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Performance of the Verigene Gram-Positive Blood Culture Assay for Direct Detection of Gram-Positive Organisms and Resistance Markers in a Pediatric Hospital

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The performance characteristics of the Verigene Gram-positive blood culture (BC-GP) assay were evaluated in pediatric patients. Concordance of the BC-GP assay was 95.8%, with significant decreases in turnaround time for identification and resistance detection. BC-GP is highly accurate and can be integrated into the routine workflow of the microbiology laboratory.

Bloodstream infections are one of the leading causes of death in the United States, and prompt initiation of appropriate antimicrobial therapy is essential to significantly improve patient management and reduce the rate of mortality of bacteremic patients (1). Gram-positive pathogens are implicated in >50% of all bacterial bloodstream infections, with coagulase-negative staphylococci (CoNS) being the most common Gram-positive bacteria isolated from blood culture, followed by *Staphylococcus aureus* and *Enterococcus* spp. (2–4). Similar findings have been demonstrated in our institution where approximately 64% of all positive blood cultures consist of Gram-positive organisms. The workup of pathogens in the clinical microbiology laboratory is a key contributing factor in overall mortality rate, as delays in identification and susceptibility testing have been demonstrated to directly impact patient survival (5). Previous studies have been fundamental in directly correlating rapid identification of Gram-positive pathogens with improved patient outcome, decreased hospital length of stay, and decreased hospital costs (6–9). Moreover, direct detection of the *mecA* gene in methicillin-resistant *S. aureus* (MRSA) has been demonstrated to be an effective tool for managing vancomycin usage as well as reducing both hospital length of stay and costs (10, 11).

The Verigene Gram-positive blood culture (BC-GP) assay (Nanosphere Inc., Northbrook, IL) is a microarray-based, multiplexed, molecular assay approved by the Food and Drug Administration (FDA) for rapid detection and identification of 12 Gram-positive targets (*S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Staphylococcus* spp., *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Streptococcus pneumoniae*, *Streptococcus* spp., *Enterococcus faecium*, *Enterococcus faecalis*, *Listeria monocytogenes*) and 3 resistance markers (*mecA*, *vanA*, *vanB*). Four recent studies have evaluated the performance of the BC-GP assay on different bottle types (12–15). Specifically, Sullivan et al. reported high sensitivity and specificity in pediatric patients using BacT/Alert pediatric FAN (PF) blood cultures (14). This study evaluated the performance characteristics of the BacT/Alert standard aerobic (SA) noncharcoal and PF charcoal blood culture bottles collected from pediatric patients admitted to Children's Hospital Los Angeles (CHLA), a freestanding pediatric tertiary care center. The implementation process and comparison of workflow and turnaround time (TAT) were also assessed.

(Portions of the study data were presented at the 23rd European Society of Clinical Microbiology and Infectious Diseases [ECCMID] Conference, 2013, and the 113th American Society for Microbiology [ASM] General Meeting, 2013.)

A total of 203 positive blood cultures (104 SA, 99 PF) from 203 pediatric patients submitted for routine workup to the Clinical Microbiology Laboratory at CHLA were included in the study. Verigene BC-GP assays were performed on positive blood culture bottles that demonstrated Gram-positive cocci, not Gram-positive bacilli by Gram stain. Blood culture bottles were incubated at room temperature, and testing was performed within 12 h of positivity. The BC-GP assay was performed according to the manufacturer's protocol in the package insert. "No call" or "not detected" results were repeated once for each specimen. Conventional identification and susceptibility workup were also performed on all 203 blood cultures by aseptically inoculating onto blood agar, MacConkey, and chocolate agar, followed by incubation for 18 to 24 h at 37°C and 5% CO_2. Isolates were identified using routine identification methods, such as Vitek II (bioMérieux) identification and other biochemical tests. Discordant results were confirmed by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS; BioTyper 3.0; Bruker Daltonics, Billerica, MA) or 16S rRNA sequencing. Routine susceptibility testing for *S. aureus*, *S. epidermidis*, *E. faecium*, and *E. faecalis* was performed using the Vitek II and/or Etest (bioMérieux). Methicillin-resistant staphylococci were confirmed by cefoxitin screening by broth microdilution and *mecA* PCR. Ninety-five-percent confidence intervals were calculated using binomial expansion.

