ID Learning Unit—Diagnostics Update: Current Laboratory Methods for Rapid Pathogen Identification in Patients With Bloodstream Infections

Matthew P. Rubach[1] and Kimberly E. Hanson[1,2,3]

[1]Department of Pathology, [2]Department of Internal Medicine, Division of Infectious Diseases, University of Utah School of Medicine, and [3]ARUP Laboratories, Salt Lake City, Utah

Diagnostic assays that rapidly identify bloodstream pathogens have the potential to improve patient outcomes and antibiotic stewardship efforts. Current tests are based on the detection of nucleic acids that are specific to a targeted pathogen or based on organism identification using mass spectrometry. Most rapid assays require a positive blood culture as their sample input and expedite pathogen identification by 24–72 hours. For those assays that also report detection of drug resistance markers, information on antimicrobial resistance is expedited by 48–96 hours. This learning unit reviews the basic principles of rapid microorganism identification assays for bloodstream infections with the aim of assisting clinicians in the interpretation and optimal utilization of test results.

Keywords. bacteremia; bloodstream infection; rapid diagnostics; septicemia.

A 57-year-old man is admitted to the medical intensive care unit (ICU) for evaluation and management of septic shock. The microbiology laboratory's automated blood culture incubation system detected microbial growth at 16 hours in blood culture bottles inoculated in the emergency department. An initial Gram stain result was issued stating "Gram positive cocci in clusters," and 2 hours later the laboratory reports "*Staphylococcus aureus* was detected by a FDA-approved molecular method. The *MecA* gene was not detected." An inpatient pharmacist calls you to inform you of the result, and he recommends you consider tailoring the patient's empiric vancomycin therapy to cefazolin or nafcillin for treatment of methicillin-sensitive *S aureus* bacteremia.

How do you interpret these findings? Can you trust the organism identification and prediction of methicillin susceptibility provided by the nucleic acid detection assay your hospital laboratory recently began to run on positive blood culture bottles? Is this information actionable or should you wait 48 hours until the laboratory determines the organism identification and susceptibility pattern by conventional methods?

This learning unit aims to update clinicians on recent laboratory developments for the diagnosis of bacterial or fungal bloodstream infections. In this report, we focus on the current US Food and Drug Administration (FDA)-approved tests, with a review of their clinical utility and suggested optimal use for patient care.

THE NEED FOR MORE RAPID IDENTIFICATION OF BLOODSTREAM PATHOGENS

Delays in establishing a microbiologic diagnosis and in instituting effective antimicrobial therapy for bloodstream infections lead to poor clinical outcomes. For example, a >48-hour delay in instituting effective therapy for enterococcal bacteremia carried a 5-fold increased risk in 14-day mortality [1], whereas a >12-hour delay in effective therapy for candidemia carried a 2-fold increase in hospital mortality [2]. Organism identification by conventional methods (ie, culture on solid media followed by biochemical identification) can take 12–48 hours after growth is first detected in the blood culture bottle. In addition, standard phenotypic antimicrobial susceptibility testing typically requires an additional 24–36 hours after organism isolation. Molecular and proteomic methods have held great promise for expediting organism identification and drug resistance detection. Although rapid pathogen detection directly from a blood specimen remains the ideal approach for septicemia diagnostics, most assays have lacked the analytic sensitivity required for direct detection [3]. To date, only 1 assay has been approved by the FDA for pathogen detection and identification directly from blood (see T2Candida test below). Alternatively, there are now multiple assays that enable rapid organism identification by testing aliquots from positive blood culture bottles. We will

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review these new technologies in 2 methodological groupings: nucleic acid-based detection tests and proteomic-based methods using mass spectrometry (MS). We then conclude with a brief summary of the available clinical outcomes data that demonstrate the utility and cost-effectiveness of these approaches.

