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# Evaluation of a Microarray-Based Assay for Rapid Identification of Gram-Positive Organisms and Resistance Markers in Positive Blood Cultures

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**Rapid identification of pathogens directly from positive blood cultures can play a major role in reducing patient mortality rates. We evaluated the performance of the Verigene Gram-Positive Blood Culture (BC-GP) assay (Nanosphere Inc., Northbrook, IL) for detection of commonly isolated Gram-positive organisms as well as associated resistance markers from positive blood cultures. Positive blood cultures (VersaTREK; Trek Diagnostic Systems, Independence, OH) from 203 patients with Gram-positive organism infections were analyzed using the BC-GP assay within 12 h for the detection of 12 different organisms, including staphylococci, streptococci, and enterococci, as well as for the presence of 3 resistance markers (*mecA*, *vanA*, and *vanB*). Results were compared to those of routine laboratory methods for identification and susceptibility testing. For identification of organisms and detection of resistance markers in 178 monomicrobial positive blood cultures, the BC-GP assay showed 94% and 97% concordance, respectively, with routine methods. After 25 polymicrobial cultures were included, the results showed 92% and 96% agreement for identification and resistance markers, respectively, for a total of 203 positive cultures. In 6/25 polymicrobial cultures, at least 1 isolate was not detected. Concordance levels for detection of major pathogens such *Staphylococcus aureus* ($n = 45$) and enterococci ($n = 19$) were 98% and 95%, respectively. Agreement levels for detection of resistance markers such as *mecA* and *vanA/B* were 92% and 100%, respectively. The BC-GP assay is capable of providing rapid identification of Gram-positive cocci as well as detection of resistance markers directly from positive blood cultures at least 24 to 48 h earlier than conventional methods.**

Rapid detection and identification of bloodstream pathogens are crucial to timely therapeutic intervention and patient management. Early administration of appropriate antibiotics improves survival of bacteremic patients (1–3), but improvement depends on rapid identification and susceptibility testing of pathogens from positive blood cultures. This process may take 1 to 3 days, leading to potential delays in administering appropriate therapy. This delay is significant, because each additional day required for definitive identification of pathogens in blood cultures has been shown to increase mortality rates (4). Strategies that reduce the time required for reporting positive blood cultures such as prompt Gram staining and notification processes have been demonstrated to reduce length of hospital stay and mortality (2, 5, 6). The use of molecular technologies for early identification of pathogens and resistance determinants directly from positive blood cultures may be able to reduce the time required for laboratory processes and significantly impact patient management.

Gram-positive organisms are implicated as the primary pathogens in the majority of bacteremic episodes (6). Rapid identification of these isolates directly from positive blood cultures has been shown to improve patient outcomes as well as reduce inappropriate antimicrobial therapy and decrease hospital charges (7–11). Rapid detection of nonpathogens contaminating blood cultures is also valuable. Beekmann et al. demonstrated that whereas coagulase-negative staphylococci (CoNS) represented 22% of all Gram-positive isolates from blood cultures, ~80% of the isolates were subsequently determined to represent contamination (12). Early identification of these isolates can significantly reduce unnecessary antimicrobial usage and decrease length of stay (8). In addition, rapid detection of resistance markers such as *mecA*, which confers methicillin resistance in staphylococci, directly from positive blood cultures can have a similar positive impact on mortality, hospital costs, and length of stay (10, 13).

Early efforts toward rapid detection of pathogens from positive blood cultures involved peptide nucleic acid-based fluorescence *in situ* hybridization (PNA-FISH) (7–9). Other approaches included molecular amplification methods coupled with probe-based detection (10, 13, 14). Matrix-associated laser desorption ionization–time of flight (MALDI-TOF) analysis for identification of bacterial isolates from positive blood cultures is another recent development (15–18). Each methodology has limitations, for example, the inability to detect resistance markers (PNA-FISH and MALDI-TOF) or problems with detecting polymicrobial infections (MALDI-TOF) (16, 17). The use of microarrays in this setting has been previously described and offers the advantages of being able to detect multiple targets and associated resistance markers simultaneously (19–21). Here we evaluate a microarray-based assay, the Verigene Gram-Positive Blood Culture (BC-GP) nucleic acid test (Nanosphere Inc., Northbrook, IL), which is performed on the Verigene system for detection of Gram-positive microorganisms and associated resistance markers in positive aerobic blood culture bottles. The assay is capable of detecting *Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp., *Staphylococcus au-*

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*reus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Enterococcus faecalis*, and *Enterococcus faecium*. In addition, the assay is capable of detecting the presence of *mecA* if *S. aureus* or *S. epidermidis* or both are present and *vanA* and *vanB* (*vanA/B*) if *E. faecium* and *E. faecalis* or both are detected.

