Emerging Technologies for Rapid Identification of Bloodstream Pathogens

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Technologies for rapid microbial identification are poised to revolutionize clinical microbiology and enable informed decision making for patients with life-threatening bloodstream infections. Species identification of microorganisms in positive blood cultures can be performed in minutes using commercial fluorescence in situ hybridization tests or mass spectroscopy. Microorganisms in positive blood cultures can also be identified within 1–2.5 hours using automated polymerase chain reaction–based systems that can also detect selected antibiotic resistance markers, such as methicillin resistance. When combined with antibiotic stewardship programs, these approaches improve clinical outcomes and reduce healthcare expenditures. Tests for direct detection in whole blood samples are highly desirable because of their potential to identify bloodstream pathogens without waiting 1-2 days for blood cultures to become positive. However, results for pathogen detection in whole blood do not overlap with those of conventional blood culture techniques and we are still learning how best to use these approaches.

Keywords. bloodstream infections; rapid diagnostics; molecular diagnostics; clinical microbiology; antimicrobial stewardship.

Antimicrobial therapy is a critical determinant of clinical outcome for patients with bloodstream infections (BSIs). For example, there is a mean decrease in survival of 7.6% for each hour after the onset of hypotension until initiation of effective antibiotics [1]. Use of inappropriate antibiotics within the first 6 hours after recognition of septic shock is associated with a 5-fold higher mortality risk [2]. To address this problem, initial therapy is typically broad spectrum to ensure that all potential pathogens are addressed. For this reason, rapid microbial identification methods are urgently needed to empower antibiotic stewardship programs with the information needed to deescalate antibiotic therapy. Timely and accurate reporting of microbiologic data from positive blood cultures improves clinical outcomes and reduces healthcare costs [3–5].

Pathogen identification methods have been developed to dramatically shorten the time from sample acquisition to microbiologic diagnosis of BSI, which frequently takes 48–72 hours using conventional methods. Technologies for rapid identification of bloodstream pathogens can be separated into 2 categories based on sample type: either positive blood cultures or blood samples. When combined with antimicrobial stewardship programs, the former have been shown to enable earlier implementation of targeted antimicrobial therapy and have been associated with reduced overall and sepsis-related mortality. Although the latter have not yet been approved by the Food and Drug Administration (FDA) for commercial use, they offer the potential advantage of same-day pathogen identification.

PATHOGEN IDENTIFICATION FROM POSITIVE BLOOD CULTURES

Several diagnostic systems have been developed for rapid identification of organisms found in positive blood cultures and provide results more quickly than...
conventional methods (Table 1). The FDA cleared assays discussed in this review rapidly identify organisms growing in positive blood cultures but do not eliminate the required time for the growth in the blood culture. These assays are gradually becoming less labor intensive and easier to integrate into clinical microbiology laboratory workflow, with the goal of decreasing turnaround times for pathogen identification without significantly increasing costs or laboratory personnel.

**Peptide Nucleic Acid Fluorescent In Situ Hybridization Molecular Stains**

Peptide nucleic acid (PNA) fluorescent in situ hybridization (FISH) stains (AdvanDx) have been commercially available for over 10 years for direct identification of selected pathogens from positive blood cultures. PNA-FISH methods target species-specific ribosomal RNA (rRNA), which are abundant in growing bacteria and yeast found in broth from positive blood cultures [6, 7]. PNA-FISH kits are currently available to differentiate between *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (CoNS); *Enterococcus faecalis* and *Enterococcus avulcanis*; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida parapsilosis*, *Candida glabrata*, *Candida krusei*, and *Candida tropicalis*.