RAPID PATHOGEN IDENTIFICATION BY NUCLEIC ACID DETECTION

Table 1 summarizes currently available FDA-approved diagnostic assays that utilize nucleic acid detection (ie, molecular diagnostic tests) and their performance characteristics. Peptide

Table 1. US Food and Drug Administration-Approved Diagnostic Assays That Utilize Nucleic Acid Detection for the Rapid Identification of Bloodstream Pathogens

Assay (Manufacturer)	Pathogens Targeted/Reported	Resistance Detection	Clinical Accuracy[a]	Sensitivity (SN) Specificity (SP)	Turnaround Time (Hours)[b]	References
PNA FISH (AdvanDx)[c]						
Staphylococcus aureus/ CNS	*S aureus* vs Coagulase-negative *Staphylococcus* spp	No	97%	SN 97%/SP 100%	1.5	4
GNR Traffic Light	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*	No	99%	SN 99%/SP 98%	1.5	5
Enterococcus faecalis/ OE	*E faecalis* vs other *Enterococci* spp	No	100%	SN 100%/SP 100%	1.5	6
Yeast Traffic Light[d]	*Candida albicans, Candida glabrata, Candida tropicalis*	No	96%	SN 98%/SP 83%	1.5	7
GeneOhm (BD)						
StaphSR	*S aureus*	mec insertion site	97%	SN 99%/SP 97%	2	8, 9
XPert						
(Cepheid) MRSA/SA BC	*S aureus*	mecA, attB	99%	SN 99%/SP 99%	1	9, 10
Verigene (Nanosphere)						
Gram-positive blood culture test (BC-GP)	*S aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis, Staphylococcus anginosus* Group, *Staphylococcus agalactiae, Staphylococcus pneumoniae, Staphylococcus pyogenes, E faecalis, Enterococcus faecium, Listeria* spp, *Micrococcus* spp,[e] *Staphylococcus* spp, *Streptococcus* spp	mecA, vanA, vanB	95%	SN 86%–100%/SP 99%–100%	2.5	11–15
Gram-negative blood culture test (BC-GN)	*E coli, K pneumoniae, Klebsiella oxytoca, P aeruginosa, Staphylococcus marcescens,*[e] *Acinetobacter* spp, *Citrobacter* spp, *Enterobacter* spp, *Proteus* spp	CTX-M, IMP, KPC, NDM, OXA, VIM	95%	SN 88%–100%/SP 99%–100%	2	17–19
FilmArray (Biofire)						
Blood Culture Identification Panel	*Listeria monocytogenes, S aureus, S agalactiae, S pyogenes, S pneumoniae, Enterococcus* spp, *Staphylococcus* spp, *Streptococcus* spp, *Acinetobacter baumanii, Enterobacter cloacae, E coli, Haemophilus influenzae, K oxytoca, K pneumoniae, Neisseria meningitidis, P aeruginosa, S marcescens, Proteus* spp, *Enterobacteriaciae, C albicans, C glabrata, Candida krusei, Candida parapsilosis, C tropicalis*	mecA, vanA/B, KPC	94%	SN 83%–100%/SP 99%–100%	1.2	20–23
T2MR (T2 Biosystems)						
T2Candida Panel	*C albicans, C glabrata, C krusei, C parapsilosis, C tropicalis*	No	97%	SN 91%/SP 99%	3–5	25

Abbreviations: BC, blood culture; CNS, Coagulase-negative *Staphylococci*; FDA, US Food and Drug Administration; OE, other enterococci; PNA FISH, peptide nucleic acid fluorescence in situ hybridization.

(Note: Contents of this table are not intended to be an exhaustive list, and reader should note that several additional platforms not listed here are seeking or pending FDA approval. Performance of resistance marker detection is not included in this Table. For the multiplex assays, both polymicrobial and monomicrobial culture results are included in these calculations.)

[a] Accuracy defined as agreement (concordance) with blood culture result.

[b] Time is the assay run time on the instrument.

[c] AdvanDx also now offer QuickFISH product line for many of the following PNA FISH assays. QuickFISH have reported turnaround time of 20 minutes.