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## MATERIALS AND METHODS

**Patient samples.** Clinical samples for this study were blood cultures submitted as part of routine patient care to the Henry Ford Health System (HFHS) Core Microbiology laboratory, which serves the 900-bed Henry Ford Hospital in Detroit, MI, as well as 3 acute care hospitals and 32 medical centers in southeast Michigan. Blood cultures were collected in REDOX EZ Draw 40-ml aerobic and anaerobic culture bottles (Trek Diagnostic Systems, Cleveland, OH) and incubated on a VersaTREK instrument (Trek Diagnostic Systems). The instrument detects organism growth by measuring changes in headspace pressure of the blood culture bottles. Aerobic bottles from nonconsecutive cultures that flagged positive were Gram stained and, if positive for Gram-positive cocci or bacilli, were included in the study. Only one positive blood culture per patient was included in the study. Laboratory staff performing the BC-GP assay were not aware of the organism identification at the time of testing. A total of 203 positive blood cultures containing Gram-positive organisms were included in this study. This study was approved by the Institutional Review Board at HFHS.

**Blood culture processing.** The aerobic bottles from blood cultures selected for inclusion in the study were sampled aseptically in a biosafety cabinet and inoculated into blood agar and MacConkey and chocolate agar for overnight incubation at 37°C and 5% $CO_2$. An additional aliquot of 1.5 ml was removed for study purposes, and 350 μl of the aliquot was utilized for testing on the BC-GP assay within 12 h of the blood culture flagging positive. The remainder was stored at −70°C for additional testing as needed. If a valid result was not obtained in the initial run, an additional aliquot was taken from the blood culture bottle (stored at room temperature) and retested within 24 h of the positive blood culture signal. If the test could not be performed within 24 h, an aliquot was frozen at −70°C for later testing.

Subcultured isolates were identified by the use of a combination of routine identification tests and automated platforms such as the Vitek 2 (bioMérieux, Durham, NC). Susceptibility testing was performed using the Vitek 2, disk diffusion methods (CLSI M100-S22), and E-tests (bioMérieux, Durham, NC). In the case of discordant results, a combination of routine laboratory methods such as Vitek 2 and API (bioMérieux, Durham, NC) and/or bidirectional sequencing was performed at a reference laboratory (Dynacare Laboratories, Milwaukee, WI) as needed for resolution.

**Microarray testing.** The Verigene platform includes the Verigene Processor *SP* and Verigene Reader. The Verigene Processor *SP* carries out extraction of nucleic acid from specimens using magnetic glass beads. Patient samples are loaded into an extraction tray, which is then loaded into the processor along with the utility tray, pipette tip holder assembly, and test cartridge. These items are all single-use disposable components that contain all the reagents required for testing. The Verigene Reader controls the processor and is responsible for specimen tracking, test selection, imaging, and analysis of test cartridges and display of the results.

For testing on the Verigene platform using the BC-GP assay, 350 μl of blood culture media from the positive aerobic bottle is loaded into the extraction tray, which is then placed into the processor *SP* along with all other consumables. The instrument extracts nucleic acid from the sample which is then mixed with the appropriate buffer and transferred to the test cartridge. The target analyte, if present, hybridizes to synthetic gene-specific oligonucleotide capture strands on the test cartridge substrate slide.

