Early Identification and Treatment of Pathogens in Sepsis
Molecular Diagnostics and Antibiotic Choice

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INTRODUCTION

Sepsis, severe sepsis, and septic shock are stages of increasing severity of the systemic host response to bloodstream infections.\(^1,2\) Since the 1980s, the epidemiologic burden of sepsis in the United States and other developed countries has steadily increased. Despite significant improvements in medical care and the development of newer and more broad-spectrum antibiotics for treatment, sepsis still accounts for significant morbidity and mortality in the United States, and is considered among the top 10 leading causes of death.\(^3\) In 2013, the Agency for Healthcare Research and Quality published data from the Healthcare Cost and Utilization Project, indicating that sepsis was among the top 4 conditions associated with the highest cost to hospitals in the United States.\(^4,5\) Aside from the high mortality rate and economic burden during the acute phase of the illness, sepsis and severe sepsis have

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associated indirect costs, such as health care expenditures after hospital discharge and nontrivial productivity loss among sepsis survivors (eg, work absenteeism, early retirement, and overall increased morbidity and mortality).\(^6\) In addition, several other studies described an increased risk of death lasting up to 5 years after survival of 1 episode of sepsis, although many of the mechanisms of the increased morbidity and mortality years after surviving sepsis remain unclear to date. All stages of sepsis combined pose a significant financial and humanistic burden, not only to the individual patients but also to society as a whole.

Despite the technical improvements of continuous monitoring blood culture systems, the value of blood cultures for confirming the clinical suspicion of sepsis has been shown to be suboptimal. The diagnostic limitations and uncertainties of blood cultures related to a low sensitivity (ie, positivity rate), prolonged time to pathogen detection and turn-around-times (TAT) of results, and frequent contamination of blood cultures by patients’ skin microbiota during blood culture procurement, are often compensated by the liberal use of broad-spectrum antimicrobial therapy.\(^2\)

However, considering rising antimicrobial resistance rates and the emergence of novel antimicrobial resistance types (eg, carbapenem-resistant Enterobacteriaceae), it is important to exercise a judicial approach to the use of broad-spectrum antimicrobial therapy. The major role of antimicrobial stewardship programs (ASPs) is to optimize the overall utilization, selection, and dosing of antimicrobial agents to minimize adverse events and prevent the emergence of antimicrobial resistance in the healthcare setting. During the past decade, development of rapid, often molecular, detection methods for both pathogens and antimicrobial resistance markers has seen a significant upsurge. The implementation of such rapid diagnostic technologies in the clinical microbiology laboratory is critical not only for the confirmatory diagnosis of sepsis, but also for support of ASPs. This article provides an update and assessment of recent improvements in pathogen and antimicrobial resistance detection in sepsis, focusing primarily on diagnostic molecular technologies.

**MOLECULAR METHODS FOR DETECTION OF BACTEREMIA**

Tremendous progress has been made over the last 2 decades in the development of diagnostic assays to speed up the detection of bacteria and yeast in blood. The initial wave of these assays involved testing positive blood culture bottles at the time of positivity with molecular methods that determine both the identity of a pathogen or pathogens and associated resistance markers. The second wave, direct from whole blood testing, has been ushered in by the T2 Biosystems Candida assay (described in more detail elsewhere in this article), the first such assay to obtain US Food and Drug Administration (FDA) approval; Table 1 summarizes the assays discussed in this review. Several other assays are in development or have progressed to the clinical trial stage. This section of the review discusses the existing molecular platforms and their performance characteristics. The next section discusses the impact of these methods on antimicrobial stewardship activities and patient outcomes where available.

**NONAMPLIFIED, GROWTH-DEPENDENT METHODS**

Rapid pathogen detection is of pivotal importance for the diagnosis of sepsis, and a variety of molecular techniques have been developed over time for the detection of specific pathogens. However, many of these technologies still require an initial growth of the pathogen(s) in blood culture bottles. Fluorescence in situ hybridization (FISH) technologies were among the earliest developed and most studied techniques for the detection of pathogens from positive blood cultures.\(^2,7\) These technologies are typically pathogen specific, and allow for the detection of only a small number (1–3) of organisms per test. Broad-based methods on the other hand detect various pathogens from positive blood culture bottles without nucleic acid amplification; these multiplex, automated methods allow for the identification of genus, species, and specific resistance determinants for the most common organisms implicated in bloodstream infections.