[d] PNA FISH also have FDA-approved assays for rapid identification of *C albicans* and *C albicans* vs *C glabrata*.

[e] *Micrococcus* spp is not an FDA-approved analyte on the Verigene BC-GP panel, and *S marcescens* is not an FDA-approved analyte on the Verigene BC-GN panel.

nucleic acid fluorescence in situ hybridization (PNA FISH) was one of the first methods deployed in the clinical laboratory for the identification of organisms detected on the Gram stain from a positive blood culture bottle. Peptide nucleic acid FISH utilizes a DNA probe that specifically hybridizes to target pathogen ribosomal RNA [4]. Compared with conventional identification methods, the AdvanDx (Woburn, MA) PNA FISH tests have demonstrated excellent clinical accuracy for *Staphylococcus aureus* [4], *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* [5], *Enterococcus faecalis*, and other *Enterococci* [6] as well as *Candida albicans*, *Candida glabrata*, and *Candida tropicalis* [7] (Table 1). Of note, PNA FISH test results must be determined by a trained technologist's review of a stained slide under a fluorescent microscope, and these assessments can be subject to interreader variability in visual discrimination of color fluorescence.

There are also 2 FDA-approved real-time polymerase chain reaction (PCR) assays that detect *S aureus* and methicillin-resistant *S aureus* from positive blood cultures: the GeneOhm StaphSR (BD, Sparks, MD) and the Xpert MRSA/SA (Cepheid, Sunnyvale, CA) assays. Compared with blood culture, both tests have clinical accuracy >97% for detection of *S aureus* and differentiation of methicillin-resistance (Table 1) [8–10].

The Verigene assay (Nanosphere, Northbrook, IL) automates nucleic acid extraction from positive blood culture broth followed by pathogen detection via hybridization onto a microarray containing complementary nucleic acid targets for multiple bacterial pathogens. Verigene has a Gram-positive microarray panel (Gram-positive blood culture [BC-GP]) that includes detection of *mecA* for *Staphylococci* and *vanA/vanB* for *Enterococci* as well a Gram-negative microarray panel (Gram-negative blood culture [BC-GN]) that likewise includes genotypic detection of drug resistance (Table 1). The clinical laboratory selects which panel to test based on the Gram stain morphology observed from the blood culture bottle. The BC-GP has demonstrated robust concordance with conventional identification methods [11, 13–15]. However, multiple studies have described misidentifications of various *Streptococcus* species (spp) as *Streptococcus pneumoniae* (ie, false-positive for *S pneumoniae*) [11, 14, 16]. The BC-GN has demonstrated robust concordance with conventional identification methods in 3 of the larger representative studies (Table 1) [17–19]. Polymicrobial bloodstream infections pose a challenge for current rapid diagnostics. The BC-GP and BC-GN typically detect at least 1 organism in mixed infections, and they identify all organisms in approximately 60%–76% [11, 13, 14] and 55%–57% of polymicrobial broths, respectively [17, 18]. Regarding resistance detection, BC-GP has demonstrated accuracy of 97%–100% for *mecA* detection and 96%–100% accuracy for detection of *vanA/vanB* [11, 13, 14, 16]. The BC-GN showed 94%–100% sensitivity and >99.9% specificity for the 6 resistance genes included in the panel when compared with laboratory-developed PCR and bidirectional sequencing [18].

The FilmArray Blood Culture Identification Panel ([BCID] Biofire Diagnostics, Salt Lake City, UT) uses a pouch-based platform to perform a closed system multiplex PCR. The positive blood culture broth sample undergoes fully automated nucleic acid extraction, followed by PCR amplification using a pool of nucleotide primers for the >24 pathogens targeted by the assay (Table 1). Like the other platforms, the FilmArray BCID also has demonstrated robust clinical accuracy compared with conventional identification methods [20–22]. In cases of polymicrobial bloodstream infection, BCID usually detects at least 1 organism in the mixture and may correctly identify all organisms 50%–80% of the time [21–23]. The BCID has demonstrated high accuracy of resistance detection of *mecA* (94%–100%) and *vanA/vanB* (100%), but kpc-harboring organisms have not been well represented in the published assessments to date [20–23].