**TABLE 1** Distribution of isolates encountered during the study and numbers of isolates correctly identified, misidentified, or not detected

| Organism | Total no. of isolates | No. (%) of isolates correctly identified[c] | No. (%) of isolates not detected | No. (%) of isolates incorrectly identified |
|---|---|---|---|---|
| *Staphylococcus aureus* | 45 | 44 (98) | 1 (2) | |
| MSSA | 14 | 13 (93) | 1 (7) | |
| MRSA | 31 | 31 (100) | | |
| CoNS | 117 | 107 (91) | 10 (9) | |
| *S. epidermidis* | 54 | 47 (87) | 7 (13)[d] | |
| Other CoNS | 61 | 59 (97) | 2 (3) | |
| *S. lugdunensis* | 2 | 1 (50) | 1 (50) | |
| Enterococci | 19 | 18 (95) | 1 (5) | |
| *Enterococcus faecalis* | 15 | 14 (93) | 1 (7) | |
| *Enterococcus faecium* | 4 | 4 (100) | | |
| Streptococci | 26 | 24 (92) | | 2 (8) |
| *Streptococcus pneumoniae* | 6 | 6 (100) | | |
| *Streptococcus pyogenes* | 1 | 1 (100) | | |
| *Streptococcus agalactiae* | 2 | 2 (100) | | |
| *Streptococcus anginosus* group | 1 | 1 (100) | | |
| Other streptococci | 16 | 14 (88) | | 2 (13) |
| Total | 207 | 193 (93) | 12 (6) | 2 (1) |
| Other | | | | |
| Gram-positive bacilli[a] | 13 | | 13 (100) | |
| Other[b] | 5 | | 4 (80) | 1 (20) |
| *Granulicatella* spp. | 2 | | 2 (100) | |
| Total isolates | 227 | | | 3 (1) |

[a] Includes *Bacillus* spp., *Corynebacterium* spp., etc.
[b] Includes one isolate each of *E. casseliflavus*, *Dermacoccus* spp., and *Leuconostoc* spp. and two isolates of *Rothia* spp.
[c] Correct identification to the species level.
[d] A total of 4 of 7 isolates of *S. epidermidis* were correctly identified to the genus level but not to the species level.

Another synthetic mediator target-specific nucleotide is introduced to form a hybridization sandwich with the gene of interest. At this point, a gold nanoparticle-labeled probe is introduced with oligonucleotides complementary to the intermediate oligonucleotide bound to the gene of interest. Finally, the gold nanoparticles are coated with silver to enhance the optical signal. The test cartridge is then removed from the Processor *SP*, and the substrate slide is inserted into the Verigene Reader for analysis. The Verigene Reader projects white light across the substrate slide, detects the relative brightness of each spot due to gold nanoparticles bound to target-specific probes and provides a "Detected" or "Not Detected" result for each of the panel members.

**Statistical analysis.** Results of routine laboratory testing were not available to laboratory testing personnel at the time of BC-GP testing. Final discrepant result analysis was also not made available until the conclusion of sample testing. Concordance was determined in comparison to results of local laboratory methods, with discrepancies resolved by testing at a reference laboratory using either routine laboratory methods or bidirectional sequencing as described previously.

## RESULTS

A total of 203 positive blood cultures containing 227 Gram-positive organisms were tested using the BC-GP assay on the Verigene system. Over 12% (25/203) of positive samples yielded two or more organisms during routine laboratory culture. The distribution of microorganisms that were isolated from the positive blood cultures is shown in Table 1. At least 91% (207/227) of the isolates were members of the panel of targets on the BC-GP assay. Staph-

**TABLE 2** Performance of the BC-GP assay for detection of resistance markers in positive blood cultures

| Isolate category | Total no. of isolates | No. (%) of isolates with gene detected | No. (%) of isolates with gene not detected |
|---|---|---|---|
| *mecA* positive | | | |
| *S. aureus* | 31 | 30 (97) | 1 (3) |
| *S. epidermidis* | 42 | 37 (88) | 5 (12) |
| Total *mecA* positive | 73 | 67 (92) | 6 (8) |
| Total *vanA*/*B* positive | 9 | 9 (100) | 0 (0) |
| Total | 82 | 76 (93) | 76 (7) |

**TABLE 3** Agreement between the BC-GP assay and reference methods for identification of positive blood cultures

| Blood culture category and parameter | Total no. of cultures | No. (%) of cultures with concordant results | No. (%) of cultures with discrepant results |
|---|---|---|---|
| Monomicrobial | 178 | | |
| Identification | 178 | 168 (94) | 10 (6) |
| Resistance markers | 178 | 173 (97) | 5 (3) |
| Polymicrobial | 25 | | |
| Identification | 25 | 19 (76) | 6 (24) |
| Resistance markers | 25 | 21 (84) | 4 (16) |
| Total agreement | | | |
| Identification | 203 | 187 (92) | 16 (8) |
| Resistance markers | 203 | 194 (96) | 9 (4) |

ylococci, enterococci, and streptococci represented 72% (162/227), 8% (19/227), and 11% (26/227) of total isolates, respectively. No *Listeria* spp. were detected during the course of this study.

