Advances in Rapid Molecular Blood Culture Diagnostics: Healthcare Impact, Laboratory Implications, and Multiplex Technologies

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Background: For far too long, the diagnosis of bloodstream infections has relied on time-consuming blood cultures coupled with traditional organism identification and susceptibility testing. Technologies to define the culprit in bloodstream infections have gained sophistication in recent years, notably by application of molecular methods.

Content: In this review, we summarize the tests available to clinical laboratories for molecular rapid identification and resistance marker detection in blood culture bottles that have flagged positive. We explore the cost–benefit ratio of such assays, covering aspects that include performance characteristics, effect on patient care, and relevance to antibiotic stewardship initiatives.

Summary: Rapid blood culture diagnostics represent an advance in the care of patients with bloodstream infections, particularly those infected with resistant organisms. These diagnostics are relatively easy to implement and appear to have a positive cost–benefit balance, particularly when fully incorporated into a hospital's antimicrobial stewardship program.

IMPACT STATEMENT

Understanding the value and role of rapid molecular blood culture diagnostics in the healthcare system is vital to the practice of clinical laboratories and providers.

Diagnosis of bloodstream infection (BSI)[3] caused by routine bacteria and yeast with traditional identification and susceptibility methods requires, above all things, time. Blood culture bottles are inoculated with patient blood and then incubated on an automated microorganism growth detection system. After a bottle flags positive, the blood culture bottle is removed, the broth is Gram stained, and then an aliquot is subcultured to solid media. Overnight growth allows organisms to become sufficiently visible to distinguish pure from mixed infections and to be used for conventional

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DOI: 10.1373/jalm.2018.027409

[3] **Nonstandard abbreviations:** BSI, bloodstream infection; AST, antimicrobial susceptibility testing; MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum beta-lactamase; FDA, Food and Drug Administration; MDRO, multidrug resistant organism; MSSA, methicillin-susceptible *Staphylococcus aureus*.

biochemical identification. Antimicrobial susceptibility testing (AST), the method of which depends on the organism identification, requires at least an additional day. Clearly, the limitations in turnaround time were apparent decades ago to clinicians, who grew accustomed to using empiric therapy for prolonged periods. Consequently, clinical microbiologists devised means for rapid organism characterization by biochemical or serological methods. Direct from positive-bottle coagulase testing, for example, could be performed on small aliquots of broth containing gram-positive cocci for expedited identification of *Staphylococcus aureus* and coagulase-negative staphylococci (*1*, *2*). Direct inoculation of organisms to automated identification systems, such as Vitek, Microscan, and others, has long been validated and used by some clinical laboratories, with a turnaround time of as little as 4 h for identification and 6–8 h for susceptibility testing (*3*–*5*). Technology has advanced the early characterization of blood culture isolates from nucleic acid probe methods toward sample-to-answer molecular assays (Table 1). In this review, we will describe the effect that these molecular technologies have had on healthcare, discuss factors to be considered for laboratories looking to implement these tests, and present the platforms currently available for clinical laboratories.

HEALTH EFFECT CONSIDERATIONS

The Gram stain, one of the most fundamental methods in microbiology, by itself provides immediately actionable information for providers and has been shown to decrease mortality from BSI the quicker the results are delivered (*6*, *7*). Direct-from-bottle susceptibility, which has been validated by laboratories for decades, has been shown to result in healthcare cost savings and adjustments to directed therapy sooner than the conventional setup (*5*). Because implementation of molecular techniques would represent significant added costs to the laboratory, it is vital to consider how these incremental advances in technology can positively affect the provision of care even further for BSI while decreasing overall healthcare costs.

For BSIs, clinicians will always want fast, actionable, and reliable results. Nucleic acid–based methods can provide more definitive results than biochemical methods, making them attractive tools for BSI diagnosis. Intuitively, these rapid tests have potential to affect patient care, but the extent of the effect cannot be quantified without clinical studies. Common metrics that studies have used to determine if rapid identification technologies are clinically useful include days on broad-spectrum antibiotics, days until start of directed therapy, patient mortality, and length of stay in hospital or intensive care unit. Retrospective studies at individual institutions suggest that the implementation of rapid blood culture identification assays has improved patient care. On average, providers are initiating appropriate antibiotics more promptly while limiting patient exposure to broad-spectrum agents, with some studies demonstrating an effect on patient mortality and decreased healthcare costs (*8*–*10*). Clinician judgment and hospital culture affect the interpretation of test results and thus likely confound the results and explain variations. As technologies progress to lessen turnaround time of test results while becoming less expensive, the positive effect on the delivery of healthcare will continue to grow.

Barriers to achieving the desired outcome when providing rapid identification and resistance determinants have also been studied. Providers are not typically well informed about how these new technologies fit with existing procedures or how to correctly interpret a microbiology laboratory report of the results (*11*). The utility of organism identification without similarly rapid AST results could be variable by institution in terms of patient complexity and the local antibiogram. Broad-spectrum antibiotics may be continued despite rapid organism

Table 1. Description of 4 FDA-approved multiplexed assays for rapid identification of microorganisms from positive blood cultures.