The T2Dx platform's T2Candida test (T2 Biosystems, Lexington, MA) is the first FDA-approved assay for rapid identification of bloodstream infections that detects the pathogen directly from patient whole blood specimens, without requiring incubation in blood culture broth. This platform detects the 5 most common *Candida* spp (Table 1) and integrates automated DNA extraction followed by PCR amplification of *Candida*-specific ribosomal RNA targets. The amplified nucleic acid product is detected by a novel method involving amplicon-induced agglomeration of supermagnetic particles that is measured by T2 magnetic resonance relaxation [24]. The assay's limit of detection for *Candida* spp is comparable with blood culture (ie, 1 colony-forming unit [CFU]/mL for *C tropicalis* and *Candida krusei*, 2 CFU/mL for *C albicans* and *C glabrata*, and 3 CFU/mL for *Candida parapsilosis*). In a prospective clinical trial, assay sensitivity was 91% and specificity was >99% [25].

As with any laboratory test, the impact of T2Candida results on clinician management should depend upon the prevalence of the condition in a given patient population. Figure 1 demonstrates how even with a highly sensitive and specific test, the negative and positive predictive values of a test result depend upon disease prevalence among a given patient population. If the prevalence of candidemia is 3% in a typical ICU [26], then the positive predictive value of the T2Candida test (ie, the probability that the disease is present when the test is positive) is close to 80%. Alternatively, a negative T2Candida result has a much higher negative predictive value (≈99.7%). In the 3% prevalence scenario, a positive test requires confirmation, and negative results could potentially inform a decision to withhold empiric antifungal therapy. Such considerations are crucial to proper utilization of these rapid identification platforms.
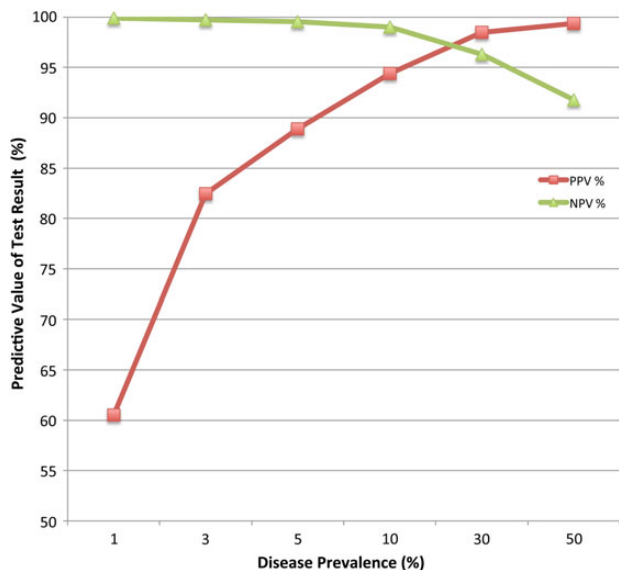
Figure 1. Predictive values of T2Candida test by function of disease prevalence in the tested patient population. Using the published clinical sensitivity (91%) and specificity (99%) of the T2Candida test, this graph plots the variation of the negative predictive value (NPV [triangle plots]) and positive predictive value (PPV [square plots]) across a range of disease prevalence.