Blood cultures positive for Gram-positive cocci were automatically reflexed to the Verigene BC-GP assay 24 h a day, 7 days a week. The turnaround times for identification of the targeted bac-

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TABLE 1 Distribution of bacteria identified from BacT/Alert SA or BacT/Alert PF blood culture bottles and performance of resistance marker detection by the Verigene BC-GP assay

Organism isolated from blood culture bottles	BacT/Alert SA		BacT/Alert PF		% (95% CI)	
	No. of isolates	No. (%) of isolates correctly identified by BC-GP	No. of isolates	No. (%) of isolates correctly identified by BC-GP	Sensitivity	Specificity
Staphylococcus aureus	13	13 (100)	23	23 (100)[a]	100 (88.5–100)	100 (97.6–100)
Methicillin resistant	1	1 (100)	9	9 (100)	100 (67.9–200)	100 (84.8–100)
Methicillin susceptible	12	12 (100)	14	14 (100)		
Staphylococcus epidermidis	31	31 (100)	34	33 (97.1)	100 (93.2–100)	99.4 (96.2–99.9)
Methicillin resistant	22	22 (100)	29	28 (96.6)	98 (88.7–99.9)	92.9 (92.9–99.9)
Methicillin susceptible	9	9 (100)	5	4 (80.0)		
Staphylococcus lugdunensis	0	NA	1	1 (100)	100 (16.8–100)	100 (97.9–100)
Other staphylococci	12	12 (100)	25	25 (100)[a]	100 (88.9–100)	100 (97.6–100)
Enterococcus faecalis	9	9 (100)[a]	4	3 (75.0)	92.3 (64.6–99.9)	100 (97.9–100)
Enterococcus faecium	13	13 (100)	2	2 (100)	100 (76.4–100)	100 (97.8–100)
Vancomycin resistant	6	6 (100)	1	1 (100)	100 (59.6–100)	100 (62.8–100)
Vancomycin susceptible	7	7 (100)	1	1 (100)		
Streptococcus pneumoniae	4	4 (100)	1	1 (100)	100 (98.5–100)	100 (98.5–100)
Streptococcus pyogenes	3	3 (100)[a]	3	2 (66.7)[a]	83.3 (41.8–98.9)	100 (97.9–100)
Streptococcus agalactiae	2	2 (100)	4	4 (100)	100 (55.7–100)	100 (97.9–100)
Other streptococci[b]	23	19 (82.6)[a]	8	6 (75.0)	96.8 (79.6–99.9)	97.5 (94.0–99.1)
Other[c]	5	0 (0)	3	0 (0)		
Total	110[d]	106	105[e]	100		

[a] One isolate required repeat testing.
[b] Five *S. mitis/oralis* isolates (4 SA, 1 PF) were misidentified as *S. pneumoniae*.
[c] Isolates not included in the BC-GP panel; includes three isolates of *Micrococcus* species and one isolate each of *Abiotrophia*, *E. coli*, *A. baumannii* complex, *Leclercia* species, and *K. pneumoniae* that were mixed with Gram-positive organisms.
[d] Total number of organisms on the BC-GP panel isolated from 104 BacT/Alert SA bottles.
[e] Total number of organisms on the BC-GP panel isolated from 99 BacT/Alert PF bottles.

teria were compared in 4-month periods for a conventional workup (preimplementation, February to May 2012) and for Verigene BC-GP (postimplementation, February to May 2013). In addition, the turnaround times for susceptibility reporting for *S. aureus*, *S. epidermidis*, *E. faecalis*, and *E. faecium* were compared to the TAT for detecting resistance determinants during the same time periods. The results from the Verigene BC-GP assay were compared to those of routine methods, and a paired *t* test was used to calculate the statistical significant difference in turnaround time between the Verigene BC-GP and conventional methods for identification and resistance detection.