Assay	Organisms targeted	Resistance mechanisms targeted	Method	Run time
Verigene BC-GP (Luminex Corp.)	*S. aureus*	*mecA*	DNA microarray	2.5 h
	S. epidermidis	*vanA*		
	S. lugdunensis	*vanB*		
	S. pneumoniae			
	S. pyogenes			
	E. faecalis			
	E. faecium			
	L. monocytogenes			
Verigene BC-GN (Luminex Corp.)	*E. coli*	bla_{CTX-M}	DNA microarray	2.5 h
	K. pneumoniae	bla_{KPC}		
	K. oxytoca	bla_{OXA-48}		
	Citrobacter spp.	bla_{-IMP}		
	Enterobacter spp.	bla_{VIM}		
	Proteus spp. *Acinetobacter* spp.	bla_{NDM}		
	P. aeruginosa			
FilmArray BCID (bioMérieux)	*Staphylococcus*	*mecA*	Nested PCR	~1 h
	S. aureus	*vanA/B*		
	Streptococcus	bla_{KPC}		
	S. agalactiae			
	S. pyogenes			
	S. pneumoniae			
	Enterococcus			
	L. monocytogenes			
	A. baumannii			
	H. influenzae			
	N. meningitidis			
	P. aeruginosa			
	Enterobacteriaceae			
	E. coli			
	E. cloacae complex			
	K. oxytoca			
	K. pneumoniae			
	Proteus			
	S. marcescens			
	C. albicans			
	C. glabrata			
	C. krusei			
	C. parapsilosis			
	C. tropicalis			
iC-GPC (iCubate)	*S. aureus*	*mecA*	Amplicon rescue multiplex PCR	4–5 h
	S. epidermidis	*vanA*		
	S. pneumoniae	*vanB*		
	E. faecalis			
	E. faecium			

identification for a variety of reasons, such as need for increased confidence in full AST profile before deescalation or before patient discharge or empiric treatment of suspected infection localized elsewhere (*8*, *12*, *13*). For gram-negative organisms, deescalation may be especially difficult because the antibiotic resistance mechanisms are diverse and complex, well beyond what is targeted by available assays.

Other barriers include available resources for intensive antimicrobial stewardship support and the high cost of sample-to-answer kits compared to nonmolecular methods such as MALDI-TOF mass spectrometry (*14*). Maximal clinical and cost benefits of these molecular assays for BSI cannot be achieved without collaboration between the laboratory and its clinical partners. Implementation of new testing alone may decrease turnaround time for the laboratory result and yet not result in improvements in clinical outcomes without antimicrobial stewardship intervention (*15*). Conversely, antimicrobial stewardship alone has been shown to decrease time to effective or optimal therapy for BSI (*16*), but rapid laboratory testing can greatly augment the effect (*8*, *9*, *17*, *18*). Some studies on the effect of BSI diagnostics have paired implementation of rapid molecular diagnostics with intensive antimicrobial stewardship (*8*, *19*, *20*). However, a stewardship service operating without interruption in coverage may not be an achievable reality in many hospitals. Recent clinical guidelines for the creation of antimicrobial stewardship programs from the Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America include provisions to further research and develop rapid diagnostic tests to help limit antimicrobial use (*21*).

For a typical 500-bed community hospital, additional multiplex testing of positive blood cultures could cost upwards of a $500 000/year in reagents alone. An informed cost analysis, weighing the high cost of implementation against the cost benefits to the hospital, is essential to choosing a diagnostic test wisely. One 2008 economic analysis reasonably questioned the cost-effectiveness of molecular identification of *S. aureus* from positive blood cultures compared to tube coagulase testing (*22*). Such questions continue today as testing becomes more advanced and highly multiplexed and hence, generally more expensive. A recent comprehensive cost-effectiveness study using evidence-based modeling found that out of 12 strategies for BSI diagnosis, MALDI-TOF from blood culture broth combined with antimicrobial stewardship was the most cost-effective strategy, although some of the molecular tests discussed in this review were also considered cost-effective options (*14*). The assumptions made in a broad analysis do not all apply to a given institution. As such, calculations must be individualized on the basis of institution-specific factors.

LABORATORY CONSIDERATIONS

Vital factors to consider in assessing feasibility of implementation include laboratory staffing, skill level of technologists, and diversity of test menu available on a platform. A laboratory that does not have third-shift personnel who are proficient at reading Gram stains may consider implementing a blood culture panel that does not require a Gram stain interpretation before testing, such as BioFire BCID (bioMérieux). If a laboratory's staffing level cannot accommodate on-demand molecular testing but requires batch testing, the opportunity for clinical effect is much diminished. Such may be the case for testing from blood culture bottles, which requires more hands-on time, e.g., peptide nucleic acid fluorescence in situ hybridization or MALDI-TOF.

With the increasing number of options for molecular testing from positive blood culture bottles, the laboratory plays an important role in selection of the optimal platform for its patients. Different patient populations will see a different spread of the common BSI pathogens (Table 2). Tests with

Table 2. Summary of causative agents of BSIs, based on patient population, each reported as a percentage of total.

Pathogen	Nosocomial BSI in 24 179 adult patients from national surveillance study, % (*26*)[a]	BSI in 1470 adult patients from 9 US community hospital, % (*64*)[c]	Community-acquired bacterial BSI in 1158 (+) cultures from adult HIV+ patients, US and Europe, % (*65*)	Nosocomial BSI in 2340 adult oncologic patients, US, % (*66*)[d]	BSI in 1484 newborn infants, US, % (*67*)
Gram positive					
S. aureus	20.2	28.3	19.6	11.5	3.5
Other *Staphylococcus* species	31.3	9.8	7.3	30.2	
Enterococcus species	9.4	3.4	2.9	11.6	3.1
Streptococcus species	See footnote[b]	4.8	*S. pneumoniae:* 22.8	6.0	*S. agalactiae*: 35.8
			Other: 7.8		Viridans group: 18.9
					Group D: 1.4
					S. pneumoniae: 0.9
Other			*Rhodococcus equi*: 0.9	1.2	*Listeria monocytogenes*: 1.3
Gram negative					
E. coli	5.6	23.7	10.5	7.6	24.8
Klebsiella species	4.8	6.6	1.2	6.4	0.9
Enterobacter species	3.9	2.0	2.6	3.0	
Serratia species	1.7	0.9			
Pseudomonas species	4.3	3.4	7.1	4.4	
Acinetobacter species	1.3	0.7	1.5		
Other		*Proteus* spp.: 3.8	*Salmonella* spp.: 12.4	3.2	*H. influenzae*: 4.5
		Citrobacter spp.: 0.7	*H. influenzae*: 2.5		
			Campylobacter spp.: 1.5		
Anaerobes	1.3	0.9		3.4	
Fungi					
Candida species	9.0	0.7	Not reviewed	8.5	
Polymicrobial	13.2	4.1	Not reviewed	14.1	1.6