**Pathogen-Specific Methods**

Peptide nucleic acid PNA-(FISH) technology (AdvantDx, Woburn, MA) is probably the most studied commercially available technology suitable for the detection of pathogens from positive blood cultures.\(^7,8\) The first technology to be commercially available for rapid organism detection, it is now, becoming rapidly surpassed by other molecular detection methods. PNA FISH technology uses fluorescein-labeled probes that target pathogen-specific 16S rRNA of bacteria or 26S rRNA of yeast. FDA-approved PNA FISH probes are available for the following common pathogens implicated in BSIs: *Staphylococcus aureus* and coagulase-negative staphyloccoci (CoNS); *Enterococcus faecalis* and other *Enterococcus* spp.; *Escherichia*
<table>
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<th>Assay</th>
<th>Manufacturer</th>
<th>Principle</th>
<th>Pathogens Detected</th>
<th>Resistance Markers</th>
<th>Sensitivity (%)</th>
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<tr>
<td>PNA FISH</td>
<td>AdvanDx, USA</td>
<td>Fluorescence-based hybridization with PNA probes</td>
<td>Staphylococcus aureus (green)/CoNS (red) Enterococcus faecalis (green)/other enterococci (red) Escherichia coli (green)/Klebsiella pneumoniae (yellow)/Pseudomonas aeruginosa (red) Candida albicans and Candida parapsilosis (green)/Candida tropicalis (yellow)/Candida glabrata and Candida krusei (red)</td>
<td>mecA (mecAXpressFISH)</td>
<td>94–100</td>
<td>87–100</td>
<td>9–12</td>
<td>1.5–3</td>
<td>CE/FDA</td>
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<td>PNA FISH: GNR Traffic Light assay</td>
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<td>PNA FISH: yeast traffic light assay</td>
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<td>QuickFISH</td>
<td></td>
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<td>S aureus (green)/CoNS (red)</td>
<td>None</td>
<td>98–100</td>
<td>89–100</td>
<td>13–16</td>
<td>0.5</td>
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<th>Assay</th>
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<td><strong>Pathogen specific real-time PCR methods</strong></td>
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<tr>
<td>Staph SR</td>
<td>BD GeneOhm, USA</td>
<td>Real-time PCR</td>
<td><em>S. aureus</em> (MRSA/MSSA)</td>
<td><em>mecA</em></td>
<td>94–99</td>
<td>96.5</td>
<td>25,26</td>
<td>2.5–3</td>
<td>CE/FDA</td>
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<td>Xpert</td>
<td>Cepheid, USA</td>
<td>Real-time PCR</td>
<td><em>S. aureus</em> (MRSA/MSSA)</td>
<td><em>mecA</em></td>
<td>98.1–99.6</td>
<td>99.5</td>
<td>25,26</td>
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<td>CE/FDA</td>
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<td><strong>Broad Based Technologies</strong></td>
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<td>Prove-It</td>
<td>MobiDiag, Finland</td>
<td>Multiplex PCR plus microarray</td>
<td>50 different pathogens</td>
<td><em>mecA</em></td>
<td>95–99</td>
<td>98</td>
<td>28,29</td>
<td>3</td>
<td>CE</td>
</tr>
<tr>
<td>SeptiFast</td>
<td>Roche, Germany</td>
<td>Multiplex real-time PCR</td>
<td>25 different pathogens</td>
<td><em>mecA</em></td>
<td>68–75</td>
<td>86–92</td>
<td>41,42</td>
<td>3–30</td>
<td>CE</td>
</tr>
<tr>
<td>SepsiTest Molzym, Germany</td>
<td>Broad-range PCT followed by sequencing</td>
<td>&gt;300 different pathogens</td>
<td>None</td>
<td>87</td>
<td>85.5</td>
<td>43</td>
<td>8–12 CE</td>
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<td>Vyoo Assay SIRS-Lab, Germany</td>
<td>Multiplex PCR with gel electrophoreses</td>
<td>40 different pathogens (including gram-positive, gram-negative, yeast, and Aspergillus fumigatus)</td>
<td>mecA, vanA, vanB, vanC, blaSHV</td>
<td>60</td>
<td>75</td>
<td>44</td>
<td>8 CE</td>
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<tr>
<td>T2 Candida magnetic resonance assay T2 Biosystems Lexington MA</td>
<td>Magnetic resonance assay</td>
<td>5 Candida spp. reported as 3 independent results: C albicans/C tropicalis C glabrata/C krusei, and C parapsilosis</td>
<td>None</td>
<td>88–94</td>
<td>98.9–99.9</td>
<td>47,48</td>
<td>3–5 FDA</td>
<td></td>
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<td>IRIDICA BAC BSI Abbott Molecular, Carlsbad, CA</td>
<td>PCR coupled with electrospray ionization mass spectrometry</td>
<td>Theoretically up to hundreds of organisms</td>
<td>mecA, vanA, vanB, blaKPC</td>
<td>83–91</td>
<td>94–99</td>
<td>49–53</td>
<td>8 Neither</td>
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Abbreviations: CE, Conformité Européenne; FDA, Food and Drug Administration; PCR, polymerase chain reaction; PNA, peptide nucleic acid.

coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa; Candida albicans, Candida parapsilosis, Candida tropicalis, Candida glabrata, and Candida krusei.8–12 These PNA FISH assays typically differentiate between 2 and 5 different key pathogens in a blood culture broth by using 2 or 3 different fluorescent dyes. In the S aureus/CoNS assay, S aureus appears green, and CoNS appears red; for the Yeast-Traffic Light assay, C albicans/ C parapsilosis appear green, C tropicalis is yellow, and C glabrata/C krusei appear red. Although all PNA FISH probes have been approved for use with all commercially available continuous monitoring blood culture systems, not all assays have been subject to thorough evaluation using all continuous monitoring blood culture systems. Based on studies published to date, sensitivities and specificities for these 4 assays range from 94% to 100% and 87% to 100%, respectively.9–12 The original version of the PNA FISH assay required approximately 10 minutes of hands-on time and 90 minutes of incubation time to results reporting. Recently, a more rapid version of the assay, QuickFISH, providing results within 30 minutes with less than 5 minutes of hands-on time has been introduced. This improved TAT has been very attractive to both laboratories and clinicians, and all the assays for all the mentioned organisms except the yeast have been cleared by the FDA. In a recent multicenter study, investigating 722 positive blood cultures from the BactT/ALERT systems, the QuickFISH assay had a sensitivity of 99.5% for reporting S aureus and 98.8% for CoNS, with a combined specificity of 89.5%.13 In another study, using 173 positive blood cultures from the BACTEC system, sensitivity and specificity for detection of S aureus was 100%, and for CoNS 98.5% and 100%, respectively.14 Another study compared the Candida QuickFISH blood culture assay against conventional laboratory methods.15 In this study, the investigators describe an overall agreement of 99.3% between the QuickFISH and conventional methods for 3 major Candida species (C albicans, C glabrata, and C parapsilosis). The overall sensitivity was 99.7%, and the specificity was 98.0%.15 Studies investigating the economic and antimicrobial stewardship impact of the PNA FISH and QuickFISH technology have been published and will be discussed in a subsequent section in this review. Last, the addition of the mecAXpressFISH assay (AdvanDx), which is designed for use in conjunction with the Staphylococcus QuickFISH assay, allows for the detection of methicillin-resistant S aureus (MRSA). In a multicenter study by Salimnia and colleagues,16 the authors reported a 99.1% sensitivity and 99.6% specificity for detection of methicillin resistance in S aureus by this assay. The authors furthermore demonstrated that the mecAXpressFISH assay showed high reproducibility among 6 operators over 5 days of testing, with a 98.9% level of agreement.16 The authors concluded that this assay would require very little effort for implementation among laboratories that already use any of the currently available S aureus PNA FISH tests, and that it has the potential of leading to further improvements of patient outcomes and antibiotic utilization, when applied immediately after the Staphylococcus QuickFISH test.16

