antistaphylococcal therapy when a probable contaminant was identified (7, 8). The daily cost of 2 vancomycin doses per day at our institution was calculated to be approximately $35.00. This includes drug cost together with intravenous (i.v.) preparation and nursing administration costs. Based on a total of approximately 500 patient blood culture isolates with CoNS seen at our institution in 2012, potential savings may be realized if empirical vancomycin therapy was removed/discontinued at least 1 day sooner. Additional savings due to reduced length of stay related to faster resolution of blood culture contamination may also occur. BNSA test results on 60 inpatients (30 with CoNS and 30 with *S. aureus*) were reviewed to determine physician same-day actions with respect to antimicrobial therapy. Eighty-seven percent and 90% of patients in the groups with CoNS and *S. aureus*, respectively, were on antimicrobial therapy at the time of result reporting and 69% versus 83% were on therapy that covered MRSA. BNSA results from patients with CoNS led to few immediate therapy changes. Only one patient, who was receiving vancomycin, had therapy stopped, and 2 patients not on any therapy were not started on therapy. The impact that BNSA test reporting had on nonadmitted patients could not be determined, since the antimicrobial stewardship team could not review these patients. The impact that BNSA results had on inpatients with *S. aureus* bacteremia, however, was more evident. Of the 30 inpatients with *S. aureus* reviewed, 13 (43.3%) had changes in their antibiotic regimens on the day that the BNSA results were reported. Five (16.7%) were either on no antibiotic ($n = 3$) or on an antibiotic that did not cover MRSA ($n = 2$) at the time of reporting. All 5 of these patients were either started on or changed to an antibiotic that covered MRSA.

DISCUSSION

S. aureus is a major cause of morbidity and mortality in both health care and community settings. Over half of all nosocomial bloodstream infections have been attributed to GPCCs. Rapid identification and differentiation of *S. aureus* from CoNS in blood culture bottles is reported to have a major impact on improving patient outcomes, decreasing length of hospital stays, and reducing health care expenses (8–11). Conventional identification of *S. aureus* from blood cultures requires isolated colonies and generally takes 18 to 24 h after positive signaling on continuously monitored automated blood culture systems. A number of biochemical, immunological, and molecular methods have been used to decrease identification times. Many of these methods have been shown to either lack appropriate sensitivity or have high reagent, instrument, or labor costs. Several variations of the DTCT with sensitivities of ≥80% have been reported in the literature. Performing the DTCT on organisms concentrated from blood culture bottles may increase test sensitivity (12–14). However, fear of a false-negative *S. aureus* test result and its clinical implications has prevented this rapid and cost-effective test from gaining wider use. Peptide nucleic acid (PNA) fluorescence *in situ* hybridization (FISH) provides accurate same-day results but at a higher cost than the DTCT. Previous studies using PNA FISH have demonstrated trends in the reduction of antibiotic use and mortality (7, 8). Newer molecular amplification methods can provide accurate same-day results together with methicillin susceptibility but at a significantly higher cost in reagents and instrumentation. Since the majority of GPCCs from blood culture bottles turn out not to be clinically important, use of expensive molecular testing procedures may not be a cost-effective approach if less costly but accurate methods are available. The BNSA rapid immunochromatographic test is a relatively inexpensive nonmolecular method for differentiating *S. aureus* from other GPCCs. The supply cost is approximately $10.00 per test and tests can be performed individually or in small batches within 30 min. It is critical that careful attention is paid to pellet loss during processing, and if a pellet is not seen, test results should not be reported. Four of eight potential false-negative *S. aureus* results were avoided by this observation. In addition, the DTCT should be performed together with the BNSA test, since two of four reported false-negative BNSA results in our study set were DTCT positive and recognized within 4 h. Combined use of the DTCT with the BNSA test, as is our current laboratory procedure, increased sensitivity of *S. aureus* detection within 4 h from 96% to 97.9%. Since 31.3% of *S. aureus* isolates were identified within 1 h and another 36.5% by 2 h using the DTCT, the BNSA test may not have to be performed on all blood cultures with GPCCs. DTCT- or BNSA-positive samples for *S. aureus* could be reflexed to a molecular test method that also detects *mecA*, thereby further reducing laboratory costs compared to testing all specimens with GPCCs by a molecular method but providing the added value of a methicillin susceptibility result.

Based on seeded studies, *Staphylococcus schleiferi* and coinfections of CoNS with *Clostridium perfringens*, *Clostridium bifermentans*, or *Clostridium histolyticum* have been reported to potentially cause false-positive BNSA results. Only one false-positive BNSA result was observed in our study set, with a presumptive but not confirmed *S. lugdunensis* isolate. One false-negative BNSA test produced a faint band beyond the 10-min incubation period. The specimen had mixed morphotypes of regular golden colonies mixed with second predominant tiny whitish colonies, both of which were identified as *S. aureus*. Repeat BNSA testing was positive on isolated colonies for both morphotypes. The analytical limit of detection for the BNSA test is 5.42×10^8 cells/ml, and this accuracy has not been established in the presence of coinfections with other bacteria. Since VersaTREK bottles are approved for lower blood volumes, the BNSA test may have missed this isolate in a mixed population at a lower threshold. Also, an *S. aureus* strain (ATCC 14993) has been reported to produce false-negative results, so this test may miss rare clinical strains that may not have been tested during validation. These limitations are noted in the manufacturer's product insert. We emphasize being cautious during the washing steps, as loss of pellet during processing can lead to a false-negative test for *S. aureus* and these results should not be reported.

