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Evaluation of the Nanosphere Verigene Gram-Positive Blood Culture Assay with the VersaTREK Blood Culture System and Assessment of Possible Impact on Selected Patients

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The Verigene Gram-positive blood culture (BC-GP) assay (Nanosphere, Northbrook, IL) is a molecular method for the rapid identification of Gram-positive organisms and resistance markers directly from blood culture bottles. A total of 148 VersaTREK REDOX 1 40-ml aerobic bottles demonstrating Gram-positive bacteria were tested. Results were compared with those from conventional biochemical and matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) identifications. We obtained isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) (24), methicillin-susceptible *Staphylococcus aureus* (MSSA) (14), methicillin-resistant *Staphylococcus epidermidis* (MRSE) (17), methicillin-susceptible *Staphylococcus epidermidis* (MSSE) (9), other coagulase-negative staphylococci (19), *Streptococcus salivarius* (5), *Streptococcus parasanguinis* (2), *Streptococcus sanguinis* (1), *Streptococcus cristatus* (1), the *Streptococcus bovis* group (5), *Streptococcus agalactiae* (9), the *Streptococcus anginosus* group (1), *Streptococcus pneumoniae* (6), vancomycin-resistant *Enterococcus faecium* (VRE FCM) (16), vancomycin-susceptible *Enterococcus faecalis* (3), *Aerococcus viridans* (2), *Bacillus* (6), *Corynebacterium* (8), *Lactobacillus* (2), *Micrococcus* (2), *Neisseria mucosa* (1), *Escherichia coli* (3), *Candida tropicalis* (1), *Propionibacterium* (1), and *Rothia* (1). Overall agreement with the culture results was 95%. A total of 137 of 138 (99%) monomicrobial cultures were concordant. We tested 9 polymicrobial samples and found 33% agreement. A chart review of 31 patients with MRSA, MSSA, or VRE demonstrated that the Nanosphere BC-GP assay might have led to more appropriate antibiotic selection for these patients an average of 42 h earlier. Additionally, contact isolation could have been initiated an average of 37 h earlier for patients with MRSA or VRE. The BC-GP assay may have a positive impact on patient care, health care costs, and antibiotic stewardship.

Gram-positive organisms remain the most common pathogens in bacteremia, but Gram-negative organisms are also seen (1). Bacteremia is fatal in >25% of cases and is associated with high costs and long lengths of hospital stays (2). Outcomes are determined by how quickly treatment is started, and the risk of mortality increases with every hour from presentation to initiation of antibiotics (3). Current methods for the identification and susceptibility testing of blood culture isolates may take up to 72 h. Newer technologies allow identification of bacteria directly from blood culture bottles before growth is visible on solid media. Identifying the etiologic agent of the bacteremia in a more timely fashion (4) allows targeted antimicrobial therapy to be instituted much more rapidly. This is important, as studies have shown that mortality increases with each additional day of inappropriate therapy (5). The use of molecular assays for identification of microorganisms has led to early initiation of appropriate antibiotics, improved clinical outcomes, reduced costs, decreased spread of organisms, and possibly prevention of further progression of bacterial resistance (6, 7).

Methods for the rapid detection of bacteria from positive blood culture bottles include matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) analysis, PCR, peptide nucleic acid-based fluorescence *in situ* hybridization (PNA FISH), and probe-based direct detection (8, 9). Each system has its benefits and limitations. MALDI-TOF MS offers the advantage of a huge database of organisms, but it, like PNA FISH, is limited by the inability to detect resistance markers. Probe-based detection systems and PCR have the ability to detect resistance genes (10) but are limited by relatively small panel sizes; however, the clinical significance of this limitation is unknown.