Broad-Based Methods

The Verigene Blood Culture Nucleic Acid Test (Nanosphere, Northbrook, IL) is a nonamplified, qualitative, multiplex assay that uses gold nanoparticle technology for the detection of bacterial pathogens directly from positive blood culture bottles.2,17 This system consists of a Verigene bench-top Processor SP and a Verigene Reader.17 Additional components are the extraction tray, utility tray, and the test cartridge, which are inserted into the Processor SP. After vortexing a sample from a patient’s positive blood culture bottle, 350 μL of blood are inoculated into the sample well of the extraction tray; from that point forward, the remaining testing process is automated. Using a magnetic bead-based procedure, bacterial DNA is extracted, fragmented, and denatured in the extraction tray and transferred subsequently by pipette tips to the self-contained test cartridge. Pathogen-specific capture probes are already immobilized on a glass slide. If target DNA is present in the sample, the extracted nucleic acids within the sample hybridize to complimentary sequence-specific capture oligonucleotides that are arranged on the glass slide. A second set of mediator DNA oligonucleotides, which are conjugated to gold nanoparticles, contains complementary sequences to the target DNA and hybridizes with the target DNA present in the sample forming a sandwich. In the final step, elemental silver is deposited onto the gold nanoparticle through a catalytic process, resulting in an amplified signal facilitating the detection of the bound nucleic acids. At the end of the test cycle, the glass slide in its holder is removed and placed into the compartment of the Verigene Reader. Inside the reader, the presence of hybridized probes is determined using optical array scanning technology, and results are reported as detected or not detected for all targets present on the slide. The TAT from positive blood culture to pathogen identification is approximately 2.5 hours. There are 2 FDA-approved panels available for the Verigene
system: the Gram-positive Blood Culture (BC-GP) and the Gram-negative Blood Culture (BC-GN) nucleic acid test. In these studies, the sensitivity and specificity for the BC-GP assay were 92.6% to 100% and 95.4% to 100%, respectively; the sensitivity for the BC-GN assay was found to be 89% to 100%, and the specificity 93% to 100%. Although both Verigene assays demonstrated overall high levels of sensitivity, specificity, and accuracy (≥95% accuracy for BC-GP; 85%–100% for BC-GN) for monomicrobial BSIs, a significant difference in performance was identified for polymicrobial blood cultures. Samuel and colleagues reported a 94% concordance of the BC-GP assay compared with traditional blood culture organism identification for monomicrobial blood cultures, but a 76% concordance for polymicrobial blood cultures. Similar differences were reported by other investigators. Likewise the detection of resistance markers performed better for monomicrobial blood cultures than for polymicrobial blood cultures. In the study by Samuel and colleagues, the BC-GP panel detected 93% of resistance markers (vanA/B and mecA) in positive blood cultures with S aureus, S epidermidis, Enterococcus faecium, and/or E faecalis. The level of concordance for monomicrobial blood cultures was 97%, compared with 84% for polymicrobial blood cultures. Although both assays have difficulties detecting all organisms in polymicrobial blood culture samples, it is also important to recognize that the assay does not link the resistance marker to the detected organisms in polymicrobial samples; this issue is of particular importance for the detection of mecA in samples containing both S aureus and CoNS. Although several studies described similar problems with polymicrobial blood cultures, it should be recognized that true polymicrobial sepsis is thought to be uncommon, estimated to occur in only 5% of all sepsis episodes, although in the authors’ own institution the rates are somewhat higher (10%; Karen Carroll, personal communication, 2015). Nonetheless, careful judgment is warranted when interpreting results of the Verigene panels from blood culture samples containing multiple organisms. To date, only a few studies have been performed analyzing the impact of utilization of the Verigene system with respect to antimicrobial stewardship, patients’ clinical outcomes, and cost effectiveness; these studies are discussed in a subsequent section of this review.

### AMPLIFIED METHODS: GROWTH REQUIRED

#### Pathogen-Specific Real-time Methods

There are numerous nucleic amplification assays that identify S aureus in blood culture bottles positive for gram-positive cocci in clusters. All of them also detect the mecA gene for the distinction between methicillin-susceptible S aureus and MRSA.

The BD GeneOhm StaphSR assay (BD Diagnostics, Sparks, MD) is a real-time polymerase chain reaction (PCR) assay that uses molecular beacon technology to amplify 2 targets—a proprietary species-specific target for S aureus identification and SCCmec for the detection of MRSA. The assay is performed on the SmartCycler instrument (Cepheid, Sunnyvale, CA, USA) and requires manual extraction. One instrument can test 16 samples. This assay takes approximately 2.5 hours to perform and reports results as positive for MRSA, positive for S aureus, or negative.