Rapid differentiation of *S. aureus* from other GPCCs from blood culture bottles potentially saves only 1 day over traditional subculture. By the next day, subcultured staphylococcal colonies growing on solid media can be identified easily by latex agglutination, and methicillin susceptibility can be determined on *S. aureus* colonies using rapid penicillin binding protein 2a (PBP2a) detection methods. Previous studies evaluating rapid methods have used testing in conjunction with direct physician reporting and have compared this to traditional culture without direct reporting of organism identification (7, 11). This approach makes it difficult to independently evaluate the roles of rapid testing versus direct reporting and potentially widens the reporting time interval between rapid test and traditional culture results when comparative data analysis is performed. If microbiology laboratories reported

next-day subculture identification results directly to physicians, the cost differential between rapid and traditional methods may be less than previously reported.

Our review of 30 patients with CoNS showed that only 3 patients (10%) were impacted by rapid identification. Reasons for not stopping empirical vancomycin therapy may include the following: (i) if non-*S. aureus* GPCCs were detected in two of two blood culture sets, the possibility of true infection was still under consideration and (ii) if non-*S. aureus* GPCCs were detected in one of two blood culture sets, physicians may wait to see if the second set becomes positive later in the day before making a treatment change. The role of our antimicrobial stewardship team upon identification of patients with *S. aureus* bacteremia is to ensure that the patient is receiving the correct empirical treatment and to recommend an infectious diseases consult. More than 99% of inpatients with *S. aureus* bacteremia at our institution are treated by an infectious diseases physician.

In conclusion, this is the first study that evaluated the performance of the BNSA test using the VersaTREK blood culture system. The BNSA test is a rapid, cost-effective test that can identify *S. aureus* and differentiate it from other GPCCs directly from positive blood cultures within 30 min. Early notification of results in conjunction with an antimicrobial stewardship program can potentially reduce unnecessary antimicrobial costs and improve care, especially for inpatients with *S. aureus* bacteremia.

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REFERENCES

1. **Annane D, Bellissant E, Cavaillon JM.** 2005. Septic shock. Lancet **365:** 63–78.
2. **Qian Q, Eichelberger K, Kirby JE.** 2007. Rapid identification of *Staphylococcus aureus* in blood cultures by use of the direct tube coagulase test. J. Clin. Microbiol. **45:**2267–2269.
3. **Chapin K, Musgnug M.** 2003. Evaluation of three rapid methods for the direct identification of *Staphylococcus aureus* from positive blood cultures. J. Clin. Microbiol. **41:**4324–4327.
4. **Oliveira K, Brecher SM, Durbin A, Shapiro DS, Schwartz DR, De Girolami PC, Dakos J, Procop GW, Wilson D, Hanna CS, Haase G, Peltroche-Llacsahuanga H, Chapin KC, Musgnug MC, Levi MH, Shoemaker C, Stender H.** 2003. Direct identification of *Staphylococcus aureus* from positive blood culture bottles. J. Clin. Microbiol. **41:**889–891.
5. **Carretto E, Bardaro M, Russello G, Mirra M, Zuelli C, Barbarini D.** 2013. Comparison of the *Staphylococcus* QuickFISH BC test with the tube coagulase test performed on positive blood cultures for evaluation and application in a clinical routine setting. J. Clin. Microbiol. **51:**131–135.
6. **Kelley PG, Grabsch EA, Farrell J, Xie S, Montgomery J, Mayall B, Howden BP.** 2011. Evaluation of the Xpert MRSA/SA blood culture assay for the detection of *Staphylococcus aureus* strains with reduced vancomycin susceptibility from blood culture specimens. Diagn. Microbiol. Infect. Dis. **70:**404–407.
7. **Forrest GN, Mehta S, Weekes E, Lincalis DP, Johnson JK, Venezia RA.** 2006. Impact of rapid *in situ* hybridization testing on coagulase-negative staphylococci positive blood cultures. J. Antimicrob. Chemother. **58:**154–158.
8. **Ly T, Gulia J, Pyrgos V, Waga M, Shoham S.** 2008. Impact upon clinical outcomes of translation of PNA FISH-generated laboratory data from the clinical microbiology bench to bedside in real time. Ther. Clin. Risk Manag. **4:**637–640.
9. **Frye AM, Baker CA, Rustvold DL, Heath KA, Hunt J, Leggett JE, Oethinger M.** 2012. Clinical impact of real-time PCR assay for rapid identification of staphylococcal bacteremia. J. Clin. Microbiol. **50:**127–133.
10. **Parta M, Goebel M, Thomas J, Matloobi M, Stager C, Musher DM.** 2010. Impact of an assay that enables rapid determination of *Staphylococcus* species and their drug susceptibility on the treatment of patients with positive blood culture results. Infect. Control Hosp. Epidemiol. **31:**1043–1048.
11. **Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA.** 2010. An antimicrobial stewardship program's impact with rapid polymerase chain reaction methicillin-resistant *Staphylococcus aureus*/*S. aureus* blood culture test in patients with *S. aureus* bacteremia. Clin. Infect. Dis. **51:**1074–1080.
12. **McDonald CL, Chapin K.** 1995. Rapid identification of *Staphylococcus aureus* from blood culture bottles by classic 2-hour tube coagulase test. J. Clin. Microbiol. **33:**50–52.
13. **Ozen NS, Ogunc D, Mutlu D, Ongut G, Baysan BO, Gunseren BF.** 2011. Comparison of four methods for rapid identification of *Staphylococcus aureus* directly from BACTEC 9240 blood culture system. Indian J. Med. Microbiol. **29:**42–46.
14. **Sturm PDJ, Kwa D, Vos FJ, Bartels CJM, Schulin T.** 2008. Performance of two tube coagulase methods for rapid identification of *Staphylococcus aureus* from blood cultures and their impact on antimicrobial management. Clin. Microbiol. Infect. **14:**510–513.