### Table 1. Tests for Rapid Identification of Bloodstream Pathogens

<table>
<thead>
<tr>
<th>Blood Culture Assay</th>
<th>Pathogens Detected</th>
<th>Resistance Markers</th>
<th>Turnaround Time (After Blood Cultures Turn Positive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>QuickFISH</td>
<td><em>S. aureus</em>, CoNS, <em>E. faecalis</em>, other enterococci, <em>E. coli</em>, <em>K. pneumoniae</em>, <em>P. aeruginosa</em></td>
<td>No</td>
<td>&lt;30 min</td>
</tr>
<tr>
<td>MALDI-TOF&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Gram-positive and gram-negative bacteria, yeast, fungi, filamentous fungi, mycobacteria</td>
<td>In development</td>
<td>10–30 min</td>
</tr>
<tr>
<td>Gene Xpert MRSA/SA</td>
<td><em>S. aureus</em></td>
<td>mecA</td>
<td>&lt;1 h</td>
</tr>
<tr>
<td>Verigene gram-positive blood culture (BC-GP)</td>
<td><em>S. aureus</em>, <em>Staphylococcus epidermidis</em>, <em>Staphylococcus lugdunensis</em>, <em>Streptococcus anginosus group</em>, <em>Streptococcus agalactiae</em>, <em>Streptococcus pneumoniae</em>, <em>Streptococcus pyogenes</em>, <em>E. faecalis</em>, <em>Enterococcus faecium</em>, <em>Staphylococcus spp.</em>, <em>Streptococcus spp.</em></td>
<td>mecA, vanA, vanB</td>
<td>2.5 h</td>
</tr>
<tr>
<td>Verigene gram-negative blood culture (BC-GN)&lt;sup&gt;b&lt;/sup&gt;</td>
<td><em>Escherichia coli</em>, <em>Shigella spp.</em>, <em>K. pneumoniae</em>, <em>Klebsiella oxytoca</em>, <em>P. aeruginosa</em>, <em>Serratia marcescens</em>, <em>Acinetobacter spp.</em>, <em>Proteus spp.</em>, <em>Citrobacter spp.</em>, <em>Enterobacter spp.</em></td>
<td>KPC, NDM, CTX-M, VIM, IMP, OXA</td>
<td>2 h</td>
</tr>
<tr>
<td>Whole blood assays</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light Cycler&lt;sup&gt;c&lt;/sup&gt; SeptiFast</td>
<td><em>S. aureus</em>, CoNS, <em>Streptococcus spp.</em>, <em>S. pneumoniae</em>, <em>E. faecalis</em>, <em>E. faecium</em>, <em>E. coli</em>, <em>K. pneumoniae</em>, <em>K. oxytoca</em>, <em>P. aeruginosa</em>, <em>Enterobacter cloacae/aerogenes</em>, <em>S. marcescens</em>, <em>A. baumanii</em>, <em>Proteus mirabilis</em>, <em>Stenotrophomonas maltophilia</em>, <em>C. albicans</em>, <em>C. glabrata</em>, <em>C. krusei</em>, <em>C. parapsilosis</em>, <em>C. tropicalis</em>, <em>Aspergillus fumigatus</em></td>
<td>No</td>
<td>6 h</td>
</tr>
<tr>
<td>SeptiTest&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&gt;300 pathogens</td>
<td>No</td>
<td>8–12 h</td>
</tr>
<tr>
<td>T2 Candida&lt;sup&gt;d&lt;/sup&gt;</td>
<td><em>C. albicans</em>, <em>C. glabrata</em>, <em>C. krusei</em>, <em>C. parapsilosis</em>, <em>C. tropicalis</em></td>
<td>No</td>
<td>3 h</td>
</tr>
</tbody>
</table>

Abbreviations: CoNS, coagulase-negative staphylococci; FDA, Food and Drug Administration; KPC, *K. pneumoniae* carbapenemase; MALDI-TOF, matrix-assisted laser desorption-ionization/time-of-flight mass spectroscopy; MRSA, methicillin-resistant *S. aureus*; NDM, New Delhi metallo-beta-lactamase; PNA-FISH, peptide nucleic acid fluorescent in situ hybridization.

<sup>a</sup> Biomerieux instrument approved by the FDA, Bruker instrument pending approval.

<sup>b</sup> Pending Food and Drug Administration approval.