*S. aureus* represented 28% (45/162) of the staphylococcal isolates detected. The BC-GP assay accurately detected 98% (44/45) of these isolates (Table 1). The remaining staphylococcal isolates were *S. epidermidis* isolates (54/117), *S. lugdunensis* isolates (2/117), or other CoNS isolates (61/117). The BC-GP assay correctly identified 87% (47/54) of the *S. epidermidis* isolates. Of the remaining 7 isolates, 4/7 were correctly identified as being staphylococci but were not identified to species level and 3/7 isolates were not detected (Table 1). Only 2 isolates of *S. lugdunensis* were encountered during the course of the study, and 1/2 was present in a polymicrobial culture and was not detected. The remaining CoNS isolates were identified to the genus level, with only 3% (2/61) of isolates not detected (Table 1).

A total of 19 enterococcal isolates were detected using the BC-GP assay, including 4/19 isolates of *E. faecium* and 15/19 isolates of *E. faecalis* (Table 1). At least 95% (18/19) of the enterococcal isolates were captured by the BC-GP assay, with the sole exception being an *E. faecalis* isolate that was present in a mixed culture with *Escherichia coli*. A total of 26 streptococcal isolates were also encountered during the course of the study, and 92% (24/26) were detected and accurately identified to either the genus or species level (Table 1). The remaining isolates (2/26) were identified by the reference laboratory as *Streptococcus oralis* and *Streptococcus mitis* but were misidentified by the BC-GP assay as *S. anginosus* group and *S. pneumoniae*, respectively. Other streptococcal isolates that were correctly identified included *S. pneumoniae* (6/6), *S. pyogenes* (1/1), *S. agalactiae* (2/2), and *S. anginosus* group (1/1). The positive blood cultures tested using the BC-GP assay included 20 Gram-positive isolates that were not part of the bacterial panel of targets in the assay. These isolates included Gram-positive bacilli ($n = 13$), *Granulicatella* spp. ($n = 2$), *Rothia* spp. ($n = 2$), and one each of *Dermacoccus nishinomiyaensis*, *Enterococcus casseliflavus*, and *Leuconostoc mesenteroides* subsp. *cremoris* (Table 1). None of these isolates were detected by the BC-GP assay, as anticipated, with the exception of the *Leuconostoc* isolate, which was misidentified as *S. epidermidis*.

The BC-GP assay was also evaluated for its ability to detect the presence of the resistance markers *mecA* and *vanA*/*B*. The presence of *mecA* was confirmed by routine laboratory methods in 69% (31/45) and 78% (42/54) of isolates of *S. aureus* and *S. epidermidis*, respectively (Table 2). The BC-GP assay detected the presence of *mecA* in 97% (30/31) and 88% (37/42) of blood cultures positive for *S. aureus* and *S. epidermidis*, respectively (Table 2). The BC-GP assay also detected the presence of *vanA*/*B* in 100% (9/9) of blood cultures positive for enterococcal isolates that were later determined to be vancomycin resistant by routine laboratory methods (Table 2).

The BC-GP assay showed overall concordance of 93% (193/207) for detection and identification of Gram-positive bacterial isolates in positive blood cultures (Table 1). Agreement was better with monomicrobial blood cultures (94%, 168/178) than with polymicrobial blood cultures (76%, 19/25) (Table 3). The BC-GP assay detected 93% (75/81) of resistance markers (*mecA* and *vanA*/*B*) in positive blood cultures with *S. aureus*, *S. epidermidis*, or *E. faecium* or *E. faecalis* (Table 2). Concordance levels for detection of resistance markers in monomicrobial and polymicrobial cultures were 97% and 84%, respectively.