The Verigene Gram-positive blood culture (BC-GP) assay (Nanosphere, Inc., Northbrook, IL) is a molecular method for rapid identification of Gram-positive organisms directly from blood culture bottles. The targets encompass the commonly seen blood pathogens, including both genus- and species-specific targets. It detects *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus anginosus* group, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Enterococcus faecalis*, and *Enterococcus faecium* as well as *Staphylococcus*, *Streptococcus*, and *Listeria* genera. It also detects three resistance markers, *mecA*, *vanA*, and *vanB*. The test takes approximately 10 min of hands-on time and 2.5 h of run time. The purpose of this study was to determine if the BC-GP assay correctly identifies bacteria from the VersaTREK blood culture bottles. In addition, we wanted to determine the possible impact of the results on antibiotic therapy in patients with methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA), and vancomy-

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cin-resistant enterococci (VRE) and on initiation of contact isolation for those with MRSA and VRE. To our knowledge, only one other study that evaluated the use of the Nanosphere assay with the VersaTREK blood culture system has been published (11). Our study has additional data from assessment of the possible impact of the results on therapeutic choices and initiation of contact isolation.

(A portion of the data from this study was presented at the 113th General Meeting of the American Society for Microbiology, Denver, CO, 2013.)

MATERIALS AND METHODS

Blood culture samples and processing. Samples included in this study were submitted as a part of routine patient care to University Hospitals Microbiology Laboratory, which serves St. Paul University Hospital (300 beds), Zale Lipshy University Hospital (152 beds), an emergency room, and several outpatient clinics. A majority of the samples in the study were consecutive; however, later in the study, samples were chosen based on recovered organisms, but the organism identification was blinded to the tester. Blood culture bottles were tested irrespective of the results of the anaerobic bottle in the same set or results of bottles in other sets. In most cases, only a single sample from each patient was tested. A total of 148 samples from 110 patients were collected in VersaTREK REDOX 1 40-ml aerobic bottles (Trek Diagnostic Systems, Cleveland, OH) and incubated on the VersaTREK instrument (Trek Diagnostic Systems). Bottles that were flagged positive on the instrument and demonstrated Gram-positive bacteria by Gram staining were included in this study. As part of routine practices, subcultures from positive aerobic bottles were inoculated onto blood agar, MacConkey agar, and chocolate agar plates and incubated overnight at 37°C in 5% CO_2. Standard operating procedures for identification and susceptibility testing of blood culture isolates were followed, and these included use of a catalase test, Pastorex Staph-Plus (Bio-Rad Laboratory Diagnostics Group, Redmond, WA), CAMP test, optochin disk (Oxoid, United Kingdom), BactiCard Strep (Remel, Lenexa, KS), RapID ANA II system (Remel, Lenexa, KS), and an automated platform, the Microscan WalkAway plus system (Siemens Medical Solutions USA, Inc., Malvern, PA) using the PC-33 panel. In some instances, such as single blood culture sets with coagulase-negative staphylococci, definitive identifications were performed for study purposes only.

Nanosphere BC-GP assay. A 1-ml aliquot of blood culture medium was drawn from each blood culture bottle included in the study. Using the Verigene Gram-positive blood culture (BC-GP) assay (Nanosphere Inc., Northbrook, IL), we tested 46 samples (31%) in real time within 90 min following the Gram staining, and the remaining samples were frozen at −80°C and tested at a later date. Five medical technologists were trained and participated in the testing.

The tests were completed according to the manufacturers' instructions (12, 13). We ran the tests on 2 processors and 1 reader. Briefly, the extraction tray, utility tray, and Verigene test cartridge and a tip holder assembly were loaded into the Verigene Processor SP, and then 350 μl of blood culture broth was added to the sample well in the extraction tray. Hands-on time to begin the assay was about 10 min. Within the processor, bacterial DNA was extracted, fragmented, and denatured. The DNA was then exposed to the microarray, consisting of capture oligonucleotides arranged as a grid on a glass slide, which is part of the test cartridge. These oligonucleotides are complementary to known bacterial genes that are sequence specific to certain species, genera, or resistance markers. Bacterial DNA sequences hybridize to complementary capture oligonucleotides, if present. A second oligonucleotide, the mediator oligonucleotide, contains sequences complementary to bacterial DNA and a second domain, which will bind an oligonucleotide attached to a gold nanoparticle. This process takes 2.5 h. The test cartridge was then removed from the processor and placed on the reader, where the relative densities of probes on the microarray were assessed optically. The reader provided results after approximately 5 min. All analyses were completed within the reader, and positive reports included one or more genera, species, and resistance markers, if present.