The latest version of the Xpert MRSA/SA blood culture assay (Cepheid) uses proprietary primers and probes to target spa (identifies S aureus), mecA (detection of methicillin resistance), and the SCCmec-orfX junction in a multiplex real-time PCR assay. All 3 genes must be amplified for a call of MRSA. Methicillin-sensitive S aureus is defined as the presence of spa alone or in conjunction with SCCmec (but not mecA), or the presence of spa and mecA but the absence of SCCmec-orfX. If spa is not detected then the results are interpreted as negative for S aureus despite the presence of the other genes. The first step involves transferring a 50-µL aliquot of the positive blood culture bottle into an elution reagent vial; after vortexing it for 10 seconds, the entire contents of the vial are inoculated into the test cartridge. The test cartridge is then sealed and inserted into a GeneXpert instrument. These instruments are modular and are available in platforms that contain 1, 2, 4, 16, 28, and 80 individual units. Time to results is about 75 minutes, including sample preparation.

A recent multicenter study (8 sites) compared the current version of the BD GeneOhm StaphSR assay with the Xpert MRSA/SA assay on positive blood culture bottles from the 3 major manufacturers following the manufacturers’ recommendations. Both systems were compared with phenotypic methods of identification and cefoxitin disk diffusion for detection of methicillin resistance. A total of 795 blood culture bottles containing gram-positive cocci in clusters were evaluated. The sensitivity and specificity for the
GeneOhm Staph SR and the Xpert MRSA/SA for the detection of \textit{S. aureus} compared with routine methods were 99.2% and 96.5%, and 99.6% and 99.5%, respectively.\textsuperscript{26} There was greater variability between the 2 assays for the detection of MRSA. The GeneOhm Staph SR missed 6 cultures that contained MRSA for an overall sensitivity of 94.3% whereas the GeneXpert missed 2, for a sensitivity of 98.1%.\textsuperscript{26} The Xpert assay had greater specificity 99.5% compared with the GeneOhm assay (96.5%), which demonstrated 15 false positives.\textsuperscript{26}

\textbf{Broad-based Technologies}

The Prove-It Sepsis (Mobidiag, Helsinki, Finland) is not available in the United States but was launched in Europe in 2013. The initial assay used front-end multiplex PCR followed by detection of amplified products in a proprietary tube that contains the microarray at the bottom of the vial.\textsuperscript{2,27,28} Subsequently, the assay has been modified into a strip format (StripArray), which consists of 8 successive reaction vials with a chip microarray on the bottom. The test menu now includes 60 bacterial targets and \textit{vanA}, \textit{vanB} resistance markers in addition to \textit{mecA} and also includes 8 of the most clinically relevant \textit{Candida} spp., namely \textit{C. albicans}, \textit{Candida dubliniensis}, \textit{C. glabrata}, \textit{C. parapsilosis}, \textit{C. tropicalis}, \textit{C. guilliermondii}, \textit{Candida lusitaniae}, and \textit{C. krusei}.\textsuperscript{29}

The array also includes a pan-yeast probe that can detect but not differentiate among 6 other yeasts (5 \textit{Candida} spp. and \textit{Saccharomyces cerevisiae}). Total assay time takes 3.5 hours. In a study performed on spiked BactAlert SA standard blood culture bottles, using 69 different clinical isolates representing 8 \textit{Candida} spp., there was 100% concordance by the microarray analysis with the original identification.\textsuperscript{29} An additional evaluation of 62 samples containing yeast DNA was also performed. The array identified yeast species in 93.5% of the samples.\textsuperscript{29} Two of the missed samples were not contained in the database and the 2 others were not identified by conventional methods.\textsuperscript{29} All of the negative cultures and positive bacterial samples yielded a negative yeast result.\textsuperscript{29}

The FilmArray Blood Culture Identification (BioFire Diagnostics, Inc Salt Lake City UT) assay consists of a self-contained pouch that stores reagents for sample extraction and purification and nested multiplex PCR. The pouch is inoculated with 200 \(\mu\)L of positive blood culture broth on one end and is hydrated on the opposite end with buffer before placement in the FilmArray instrument. The assay has primers and probes that detect members of the family \textit{Enterobacteriaceae}, 3 Gram-positive and 1 Gram-negative genera, 5 Gram-positive species, 9 Gram-negative species and 5 \textit{Candida} spp. (24 agents in total). In addition, the \textit{mecA} (\textit{S. aureus}), \textit{vanA}/\textit{vanB} (enterococci), and \textit{blaKPC} genes (\textit{Enterobacteriaceae}) are also detected. Hands-on time is 2 minutes and results are available within 1 hour.

There have been several publications from single institution studies on the performance of this assay and 1 very large multicenter study.\textsuperscript{30–35} Among these studies conducted at 13 institutions, the pouch identified 87% to 92% of all pathogens routinely recovered in positive blood cultures. The results of these studies, which are quite variable in their study designs, indicate that the overall accuracy for detection of the genera and species in the panel compared with conventional methods ranged from 94% to 100%.\textsuperscript{30–35} The performance for accurately detecting resistance markers ranged from 80.4% to 100%. In 1 study, the FilmArray had difficulty in detecting \textit{mecA} among coagulase-negative staphylococci accounting for the low sensitivity.\textsuperscript{35} This was either not observed or not reported in the other studies.\textsuperscript{30–34}

Also, like the Verigene assay, in mixed cultures containing \textit{S. aureus} and CoNS, correct assignment of a \textit{mecA} gene positive result to the particular species cannot occur. In all of the studies, detection of \textit{mecA} in \textit{S. aureus} ranged from 98% to 100%. The FilmArray assay can distinguish \textit{Klebsiella oxytoca} from \textit{Raoultella ornithinolytica}, whereas phenotypic methods cannot. One of the major limitations of this assay, similar to the Verigene assays, is the inability to detect all organisms in polymicrobial cultures.\textsuperscript{30–35} Also of note, in the study by Ward and colleagues,\textsuperscript{35} a significant number of false-positive results for \textit{P. aeruginosa} were observed. It was determined, and the manufacturer, bioMérieux, has confirmed, that this is owing to contamination of the BactAlert standard anaerobic blood culture bottles with nucleic acid from nonviable organisms.\textsuperscript{36} In May 2014, BioFire released an advisory note regarding the possibility of detecting false positive results for \textit{P. aeruginosa} and \textit{Enterococcus} spp. when testing bioMérieux BactAlert standard anaerobic bottles.\textsuperscript{37}