RAPID PATHOGEN IDENTIFICATION BY MASS SPECTROMETRY

In the past 10 years, organism identification in the clinical microbiology laboratory has been revolutionized by methods that utilize MS to identify a microbe's unique ribosomal protein profiles. The most widely adopted MS approach in clinical microbiology is matrix-assisted laser desorption ionization, time-of-flight MS (MALDI-TOF MS, reviewed in [27]). The most common application of MALDI-TOF MS is the identification of pure microbial isolates grown by culture. Protein pattern matching by MALDI-TOF MS is more accurate than conventional biochemical phenotypic testing and is faster and less expensive than 16S DNA sequencing [28]. Two MALDI-TOF instruments are currently approved by the FDA for the identification of bacterial isolates from conventional culture on solid media (Microflex Biotyper; Bruker Daltonics, Billerica, MA) and Vitek Mass Spectrometry System (Vitek MS2; bioMerieux, Lille, France).

Although not an FDA-approved application, numerous studies have shown that MALDI-TOF MS can be applied to broth media from a positive blood culture bottle, with a diagnostic yield approximately 80% (range, 74%–98%) and a turnaround time of 20–60 minutes [29–33]. Of note, the identification of yeast in positive blood culture broth by MALDI-TOF MS has been more challenging than identification of bacteria [34], although some studies show yeast identification can be optimized with more involved protein extraction protocols [35]. Regarding performance in the setting of polymicrobial bloodstream infections, MALDI-TOF MS often detects at least 1 of the organisms, but it rarely (<10% of cases) detects all organisms [23, 29–32].

Although current MALDI-TOF MS systems cannot directly detect antimicrobial resistance, MALDI-TOF MS can assess for β-lactamases by incubating a cultured isolate with a given antibiotic and then measuring drug metabolites of β-lactamase-mediated antibiotic degradation [36, 37]. The MALDI-TOF MS has also been incorporated into rapid antimicrobial susceptibility testing algorithms. In these laboratory-developed protocols, centrifuged pellets [38] or filtered lysates [39] of broth from a positive blood culture are processed for MS identification and simultaneously inoculated into an FDA-approved automated susceptibility instrument.

COST-EFFECTIVENESS AND CLINICAL OUTCOMES DATA

Adoption of the above-mentioned rapid diagnostic assays requires a considerable capital investment for the clinical laboratory, and the cost-per-test of the nucleic acid detection assays is typically higher than the cost of conventional microbiologic methods. Despite these differences, cost savings are potentially derived from targeted de-escalation of empiric broad-spectrum antimicrobial therapy (ie, a decrease in pharmacy costs) [40–42] and from decreased hospital length of stay [41, 43]. Most studies demonstrating cost savings have systematically integrated rapid pathogen identification into an antimicrobial stewardship program [41, 43]. In fact, a recent randomized study showed improved antimicrobial de-escalation with FilmArray BCID coupled to real-time stewardship compared with FilmArray BCID coupled to standard laboratory results reporting alone [44]. Although more clinical outcomes data are needed, at least 2 studies have demonstrated a mortality benefit for rapid pathogen identification direct from positive blood culture [6, 45], and this is consistent with the mortality benefit of expedited diagnosis of Gram-negative bacteremia using MALDI-TOF on blood culture isolates [46].

CONCLUSIONS

Rapid identification of bloodstream pathogens is now a reality with the various laboratory systems discussed in this review. Most of these modalities still require growth detection in an incubated blood culture, but novel FDA-approved nanotechnologies, such as the T2Dx, or other technologies on the horizon, such as PCR-electrospray ionization MS and NanoString, hold promise for the detection of bloodstream pathogens directly from whole blood patient samples. More patient outcomes data are needed to assess the clinical impact of rapid identification systems, but studies to date show these assays are cost-effective and are associated with a mortality benefit

when formally integrated into antibiotic stewardship programs that act on the test results in near real-time.

Returning to the 57-year-old patient with methicillin-sensitive *S aureus* bacteremia detected from his positive blood culture by the hospital laboratory's rapid molecular identification platform: given the documented test performance for both organism identification and resistance detection of the currently FDA-approved tests, these results are clinically actionable, and tailoring his antimicrobial therapy from empiric vancomycin to cefazolin or nafcillin is indicated [47].

Acknowledgments

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