<sup>c</sup> Not available in the United States.

<sup>d</sup> Research use only.
The QuickFISH assay has excellent sensitivity and specificity results, similar to those seen with the original PNA-FISH assays.

Mass Spectroscopy
Matrix-assisted laser desorption-ionization/time-of-flight (MALDI-TOF) mass spectroscopy is a relatively new approach to microbial identification [9]. Acquisition of these instruments may be cost prohibitive for smaller laboratories, but ongoing performance costs are quite low. Colonies from an agar plate are spotted onto a target plate, a drop of matrix solution is added and dried, and then the target is loaded into the specimen ionization chamber, where the sample-matrix mixture is pulsed with a laser. Ionized particles produced by the laser pulse differ by size, which determines the time required to travel to the mass analyzer. The distribution of particle sizes, or mass spectrum, is unique to each organism and can be identified by comparison with a library of standard reference spectra [7]. The system allocates a numerical score to each identification, with a log score $\geq 2.0$ indicating acceptable identification to the species level and a log score $> 1.7$ but $< 2.0$ identification to the genus level [10]. Results are typically available within 10–30 minutes, depending on the number of samples being tested. MALDI-TOF is also being examined as a method to identify bacterial virulence factors and antibiotic resistance markers, but this work is still in the developmental stage [11].

Typically, MALDI-TOF is applied to organisms isolated as single colonies on an agar plate. However, several studies have examined MALDI-TOF analysis of direct positive blood cultures. A washing/centrifugation sample preparation protocol is required to purify the bacteria from erythrocytes and other blood culture components. This sample preparation method involves differential centrifugation, followed by erythrocyte lysis and a subsequent washing step [10]. Identification can be achieved in approximately 75% of the positive blood cultures, but mixed cultures currently present a problem for this technique. Use of this protocol allows identification within approximately 1 hour, a major advantage compared with waiting for overnight growth of bacteria.

Polymerase Chain Reaction–Based Detection Systems
*Staphylococcus aureus* is a cause of 20% of nosocomial BSI in the United States [12]. Because >60% of all *S. aureus* catheter-associated BSI are caused by methicillin-resistant *S. aureus* (MRSA) [13], empiric therapy with vancomycin is typically prescribed for patients suspected of having *S. aureus* bacteremia. However, several studies have shown that $\beta$-lactam antibiotics are superior to vancomycin in the treatment of methicillin-susceptible *S. aureus* (MSSA) bacteremia [14–16]. Therefore, rapid determination of $\beta$-lactam antibiotic susceptibility of *S. aureus* isolates in positive blood culture would be likely to improve both treatment and clinical outcomes in such patients.

The Xpert MRSA/SA (Cepheid) assay is a rapid, automated polymerase chain reaction (PCR) test for *S. aureus* and methicillin resistance in positive blood cultures containing gram-positive cocci in clusters. This test is a novel multiplex real-time assay for 2 genes: the staphylococcal protein A (spa) gene, which is broadly conserved among and specific for *S. aureus*; and the mecA gene which encodes the penicillin-binding protein 2a protein that is resistant to $\beta$-lactam antibiotics. The MRSA/SA test has 98.3%–100% sensitivity and 98.6%–99.4% specificity for MSSA and MRSA identification [17] and can be completed on the GeneXpert platform (Cepheid) in <1 hour.