\textbf{BROAD-BASED TECHNOLOGIES DIRECTLY FROM WHOLE BLOOD}

The greatest impact on patient outcomes is likely to occur with assays that offer direct from whole blood testing. There are many challenges to detecting organisms directly from whole blood. The first of these is sensitivity. Often patients
with bacteremia will have less than 1 colony forming unit per milliliter (CFU/mL) of bacteria in blood.\textsuperscript{27,38} Detecting such low quantities of organisms is challenging even in the era of ultrasensitive nucleic acid amplification techniques. In addition, the repertoire of organisms that can cause bacteremia continues to expand as opportunistic organisms cause serious disease in extremely immunocompromised patients. Clinical specificity is challenged by the possibility of detecting the nucleic acid of dead organisms, random nucleic acid that appears in blood transiently, and also reagent contamination from environmental organisms.\textsuperscript{27,36,39} When comparing such assays to conventional blood culture methods, the optimum “gold standard” is unclear. Although these are obstacles, great progress is being made and some of these methods hold promise for detecting noncultivable or difficult to cultivate pathogens that continue to allude current blood culture methods.

Several assays that detect pathogens in whole blood have been available outside the United States for a number of years. Table 1 lists the features of these assays and several reviews describe their performance in more detail.\textsuperscript{2,27,40}

The LightCycler SeptiFAST test (Roche Diagnostics, Mannheim Germany) is a 6- to 8-hour assay that detects up to 25 pathogens and meca from whole blood in an assay combining multiplex real-time PCR using dual-fluorescence energy transfer probes (FRET) and melt curve analysis for pathogen differentiation.\textsuperscript{2} This assay detects 10 bacteria to species level, several others to the genus level, 5 Candida spp., and Aspergillus fumigatus, and it has been evaluated extensively in the literature in adult and pediatric patients including 2 recent metaanalyses of its performance.\textsuperscript{21,27,41,42} Only the results of the metaanalyses are summarized herein. The systematic review and metaanalysis by Chang and colleagues\textsuperscript{41} included 34 primary studies through October 2012. These 34 studies collectively evaluated 6012 patients (8438 episodes), 22.8% of which were confirmed bacterial or fungal infections.\textsuperscript{41} Of note, few of the studies were blinded and a wide range of reference standards were applied—some clinical and laboratory, others laboratory based alone. With these shortcomings in mind, the pooled sensitivity was 75% (95% CI, 65%–83%) and the pooled specificity was 92% (95% CI, 90%–95%).\textsuperscript{41} The analyses examined the overall positive and negative likelihood ratios (high and moderate, respectively), performance for bacteria and fungi independently, and subgroup analyses examining bacteremic and fungemic patients in studies that had similar settings (such as patients in the intensive care unit (ICU)) and reference methods. The authors concluded that the SeptiFAST assay is of high “rule-in” value for early detection of septic patients, and in patients with low probability of sepsis, the test may be of value in excluding bacteremia and fungemia.\textsuperscript{41}

In the study by Dark and colleagues,\textsuperscript{42} the authors likewise found significant heterogeneity and variable quality among the 41 diagnostic accuracy studies evaluated in their analyses. The authors of this metaanalysis used blood culture as the reference standard and included any clinical diagnostic accuracy study that compared SeptiFAST to the reference method through April 2014.\textsuperscript{42} In total, 7727 patients, representing a wide range of age and settings, contributed 10,493 episodes of suspected sepsis in which the median prevalence of blood culture positivity was 17%.\textsuperscript{42} Similar to the results of the Chang study, the combined results for sensitivity and specificity, 68% (95% CI, 63%-73%) and 86% (95% CI, 84%-89%), respectively, showed better specificity.\textsuperscript{42} The authors concluded that positive results are likely to be of greater diagnostic utility than negative results when compared with blood cultures.\textsuperscript{42} Because the upper CI for both sensitivity and specificity did not reach 90% in this pooled analysis, standard blood cultures will still be required to detect false negatives in patients with suspected sepsis and, at least in some settings, even accounting for the superior sensitivity of molecular methods; “false positives” may lead to erroneous diagnoses.\textsuperscript{42}

The SepsITest (Molzym GmbH, Bremen, Germany) uses broad range primers targeting 16S rDNA of bacteria and 18S rDNA of fungi in a real-time PCR assay. The amplicons are sequenced to yield definitive identification of a total of 345 bacteria and fungi. Two 1-mL samples are suggested for testing in duplicate. The manufacturer provides an additional kit called Add-On10 that accommodates up to 10 mL of blood to enhance sensitivity. The assay requires at least 8 hours to complete. In the prospective, multicenter study by Wellinghausen and colleagues,\textsuperscript{43} 342 blood samples from 187 patients, mostly adults, were evaluated. Compared with reference blood cultures, the overall diagnostic sensitivity and specificity of the PCR in this study were 87.0% and 85.8%, respectively, and the concordance between the SepsITest and blood cultures was 86%.\textsuperscript{43} There were 7 samples that grew bacteria that were not detected by PCR and 41 samples that were PCR positive but negative by blood cultures. Among the latter group, 12 of the patients were believed to have true bacteremia and in 11 cases, the same species were detected in other body sites. The majority of the remaining samples
were obtained from patients who had received antibiotics. Although the performance characteristics of the assay in this study are encouraging, the assay is labor intensive. The practicality of this in a routine clinical laboratory setting is questionable.