MALDI-TOF MS. Discrepant or ambiguous bacterial identifications were resolved with MALDI-TOF MS using the Bruker Biotyper database, version 3.0 (Billerica, MA). Performance of the MALDI-TOF MS was verified through an in-lab verification study using clinical isolates, conducted at the Children's Medical Center of Dallas. Identifications were performed as previously described (14).

Statistical analysis. Concordance was determined by comparing the BC-GP assay results to those from conventional biochemical identification and susceptibility testing. Any discrepancies between the BC-GP assay and the biochemical methods were resolved by MALDI-TOF MS.

Clinical correlation. To assess the potential clinical significance of this assay, we performed a retrospective chart review on 31 adult University Hospital patients (9 with MSSA, 15 with MRSA, and 7 with VRE identified in blood cultures by the BC-GP assay and confirmed by conventional methods). In patients with multiple samples tested by the BC-GP assay, only the first sample from each patient with MSSA, MRSA, or VRE was included in this portion of the study. Data for analysis included gender, age, antibiotic allergies, dates of admission and discharge, the antibiotics ordered and times of orders, time of report of the Gram stain and culture results, infection control measures and time of initiation of isolation, and a brief patient history. We obtained University of Texas Southwestern Institutional Review Board approval for this study with a waiver for informed consent.

RESULTS

Nanosphere BC-GP assay. We obtained isolates of the following organisms from 148 cultures: MRSA (24), MSSA (14), methicillin-resistant *Staphylococcus epidermidis* (MRSE) (17), methicillin-susceptible *Staphylococcus epidermidis* (MSSE) (9), other coagulase-negative staphylococci (including *S. auricularis*, *S. capitis*, *S. haemolyticus*, *S. hominis*, *S. simulans*, and *S. warneri*) (19), *Streptococcus salivarius* (5), *Streptococcus parasanguinis* (2), *Streptococcus sanguinis* (1), *Streptococcus cristatus* (1), *Streptococcus bovis* group (5), *Streptococcus agalactiae* (9), *Streptococcus anginosus* group (1), *Streptococcus pneumoniae* (6), vancomycin-resistant *Enterococcus faecium* (VRE FCM) (16), vancomycin-susceptible *Enterococcus faecalis* (3), *Aerococcus viridans* (2), *Bacillus* spp. (6), *Corynebacterium* spp. (8), *Lactobacillus* spp. (2), *Micrococcus* spp. (2), *Neisseria mucosa* (1), *Escherichia coli* (3), *Candida tropicalis* (1), *Propionibacterium* spp. (1), and *Rothia* spp. (1) (as shown in Table 1). Cultures with ≥2 organisms were counted in each appropriate category.

Of 138 monomicrobial cultures, 137 (99%) were concordant. The only discrepancy was viridans group streptococci not detected by the BC-GP assay but identified by MALDI-TOF MS as *S. salivarius*. There were no incorrect identifications by the BC-GP assay. One or more expected targets (*Staphylococcus* spp., *S. aureus*, *mecA*, *S. epidermidis*, *E. faecalis*, *vanA* or *vanB*, *Streptococcus* spp., and *S. anginosus* group) were undetected in 6 of 9 polymicrobial cultures, as shown in Table 2. The percent agreement for polymicrobial cultures was 33% (3/9).

For monomicrobial and polymicrobial cultures combined, the percent agreement was 95% (140/147). This excludes one sample that failed twice. The percent agreement was calculated by determining if the BC-GP assay obtained all targets that were expected based on the culture results.

