Robert A. Weinstein, Section Editor

Emerging Technologies for Rapid Identification of Bloodstream Pathogens

Atul Kothari,[1] Margie Morgan,[2] and David A. Haake[1,3]

[1]Division of Infectious Diseases, VA Greater Los Angeles Healthcare System, [2]Department of Pathology and Laboratory Medicine, Cedars-Sinai Medical Center, and [3]Departments of Medicine, Urology, and Microbiology, Immunology, and Molecular Genetics, David Geffen School of Medicine at UCLA, Los Angeles, California

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

Received 13 February 2014; accepted 9 April 2014; electronically published 24 April 2014.

Correspondence: Atul Kothari, MD, Infectious Diseases Section, VA Greater Los Angeles Healthcare System, 111F, 11301 Wilshire Blvd, Los Angeles, CA 90073 (dratulkothari@gmail.com).

Clinical Infectious Diseases 2014;59(2):272–8

DOI: 10.1093/cid/ciu292

Table 1. Tests for Rapid Identification of Bloodstream Pathogens

Blood Culture Assay	Pathogens Detected	Resistance Markers	Turnaround Time (After Blood Cultures Turn Positive)
PNA-FISH	*Staphylococcus aureus*, CoNS, *Enterococcus faecalis*, other enterococci, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida albicans, Candida parapsilosis, Candida glabrata, Candida krusei, Candida tropicalis*	No	1.5–3 h
*Quick*FISH	*S. aureus*, CoNS, *E. faecalis*, other enterococci, *E. coli, K. pneumoniae, P. aeruginosa*	No	<30 min
MALDI-TOF[a]	Gram-positive and gram-negative bacteria, yeast, fungi, filamentous fungi, mycobacteria	In development	10–30 min
Gene Xpert MRSA/SA	*S. aureus*	*mecA*	<1 h
Verigene gram-positive blood culture (BC-GP)	*S. aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Streptococcus anginosus group, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, E. faecalis, Enterococcus faecium, Staphylococcus* spp., *Streptococcus* spp., *Listeria* spp.	*mecA, vanA, vanB*	2.5 h
Verigene gram-negative blood culture (BC-GN)[b]	*Escherichia coli, Shigella* spp., *K. pneumoniae, Klebsiella oxytoca, P. aeruginosa, Serratia marcescens, Acinetobacter* spp., *Proteus* spp., *Citrobacter* spp., *Enterobacter* spp.	KPC, NDM, CTX-M, VIM, IMP, OXA	2 h
FilmArray blood culture identification (BC ID)	*S. aureus, Staphylococcus* spp., *S. agalactiae, S. pneumoniae, S. pyogenes, Streptococcus* spp., *Enterococcus* spp., *Listeria monocytogenes, Hemophilus influenza, Neisseria meningitides, Enterobacter cloacae* complex, *E. coli, K. pneumoniae, K. oxytoca, P. aeruginosa, Serratia marcescens, Acinetobacter baumanii, Proteus* spp., *C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*	*mecA, vanA, vanB*	1 h
Whole blood assays			
Light Cycler[c] SeptiFast	*S. aureus*, CoNS, *Streptococcus* spp., *S. pneumoniae, E. faecalis, E. faecium, E. coli, K. pneumoniae, K. oxytoca, P. aeruginosa, Enterobacter cloacae/aerogenes, S. marcescens, A. baumanii, Proteus mirabilis, Stenotrophomonas maltophilia, C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Aspergillus fumigatus*	No	6 h
SeptiTest[c]	>300 pathogens	No	8–12 h
T2 *Candida*[d]	*C. albicans, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis*	No	3 h

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 metallobetalactamase; PNA-FISH, peptide nucleic acid fluorescent in situ hybridization.

[a] Biomerieux instrument approved by the FDA, Bruker instrument pending approval.

[b] Pending Food and Drug Administration approval.

[c] Not available in the United States.

[d] Research use only.