Another 8-hours platform, also manufactured in Germany, is the VYOO assay (SIRS-LAB GmbH, Jena, Germany), which combines 16S rRNA gene multiplex PCR with highly specific melting point analysis to identify 34 of the most common bacterial species and 6 fungal species causing bloodstream infections. In addition, 5 resistance markers are also detected: mecA, vanA, vanB, and variants of \textit{bla}\textsubscript{SHV} and \textit{bla}\textsubscript{CTX}, extended spectrum \beta-lactamase genes. This assay accommodates 5 mL of ethylenediaminetetraacetic acid (EDTA) whole blood.

In the study by Bloos and colleagues, 336 blood samples collected from 245 adult ICU patients were collected for PCR simultaneously with standard blood cultures (311 pairs). The concordance between the PCR assay and standard blood cultures was 72.7%; 67 samples were positive by PCR but negative by standard cultures and 18 were positive by blood cultures that were negative by PCR. Seven of the 18 “false-negative” PCR tests were pathogens not included in the VYOO assay. Two independent arbitrators reviewed the charts of patients with positive PCR results and concluded that 94% of the PCR results yielded clinically meaningful results (ie, not suggestive of contamination), including detection of 12 pathogens that were not treated by initial empiric therapy. In this study, owing to staffing limitations, the time for PCR results was 24.2 hours (not the 8 hours possible by the technology) compared with 68.8 hours for standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures. The overall sensitivity of 60% and specificity of 75% emphasizes the fact that this assay will remain an adjunct to standard blood cultures.

Seegene (Seoul, Korea) has developed an assay (MagicPlex Sepsis Test) that screens for up to 90 pathogens and 3 resistance markers and definitively identifies 21 bacterial species and 6 fungal species. After DNA extraction from 1 mL of fresh whole blood (EDTA), the first conventional PCR creates an amplicon bank. Real-time PCR using the Magicplex Screening kit screens for the presence of bacteria and fungi. Species identification is then performed on the screen positive samples using the Magicplex ID1-ID-9 real-time detection kit. The assay requires about 7 hours to perform including the DNA extraction step. There are 2 studies that have evaluated the MagicPlex assay. In the study by Carrara and colleagues, the authors compared the Magicplex Sepsis Test to standard blood cultures on 267 patients from an ICU, the emergency department, and a hematology unit. Clinical data were also included in the analysis. The agreement between standard blood cultures and the PCR assay was 73%. Of the 63 positive specimens considered true BSI by clinical assessment, 23 were positive by PCR and standard methods, 18 by PCR only, and 22 by blood cultures only. There was a high rate of contamination by both PCR and standard blood cultures making it difficult to interpret the results that were positive by 1 method alone. The authors concluded that the Magicplex assay shows high specificity overall (92%) but lacks sensitivity (65%) and they recommended technical improvements to the assay.

In the Ljungstrom study, 375 patients who had 383 episodes of sepsis were analyzed by blood cultures, the Magicplex assay performed on whole blood and the Prove-it microarray based assay on positive blood culture bottles. The sensitivity of the Magicplex assay adjudicated by clinical data was 62%, a value similar to the Carrara study. In the study by Ljungstrom and colleagues, the specificity was 96% and the concordance with blood cultures was only moderate (kappa 0.50). Also of note in this study was the initial invalid rate of 12% for the Magicplex assay. However, this study was not able to accurately measure the turnaround times because of the variability in the times of the assay performances.

Although such assays, such as SeptiFAST and others have been available in Europe, until recently there has not been an FDA-cleared assay available in the United States. Two systems, one that is FDA-cleared and currently available for detection of \textit{Candida} species in blood and a second more broad-based technology that is in clinical trials in the United States are discussed.

\textbf{T2 Candida Magnetic Resonance Assay}

The T2Dx instrument (T2 Biosystems, Lexington, MA) lyses the \textit{Candida} cells by standard mechanical bead beating, amplifies the DNA using a proprietary thermostable polymerase and primers targeting the intervening transcribed spacer2 (ITS2) region within the \textit{Candida} ribosomal operon, and detects the amplification products by hybridization to supermagnetic nanoparticles that are detected by magnetic resonance. The assay has an internal control and requires positive and negative external controls. Whole blood from patients suspected of having candidemia is collected into K\textsubscript{2}EDTA plastic blood collection vacutainers.
detects 5 yeasts reported as 3 independent results. C albicans/C tropicalis, C glabrata/C krusei, and C parapsilosis in 3 to 5 hours. This reporting arrangement is designed to assist with determining optimum treatment. Preclinical testing revealed a limit of detection for each of these species in whole blood between 1 and 3 CFU/mL. In the clinical trial, performed at 12 centers, 300 contrived samples representing 250 whole blood samples from patients spiked with known concentrations of the 5 targeted Candida species and 50 uninoculated whole blood samples were tested as negative controls. In addition, for the prospective arm of the study, 1501 patients were enrolled who had blood cultures drawn concurrently for standard of care testing and the T2MR Candida assay.48 For the contrived samples, the overall sensitivity was 91% with a range of 88.1% for C krusei/C glabrata to 94.2% for C albicans/ C tropicalis.48 The specificity results were overall 99.4% with a range of 98.9% (C albicans/C tropicalis) to 99.9% (C krusei/C glabrata).48 In the prospective arm of this study, there were only 4 patients who had concomitantly positive T2MR and standard of care blood culture results. There were 31 discordant results, 29 of which were detected only by the T2MR assay and 2 that were T2MR negative but grew Candida species in blood cultures. In 5 of these 29 cases, the same Candida species grew from other clinical samples and in 6 cases the patients were on antifungal therapy at the time of blood draw. The mean time for completion of standard blood cultures to species level identification in this study was 129.9 ± 26.3 hours and for the T2MR the mean time to species identification was 4.4 ± 1 hours (P<.001).48 Although the negative predictive value of 99.4% may allow for cessation of empiric antifungal therapy for the organisms detected by the assay, it remains to be determined whether this will actually occur among populations at risk for invasive fungal disease beyond Candida species. The company is pursuing expanding its menu beyond detection of Candida species to include bacterial pathogens.