## DISCUSSION

The use of microarray- or DNA probe-based assays for the detection of bacterial pathogens and resistance markers in positive blood cultures has been previously described (19–23). We evaluated the ability of the BC-GP assay to detect 12 different Gram-positive bacterial targets and 3 resistance markers in 203 positive aerobic REDOX 40-ml blood culture bottles. The BC-GP assay is run on the Verigene platform, which is comprised of one or more processor units linked to a reader unit that also serves as the user interface. The testing is cartridge based, making it more amenable to random-access testing rather than batched processing. Each unit can run one sample in 2.5 h, with hands-on time of 5 to 10 min. Timely use of the BC-GP assay on positive blood cultures for identification of blood culture isolates and detection of resistance markers offers a potential time savings of 1 to 3 days over routine laboratory methods. The assay showed overall agreement of 93% and 93% for the detection and identification of Gram-positive bacterial isolates and associated resistance markers, respectively, compared to reference methods.

The ability to identify potential contaminants such as CoNS directly from blood cultures offers the potential advantages of de-escalation of therapy (8). The BC-GP assay was able to identify 91% (107/117) of CoNS isolates to either the species or genus level. Of the 10 discrepant results in this group, 4/10 were *S. epidermidis* isolates that were correctly identified as staphylococci but

were not identified to the species level. The assay performed well with regard to detection of *S. aureus*, with 98% (44/45) of isolates correctly identified (Table 1). The performance of the BC-GP assay for detection of *mecA* was better in *S. aureus* than in *S. epidermidis* (97% versus 88%) (Table 2). This may be accounted for by the fact that *S. epidermidis* was more likely to be present in polymicrobial positive cultures than *S. aureus*. Three of seven cultures with *S. epidermidis* and discrepant *mecA* results were polymicrobial. In addition, 3 positive cultures containing *S. epidermidis* were falsely positive for *mecA* using the BC-GP assay. A potential limitation of the assay is that in mixed cultures containing both *S. aureus* and *S. epidermidis*, the *mecA* gene target, if positive, cannot be assigned specifically to isolates of either organism.

The assay performed well for the detection of *E. faecium* and *E. faecalis* and *vanA*/*B*. Only 1 isolate of *E. faecalis* in a polymicrobial positive culture was not detected, and 9/9 *vanA*/*B*-positive cultures were detected (Table 2). Another limitation of the assay is that it does not detect enterococci other than *E. faecium* or *E. faecalis*, as was evidenced in a positive blood culture that contained *E. casseliflavus*. The assay also accurately identified 24/26 streptococcal isolates, with the most notable exception being an *S. mitis* isolate that was misidentified as *S. pneumoniae*. The difficulty in distinguishing between these two species has been noted with other rapid blood culture identification methods such as MALDI-TOF analysis (16, 17) and is another potential limitation, although in this study, 6/6 *S. pneumoniae* isolates were accurately identified (Table 1).

Other rapid methods for detection of pathogens in positive blood cultures such as MALDI-TOF analysis perform better with Gram-negative than Gram-positive isolates; they show significant variability in performance, with percentages of isolates accurately detected and identified ranging from 37% to 86.3% depending on the protocol (16–18). Similar issues have been associated with DNA probe-based assays that target multiple pathogens, with discordant results ranging from 11% to 21% for Gram-positive cocci (22, 23). The microarray-based Prove-it sepsis assay (Mobidiag Ltd., Helsinki, Finland) was able to detect 93% (123/133) of CoNS isolates from positive blood cultures, but an additional 24 CoNS isolates were isolated by the reference method that were not part of the sepsis panel targets (21). The BC-GP assay was able to correctly identify 93% (193/207) of isolates for which targets were available on the panel (Table 1). Another 7 isolates were correctly identified to the genus level (data not shown), and only 1% (3/227) of the total isolates were misidentified (Table 1).