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 staphylococci (CoNS); *Enterococcus faecalis* and *Enterococcus* species; *Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*; and *Candida* species. The turnaround time for these PNA-FISH assays is approximately 90 minutes. PNA-FISH has demonstrated sensitivities and specificities ranging from 96% to 100% [8]. In 2013, a faster and less labor-intensive *Quick*FISH (AdvanDx) assay was introduced with a turnaround time of 20

minutes [8]. The *Quick*FISH 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 ≥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 β-lactam antibiotics are superior to vancomycin in the treatment of methicillin-susceptible *S. aureus* (MSSA) bacteremia [14–16]. Therefore, rapid determination of β-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 β-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

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

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

[a] Difference not statistically significant.

[32]. A similar study evaluated clinical and economic outcomes of the use of the GeneXpert MRSA/SA in cases of *S. aureus* bacteremia together with stewardship intervention in a 1150-bed tertiary care hospital [26]. This study found a significantly shorter LOS (6.2 days) and a cost saving of $21 387 per patient, with a trend toward lower mortality rates (18% vs 26%) [26].

Clinical microbiology laboratories have begun examining the clinical and economic impact of MALDI-TOF for direct identification of organisms in positive blood cultures. In a study from a 1000-bed quarternary care academic medical center, Perez and colleagues [27] found that using near real time antimicrobial stewardship based on MALDI-TOF results, time to adjustment of antimicrobial therapy was shortened by 46 hours in BSI. They also showed a significant decrease in LOS in the intensive care unit by 1.2 days and in the hospital by 1.8 days, along with a cost saving of $19 547 per patient when using the MALDI-TOF for diagnosis of BSI [27].

A prospective observational study from Switzerland on the sequential impact of reporting Gram stain and MALDI-TOF results in patients with gram-negative bacteremia showed that MALDI-TOF led to more appropriate antimicrobial therapy in 42.2% of cases [33]. The implications of these results were limited by the fact that Switzerland has a low prevalence of extended-spectrum β-lactamases and multidrug-resistant organisms. A separate study by Huang and colleagues [28] looking at 501 patients with bacteremia and candidemia showed that combining MALDI-TOF with antimicrobial stewardship interventions shortened the time to effective antibiotic therapy by 9.7 hours, and the time to optimal therapy by 43 hours. The intensive care unit LOS was reduced by 6.6 days, and the mortality rate was reduced from 20.3% to 12.7% after the intervention [28].

There are limited data evaluating clinical outcomes using PCR-based assays, such as Verigene and FilmArray. A small chart review of 31 patients with MRSA, MSSA, or vancomycin-resistant enterococcal bacteremia showed that application of the Verigene BC-GP assay would have led to more appropriate antibiotic selection a mean of 42 hours earlier per patient [34]. In a separate quasi-experimental preintervention/postintervention study among inpatients with enterococcal bacteremia, the Verigene BC-GP assay led to a significantly faster introduction of appropriate antimicrobials (mean difference, 31.1 hours), reduced the LOS by 21.7 days, and lowered hospital costs by $60 729 [29].

DIRECT DETECTION FROM BLOOD

Molecular techniques applied directly to whole blood samples provide the advantage of same-day identification of sepsis pathogens and early pathogen-specific targeting of antimicrobial therapy. These methods (summarized in Table 1) can provide clinically relevant information before detectable numbers of 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 SepsiTest (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; T2Biosysystems) 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

Septicemia remains a major cause of hospital mortality. Rapid pathogen identification in BSI can lead to improved clinical

outcomes, shorter hospital stays, and dramatically lower healthcare 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 *Quick*FISH 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 *Quick*FISH 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, SepsiTest, 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 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

Acknowledgments. Thanks to Matthew B. Goetz, MD, and Christopher Graber, MD, for helpful discussions.

Potential conflicts of interest. D. A. H. has relevant intellectual property and is a shareholder in Qvella Corporation (Toronto, Ontario, Canada). All other authors report no potential conflicts.