**IRIDICA BAC-BSI Assay**

This commercial assay (IRIDICA BAC-BSI Assay, Ibis Biosciences, Abbott Molecular, Carlsbad, CA), which has undergone numerous iterations, uses broad range multiplex PCR coupled with electrospray ionization mass spectrometry.49–53 Electrospray ionization mass spectrometry measures the mass of the A, G, C, and T composition of the PCR amplicons.49 Comparing the base composition of the detected organisms with those of organisms in the database, bacteria and Candida species in whole blood can be identified with high accuracy in about 6 to 8 hours. The assay requires 5 mL of whole blood.53 The current BAC-BSI assay, which is in clinical trials at the time of this publication, definitively detects 48 bacterial pathogens, 5 yeast (C albicans, C dubliniensis, C glabrata, C parapsilosis, and C tropicalis), and 4 resistance markers vanA, vanB, mecA, and blaKPC, but it has the capacity to detect many more species. A preliminary study by Bacconi and colleagues describes optimization of the assay in the presence of high levels of human DNA. In the clinical phase of the study, 331 prospectively obtained patient samples (5 mL) were tested by conventional cultures (2 sets each consisting of 1 BD Bactec Plus aerobic/F bottle and 1 BD Bactec anaerobic/F bottle; BD Diagnostics).53 The BAC-BSI detected 35 positives compared with 18 by standard methods. Fifteen samples were positive by both methods for an overall accuracy compared with standard culture positives of 94% (15/16). Overall, the sensitivity and specificity were 83% and 94%, respectively. Repeat testing of a second whole blood sample improved the sensitivity and specificity to 91% and 99%, respectively.

In Rapid Diagnosis of Infections in the Critically Ill (RADICAL), an observational study performed in 9 ICUs in 6 European countries, the PCR-ESI MS assay was used to test 616 whole blood samples, 185 respiratory samples, and 110 sterile fluid and tissue samples from 529 patients.54 Only the results for the bloodstream infections are presented herein. PCR-ESI MS detected a pathogen in 228 cases (37%) compared with 68 (11%) using culture. In 13 cases, culture was positive and PCR-ESI MS was negative, and both were negative in 384 cases. Clinical analysis performed by independent investigators suggested that altered treatment would have potentially occurred as a result of the 6-hour availability, enhanced sensitivity, and high negative predictive value (97.5%) of the PCR-ESI MS.

Although the broad spectrum nature of this assay is the best among the platforms described to date, the footprint of this assay and the costs are likely to make implementation of this assay possible for only the largest reference laboratories.

**POTENTIAL ECONOMIC IMPACT OF RAPID MOLECULAR DIAGNOSTIC METHODS AND BENEFITS FOR ANTIMICROBIAL STEWARDSHIP PROGRAMS**

Sepsis has been identified as the 10th leading cause of death in the United States, and the overall
humanistic and economic burden is extremely high and growing. The time interval to appropriate/optimal antimicrobial therapy has been recently described as one of the most important factors influencing clinical outcomes and mortality rates in sepsis patients. When considering the goal to shorten the time of empiric, broad-spectrum antimicrobial therapy toward optimal therapy, one must also recognize the globally rising rates of antimicrobial resistance. Infections involving organisms with resistance to various antimicrobial agents have been shown to increase hospital length of stay (LOS), health care costs, and mortality. Furthermore, the connection between “unrestricted” broad-spectrum antimicrobial use and the subsequent development of antimicrobial resistance has now been widely accepted. ASPs have been developed as a form of multidisciplinary interventions to allow for selection of the most optimal antimicrobial agent, dosage, and length of therapy to treat an infection. Decreasing the time to appropriate/optimal antimicrobial therapy has been shown to decrease (unnecessary) antimicrobial exposure and can result in cost avoidance. As stated, the use of blood cultures and routine laboratory methods for organism identification to confirm the clinical suspicion/diagnosis of sepsis has been shown to be suboptimal. Furthermore, studies demonstrated that only 5% to 15% of blood cultures drawn for any reason, and approximately 50% to 60% of the blood cultures collected from patients with clinical signs of septic shock gave positive results using current, continuously monitoring blood culture systems. In this review, we presented data on various rapid molecular pathogen detection methods and their performance; although the majority of the methods described in this review demonstrate an acceptable performance and accuracy for implementation in everyday clinical use and can decrease the TAT for reporting results for organism identification and limited AST (ie, presence of predetermined, important genetic resistance markers), only a few studies have been published to date that evaluate the impact of these methods on clinical outcomes, mortality, and costs of health care. The timeline summarized in Fig. 1 illustrates the potential impact on improving TAT for organism ID by these various detection methods.