In this study, the BC-GP assay had an overall concordance rate of 95.8% (206/215) for identification of organisms on the panel. Of the 203 bottles tested, 92.1% (187/203) grew one organism and 7.9% (16/203) grew >1 organisms. The list of targets included in the BC-GP assay covered 96.4% (215/223) of all isolates recovered in the study. Organisms not included in the panel were reported as "not detected" by the system (Table 1). A total of 209 (97.2%) of the 215 isolates detected by BC-GP were correctly identified, except for 5 *Streptococcus mitis/oralis* isolates misidentified as *S. pneumoniae* and 1 *S. epidermidis* isolate misidentified as *Staphylococcus* spp. In addition, the BC-GP assay failed to detect the presence of *E. faecalis* and *Streptococcus* spp. in two individual BacT/Alert PF bottles. The *E. faecalis* isolate was present in a mixed culture with *Escherichia coli*, while the *Streptococcus* species isolate was isolated from a monomicrobial blood culture bottle that was found to be a slow-growing streptococcus.

Throughout the study period, only 3.0% (6/203; 3 SA, 3PF) of blood cultures required repeat testing due to invalid results related to the internal processing control and all but 1 bottle were correctly identified as *E. faecalis*, *S. pyogenes*, *Streptococcus* spp., *S. aureus*, and *Staphylococcus* spp. after initial repeat, resulting in an overall accurate identification rate of 97.0%. The final PF bottle, identified as *S. pyogenes* by routine laboratory methods, continued to yield "no call" results by BC-GP.

The ability of the BC-GP assay to detect the presence of the *mecA* gene in *S. aureus* and *S. epidermidis* and the *vanA* and *vanB* genes in *E. faecium* and *E. faecalis* was also determined (Table 1). The BC-GP assay is highly accurate, with correct detection of the *mecA* gene in 100% of MRSA and 98.0% of methicillin-resistant *S. epidermidis* (MRSE) isolates. One *S. epidermidis* isolate with *mecA* detection by BC-GP assay was confirmed to be cefoxitin screen negative and *mecA* PCR negative, and 1 isolate with no *mecA* detection was confirmed to be cefoxitin screen positive and *mecA* PCR positive. The BC-GP assay also correctly identified 100% of vancomycin-resistant *E. faecium* isolates. No *E. faecalis* isolates were found to be vancomycin resistant during the course of this study. Our group previously evaluated the BC-GP assay on clinical and seeded specimens and confirmed *vanA* and *vanB* detection in vancomycin-resistant *E. faecalis* isolates with 100% concordance (16). Buchan et al. reported similar findings, where detection of *vanA* and *vanB* in *E. faecium* or *E. faecalis* isolates was 99.5% sensitive and 97% specific (12).

Polymicrobial bacteremias account for 5 to 20% of all bloodstream infections, with most cases linked to patients with underlying conditions (17–20). Similarly, 7.9% (16/203) of all positive blood cultures collected from pediatric patients ranging from 5 months to 16 years of age in this study were polymicrobial, with

TABLE 2 Agreement between the BC-GP assay and standard laboratory methods for polymicrobial blood cultures