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

1. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med **2006**; 34:1589–96.
2. Kumar A, Ellis P, Arabi Y, et al. Initiation of inappropriate antimicrobial therapy results in a fivefold reduction of survival in human septic shock. Chest **2009**; 136:1237–48.
3. Barenfanger J, Graham DR, Kolluri L, et al. Decreased mortality associated with prompt Gram staining of blood cultures. Am J Clin Pathol **2008**; 130:870–6.
4. Beekmann SE, Diekema DJ, Chapin KC, Doern GV. Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. J Clin Microbiol **2003**; 41:3119–25.
5. Doern GV, Vautour R, Gaudet M, Levy B. Clinical impact of rapid in vitro susceptibility testing and bacterial identification. J Clin Microbiol **1994**; 32:1757–62.
6. Oliveira K, Procop GW, Wilson D, Coull J, Stender H. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. J Clin Microbiol **2002**; 40:247–51.
7. Paolucci M, Landini MP, Sambri V. Conventional and molecular techniques for the early diagnosis of bacteraemia. Int J Antimicrob Agents **2010**; 36 (Suppl 2):S6–16.
8. Deck MK, Anderson ES, Buckner RJ, et al. Multicenter evaluation of the *Staphylococcus Quick*FISH method for simultaneous identification of *Staphylococcus aureus* and coagulase-negative staphylococci directly from blood culture bottles in less than 30 minutes. J Clin Microbiol **2012**; 50:1994–8.
9. Seng P, Drancourt M, Gouriet F, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis **2009**; 49:543–51.
10. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiol Rev **2012**; 36:380–407.
11. Hrabak J, Chudackova E, Walkova R. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry for detection of antibiotic resistance mechanisms: from research to routine diagnosis. Clin Microbiol Rev **2013**; 26:103–14.
12. Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis **2004**; 39:309–17.
13. Burton DC, Edwards JR, Horan TC, Jernigan JA, Fridkin SK. Methicillin-resistant *Staphylococcus aureus* central line-associated bloodstream infections in US intensive care units, 1997–2007. JAMA **2009**; 301: 727–36.
14. Chang FY, Peacock JE, Musher DM, et al. *Staphylococcus aureus* bacteremia: recurrence and the impact of antibiotic treatment in a prospective multicenter study. Medicine (Baltimore) **2003**; 82:333–9.
15. Gonzalez C, Rubio M, Romero-Vivas J, Gonzalez M, Picazo JJ. Bacteremic pneumonia due to *Staphylococcus aureus*: a comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms. Clin Infect Dis **1999**; 29:1171–7.
16. Kim SH, Kim KH, Kim HB, et al. Outcome of vancomycin treatment in patients with methicillin-susceptible *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother **2008**; 52:192–7.
17. Wolk DM, Struelens MJ, Pancholi P, et al. Rapid detection of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) in wound specimens and blood cultures: multicenter preclinical evaluation of the Cepheid Xpert MRSA/SA skin and soft tissue and blood culture assays. J Clin Microbiol **2009**; 47:823–6.
18. Buchan BW, Ginocchio CC, Manii R, et al. Multiplex identification of gram-positive bacteria and resistance determinants directly from positive blood culture broths: evaluation of an automated microarray-based nucleic acid test. PLoS Med **2013**; 10:e1001478.
19. Altun O, Almuhayawi M, Ullberg M, Ozenci V. Clinical evaluation of the FilmArray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. J Clin Microbiol **2013**; 51:4130–6.