One of the first studies investigating the impact of rapid diagnostic tests in conjunction with an ASP on earlier appropriate antimicrobial therapy, patient outcomes, and hospital costs was published by Forrest and colleagues, using the PNA FISH S aureus/CoNS on blood cultures with gram-positive cocci in clusters from ICU patients with suspected sepsis. PNA FISH results for 139 blood cultures were reported in real time to an ASP for assessment of continued need for vancomycin therapy. The investigators in this study compared the results with a control group of 84 blood cultures with gram-positive cocci in clusters from patients with presumed sepsis for whom PNA FISH was not used. In this study, there was a significant decrease in medium length of hospital stay (approximately 2 days per sepsis episode), a trend toward reduced vancomycin use (approximately 5% decrease), and a decrease in associated hospital cost (approximately $4000 per patient). Subsequently, other studies reported similar data for the PNA FISH S aureus/CoNS and the E faecium/Enterococcus spp. probes. Similarly, a study investigating the role of PNA FISH Candida probes demonstrated significant cost savings, mainly attributed to a decreased use of echinocandins. Subsequently, Holtzman and colleagues published results from their study demonstrating the absence of a benefit of the PNA FISH assay(s) with respect to LOS and antimicrobial use without implementation of active notification and antimicrobial stewardship interventions. Few studies investigated the impact of real-time PCR assays in conjunction with an ASP on patient outcomes, antimicrobial use, and cost of patient care. Bauer and colleagues published data from a single-center study, investigating the impact of the Xpert MRSA/SA assay on patient outcomes and associated cost for patients with blood cultures positive for gram-positive cocci in clusters. The investigators reported an overall decrease in hospital LOS by 6.2 days with an associated cost saving of approximately $21,000. Finally, only a few studies using broad-based multiplexed platforms for the assessment of impact on antimicrobial stewardship, LOS, and clinical outcome have been performed to date. Sango and colleagues investigated the impact of Verigene BC-GP platform combined with ASP interventions on patients with enterococcal sepsis. The results of this study, comparing the Verigene BC-GP assay with conventional laboratory methods, demonstrated a decrease in time to effective antimicrobial therapy, together with a decrease in LOS (average, 21.7 days). Bork and colleagues performed a “theoretic antibiotic therapy comparison” study after validation of the Verigene BC-GN assay; in this study, assuming that 100% of the AST/ASP recommendations would be followed by clinicians, the investigators described a statistically significantly shorter time (P<.01) from detection of positive blood cultures to effective and/or optimal antimicrobial therapy. The authors
Fig. 1. Turnaround time for conventional blood culture results and impact of rapid diagnostic methods. AST, antimicrobial susceptibility testing; ID, organism identification; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; PNA FISH, Peptide nucleic acid fluorescence in-situ hybridization; SA, *S aureus*; TAT, turn-around-time.
therefore concluded, that the use of this assay could potentially decrease to both effective and optimal antimicrobial therapy and significantly improve clinical outcomes in sepsis patients. When reviewing the existing evidence all together, a few limitations must be mentioned here; 2 of these studies were retrospective in design, 3 studies did not specifically investigate the impact of the rapid molecular test method on cost savings per patient, 1 one study was based on a computer-modelling approach to test interventions. Furthermore, all clinical studies were limited by their relatively small sample sizes and absence of a comprehensive assessment of mortality.

To our knowledge, at the time of writing this review only one prospective, randomized controlled trial evaluating clinical outcomes associated with use of a rapid multiplex PCR method for the detection of bacteria, fungi, and resistance genes directly from positive blood cultures has been published. In this study, Banerjee and colleagues investigated the usefulness of a rapid multiplex PCR method (FilmArray Blood Culture Identification; BioFire Diagnostics, Inc) in conjunction with an ASP, compared with the use of rapid multiplex PCR without ASP, and their standard approach to organism ID and AST. In this study, both intervention groups, when compared with the control group, showed a significant decreased use of broad-spectrum antibiotics, less treatment of contaminant organisms, and much shorter time to deescalation of antimicrobials used for treatment. However, the authors did not identify any statistically significant differences in LOS, mortality, or associated cost for treatment.

Finally, 1 study has been published to date investigating the economic impact of a rapid diagnostic method for use on whole blood samples. This study by Bilir and colleagues is a decision tree modeling analysis for the impact of the T2-Candida assay compared with the use of blood culture alone for diagnosis of fungemia in sepsis patients. This study is a computer-based modeling analysis of the economic impact (cost savings and LOS) of the T2 Candida assay over a 1-year period in a hypothetical hospital patient care setting assuming rates of candidemia, approach to intervention, and time to positivity and time to intervention for positive blood cultures based on historical data. In this study, the authors conclude that a hospital could realize a significant cost savings, decreased LOS, and potentially lower mortality rates, when using the T2 Candida assay. At this point, additional, more comprehensive, and preferably prospective studies are necessary to provide more evidence and a better understanding of the true impact of rapid molecular tests on clinical patient outcomes, mortality, LOS, and cost savings.

**SUMMARY**

The use of direct detection and identification methods for pathogens from positive blood cultures or whole blood is a promising approach to improve clinical outcomes in sepsis patients, specifically when these methods are combined with ASPs. The number of commercially available molecular test methods for the diagnosis of sepsis has increased rapidly over the past 10 years. Despite their excellent performance for identification of key pathogens and resistance genes from positive blood cultures, none of these methods by itself has shown sufficient diagnostic accuracy, sensitivity, and/or specificity to fully replace the use of blood cultures and other standard laboratory methods for identification of all organisms potentially implicated as causes of sepsis. Caution must be exercised specifically when considering the performance of these methods for polymicrobial blood cultures. To date, various studies have demonstrated that the rapid molecular tests clearly result in improvements of TAT for identifying key pathogens and resistance genes from positive blood cultures, with the potential for a significant impact on time to optimal antimicrobial therapy, clinical outcomes, and patient mortality. Although these molecular technologies for organism and resistance gene identification, are more expensive to the laboratory when compared with the current conventional methods for ID and AST, there is emerging evidence that the cost for such technologies could be offset by cost savings and cost avoidance in other areas of health care through improvements in clinical care, implementation of earlier, optimal antimicrobial therapy, and decreased ICU LOS and/or overall hospital LOS. Currently ongoing as well as future prospective clinical trials will hopefully provide the evidence to support broader implementation of rapid molecular diagnostic methods in a variety of health care settings.

**REFERENCES**