[a] Except for anaerobes and polymicrobial BSI, percentages given are for monomicrobial episodes (n = 20 978).
[b] Composite data not provided; viridans group streptococci accounted for 0.5% of nonneutropenic patients and 2.0% of neutropenic patients.
[c] Except for polymicrobial BSI, percentages given are for all isolates (n = 1514).
[d] Except for polymicrobial BSI, percentages given are for all isolates (n = 2711).

the biggest effect on patient care will identify resistance determinants or organism species that would alter management and do so in a large proportion of the institution's BSI cases. Choice of panel could therefore depend on the local prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci, and extended-spectrum β-lactamase (ESBL)-producing and carbapenemase-producing gram-negative organisms. This prevalence would determine the priority for detection of certain resistance determinants, e.g., *mecA, vanA/B,* bla_{CTX-M}, bla_{KPC}, etc., which at this time differ by assay. Gram-positive organisms generally cause most positive blood cultures, so the volume of gram-negative or yeast isolates from blood cultures could also help decide the most cost-effective molecular panels to implement.

Like any method, molecular tests also bear technical limitations that the laboratory should be familiar with before their implementation. Considerations could include the likelihood of DNA contamination, level of cross-reactivity among genetically similar organisms, and reliability of genotype in predicting phenotype. For example, with molecular methods it is not possible to reliably distinguish between *Escherichia coli* and *Shigella* spp., and some methods do not reliably distinguish between *Streptococcus pneumoniae* and other *S. mitis* group members. For *S. aureus*, most methicillin resistance is attributable to *mecA* but could also be associated with the less common *mecC* and newly described *mecB* (*23*). In addition, there are many genetic variants in the frequently targeted staphylococcal cassette chromosome *mec* (SCC*mec*), which molecular assays in the past have not always captured (*24*). Laboratories must decide whether to confirm identifications after growth on solid media in light of these caveats. Another common limitation of BSI panels is their decreased ability to detect mixed infections, which may comprise a significant percentage of BSIs depending on the patient population (Table 2). These factors highlight the importance of comprehensive assay validation before implementation.

The consideration that ought to go into creation of interpretive comments for these rapid BSI assays should not be overlooked. Laboratories need to finely balance the interpretive information that they give providers and take into account the Food and Drug Administration (FDA)-cleared claims of the assay in question. On the one hand, caveats such as those mentioned above are important to communicate so that providers understand the limitations of the testing. On the other hand, results with caveats and disclaimers that cast too much uncertainty may preclude clinical action and work against stewardship efforts. A report of "presumptive methicillin-susceptible *S. aureus* (MSSA)" may not be worded strongly enough to convince a provider to deescalate from vancomycin and may lead some providers to simply wait for confirmatory test results. The laboratory director has the task of deciding, on the basis of the laboratory's own data and the package insert information, how to couch the result in a way that is both accurate and effective at informing their clinicians.

It is expected that phenotypic AST results will be discordant with genotype results on occasion, and laboratories must be prepared for these occurrences. Examples of discordant results that are encountered by laboratories are a *mecA*-positive result on a *S. aureus* isolate that tests susceptible to cefoxitin or oxacillin or an *E. coli* that is positive for bla_{CTX-M} but tests as indeterminate or negative by ESBL phenotype testing. Guidance from the Clinical and Laboratory Standards Institute on resolving these discordant results is available. The general approach is to confirm results of the discordant tests, with final recommendations based on evidence from peer-reviewed literature (*25*).

Undeniably, laboratories play an increasingly central role in efforts against multidrug resistant organisms (MDRO). Large-scale studies have shown that the burden of antibiotic-resistant organisms causing BSI has increased in recent years,

and this increase is in addition to the steady baseline level of BSI due to antibiotic-susceptible organisms (*26*, *27*). Multiplexed panels on positive blood cultures have introduced many laboratories to the routine identification of genetic mechanisms of drug resistance of epidemiological importance. Working with infection preventionists is key to capitalizing on these data for prompt patient isolation and MDRO tracking. Integrative decision support systems linked to laboratory results and the electronic medical record are further being used to assist in the rapid capture of identifying these highly resistant organisms (*28*). Laboratories should understand and help decide how rapid detection of an ESBL or carbapenem resistance determinant would affect infection prevention practices. This determination is facilitated by having collaborative discussions with infectious diseases specialists, infection preventionists, and antimicrobial stewardship team members.

Laboratories should also be prepared for reporting potentially unusual genotypic resistance results. A thorough assay verification study involving a wide variety of MDROs will serve as a foundation for the laboratory's ability to report reliable results. Strains of well-characterized MDROs are available from many sources, but the Centers for Disease Control and Prevention AR Bank is a particularly valuable resource (https://wwwn.cdc.gov/arisolatebank/). As laboratories gain confidence in the reliability of these assays, they can more confidently report results, for instance a *Pseudomonas aeruginosa* unexpectedly harboring bla_{KPC} or detection of a carbapenemase gene of low local prevalence.

NUCLEIC ACID PROBE-BASED METHODS

Because a positive blood culture bottle contains amplified numbers of organisms, an analytically sensitive method is not necessarily desirable or a prerequisite for further organism characterization, as exemplified by the Gram stain. Advantages of probe-based methods include the obviation of DNA contamination events leading to false-positive results and potentially decreased reagent and capital expenses. Currently, FDA-cleared probe-based methods require somewhat more hands-on time than nucleic acid amplification methods, and they require prior Gram stain interpretation before testing.