Multiplex Detection of Pathogens With Resistance Determinants
The Verigene gram-positive blood culture test (BC-GP; Nanosphere) is a novel microarray test for identification of 12 gram-positive bacterial organisms and 3 genetic markers of antibiotic resistance directly from positive blood culture medium [18]. This test can identify *Staphylococcus* spp., *S. aureus*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Streptococcus* spp., *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus anginosus* group, *Streptococcus pneumoniae*, *E. faecalis*, *Enterococcus faecium*, and *Listeria* spp. The Verigene test can also identify the mecA, vanA/B genes, markers for methicillin and vancomycin resistance, respectively. Sensitivity and specificity for the 12 genus- or species-specific targets ranged between 92.6%–100% and 95.4%–100% [18]. Sensitivity for resistance markers ranged from 98.6% to 100%, with a specificity of 94.3%–100% [18]. The test involves an automated sample processing step (nucleic acid extraction and array hybridization) in the Verigene processor followed by a microarray reading in the Verigene Reader. The total time taken for the test is 2.5 hours. A gram-negative panel for positive blood cultures is currently in development, which has 9 bacterial targets, including *Acinetobacter* and *P. aeruginosa*, and 6 resistance markers, including the more common *K. pneumoniae* carbapenemase and New Delhi metallobetalactamase genes. Although antibiotic resistance cannot be excluded, positive detection of resistance genes is extremely useful in guiding therapy.

A second multiplex PCR-based platform, the Biofire FilmArray system (bioMerieux) uses nested multiplex PCR to identify 19 bacterial pathogens, 5 yeast and 3 resistance markers directly from positive blood cultures. The FilmArray blood culture identification panel includes 27 targets, including staphylococci, streptococci, *Enterococcus*, *Listeria*, *Acinetobacter*, *Neisseria meningitidis*, *P. aeruginosa* and members of the Enterobacteriaceae family, as well as different *Candida* spp. In addition to pathogen identification, this assay also tests for the presence of mecA, vanA/B, and *K. pneumoniae* carbapenemase genes in the blood culture broth. Reported sensitivities for pathogen identification are >90% [19, 20] and for resistance markers is 100% [20]. The turnaround time for the test is 1 hour. The
ability of this assay to identify a broad range of gram-positive, gram-negative, and fungal pathogens, as well as important resistance markers directly from positive blood culture media, may allow the Gram staining step to be bypassed.

An approach to the identification of organisms that combines PCR and mass spectrometry in an automated, high-throughput platform is the PCR–electrospray ionization mass spectrometry. Although not yet FDA approved, this technique can identify nearly all human pathogens and selected antibiotic resistance markers either from cultured material or directly from clinical specimens, including blood samples. Actionable information would be available approximately 8 hours after receipt of the blood specimen, hours before a blood culture would show growth. Kaleta et al [21] compared this technology to conventional identification methods to identify microorganisms from positive blood cultures and demonstrated high concordance between the 2 methods. This platform may not be appropriate for most clinical laboratories owing to the costs of the system and its assay kits but could be useful in large reference facilities.

CLINICAL AND ECONOMIC IMPACTS OF RAPID PATHOGEN IDENTIFICATION IN POSITIVE BLOOD CULTURES

Before recommending that a clinical microbiology laboratory adopt one of these techniques, it is important to consider whether their application results in significant improvements in clinical outcomes and reductions in healthcare costs (summarized in Table 2). Many blood cultures containing gram-positive cocci in clusters contain CoNS, frequently considered a contaminant, resulting in unnecessary treatment with vancomycin and increased length of hospital stay [4, 30, 31]. For this reason, tests that can rapidly differentiate between S. aureus and CoNS are likely to positively affect clinical decision making. A retrospective study at a 650-bed academic medical center evaluated the clinical outcomes and cost-effectiveness of early identification of CoNS using PNA-FISH in conjunction with an antimicrobial therapy team [22]. The study showed a significant decrease in median length of stay (LOS) by 2 days and cost savings of $4005 per patient during the intervention period [22]. In the only prospective randomized controlled study on the impact of PNA-FISH and an antimicrobial stewardship program on clinical outcomes in S. aureus bacteremia, notifying clinicians of test results was shown to decrease the mortality rate and reduce cost by $19 441 per patient [25]. This result was in stark contrast to findings of another study evaluating the role of PNA-FISH without active notification or an antimicrobial stewardship intervention [23]. In that study, a pre- and postimpact analysis showed no benefit on LOS or vancomycin usage, emphasizing the need to rapidly communicate microbiology data to individuals with the ability to make clinical interventions.