Forty *S. aureus* and *S. epidermidis* isolates with methicillin resistance were detected by conventional methods. The BC-GP assay reported *mecA* in 38 (93%) of these samples. The 2 discrepant samples were polymicrobial, and the species-specific

TABLE 1 BC-GP assay and conventional identification of bacterial isolates in 148 blood cultures

BC-GP assay target	No. of isolates detected by: BC-GP assay[a]	Conventional identification[b]	% agreement
Staphylococcus spp.[c]	78	81	96
S. aureus	36	38	95
S. epidermidis	25	26	96
Enterococcus faecalis	2	3	67
Enterococcus faecium	15	16	94
Streptococcus spp.[d]	29	30	97
S. agalactiae	9	9	100
S. anginosus group	1	1	100
S. pneumoniae	6	6	100
Nontarget organisms[e]	26	26	100
Overall target agreement[f]			96

[a] Includes isolates that were positive on repeat testing after failures in initial testing.
[b] Samples with more than one organism corresponding to the same target were counted once per target. Reasons for discrepancies in the numbers between the BC-GP assay results and conventional identification included polymicrobial samples, one repeat test failure, and one failure of the BC-GP assay to detect the target.
[c] BC-GP assay target includes *S. aureus* and *S. epidermidis*.
[d] BC-GP assay target includes *S. agalactiae*, *S. pneumoniae*, *S. pyogenes*, and *S. anginosus* group.
[e] Blood sample organisms that were not intended targets of the BC-GP assay included *Aerococcus viridans* ($n = 2$), *Bacillus* spp. ($n = 6$), *Corynebacterium* spp. ($n = 8$), *Escherichia coli* ($n = 2$), *Lactobacillus* spp. ($n = 2$), *Micrococcus* spp. ($n = 2$), *Neisseria* spp. ($n = 1$), *Propionibacterium* spp. ($n = 1$), *Rothia* spp. ($n = 1$), and *C. tropicalis* ($n = 1$).
[f] Overall target agreement is the percentage of targets which correlated with organism identification. Resistance markers were not included in this calculation.

targets (*S. aureus* and *S. epidermidis*) were not detected. We detected 16 VRE for which the BC-GP assay detected *vanA* in 13 and *vanB* in 2, for a 94% agreement. The BC-GP assay did not detect either *vanA* or *vanB* in a polymicrobial sample that contained VRE FCM and *E. coli*, and the *E. faecium* target was not detected by the BC-GP assay.

Test failures. We encountered 6/148 (4%) test failures with the BC-GP assay. One blood culture with *Enterococcus faecium* positive for *vanA* and one with *Bacillus cereus* showed error messages on their initial runs that indicated there was an "inability to obtain the test result because of high variability in the target-specific signals" (12). Two samples, one with MRSA and one with *S. auricularis*, showed a message indicating that the internal control did not work, which may happen due to "processing and/or lysis/extraction issues" (12). On repeat testing, only the sample with MRSA failed again and showed the same error message. One trial showed an error message that occurs if the analyzer cannot accurately visualize the test substrate, and the organism was subsequently correctly identified as *Staphylococcus* spp. One assay of an MRSE-positive bottle reported a high-pressure failure, which occurs if pressure criteria are not met at any portion of the procedure. All of these samples were tested again; 5 of 6 reruns produced results that agreed with conventional methods.

Clinical correlation. After excluding patients with previously positive blood cultures, we examined the charts of 31 patients (9 patients with MSSA, 15 patients with MRSA, and 7 patients with VRE detected by the BC-GP assay). The average time between placement of the blood culture bottle on the blood culture instrument and release of Gram stain results was 17 h (range, 8 to 57 h). The average time between placement on the blood culture instrument to release of organism identification and susceptibility results was 62 h (range, 28 to 170 h). If the BC-GP assay had been performed immediately after the Gram staining, the average time to identification would have been approximately 20 h. The organism identification and susceptibility would have been available approximately 42 h earlier than with conventional methods.