One of the earliest molecular methods for rapid bloodstream pathogen identification was PNA FISH (AdvanDx). The procedure is performed on a slide spotted with the positive blood culture broth. The fluorescently labeled, synthetic PNA probe diffuses through the pathogen cell wall and binds to ribosomal RNA. The FISH label provides visualization of bound PNA-RNA hybrids under fluorescent microscopy. The method has excellent accuracy, with 95%–100% sensitivity and specificity for *Staphylococcus* spp., *Enterococcus* spp., *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *Candida* spp. from positive blood culture broths (*29*–*32*). The original process consisted of a series of stains, incubations, and washes, consuming much hands-on time of licensed personnel. Subsequently, a more streamlined method (*Quick*FISH) was developed without loss in performance (*33*–*35*). The need for fluorescent microscopy, a luxury in some laboratories, still precludes widespread use. PNA FISH tests are available separately for *Staphylococcus*, *Enterococcus*, gram-negative bacilli, and *Candida* spp. Particularly impactful are the panels for *Candida*, including the *C. albicans*/*C. glabrata* and the Yeast Traffic Light PNA FISH systems. Because the antifungal susceptibility profile of clinically significant *Candida* spp. is fairly predictable, provision of an identification, grouped by typical susceptibility to fluconazole, can provide valuable preliminary information (*36*, *37*). Cross-reactivity of the yeast PNA FISH probes with less commonly encountered yeast species may be seen, for example, between *C. parapsilosis* and the closely related *C. orthopsilosis* and *C. metapsilosis* (manufacturer's data), between *C. glabrata* or *C. krusei* and *Rhodococcus mucilaginosa,* or *C. albicans* or *C. parapsilosis* and *C.*

lambica (*31*). Positive clinical outcomes have also been associated with use of other PNA FISH tests, including for enterococci and staphylococci (*32*, *38*). For example, among cancer patients at a tertiary care center, the *Staphylococcus* QuickFISH test was associated with less vancomycin use and vancomycin-level monitoring, shortened median antibiotic duration by 1 day, and half as many central venous catheter removals compared to the control group (*38*).

The Verigene system (Luminex Corp.) was FDA cleared in 2012 for its gram-positive panel test (BC-GP) and in 2014 for its gram-negative panel (BC-GN). An aliquot of positive blood culture broth is loaded onto a cartridge and placed on the processor, where organism DNA is sheared and then captured on a DNA microarray. The cartridge requires manual transfer to a reader for signal detection, which is based on interaction of silver and gold nanoparticles. The analytical time totals close to 2.5 h.

The BC-GP distinguishes coagulase-negative staphylococci from *S. aureus*, MRSA from MSSA, vancomycin-resistant enterococci from vancomycin-susceptible enterococci, and several clinically significant streptococci to species level. Overall accuracy in identification of panel targets, including *mecA, vanA,* and *vanB*, is reported to be more than 95% (*39*, *40*). Limitations include decreased sensitivity in identifying panel targets in polymicrobial blood cultures, with 1 large study reporting that the BC-GP missed 28% of these (*39*). Mixed cultures with more than 1 *Staphylococcus* spp. along with a positive result for *mecA* should be interpreted with caution as the assay cannot determine which *Staphylococcus* spp. harbors the *mecA* gene. Also, downstream confirmation of *S. pneumoniae* results are particularly necessary owing to the cross-reactivity within the *S. mitis* group, leading to false-positive *S. pneumoniae* results (*40*, *41*). Coupled with antimicrobial stewardship, use of the BC-GP has been found by clinical studies to be associated with quicker antibiotic optimization (12.5–27 h faster vs preintervention) and shortened antibiotic treatment duration for gram-positive blood culture contaminants (18–37 h reduction), but conclusions differ on whether mortality or hospital length of stay is decreased (*10*, *42*, *43*). One study did find that for enterococcal BSI specifically, implementation of the panel significantly correlated with a 21.7-day reduction in length of stay and more than $60 000 reduction in mean hospital costs (*44*).

The Verigene gram-negative panel BC-GN covers common gram-negative organisms in addition to the resistance genes $bla_{CTX\text{-}M}$ for ESBL and 5 plasmid-mediated carbapenemase genes (Table 1). The inclusion of the most common ESBL mechanism in the US ($bla_{CTX\text{-}M}$) provides information that was not previously available by molecular testing in an FDA-cleared assay. In a large multicentered study of 1847 blood cultures containing gram-negative bacteria, the BC-GN had an overall sensitivity of 98% and specificity of 100% in organism identification from monomicrobial blood cultures. In polymicrobial cultures, target organisms were detected in only 54% of cases. Users may encounter discordance in identification of *K. variicola* because the BC-GN will not detect *K. variicola*, whereas MALDI-TOF and biochemical panels misidentify these isolates as *K. pneumoniae* (*45*). Analytical sensitivity in detecting resistance determinants ranged from 94.3% to 100% depending on the target (*45*, *46*). The predictive value of negative $bla_{CTX\text{-}M}$ for susceptibility to third-generation cephalosporins can be examined by clinical laboratories that use this panel and can potentially be exploited to help guide therapy (*47*). Studies have found that compounded with antimicrobial stewardship, the BC-GN panel can help decrease time to optimal therapy by 11–14 h but vary in findings terms of effect on length of stay or hospital costs (*12*, *48*, *49*).