Another limitation of the BC-GP assay relates to its performance with polymicrobial blood cultures. The assay correctly identified all isolates in 76% (19/25) of the polymicrobial blood cultures and at least one isolate in 83% (5/6) of the remaining polymicrobial cultures (Table 3). In addition, 4/9 of the discrepancies relating to detection of resistance markers were in polymicrobial blood cultures (Table 3). The issues that hinder accurate identification of all isolates in positive polymicrobial blood cultures are not, however, limited to this assay. A study by Kok et al., using MALDI-TOF analysis for direct identification of polymicrobial blood cultures, yielded no isolates detected in 32% of cultures and only 1 isolate detected in the remaining cultures (16). Similar studies by Schubert et al. and La Scola and Raoult using probe-based methods yielded a single organism identification in 25/27 and 18/22 positive polymicrobial blood cultures, respectively, with no organisms detected in the remaining cultures (17, 18). The microarray-based Prove-it sepsis assay failed to identify any bacteria in 25% of polymicrobial blood cultures and did not detect all isolates in almost 50% of these cultures (21). The majority of these discrepancies were related to the presence of Gram-positive cocci, including *S. aureus* and enterococci (21). While polymicrobial blood cultures may represent contamination, the potential for significant mortality, particularly in the presence of known pathogens such as *S. aureus*, cannot be dismissed (24). In order for physicians to adopt and act upon results generated by these technologies, performance with regard to polymicrobial blood cultures needs to show improvement. Still, the BC-GP assay performed favorably compared to other rapid identification assays for detection of isolates involved in polymicrobial bacteremia.

A limitation of this study is that the majority of isolates (72%) were staphylococci and the remaining BC-GP panel targets were underrepresented in the final tally (Table 1). In addition, only aerobic bottles were tested, although the manufacturer's package insert indicates that anaerobic bottles demonstrate adequate analytic performance. In comparison to DNA amplification- and probe-based assays, the BC-GP assay appears to have the advantage of ease of use due to automation, with minimal hands-on time. This convenience, along with the random-access nature of the platform, reduces the need for batch processing and thus facilitates the rapid identification of positive blood cultures with Gram-positive organisms within 2.5 h from the time of positive blood culture. In addition, the assay performed better than its peers in detection of Gram-positive cocci and resistance markers, including those in polymicrobial positive cultures, although the need for improvement remains. Unlike some of the comparator methods, including MALDI-TOF analysis, the BC-GP assay is dependent on initially obtaining the Gram stain and the results are limited to Gram-positive organisms and resistance markers present on the panel. The MALDI-TOF approach, however, does not currently have a rapid and reliable means for detection of resistance markers from positive blood cultures. In conclusion, the BC-GP assay represents a useful tool for the rapid and accurate detection of Gram-positive pathogens and resistance markers in positive blood cultures.

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## REFERENCES

1. **Leibovici L, Shraga I, Drucker M, Konigsberger H, Samra Z, Pitlik SD.** 1998. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. J. Intern. Med. **244**:379–386.
2. **Cunney RJ, McNamara EB, Alansari N, Loo B, Smyth EG.** 1997. The impact of blood culture reporting and clinical liaison on the empiric treatment of bacteraemia. J. Clin. Pathol. **50**:1010–1012.
3. **Gaieski DF, Mikkelsen ME, Band RA, Pines JM, Massone R, Furia FF, Shofer FS, Goyal M.** 2010. Impact of time to antibiotics on survival in patients with severe sepsis or septic shock in whom early goal-directed therapy was initiated in the emergency department. Crit. Care Med. **38**: 1045–1053.
4. **Bouza E, Sousa D, Munoz P, Rodriguez-Creixems M, Fron C, Lechuz JG.** 2004. Bloodstream infections: a trial of the impact of different methods of reporting positive blood culture results. Clin. Infect. Dis. **39**:1161–1169.
5. **Barenfanger J, Graham DR, Kolluri L, Sangwan G, Lawhorn J, Drake CA, Verhulst SJ, Peterson R, Moja LB, Ertmoed MM, Moja AB, Shevlin DW, Vautrain R, Callahan CD.** 2008. Decreased mortality associated