In total, 13 of the 31 patients (42%) could have been placed on more appropriate antibiotics approximately 2 days earlier, if the BC-GP assay results had been released and acted upon. Six of the 9 MSSA-positive patients' antibiotics were narrowed from empirical broad-spectrum therapy (vancomycin or daptomycin) to nafcillin or were started on appropriate beta-lactam therapy at the time of culture release. Six of the 15 MRSA-positive patients' and 1 of the 7 VRE-positive patients' antibiotics were narrowed by discontinuation of Gram-negative coverage (piperacillin-tazobactam) at the time of susceptibility results.

At our institution, contact isolation is initiated for patients found to have VRE or MRSA. Fifteen of the 19 (79%) inpatients with MRSA or VRE detected by the BC-GP assay were not in contact isolation when the blood culture result became available. The average time from placement of blood culture bottles on the instrument to isolations was 61 h. Patient isolation protocols were implemented approximately 4 h after culture and sensitivity results were released. With the use of BC-GP assay, these patients could have been isolated 37 h earlier than with the current process.

TABLE 2 BC-GP assay results for 9 specimens with multiple organisms

Organisms identified by conventional methods	Targets detected by BC-GP assay	Missed target(s)
MRSA, *S. simulans*	*Staphylococcus* spp.	*S. aureus*, *mecA*
MRSE, *C. tropicalis*[a]	*Staphylococcus* spp.	*S. epidermidis*, *mecA*
Streptococcus gallolyticus, vancomycin-sensitive *Enterococcus faecalis*	*Streptococcus* spp.	*E. faecalis*
S. parasanguinis, *Neisseria mucosa*[a]	*Streptococcus* spp.	None
MSSA, *Streptococcus agalactiae*	*Staphylococcus* spp., *S. aureus*, *Streptococcus* spp., *S. agalactiae*	None
E. coli, *S. haemolyticus*, vancomycin-resistant *Enterococcus faecium*[b]	*Enterococcus faecium*, *vanB*	*Staphylococcus* spp.
MSSE, oxacillin-resistant *Staphylococcus capitis*	*Staphylococcus* spp., *S. epidermidis*, *mecA*	None
Vancomycin-resistant *Enterococcus faecium*, *E. coli*[a]	None	*vanA* or *vanB*, *Enterococcus faecium*

[a] Not an intended target of the BC-GP assay.
[b] Two samples from this patient were included.

DISCUSSION

In patients with bacteremia, accurate bacterial identification and susceptibility results are necessary to guide appropriate treatment. Additionally, unnecessary treatment and hospital stays for patients who are found to have a blood culture contaminant can be avoided. Current techniques for the identification of organisms in blood cultures can take several days. A rapid, simple assay for these purposes would be advantageous to the clinical microbiology laboratory, treatment providers, and infection control practitioners.

This assay produced reliable results, with an overall agreement of 95% in our hands. This result is similar to those of another VersaTREK study with 93% agreement (11) and a BacT/Alert study with 94.6% (15). The targets included the major Gram-positive organisms that cause bacteremia. Our agreement percentages for the most commonly seen organisms in blood cultures, *S. aureus* and *S. epidermidis*, were 95% and 96%, respectively. Samuel et al. (11) reported 98% agreement for 45 *S. aureus* isolates and 87% agreement for 54 *S. epidermidis* isolates. The authors attributed this lower concordance rate for *S. epidermidis* to the presence of this organism in a higher proportion of polymicrobial cultures, in which they were less likely to be detected. Our concordance rate for enterococci was 89%. This includes 2 mixed cultures in which enterococci were undetected. No enterococci were missed in monomicrobial cultures.

Resistance markers were detected with accuracy in this assay, with agreement percentages of 93% and 94% for *mecA* and *vanA* or *-B*, respectively. The two undetected *mecA* markers were in specimens with more than one organism, and the species-specific target was not detected. Nanosphere Verigene technicians examined the raw data from our instruments and found that there was a signal for *mecA* in both of these samples. According to the test design, the *mecA* target is reported only if either *S. aureus* or *S. epidermidis* is also reported. Similarly, neither *vanA* nor *vanB* was reported for a mixed sample with undetected VRE FCM and *E. coli*.