NUCLEIC ACID AMPLIFICATION-BASED METHODS

Sample-to-answer, PCR-based assays for *S. aureus* and *mecA* directly on positive blood culture broths have been implemented in many clinical

laboratories for some time and have included both laboratory-developed and FDA-cleared tests (*50*–*52*). Currently the MRSA/SA BC performed on the Xpert PCR system (Cepheid) is the only such assay that is FDA cleared for testing on positive blood cultures. Its analytical sensitivity and specificity for both *S. aureus* and MRSA detection are higher than 98% (*53*). A test that can identify *S. aureus* from coagulase-negative staphylococci and differentiate MRSA from MSSA can have high clinical effect and be particularly cost-effective. Still, it is critically important to incorporate antimicrobial stewardship efforts into such testing for MSSA and MRSA to maximize patient benefit and healthcare savings. One study illustrated that alongside antimicrobial stewardship intervention, the implementation of the MRSA/SA BC PCR resulted in directed therapy for MSSA 1.7 days sooner and mean hospital costs that were significantly lower by more than $21 000 (*51*).

The FilmArray BCID panel is a comprehensive panel that encompasses gram-positive, gram-negative, and fungal organisms. Resistance determinants are limited to *mecA, vanA or vanB*, and $blac_{KPC}$. With 27 targets, the cost per panel is higher than the other aforementioned assays. It can be performed on positive blood cultures bottles without prior Gram stain interpretation. The sample-to-answer method requires less than 1 h run time with minimal hands-on manipulation. Results from a multicentered trial showed reliability in identification of organism and resistance determinants on the panel, with sensitivity of more than 96% for all panel members after resolution testing. *Raoultella ornithinolytica* isolates led to discordant cases in which phenotypic testing misidentified them as *Klebsiella oxytoca* while the BCID panel did not (*54*). Polymicrobial cultures can present difficulty for the BCID, leading to false-positive results in 46% and false-negative results in roughly 30% of cases (*54*, *55*).

Unlike probe-based methods, the FilmArray uses nested PCR, which introduces concern of contamination with DNA from either viable or nonviable organisms in the blood culture broth or testing environment. Issues are periodically reported with false-positive results, including a recall in 2014 for false-positive *Enterococcus* and *Pseudomonas aeruginosa* results and contemporary issues with false-positive *Proteus* spp. results. These occurrences highlight the limitations of using nested PCR to amplify nucleic acid targets on positive blood culture broths. While microbiologic reagents such as blood culture bottles are sterile, they are not necessarily free of nucleic acid.

The sole randomized clinical trial examining rapid molecular testing on positive blood cultures studied the use of the BCID in a group without antimicrobial stewardship, use of BCID in a group combined with antimicrobial stewardship, and the use of standard blood culture processing (*8*). Both BCID groups were associated with more frequent directed antimicrobial use with shorter times until treatment optimization. No significant differences were found for length of hospital stay, mortality, or cost between any of the 3 groups. A retrospective study identified similar findings when comparing conventional organism identification with or without antimicrobial stewardship to BCID with stewardship (*17*). In contrast, other retrospective studies have reported decreased hospital length of stay, healthcare costs, and empirical vancomycin use for gram-positive cocci alongside antimicrobial stewardship (*56*). Another study combined the use of the BCID panel with a stewardship program and found significantly improved time to deescalation and reduction in the use of antipseudomonal agents (*20*).

The relatively new iCubate system for gram-positive cocci (iC-GPC), FDA cleared in 2017, has a more limited multiplexed panel (Table 1). It uses a patented amplicon rescue multiplex PCR (ARM-PCR) method at its core, which involves multiplex PCR with microarray hybridization followed by fluorescence-based signal detection (*57*). Like the Verigene system, the test cassette is manually

transferred between a processor and reader unit. Turnaround time is 4–5 h. Sensitivity and specificity exceeded 95% for organism identification and resistance gene detection for all target organism identifications except for *S. pneumoniae*, for which there were 4 false-negative results out of 27 cases (*58*).

OTHER METHODS

There have been other unique methods developed for detecting pathogens from positive blood culture broths. While they may not have enjoyed widespread use, they bear mentioning as creative avenues for cost-effective, rapid diagnostics. An immunochromatographic method for detection of *S. aureus* from blood cultures in less than 30 min (Binax NOW, Alere) had promising performance characteristics and could be used in conjunction with serologic PBP2a detection for MRSA (*59*, *60*). A bacteriophage-based method, the KeyPath MRSA/MSSA blood culture test (MMBT, MicroPhage, Inc) was FDA cleared in 2012 but did not sustain long-term commercial availability. It used bacteriophage specific to *S. aureus* to self-amplify and allow detection by specific antibodies in a process that required more than 5 h. Assay performance was stronger in specificity than sensitivity (*61*, *62*).

Specific Diagnostics has developed a novel method of early pathogen identification simultaneously with microorganism growth in blood culture bottles. The concept is based on detection of signature volatile organic compounds emitted from organisms that allow for their identification as demonstrated in seeded studies (*63*).

FUTURE DIRECTIONS

For the foreseeable future, use of bacterial cultures will continue to be necessary and relevant to the characterization of isolates for patient care purposes. Areas of assay development that require improvement include the ability to detect polymicrobial BSIs and miniaturization of test systems (to minimize instrument footprint) and test cartridges (to minimize hazardous waste disposal). Even as this review is written, additional platforms that improve on the first generation of highly multiplexed assays are nearing FDA clearance. Laboratories will see panels with broader pathogen coverage and more comprehensive targeting of resistance mechanisms. New paradigms for rapid BSI diagnosis have already been FDA cleared and made commercially available, such as direct from peripheral blood pathogen detection (T2 Biosystems) and automated AST from positive blood culture bottles with a turnaround time of less than 6 h (Accelerate Diagnostics). We will see market trends continue down this course toward faster, cheaper, and more informative microbiological results to help manage BSI.