BacT/Alert bottle type	Organism	BC-GP result	Agreement
SA	*S. epidermidis* (oxacillin resistant) *E. faecium* (vancomycin susceptible)	*Staphylococcus* spp. *S. epidermidis* *E. faecium* *mecA*[a]	Yes
SA	*Streptococcus* spp. *S. epidermidis* *S. hominis*	*Streptococcus* spp. *Staphylococcus* spp. *S. epidermidis*	Yes
SA	*S. aureus* (oxacillin susceptible) *E. faecalis* (vancomycin susceptible) *A. baumannii* complex[b] *Leclercia* spp.[b]	*Staphylococcus* spp. *S. aureus* *E. faecalis*	Yes
SA	*E. faecium* (vancomycin susceptible) *S. mitis/oralis*	*E. faecium* *Streptococcus* spp.	Yes
SA	*E. faecium* (vancomycin susceptible) *S. mitis/oralis*	*E. faecium* *Streptococcus* spp. *S. pneumoniae*	No[c]
SA	*E. faecium* (vancomycin susceptible) *S. mitis/oralis*	*E. faecium* *Streptococcus* spp.	Yes
SA	*E. faecium* (vancomycin susceptible) *S. mitis/oralis*	*E. faecium* *Streptococcus* spp.	Yes
PF	*S. aureus* (oxacillin susceptible) *K. pneumoniae*[a]	*Staphylococcus* spp. *S. aureus*	Yes
PF	*S. lugdunensis* (oxacillin resistant) *S. epidermidis* (oxacillin resistant)	*Staphylococcus* spp. *S. lugdunensis* *S. epidermidis* *mecA*[a]	Yes
PF	*S. epidermidis* (oxacillin susceptible) *S. hominis/warneri* (oxacillin susceptible)	*Staphylococcus* spp. *S. epidermidis* *mecA*[a]	No
PF	*S. epidermidis* (oxacillin resistant) *S. epidermidis* (oxacillin resistant)	*Staphylococcus* spp. *S. epidermidis* *mecA*[a]	Yes
PF	*S. aureus* (oxacillin susceptible) Coagulase-negative staphylococci[d]	*Staphylococcus* spp. *S. aureus*	Yes
PF	*E. faecalis* (vancomycin susceptible) *E. coli*	Not detected Not detected	No
PF	*S. epidermidis* (oxacillin resistant) *S. epidermidis* (oxacillin resistant)	*Staphylococcus* spp. *S. epidermidis* *mecA*[a]	Yes
PF	*S. aureus* (oxacillin susceptible) *S. pyogenes*	*Staphylococcus* spp. *S. aureus* *Streptococcus* spp. *S. pyogenes*	Yes
PF	Coagulase negative staphylococci[d] *S. mitis/oralis*	*Staphylococcus* spp. *Streptococcus* spp.	Yes

[a] The *mecA* gene is detected only in *S. aureus* or *S. epidermidis*.
[b] Organism not included in the Verigene BC-GP panel.
[c] Discordant result for *S. pneumoniae* only.
[d] Species identification and susceptibility testing was not performed.

56.3% (9/16) of the mixed cultures from BacT/Alert PF bottles (Table 2). Positive organisms not targeted in the BC-GP panel were correctly reported as "not detected" by the BC-GP assay and were identified by routine laboratory methods. A total of 14/16 polymicrobial blood cultures contained 2 isolates per bottle, and the last 2 bottles grew out 3 and 4 different isolates each. Overall, 13 (81.3%) polymicrobial blood culture bottles were correctly detected by the BC-GP assay; of the three discordant bottles, one growing *Escherichia coli* and *E. faecalis* was reported as "not detected" by the system.

The Verigene BC-GP assay was fully integrated into the 24/7 workflow in the clinical microbiology laboratory at CHLA. Prior to implementation, the average turnaround times for conventional identification and susceptibility reporting of the Gram-positive organisms were 34.2 h (range, 20.2 to 45.8 h) and 41.4 h (range, 28 to 55.3 h), respectively. Implementation of the BC-GP assay yielded an average turnaround time of 4.1 h (range, 2.5 to 15.1 h) for identification and detection of resistance markers. The mean turnaround time decreased by 30.1 h ($P < 0.0001$) for organism identification and 37.3 h ($P < 0.0001$) for detection of resistance markers in *S. aureus*, *S. epidermidis*, *E. faecalis*, and *E. faecium*.