Mixed cultures, which accounted for 6% (9/148) of our samples, will remain a challenge for this test. Polymicrobial sepsis is rare; results for one series indicated that it occurs in 4.7% of septic episodes (1). In situations where 2 organisms contribute to a blood culture bottle becoming positive on an automated instrument, one or both may be below the limit of detection for the BC-GP assay. It is important to keep in mind that the BC-GP assay is not a target amplification assay, and all of the amplification must occur in the blood culture bottle during incubation. This amplification process ends when the sum of all organisms present is enough to signal positive on the blood culture system, but the concentration of one or more organisms in a mixed bottle may still be low. Our percent agreement for mixed cultures was 33%. Wojewoda et al. (15) and Samuel et al. (11) reported 75% and 76% agreements, respectively, for polymicrobial cultures. Unlike our study with a lower percent agreement for polymicrobial cultures, the study by Wojewoda et al. restricted testing to samples demonstrating a single Gram morphology.

When more than one organism is present in a blood culture bottle, it is often a combination of a contaminant and pathogen. Three of 9 samples contained a presumed contaminant (*S. haemolyticus* [$n = 2$] and *S. simulans* [$n = 1$]); the *Staphylococcus* target was missed in the sample with *S. haemolyticus*. At the time that a blood sample is tested, a contaminating organism might be present in higher concentrations than the pathogen, preventing detection of the pathogen by the BC-GP assay. Missed targets included pathogenic organisms (*S. aureus*, *E. faecalis*, and *S. epidermidis*) and resistance markers (*mecA* and *vanA* or *-B*) in 4 of the 6 samples with discrepant polymicrobial cultures.

The *Staphylococcus* target was detected in 2 cultures, each containing 2 species of staphylococci. It was impossible to know which organism(s) contributed the genetic material needed to trigger the *Staphylococcus* target. In one of these cases, the BC-GP assay also missed the *S. aureus* and *mecA* targets. Similarly, a sample containing MRSE and *C. tropicalis* obtained only the *Staphylococcus* target on the BC-GP assay, missing the *S. epidermidis* and *mecA* targets.

In the package insert, Nanosphere acknowledges that polymicrobial cultures are a limitation (12). In some instances, users might see multiple morphologies on the Gram stain and be aware that each organism that is seen may not reach the level of detection required for identification by the BC-GP assay. Users of the assay must decide when and if to run the sample in these situations. In our 8 patients, 2 Gram stain morphologies were seen in 4 of the samples. Had we run these samples on the BC-GP assay for clinical purposes, we would have used caution when interpreting the results. For instance, in a patient with MRSE and *C. tropicalis*, the BC-GP assay detected only the *Staphylococcus* sp. target, missing the *S. epidermidis* and *mecA* targets. In practice, we would have realized that the bacterial concentrations present in the sample may have been lower than the limit of detection for species-specific and resistance gene targets. Unfortunately, selecting only samples with a single Gram morphology does not always prevent testing mixed cultures on the BC-GP assay. Wojewoda et al. (15) tested only bottles with single Gram morphology but recovered 2 or more organisms in 12 of 186 blood culture bottles. In our study, 3 patients had samples in which both organisms had the same Gram morphology. In one patient with MRSA and *S. simulans*, the BC-GP assay showed a result for a *Staphylococcus* species. This BC-GP assay result might have led clinicians to assume that the blood culture contained only a contaminant and possibly discontinue therapy.

Another limitation involves the inability to associate a resistance marker with a particular organism in polymicrobial samples that demonstrate a single Gram morphology. We had one polymicrobial blood culture in which the detected targets were *Staphylococcus* spp., *Staphylococcus epidermidis*, and *mecA*. With these results and a Gram stain that showed Gram-positive cocci in clusters, one would assume that this blood culture contained MRSE. Conventional identification and susceptibility testing revealed a methicillin-susceptible *Staphylococcus epidermidis* isolate and a second coagulase-negative staphylococcus species that was methicillin resistant (*Staphylococcus capitis*). No targets were missed or incorrectly identified, but the result was misleading. In this particular setting, the clinical significance is unclear, as coagulase-negative staphylococci often are contaminants. Wojewoda et al. (15) reported a mixed culture with *S. epidermidis* and *S. aureus* and the presence of *mecA*. The culture grew MSSA and MRSE.