with prompt Gram staining of blood cultures. Am. J. Clin. Pathol. **130:** 870–876.
6. **Beekmann SE, Diekema DJ, Chapin KC, Doern GV.** 2003. Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. J. Clin. Microbiol. **41**:3119–3125.
7. **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.
8. **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.
9. **Forrest GN, Roghmann MC, Toombs LS, Johnson JK, Weekes E, Lincalis DP, Venezia RA.** 2008. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. Antimicrob. Agents Chemother. **52**:3558–3563.
10. **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.
11. **Nguyen DT, Yeh E, Perry S, Luo RF, Pinsky BA, Lee BP, Sisodiya D, Baron EJ, Banaei N.** 2010. Real-time PCR testing for mecA reduces vancomycin usage and length of hospitalization for patients infected with methicillin-sensitive staphylococci. J. Clin. Microbiol. **48**:785–790.
12. **Beekmann SE, Diekema DJ, Doern GV.** 2005. Determining the clinical significance of coagulase-negative staphylococci isolated from blood cultures. Infect. Control Hosp. Epidemiol. **26**:559–566.
13. **Brown J, Paladino JA.** 2010. Impact of rapid methicillin-resistant Staphylococcus aureus polymerase chain reaction testing on mortality and cost effectiveness in hospitalized patients with bacteraemia: a decision model. Pharmacoeconomics **28**:567–575.
14. **Pasko C, Hicke B, Dunn J, Jaeckel H, Nieuwlandt D, Weed D, Woodruff E, Zheng X, Jenison R.** 2012. Staph ID/R: a rapid method for determining staphylococcus species identity and detecting the mecA gene directly from positive blood culture. J. Clin. Microbiol. **50**:810–817.
15. **Tan KE, Ellis BC, Lee R, Stamper PD, Zhang SX, Carroll KC.** 2012. Prospective evaluation of a matrix-assisted laser desorption ionization–time of flight mass spectrometry system in a hospital clinical microbiology laboratory for identification of bacteria and yeasts: a bench-by-bench study for assessing the impact on time to identification and cost-effectiveness. J. Clin. Microbiol. **50**:3301–3308.
16. **Kok J, Thomas LC, Olma T, Chen SC, Iredell JR.** 2011. Identification of bacteria in blood culture broths using matrix-assisted laser desorption-ionization Sepsityper and time of flight mass spectrometry. PLoS One **6**:e23285. doi:10.1371/journal.pone.0023285.
17. **La Scola B, Raoult D.** 2009. Direct identification of bacteria in positive blood culture bottles by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. PLoS One **4**:e8041. doi:10.1371/journal.pone.0008041.
18. **Schubert S, Weinert K, Wagner C, Gunzl B, Wieser A, Maier T, Kostrzewa M.** 2011. Novel, improved sample preparation for rapid, direct identification from positive blood cultures using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. J. Mol. Diagn. **13**:701–706.
19. **Cleven BE, Palka-Santini M, Gielen J, Meembor S, Kronke M, Krut O.** 2006. Identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. J. Clin. Microbiol. **44**:2389–2397.
20. **Fishbain JT, Sinyavskiy O, Riederer K, Hujer AM, Bonomo RA.** 2012. Detection of extended-spectrum beta-lactamase and Klebsiella pneumoniae carbapenemase genes directly from blood cultures by use of a nucleic acid microarray. J. Clin. Microbiol. **50**:2901–2904.
21. **Tissari P, Zumla A, Tarkka E, Mero S, Savolainen L, Vaara M, Aittakorpi A, Laakso S, Lindfors M, Piiparinen H, Maki M, Carder C, Huggett J, Gant V.** 2010. Accurate and rapid identification of bacterial species from positive blood cultures with a DNA-based microarray platform: an observational study. Lancet **375**:224–230.
22. **Karumaa S, Karpanoja P, Sarkkinen H.** 2012. PCR identification of bacteria in blood culture does not fit the daily workflow of a routine microbiology laboratory. J. Clin. Microbiol. **50**:1031–1033.
23. **Steindor M, Weizenegger M, Harrison N, Hirschl AM, Schweickert B, Gobel UB, Mackenzie CR.** 2012. Use of a commercial PCR-based line blot method for identification of bacterial pathogens and the mecA and van genes from BacTAlert blood culture bottles. J. Clin. Microbiol. **50**:157–159.
24. **Park SY, Park KH, Bang KM, Chong YP, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH, Kim YS.** 2012. Clinical significance and outcome of polymicrobial Staphylococcus aureus bacteremia. J. Infect. **65**:119–127.