Although traditional laboratory diagnosis of bloodstream infections using culture techniques remains the mainstay in the majority of microbiology laboratories, a shift toward innovative technologies that identify pathogens and resistance markers directly from blood culture is paramount. In our institution, the BC-GP assay proved to be accurate and robust compared to routine laboratory identification methods. A total of 5/6 blood cultures misidentified by the BC-GP assay were attributed to the *S. mitis/S. oralis* isolates incorrectly identified as *S. pneumoniae*, which is expected, as these organisms are well known to have more than 99% sequence homology between them (21). This finding of 45% (5/11) false-positive *S. pneumoniae* isolates is comparable to recent studies that showed misidentification rates for *S. pneumoniae* ranging from 7.7% to 60% (12, 13, 15). The importance of differentiation was emphasized within the laboratory, as viridans group streptococci may be considered contaminants, whereas *S. pneumoniae* is attributed to serious infections. The presence of lancet-shaped Gram-positive cocci typically indicative of *S. pneumoniae* on Gram stain is compulsory prior to preliminary reporting of "*S. pneumoniae* presumptive," followed by confirmation by bile solubility or optochin disk. Thus, only 1 of 5 blood cultures identified as *S. pneumoniae* by BC-GP was incorrectly reported to clinicians as *S. pneumoniae*, and the other 4 isolates were preliminarily reported as "*Streptococcus* spp." and confirmed to be *S. mitis/S. oralis* by conventional methods. Furthermore, using this prudent Gram stain criteria, 5 of 6 blood cultures correctly identified as *S. pneumoniae* by BC-GP assay were also accurately called "*S. pneumoniae* presumptive" in the preliminary report.

Recovery of CoNS from blood culture is not typically considered clinically significant (22–24), and the importance of differentiation from *S. aureus* in the pediatric population was highlighted in our study, as recovery of *S. epidermidis* and other CoNS was predominant at 46.2%. The BC-GP assay was able to correctly identify 99.3% of *S. aureus* and *S. epidermidis* to the species level and other staphylococci to the genus level, providing the potential to mitigate the use of unnecessary antimicrobial agents in our hospital setting. Moreover, correct detection of the *mecA* gene in 98.4% of MRSA and MRSE isolates from our patients can further contribute to the potential optimization of antimicrobial therapy and reduction in accumulated hospital cost (10). The ability of the BC-GP assay to also identify vancomycin-resistant enterococci and vancomycin-susceptible enterococci directly from blood cultures may further drive early targeted deescalation of antimicrobial therapy and optimize patient outcome.

Detection of polymicrobial blood cultures has been deemed challenging and was previously reported as the top reason for undetected results using several approaches, including multiplex PCR and MALDI-TOF MS (25, 26). However, the clinical significance of polymicrobial bacteremias and importance of identifying these cases in pediatric patients were highlighted in a previous study that demonstrated higher rates of polymicrobial bloodstream infections among children <1 year of age than in older children and an increase in overall case fatality rate from 4.1% to 7.4% (20). In this study, the BC-GP assay was able to successfully detect as least one organism from polymicrobial blood cultures in 93.8% of cases and correctly identify all bacterial targets and resistance markers in 81.3% of cases. This highlights the improved performance of the BC-GP assay compared to that of MALDI-TOF MS for accurate identification of organisms from polymicrobial infections, where only 67.7% of polymicrobial blood cultures were identified (26). One limitation of the BC-GP assay is the inability to differentiate between a monomicrobial or polymicrobial infection containing *Staphylococcus* spp. other than *S. aureus*, *S. lugdunensis*, and *S. epidermidis* or *Streptococcus* spp. other than *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, and *S. anginosus* group. Multiple organisms could not be deciphered from single-organism cultures solely from the BC-GP report in 25% of cases.

As expected, implementation of the Verigene BC-GP assay for the detection of Gram-positive organisms and resistance markers greatly improved turnaround time, and our results supported a recent study that reported a range of 30.5 to 41.7 h earlier than routine identification methods (15). Peptide nucleic acid-based fluorescence *in situ* hybridization (PNA-FISH) and MALDI-TOF MS are alternate approaches to rapidly identify bacteria directly from positive blood cultures (7–9, 27, 28). Pitfalls include the inability to detect resistance markers by both methods and increased hands-on time required for the MALDI-TOF method, which is more conducive to batch testing (28). The ease of use and minimal hands-on time required for the BC-GP assay allowed for its full integration into the workflow of the microbiology laboratory at CHLA, contributing to the significant reduction in turnaround time seen since implementation.

This study describes the successful use of the Verigene BC-GP assay for the identification of 12 Gram-positive organisms and 3 respective resistance markers directly from blood cultures obtained from pediatric patients. A dramatic improvement in turnaround time was observed in our institution, particularly with the identification of MRSA and VRE. Future studies are warranted to assess the effect of the BC-GP assay on antimicrobial selections, hospital costs, and patient outcome.

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