Another PNA-FISH assay with clear antimicrobial therapy implications is the Yeast Traffic Light PNA-FISH assay. This assay consists of a 3-probe system that stains Candida albicans and Candida parapsilosis green, Candida tropicalis yellow, and Candida glabrata and Candida krusei red, a color scheme designed to provide input on whether fluconazole therapy is likely to be effective. The C. albicans PNA-FISH has also been studied in the setting of candidemia. When associated with concomitant antimicrobial stewardship, intervention reduced use of echinocandins and saved $1729 per patient [24].

Several studies have assessed the impact of the GeneXpert MRSA/SA (Cepheid) system on clinical outcomes. A study from the Veterans Affairs Medical Center in Houston showed that in MSSA bacteremia, the GeneXpert MRSA/SA system reduced the mean time to initiation of appropriate therapy from 49.8 hours to 5.2 hours. In addition, the duration of unnecessary MRSA drug therapy was reduced by 61 hours per patient.

Table 2. Studies Examining Impacts on Clinical Outcomes and Healthcare Costs

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Year of Publication</th>
<th>Antimicrobial Stewardship</th>
<th>Mortality Benefit</th>
<th>Change in LOS, d</th>
<th>Cost Saving Per Patient, $</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA-FISH</td>
<td>2006</td>
<td>Yes</td>
<td>Not studied</td>
<td>−2</td>
<td>4005</td>
<td>Forrest et al, 2006 [22]</td>
</tr>
<tr>
<td>PNA-FISH</td>
<td>2011</td>
<td>No</td>
<td>Not studied</td>
<td>+2.2</td>
<td>Not studied</td>
<td>Holtzman et al, 2011 [23]</td>
</tr>
<tr>
<td>PNA-FISH</td>
<td>2006</td>
<td>Yes</td>
<td>Not studied</td>
<td>Not studied</td>
<td>1729</td>
<td>Forrest et al, 2006 [24]</td>
</tr>
<tr>
<td>PNA-FISH</td>
<td>2008</td>
<td>No</td>
<td>Yes (16.8% vs 7.9%)</td>
<td>−2*</td>
<td>19 441</td>
<td>Ly et al, 2008 [25]</td>
</tr>
<tr>
<td>Gene Xpert MRSA/SA</td>
<td>2010</td>
<td>Yes</td>
<td>Yes (18% vs 26%)b</td>
<td>−6.2</td>
<td>21 387</td>
<td>Bauer et al, 2010 [26]</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>2013</td>
<td>Yes</td>
<td>Yes (5.6% vs 10.7%)a</td>
<td>−1.8</td>
<td>19 547</td>
<td>Perez et al, 2013 [27]</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>2013</td>
<td>Yes</td>
<td>Yes (12.7% vs 20.3%)a</td>
<td>−2.8*</td>
<td>Not studied</td>
<td>Huang et al, 2013 [28]</td>
</tr>
<tr>
<td>Verigene GC-GP</td>
<td>2013</td>
<td>Yes</td>
<td>No</td>
<td>−21.7</td>
<td>60 729</td>
<td>Sango et al, 2013 [29]</td>
</tr>
</tbody>
</table>

Abbreviations: BC-GP, gram-positive blood culture; LOS, length of stay; MALDI-TOF, matrix-assisted laser desorption-ionization/time-of-flight mass spectrometry; MRSA, methicillin-resistant Staphylococcus aureus; PNA-FISH, peptide nucleic acid fluorescent in situ hybridization.

* Difference not statistically significant.
organisms are present in blood cultures, thereby avoiding the 1–2-day wait for blood cultures to become positive. Although their clinical and economic impacts are not well understood, the accuracy of pathogen/genus-specific PCR tests is well validated [35]. Investigational assays have been shown to be useful in detection of a single fastidious pathogen, such as Streptococcus pneumoniae, which may grow poorly in blood culture media [7]. Investigational PCR tests for diagnosis of candidemia and invasive aspergillosis have also been evaluated and found to have reasonably good sensitivities of 79%–100% [36–38].