CONCLUSION

The rapid identification of organisms from positive blood cultures is a critical component in providing quality healthcare. Nucleic acid probe and amplification tests have significantly improved the speed and accuracy of results in blood stream infections. Their effect on the quality of healthcare continues to evolve but overall has been positive. This positive result is especially true when the laboratory partners with antimicrobial stewardship programs to direct appropriate therapy quickly. Overall, the laboratory implementation of these molecular techniques needs to be thoughtfully adapted on the basis of a number of factors including the hospital, laboratory capabilities, clinicians, and patient population.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

REFERENCES

1. Davis TE, Fuller DD, Aeschleman EC. Rapid, direct identification of Staphylococcus aureus and Streptococcus pneumoniae from blood cultures using commercial immunologic kits and modified conventional tests. Diagn Microbiol Infect Dis 1992;15:295–300.
2. McDonald CL, Chapin K. Rapid identification of Staphylococcus aureus from blood culture bottles by a classic 2-hour tube coagulase test. J Clin Microbiol 1995; 33:50–2.
3. Weinstein MP, Mirrett S, Van Pelt L, McKinnon M, Zimmer BL, Kloos W, Reller LB. 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.
4. Malloy PJ, Ducate MJ, Schreckenberger PC. Comparison of four rapid methods for identification of Enterobacteriaceae from blood cultures. J Clin Microbiol 1983;17:493–9.
5. Trenholme GM, Kaplan RL, Karakusis PH, Stine T, Fuhrer J, Landau W, Levin S. Clinical impact of rapid identification and susceptibility testing of bacterial blood culture isolates. J Clin Microbiol 1989;27:1342–5.
6. Cunney RJ, McNamara EB, Alansari N, Loo B, Smyth EG. The impact of blood culture reporting and clinical liaison on the empiric treatment of bacteraemia. J Clin Pathol 1997;50:1010–2.
7. Barenfanger J, Graham DR, Kolluri L, Sangwan G, Lawhorn J, Drake CA, et al. Decreased mortality associated with prompt Gram staining of blood cultures. Am J Clin Pathol 2008;130:870–6.
8. Banerjee R, Teng CB, Cunningham SA, Ihde SM, Steckelberg JM, Moriarty JP, et al. Randomized trial of rapid multiplex polymerase chain reaction-based blood culture identification and susceptibility testing. Clin Infect Dis 2015;61:1071–80.
9. Bork JT, Leekha S, Heil EL, Zhao L, Badamas R, Johnson JK. Rapid testing using the Verigene Gram-negative blood culture nucleic acid test in combination with antimicrobial stewardship intervention against Gram-negative bacteremia. Antimicrob Agents Chemother 2015;59:1588–95.
10. Felsenstein S, Bender JM, Sposto R, Gentry M, Takemoto C, Bard JD. Impact of a rapid blood culture assay for Gram-positive identification and detection of resistance markers in a pediatric hospital. Arch Pathol Lab Med 2016;140:267–75.
11. Donner LM, Campbell WS, Lyden E, Van Schooneveld TC. Assessment of rapid-blood-culture-identification result interpretation and antibiotic prescribing practices. J Clin Microbiol 2017;55:1496–507.
12. Walker T, Dumadag S, Lee CJ, Lee SH, Bender JM, Cupo Abbott J, She RC. Clinical impact of laboratory implementation of Verigene BC-GN microarray-based assay for detection of Gram-negative bacteria in positive blood cultures. J Clin Microbiol 2016;54:1789–96.
13. Belknap A, Grosser DS, Hale DA, Lang BJ, Colley P, Benavides R, Dhiman N. Clinical uptake of antimicrobial stewardship recommendations following Nanosphere Verigene blood culture Gram-negative reporting. Proc (Bayl Univ Med Cent) 2017;30:395–9.
14. Pliakos EE, Andreatos N, Shehadeh F, Ziakas PD, Mylonakis E. The cost-effectiveness of rapid diagnostic testing for the diagnosis of bloodstream infections with or without antimicrobial stewardship. Clin Microbiol Rev 2018;31.
15. Cosgrove SE, Li DX, Tamma PD, Avdic E, Hadhazy E, Wakefield T, et al. Use of PNA FISH for blood cultures growing Gram-positive cocci in chains without a concomitant antibiotic stewardship intervention does not improve time to appropriate antibiotic therapy. Diagn Microbiol Infect Dis 2016;86:86–92.
16. Pogue JM, Mynatt RP, Marchaim D, Zhao JJ, Barr VO, Moshos J, et al. Automated alerts coupled with antimicrobial stewardship intervention lead to decreases in length of stay in patients with Gram-negative bacteremia. Infect Control Hosp Epidemiol 2014;35: 132–8.
17. MacVane SH, Nolte FS. Benefits of adding a rapid PCR-based blood culture identification panel to an established antimicrobial stewardship program. J Clin Microbiol 2016;54:2455–63.
18. Perez KK, Olsen RJ, Musick WL, Cernoch PL, Davis JR, Peterson LE, Musser JM. Integrating rapid diagnostics and antimicrobial stewardship improves outcomes in patients with antibiotic-resistant Gram-negative bacteremia. J Infect 2014;69:216–25.
19. 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.
20. Bookstaver PB, Nimmich EB, Smith TJ, 3rd, Justo JA, Kohn J, Hammer KL, et al. Cumulative effect of an antimicrobial stewardship and rapid diagnostic testing bundle on early streamlining of antimicrobial therapy in Gram-negative

bloodstream infections. Antimicrob Agents Chemother 2017;61:pii: e00189-17.

21. Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). Infect Control Hosp Epidemiol 2012;33:322–7.
22. Hermsen ED, Shull SS, Klepser DG, Iwen PC, Armbrust A, Garrett J, et al. Pharmacoeconomic analysis of microbiologic techniques for differentiating staphylococci directly from blood culture bottles. J Clin Microbiol 2008;46:2924–9.
23. Becker K, van Alen S, Idelevich EA, Schleimer N, Seggewiß J, Mellmann A, et al. Plasmid-encoded transferable mecB-mediated methicillin resistance in Staphylococcus aureus. Emerg Infect Dis 2018;24:242–8.
24. Thomas L, van Hal S, O'Sullivan M, Kyme P, Iredell J. Failure of the BD GeneOhm StaphS/R assay for identification of Australian methicillin-resistant Staphylococcus aureus strains: duplex assays as the "gold standard" in settings of unknown SCCmec epidemiology. J Clin Microbiol 2008;46:4116–7.
25. Clinical and Laboratory Standards Institute. Use of molecular assays for resistance detection. https://s3-ap-southeast-1.amazonaws.com/clsi/Use_of_Molecular_Assays_for_Resistance_Detection_2_3.zip (Accessed May 2018).
26. 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.
27. Ammerlaan HS, Harbarth S, Buiting AG, Crook DW, Fitzpatrick F, Hanberger H, et al. Secular trends in nosocomial bloodstream infections: antibiotic-resistant bacteria increase the total burden of infection. Clin Infect Dis 2013;56:798–805.
28. Forrest GN, Van Schooneveld TC, Kullar R, Schulz LT, Duong P, Postelnick M. Use of electronic health records and clinical decision support systems for antimicrobial stewardship. Clin Infect Dis 2014;59 Suppl 3:S122–33.
29. Della-Latta P, Salimnia H, Painter T, Wu F, Procop GW, Wilson DA, et al. Identification of Escherichia coli, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in blood cultures: a multicenter performance evaluation of a three-color peptide nucleic acid fluorescence in situ hybridization assay. J Clin Microbiol 2011;49:2259–61.
30. Chapin K, Musgnug M. Evaluation of three rapid methods for the direct identification of Staphylococcus aureus from positive blood cultures. J Clin Microbiol 2003;41: 4324–7.
31. Hall L, Le Febre KM, Deml SM, Wohlfiel SL, Wengenack NL. Evaluation of the Yeast Traffic Light PNA FISH probes for identification of Candida species from positive blood cultures. J Clin Microbiol 2012;50:1446–8.
32. Forrest GN, Roghmann MC, Toombs LS, Johnson JK, Weekes E, Lincalis DP, Venezia RA. Peptide nucleic acid fluorescent in situ hybridization for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy. Antimicrob Agents Chemother 2008;52:3558–63.
33. Morgan M, Marlowe E, Della-Latta P, Salimnia H, Novak-Weekley S, Wu F, Crystal BS. Multicenter evaluation of a new shortened peptide nucleic acid fluorescence in situ hybridization procedure for species identification of select Gram-negative bacilli from blood cultures. J Clin Microbiol 2010;48:2268–70.
34. Koncelik DL, Hernandez J. The impact of implementation of rapid QuickFISH testing for detection of coagulase-negative staphylococci at a community-based hospital. Am J Clin Pathol 2016;145:69–74.
35. Deck MK, Anderson ES, Buckner RJ, Colasante G, Davis TE, Coull JM, et al. Rapid detection of Enterococcus spp. direct from blood culture bottles using Enterococcus QuickFISH method: a multicenter investigation. Diagn Microbiol Infect Dis 2014;78:338–42.
36. Forrest GN, Mankes K, Jabra-Rizk MA, Weekes E, Johnson JK, Lincalis DP, Venezia RA. Peptide nucleic acid fluorescence in situ hybridization-based identification of Candida albicans and its impact on mortality and antifungal therapy costs. J Clin Microbiol 2006;44:3381–3.
37. Heil EL, Daniels LM, Long DM, Rodino KG, Weber DJ, Miller MB. Impact of a rapid peptide nucleic acid fluorescence in situ hybridization assay on treatment of Candida infections. Am J Health Syst Pharm 2012;69:1910–4.
38. Seo SK, Gedrimaite Z, Paskovaty A, Seier K, Morjaria S, Cohen N, et al. Impact of QuickFISH in addition to antimicrobial stewardship on vancomycin use and resource utilization in cancer patients with coagulase-negative staphylococcal blood cultures. Clin Microbiol Infect 2018;13:006.
39. Buchan BW, Ginocchio CC, Manii R, Cavagnolo R, Pancholi P, Swyers L, 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.
40. Wojewoda CM, Sercia L, Navas M, Tuohy M, Wilson D, Hall GS, et al. Evaluation of the Verigene Gram-positive blood culture nucleic acid test for rapid detection of bacteria and resistance determinants. J Clin Microbiol 2013;51:2072–6.
41. Sullivan KV, Turner NN, Roundtree SS, Young S, Brock-Haag CA, Lacey D, et al. Rapid detection of Gram-positive organisms by use of the Verigene Gram-positive blood culture nucleic acid test and the BacT/Alert pediatric FAN system in a multicenter pediatric evaluation. J Clin Microbiol 2013;51:3579–84.
42. Neuner EA, Pallotta AM, Lam SW, Stowe D, Gordon SM, Procop GW, Richter SS. Experience with rapid microarray-based diagnostic technology and antimicrobial stewardship for patients with Gram-positive bacteremia. Infect Control Hosp Epidemiol 2016; 37:1361–6.
43. Box MJ, Sullivan EL, Ortwine KN, Parmenter MA, Quigley MM, Aguilar-Higgins LM, et al. Outcomes of rapid

identification for Gram-positive bacteremia in combination with antibiotic stewardship at a community-based hospital system. Pharmacotherapy 2015;35:269–76.

44. 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.

