Published in final edited form as:

Annu Rev Med. 2018 January 29; 69: 379–394. doi:10.1146/annurev-med-052716-030320.

New Molecular Diagnostic Approaches to Bacterial Infections and Antibacterial Resistance

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INTRODUCTION

Diagnostics in infectious diseases are in the midst of a technological revolution. For more than a century, confirming a bacterial infection involved isolating a pathogen by growing it from culture from a sterile body sample. This process was slow, imprecise, and subject to errors introduced by collection conditions and growth media requirements of fastidious organisms. Spurred on by technological advances, the field of infectious disease diagnostics in general, and bacterial diagnostics in particular, are experiencing a steady stream of innovative approaches designed to provide more accurate data, sooner. In the current review, we *i*) summarize the present “state of the art” in diagnostic platforms that help us understand host responses to infection and how to decide what therapy is needed and *ii*) review contemporary technologies that help us identify pathogens and characterize their phenotype to guide empiric and definitive therapy. Our goal is to inform the general internist regarding the most promising developments that are being implemented in patient care.

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SECTION 1. HOST-FOCUSED RAPID MOLECULAR DIAGNOSTICS

Host Response Diagnostics for Infectious Disease

Most diagnostic approaches in contemporary infectious disease focus on pathogen detection and characterization. However, the host response offers a complementary strategy. When challenged with a pathogen, the host responds through the activation of multiple biological pathways to detect and control the infection. These responses are triggered at the earliest time points in infection and are highly specific to the type of infecting pathogen (e.g., viral, bacterial, protozoan, fungal).¹ This conserved nature of host response offers new approaches for diagnostic development in infectious diseases.

Until recently, most host-derived tests for infection focused on peptide-based biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT). These markers, for whom validated clinical assays already exist, correlate with infection severity and tend to be higher in patients with bacterial, as opposed to viral, infection.^{2,3} These biomarkers are reviewed elsewhere.⁴ A more recent development in host-based diagnostic development is the use of systems biology to evaluate the entirety of the host response in an unbiased manner. Instead of targeting candidate biomarkers based on known biology, systems biology approaches are agnostic: they measure all available molecules of a given type (e.g., transcriptomics, metabolomics, proteomics) and searches within that data to identify analytes diagnostic of the disease in question (Figure 1). Such measurements coupled with machine learning techniques have sparked a growing wave of new diagnostic strategies.

Study Design and Analytical Considerations

Developing a host response biomarker begins by selecting the discovery cohort in whom biomarker identification will occur. Typically, the discovery cohort should consist of patients where the test would offer a meaningful clinical distinction. For this reason, healthy controls are poorly suited for clinical assay development, as they may be readily distinguished from patients with infection on clinical grounds alone. Once identified, a host response biomarker should be validated, and ultimately employed, in the same or similar population from which it was derived. For example, there are numerous biomarkers that easily distinguish sepsis from healthy controls. However, it would be incorrect to assume such a biomarker is specific for sepsis as it could simply indicate the presence of critical illness. The more appropriate control is patients with non-infectious causes of illness. Finally, sample collection should be obtained at the clinical time point when the diagnostic is most likely to ultimately be employed. For example, a diagnostic biomarker should not be derived from patients who are several days into treatment for an acute process.

Once the samples are collected, the next step is data generation for signature derivation. Although the vast amount of data generated affords the opportunity to create disease-specific signatures, this also poses challenges. The high dimensional data is complex and generally not amenable to traditional analytic methods. This difficulty is due in part to the 'large p, small n' problem, where a small sample size can yield tens of thousands of data points for comparison.⁵ Because the methods developed to handle such data are relatively recent and may be unfamiliar to many clinicians or microbiologists, we provide a brief overview of the

commonly used approaches to “big data” analysis. Additional details regarding such methods can be found in recent reviews.^{6,7}

Reducing dimensionality is the process of reducing the number of random variables being considered in an analysis. For example, the Affymetrix U133 Plus 2.0 microarray contains probes for over 47,000 transcripts. Using dimensional reduction algorithms, these transcripts can be reduced to hundreds or even dozens of composite variables in a single patient. Many approaches to dimensional reduction have been developed and validated whereby phenotypes are matched to molecular measurements enabling both diagnostic and prognostic utility. Examples include sparse factor modelling⁸, Bayesian constructions of the elastic net⁹, sparse principal component analysis¹⁰, penalized matrix decomposition¹¹, modular transcriptional analysis¹², and the molecular distance to health¹³.

Nucleic Acid Biomarkers

Clinical Precedent—Among the most exciting sources for new diagnostic information is the host transcriptome. The expression of tens of thousands of genes and other DNA elements creates an opportunity to discriminate a multitude of clinical states more accurately than conventional peptide-based biomarkers allow. This approach is underscored by the observation that up to 93% of the human genome is transcribed, but as little as 1.5% is translated.¹⁴

Several FDA-approved gene expression-based diagnostics are available in cardiovascular disease (Corus CAD), heart transplant rejection (AlloMap), breast cancer prognosis (MammaPrint), and melanoma (myPath Melanoma).^{15–18} One test is recently FDA cleared for sepsis (SeptiCytel LAB)¹⁹, but is not yet commercially available. Gene expression-based diagnostics in infectious diseases have been limited by the need to provide rapid, simple, point-of-need results in the setting of potentially life-threatening infection. Gene expression-based diagnostics require nucleic acid extraction, amplification, and quantification with additional processing in some cases (e.g., peripheral blood mononuclear cell isolation, cDNA library preparation). While there is generally ample time to allow for such assays in coronary artery disease, heart transplant rejection monitoring, and cancer phenotyping, this is usually not the case in acute infectious diseases.