In some cases, testing samples with more than one Gram morphology can be very useful. The BC-GP assay detected all targets in a sample with MSSA and *S. agalactiae*. This BC-GP assay result was accurate and more rapid than results for conventional culture, demonstrating that the assay can be used for some mixed infections.

The clinical impact of this test cannot be understated. Other studies using different molecular blood culture identification systems have demonstrated improvement in patient care, length of hospital stay, infection prevention measures, and overall hospital

costs (6, 7). However, this may be dependent on the method of communication with the provider. While Bauer et al. (6) showed improvements in some of these areas with rapid notification of an infectious disease pharmacist in addition to the provider, Frye et al. (4) showed no improvement in their outcome measures by releasing the results of a rapid molecular *S. aureus*/MRSA test into the electronic medical record, even though there was a statistically significant decrease in the time before results were available. Similarly, Carver et al. showed that clinicians failed to utilize rapid *mecA* test results on positive blood cultures without the intervention of an infectious disease pharmacist (16). The three main hypothesized clinical benefits are earlier initiation of targeted therapy, discontinuation of unnecessary broad-spectrum therapy, and earlier initiation of contact isolation. We feel that the biggest clinical impact in our patient population would be for those with MSSA. In 67% of patients with MSSA, empirical vancomycin was narrowed to more appropriate beta-lactam therapy or beta-lactam therapy was initiated after MSSA culture and sensitivity results were released. We estimated that identification and sensitivity results would be available 42 h earlier with use of the BC-GP assay, allowing the antibiotic choices, including discontinuation of Gram-negative bacterium coverage, to be narrowed much sooner. Finally, for patients with MRSA and VRE, we estimated that isolation, which is imperative in preventing the spread of resistant organisms and progression of bacterial resistance mechanisms, would occur 37 h earlier. Our study was limited to hypothetical, extrapolated data, and larger, prospective studies are needed to confirm the clinical impact of rapid blood culture identification tests.

The Verigene BC-GP assay panel is relatively easy to use and produces results quickly. However, there are many steps in the setup process, which may contribute to some of the assay failures that were seen. For other studies (11, 15), failure rates were not reported. Three of the failures in our study occurred within the first 12 runs, and only 2 failures occurred in the 2nd half of our study. This may represent a learning curve and the benefit of experience. We also did not reanalyze a specimen for which the reader was unable to visualize the microarray. Taking certain recourse measures as outlined in the package insert might have led to a result that agreed with conventional methods, without the need to rerun the sample from the bottle. Additionally, there is no control to validate sample addition to the device. Inadvertent sample omission is possible and occurred in our investigation. Although this is intended to be a simple test, extensive training of highly knowledgeable technologists is required.

Our study showed the BC-GP assay to be reliable as we did not have any falsely reported targets. However, polymicrobial samples were not accurately identified. In other studies (11, 15), blood culture bottles containing *Streptococcus mitis/oralis* isolates were incorrectly identified on the BC-GP assay as *Streptococcus pneumoniae*. We did not have any *S. mitis/oralis* isolates in our study to confirm or refute this issue. We also did not have any *Staphylococcus lugdunensis*, *Streptococcus pyogenes*, or *Listeria* spp. in our study. These organisms are uncommon causes of bacteremia. Additional limitations of our study included the lack of data for costs and actual clinical outcomes.

In conclusion, this assay provides a rapid way to identify Gram-positive organisms from blood culture bottles. The most important and most frequent causes of bloodstream infections are included in the panel. Additionally, the most common resistance genes are targeted, offering a large advantage over other currently available assays. This combination of features offers many opportunities to improve patient care and infection control practices.

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