45. Ledeboer NA, Lopansri BK, Dhiman N, Cavagnolo R, Carroll KC, Granato P, et al. Identification of Gram-negative bacteria and genetic resistance determinants from positive blood culture broths by use of the Verigene Gram-negative blood culture multiplex microarray-based molecular assay. J Clin Microbiol 2015;53:2460–72.

46. Han E, Park DJ, Kim Y, Yu JK, Park KG, Park YJ. Rapid detection of Gram-negative bacteria and their drug resistance genes from positive blood cultures using an automated microarray assay. Diagn Microbiol Infect Dis 2015;81:153–7.

47. Pogue JM, Heil EL, Lephart P, Johnson JK, Mynatt RP, Salimnia H, Claeys KC. An antibiotic stewardship program blueprint for optimizing Verigene BC-GN within an institution: a tale of two cities. Antimicrob Agents Chemother 2018;62:e02538–17.

48. Sothoron C, Ferreira J, Guzman N, Aldridge P, McCarter YS, Jankowski CA. A stewardship approach to optimize antimicrobial therapy through use of a rapid microarray assay on blood cultures positive for Gram-negative bacteria. J Clin Microbiol 2015;53:3627–9.

49. Rivard KR, Athans V, Lam SW, Gordon SM, Procop GW, Richter SS, Neuner E. Impact of antimicrobial stewardship and rapid microarray testing on patients with Gram-negative bacteremia. Eur J Clin Microbiol Infect Dis 2017;36:1879–87.

50. Carroll KC, Leonard RB, Newcomb-Gayman PL, Hillyard DR. Rapid detection of the staphylococcal mecA gene from Bactec blood culture bottles by the polymerase chain reaction. Am J Clin Pathol 1996;106:600–5.

51. 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.

52. Snyder JW, Munier GK, Heckman SA, Camp P, Overman TL. Failure of the BD GeneOhm StaphSR assay for direct detection of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolates in positive blood cultures collected in the United States. J Clin Microbiol 2009;47:3747–8.

53. Buchan BW, Allen S, Burnham CA, McElvania TeKippe E, Davis T, Levi M, et al. Comparison of the next-generation Xpert MRSA/SA BC assay and the GeneOhm StaphSR assay to routine culture for identification of Staphylococcus aureus and methicillin-resistant S. aureus in positive-blood-culture broths. J Clin Microbiol 2015;53:804–9.

54. Salimnia H, Fairfax MR, Lephart PR, Schreckenberger P, DesJarlais SM, Johnson JK, et al. Evaluation of the Filmarray blood culture identification panel: results of a multicenter controlled trial. J Clin Microbiol 2016;54:687–98.

55. 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.

56. Pardo J, Klinker KP, Borgert SJ, Butler BM, Giglio PG, Rand KH. Clinical and economic impact of antimicrobial stewardship interventions with the Filmarray blood culture identification panel. Diagn Microbiol Infect Dis 2016;84:159–64.

57. Buchan BW, Reymann GC, Granato PA, Alkins BR, Jim P, Young S. Preliminary evaluation of the research-use-only (RUO) iCubate iC-GPC assay for identification of select Gram-positive bacteria and their resistance determinants in blood culture broths. J Clin Microbiol 2015;53:3931–4.

58. Granato PA, Unz MM, Widen RH, Silbert S, Young S, Heflin KL, et al. Clinical evaluation of the iCubate iC-GPC assay for detection of Gram-positive bacteria and resistance markers from positive blood cultures. J Clin Microbiol 2018;56.

59. Dhiman N, Trienski TL, DiPersio LP, DiPersio JR. Evaluation of the BinaxNow Staphylococcus aureus test for rapid identification of Gram-positive cocci from Versatrek blood culture bottles. J Clin Microbiol 2013;51:2939–42.

60. Heraud S, Freydiere AM, Doleans-Jordheim A, Bes M, Tristan A, Vandenesch F, et al. Direct identification of Staphylococcus aureus and determination of methicillin susceptibility from positive blood-culture bottles in a BacT/Alert system using Binax now S. aureus and PBP2a tests. Ann Lab Med 2015;35:454–7.

61. Sullivan KV, Turner NN, Roundtree SS, McGowan KL. Rapid detection of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-susceptible Staphylococcus aureus (MSSA) using the Keypath MRSA/MSSA blood culture test and the BacT/Alert system in a pediatric population. Arch Pathol Lab Med 2013;137:1103–5.

62. Bhowmick T, Mirrett S, Reller LB, Price C, Qi C, Weinstein MP, Kirn TJ. Controlled multicenter evaluation of a bacteriophage-based method for rapid detection of Staphylococcus aureus in positive blood cultures. J Clin Microbiol 2013;51:1226–30.

63. Lim SH, Mix S, Xu Z, Taba B, Budvytiene I, Berliner AN, et al. Colorimetric sensor array allows fast detection and simultaneous identification of sepsis-causing bacteria in spiked blood culture. J Clin Microbiol 2014;52:592–8.

64. Anderson DJ, Moehring RW, Sloane R, Schmader KE, Weber DJ, Fowler VG, Jr., et al.. Bloodstream infections in community hospitals in the 21st century: a multicenter cohort study. PLoS One 2014;9:e91713.

65. Huson MA, Stolp SM, van der Poll T, Grobusch MP. Community-acquired bacterial bloodstream infections in

HIV-infected patients: a systematic review. Clin Infect Dis 2014;58:79–92.

66. Wisplinghoff H, Seifert H, Wenzel RP, Edmond MB. Current trends in the epidemiology of nosocomial bloodstream infections in patients with hematological malignancies and solid neoplasms in hospitals in the United States. Clin Infect Dis 2003;36: 1103–10.

67. Schrag SJ, Farley MM, Petit S, Reingold A, Weston EJ, Pondo T, et al. Epidemiology of invasive early-onset neonatal sepsis, 2005 to 2014. Pediatrics 2016;138.