Broad-range PCR assays have been developed for the universal detection of bacteria or fungi in blood. They involve the amplification of the 16S or 23S rRNA genes of bacteria and the 18S rRNA gene of fungi. Although several different platforms have been approved for use in Europe, none is currently marketed in the United States. The best studies approach is the Light Cycler SeptiFast system (Roche Molecular Systems), which uses a real-time PCR assay to detect the most common gram-positive and gram-negative bacteria found in blood cultures, 5 Candida spp. as well as Aspergillus fumigatus. The 25 microorganisms targeted by SeptiFast cause >90% of all BSIs [39, 40]. The detection limit ranges from 3 to 30 colony-forming units/mL, and the turnaround time is 6 hours [41]. SeptiFast consistently identifies pathogens found in most positive blood culture specimens, as well as some that are blood culture negative. Interestingly, discordant (SeptiFast positive, blood culture negative) results frequently seem to be clinically significant [42]. There is room for improvement, because multiple studies have also shown that SeptiFast fails to detect pathogens in up to 20%–39% of samples with positive blood culture results [43–45], even when the pathogen is covered by a primer pair. This reinforces the conclusions that such methods cannot replace conventional blood cultures and negative results may not be actionable. A competing commercial assay is SepsitTest (Molzym) for detection of species-specific 16S and 18S rRNA of bacteria and fungi, respectively. After the initial PCR step, definitive identification is obtained by sequencing. The relatively slow turnaround time of 8–12 hours limits the clinical usefulness of this test for rapid diagnosis of BSI [41].

T2 magnetic resonance (MR; T2Biosystems) is an automated nanoparticle-based PCR assay that can detect as few as 1 colony-forming unit/mL of Candida spp. in blood in approximately 3 hours. A small initial pilot study using spiked blood samples showed a 98% positive predictive value and a 100% negative predictive value between T2 MR results and blood cultures for Candida spp. [46]. T2Biosystems is also developing an assay for the direct detection of select bacterial species from blood.

**SUMMARY**

Sepsis remains a major cause of hospital mortality. Rapid pathogen identification in BSI can lead to improved clinical
outcomes, shorter hospital stays, and dramatically lower health-care costs. Rapid identification of bloodstream pathogens with or without antibiotic resistance genes enables targeting of therapy to specific pathogens and improves the accuracy of antibiotic therapy. Several rapid pathogen identification methods, such as PNA-FISH, MALDI-TOF, PCR, and multiplex microarrays, are being applied to positive blood cultures. PNA-FISH is a well-validated method and the new QuickFISH system has reduced turnaround time to 20 minutes, enabling species-identification results to be reported in the same time frame as Gram staining. Application of MALDI-TOF directly to positive blood cultures is still in the experimental phase but has the potential to identify a much broader range of organisms than PNA-FISH. PCR-based methods, including GeneXpert (1 hour), FilmArray (1 hour), and Verigene (2.5 hours), are somewhat slower than QuickFISH and MALDI-TOF but have little or no sample processing and include selected antibiotic resistance genes.

It is now technically feasible to amplify microbial pathogen nucleic acid targets directly from blood samples obtained at the time of sepsis diagnosis. There is now a large body of literature, mostly from Europe, evaluating the accuracy of systems such as SeptiFast, SepsisTest, and, most recently, T2 MR. These data suggest that the molecular approaches provide information that is clinically relevant and complementary but not equivalent to that provided by conventional blood culture methods. Before these methods can be adopted and gain widespread acceptance, clinicians must learn how to use the information in managing BSIs. None of the methods reviewed here will replace subculturing positive blood culture isolates to agar plates for definitive identification and antimicrobial susceptibility testing. Therefore, adoption of such technologies involves additive clinical laboratory costs. Clinical and economic benefits have so far been demonstrated only when such approaches are combined with a robust antimicrobial stewardship program to help translate the results from the laboratory to the end users (ie, clinicians) and help them make informed patient care decisions.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