20. Blaschke AJ, Heyrend C, Byington CL, et al. Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system. Diagn Microbiol Infect Dis **2012**; 74:349–55.
21. Kaleta EJ, Clark AE, Johnson DR, et al. Use of PCR coupled with electrospray ionization mass spectrometry for rapid identification of bacterial and yeast bloodstream pathogens from blood culture bottles. J Clin Microbiol **2011**; 49:345–53.
22. Forrest GN, Mehta S, Weekes E, Lincalis DP, Johnson JK, Venezia RA. Impact of rapid in situ hybridization testing on coagulase-negative staphylococci positive blood cultures. J Antimicrob Chemother **2006**; 58:154–8.
23. Holtzman C, Whitney D, Barlam T, Miller NS. Assessment of impact of peptide nucleic acid fluorescence in situ hybridization for rapid identification of coagulase-negative staphylococci in the absence of antimicrobial stewardship intervention. J Clin Microbiol **2011**; 49:1581–2.
24. Forrest GN, Mankes K, Jabra-Rizk MA, et al. Peptide nucleic acid fluorescence in situ hybridization-based identification of *Candida albicans* and its impact on martality and antifungal therapy costs. J Clin Microbiol **2006**; 44:3381–3.
25. Ly T, Gulia J, Pyrgos V, Waga M, Shoham S. 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 **2008**; 4:637–40.
26. Bauer KA, West JE, Balada-Llasat JM, Pancholi P, Stevenson KB, Goff DA. 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 **2010**; 51:1074–80.
27. Perez KK, Olsen RJ, Musick WL, et al. Integrating rapid pathogen identification and antimicrobial stewardship significantly decreases hospital costs. Arch Pathol Lab Med **2013**; 137:1247–54.
28. Huang AM, Newton D, Kunapuli A, et al. Impact of rapid organism identification via matrix-assisted laser desorption/ionization time-of-flight combined with antimicrobial stewardship team intervention in adult patients with bacteremia and candidemia. Clin Infect Dis **2013**; 57:1237–45.
29. Sango A, McCarter YS, Johnson D, Ferreira J, Guzman N, Jankowski CA. Stewardship approach for optimizing antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for Enterococcus species. J Clin Microbiol **2013**; 51:4008–11.
30. Weinstein MP, Mirrett S, Van Pelt L, et al. Clinical importance of identifying coagulase-negative staphylococci isolated from blood cultures: evaluation of MicroScan rapid and dried overnight gram-Positive panels versus a conventional reference method. J Clin Microbiol **1998**; 36:2089–92.
31. Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol **2003**; 41:2275–8.
32. Parta M, Goebel M, Thomas J, Matloobi M, Stager C, Musher DM. Impact of an assay that enables rapid determination of *Staphylococcus* species and their drug susceptibility on the treatment of patients with positive blood culture results. Infect Control Hosp Epidemiol **2010**; 31:1043–8.
33. Clerc O, Prod'hom G, Vogne C, Bizzini A, Calandra T, Greub G. Impact of matrix-assisted laser desorption ionization time-of-flight mass spectrometry on the clinical management of patients with gram-negative bacteremia: a prospective observational study. Clin Infect Dis **2013**; 56:1101–7.
34. Beal SG, Ciurca J, Smith G, et al. Evaluation of the nanosphere Verigene gram-positive blood culture assay with the VersaTREK blood culture system and assessment of possible impact on selected patients. J Clin Microbiol **2013**; 51:3988–92.
35. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis **2004**; 4:751–60.
36. Einsele H, Hebart H, Roller G, et al. Detection and identification of fungal pathogens in blood by using molecular probes. J Clin Microbiol **1997**; 35:1353–60.
37. Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Clin Infect Dis **2001**; 33:1504–12.
38. Maaroufi Y, Heymans C, De Bruyne JM, et al. Rapid detection of *Candida albicans* in clinical blood samples by using a TaqMan-based PCR assay. J Clin Microbiol **2003**; 41:3293–8.
39. Kollef MH, Sherman G, Ward S, Fraser VJ. Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. Chest **1999**; 115:462–74.
40. Harbarth S, Garbino J, Pugin J, Romand JA, Lew D, Pittet D. Inappropriate initial antimicrobial therapy and its effect on survival in a clinical trial of immunomodulating therapy for severe sepsis. Am J Med **2003**; 115:529–35.
41. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M. The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev **2010**; 23:235–51.
42. Ecker DJ, Sampath R, Li H, et al. New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn **2010**; 10:399–415.
43. Reinhart K, Bauer M, Riedemann NC, Hartog CS. New approaches to sepsis: molecular diagnostics and biomarkers. Clin Microbiol Rev **2012**; 25:609–34.
44. Herne V, Nelovkov A, Kutt M, Ivanova M. Diagnostic performance and therapeutic impact of LightCycler SeptiFast assay in patients with suspected sepsis. Eur J Microbiol Immunol (Bp) **2013**; 3:68–76.
45. Dierkes C, Ehrenstein B, Siebig S, Linde HJ, Reischl U, Salzberger B. Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis. BMC Infect Dis **2009**; 9:126.
46. Neely LA, Audeh M, Phung NA, et al. T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med **2013**; 5:182ra54.