SeptiCytel, the only FDA-cleared infectious disease host gene expression diagnostic, has reduced the assay time to 4–6.5 hours, but generally remains prohibitively complex and lengthy for mainstream clinical use. SeptiCytel measures the differential expression of four genes (*CEACAM4*, *LAMP1*, *PLA2G7*, and *PLAC8*) and has been validated in observational studies among adults and children with suspected sepsis.^{19,20} However, it has not yet been used to guide real-time patient care and therefore, its clinical utility remains undefined. Ultimately, host gene expression diagnostic tests in infectious diseases will require concomitant technological advances allowing timely results in a simple-to-use format before being broadly utilized.

Technical Considerations—Although most published host response signatures based on RNA targets have utilized mRNA, non-coding RNA such as microRNA (miRNA) are increasingly recognized as potential diagnostic biomarkers. miRNAs are short (17–22

nucleotide), non-coding RNA molecules involved in post-transcriptional regulation. They inhibit translation as part of the RNA-induced silencing complex (RISC), binding complementary sequences within the 3' untranslated region of target mRNAs. miRNAs function intracellularly but can also exist stably outside cells where they are remarkably stable: they are resistant to boiling, repeated freeze–thaw cycles, or decay over time. As a result, miRNAs make attractive diagnostic targets.²¹

The study of gene expression was initially made possible through the development of microarrays. However, the scientific community is shifting to sequence-based measurements, creating new opportunities. RNA-seq, also known as whole transcriptome sequencing, is the sequencing of a sample's mRNA content. In addition to the ability to measure the level of gene expression, it provides further information on alternative splicing and non-coding RNA (such as microRNA)²². The ability to sequence the transcriptome overcomes some limitations of whole genome microarrays. Specifically, the measures are quantitative and the spectrum of detectable targets is not limited by the gene probes present on a microarray. Although the cost of sequencing is declining, it remains an impediment to more widespread use. One exciting possibility for sequence-based measurements is the prospect of simultaneous pathogen detection and host transcriptomic analysis from the same sample (*e.g.*, blood). With technological advances and reduced costs, such a combined host and pathogen detection strategy may soon be a clinical reality.

Discriminating Bacterial and Viral Etiologies of Acute Respiratory Illness—

Respiratory tract infections caused 3.2 million deaths worldwide and 164 million disability-adjusted life years lost in 2011, more than any other cause.²³ Despite a viral etiology in the majority of cases, 73% of ambulatory care patients in the U.S. with acute respiratory infection (ARI) are prescribed an antibiotic, accounting for 41% of all antibiotics prescribed in this setting.^{24,25} Even when a viral pathogen is confirmed, this does not exclude a possible concurrent bacterial infection leading to precautionary antibacterial prescribing. Therefore, the discrimination of bacterial and viral infection is particularly amenable to a host response-based test. In 2007, Ramilo et al. first described host gene expression signatures discriminating bacterial and viral infection.²⁶ Since that time, additional studies have focused on adult and pediatric cohorts^{27,28}; discriminated different types of viral infection²⁹; distinguished infection from colonization³⁰; identified pre-symptomatic disease states³¹; added non-infectious illness as more appropriate controls²⁸; and showed superiority to existing biomarkers (*i.e.*, PCT)^{28,32}. As important as each of these (and many more) studies have been, they all occurred at the pre-clinical stage of development.

In an effort to advance host response diagnostic development into the clinical domain, the Antibacterial Resistance Leadership Group (ARLG.org) has supported the RADICAL study (Rapid Diagnostics in Categorizing Acute Lung Infection).²⁸ This study has facilitated a partnership with diagnostic developers in industry to develop host gene expression signatures into a clinically relevant, timely, and simple assay. This program may provide “proof of principle” for similar diagnostic approaches in other infectious disease syndromes (*e.g.*, febrile infant, sepsis, febrile traveler).

Tuberculosis—Diagnosis of tuberculosis (TB), caused by *Mycobacterium tuberculosis*, has many well-established algorithms, yet remains challenging in clinical practice. Although slow and insensitive, the diagnostic gold standard for active pulmonary TB remains sputum culture. Newly developed PCR-based tests exhibit improved sensitivity and specificity, and are poised to replace acid fast bacilli (AFB) detection using Ziehl-Neelsen, Kinyon, or fluorescent staining or sputum culture.³³ Systems biology approaches have also been applied to TB diagnosis as well as predicting progression from latent to active disease. A number of proteomic and metabolic profiles have been identified but have yet to be validated in independent cohorts. By contrast, gene expression studies have demonstrated reproducible, validated signatures. For example, a 393-transcript signature correlated with radiographic extent of disease as well as treatment response, and was validated in two independent patient populations.³⁴ However, in subsequent studies, this signature was non-specific, demonstrating overlap with other diseases such as sarcoidosis and melioidosis.^{35,36} This limitation aside, the signature distinguished TB from community-acquired pneumonia and lung cancer, offering a mechanism to distinguish these syndromes with similar initial manifestations but with different diagnostic approaches.³⁷ Early small scale studies have also demonstrated a potential for transcriptional signatures to provide information on treatment response after 1–2 weeks of therapy rather than the current gold standard of sputum AFB smear and culture at 2 months.³⁸

Metabolomic and Proteomic Approaches

Proteomic and metabolomic analyses describe the comprehensive evaluation of proteins and metabolites produced by the host. These analyses include characterization of modifications, such as post-translational modification of proteins and molecular interactions. The techniques used to define the proteome and metabolome overlap considerably, both being largely based on mass spectrometry. However, they are also substantially varied both in terms of large scale unbiased discovery as well as targeted detection of candidate biomarkers. Such tools have been used to generate a number of infectious disease diagnostics that focus on host response (e.g., TRAIL, IP-10, and CRP to distinguish bacterial from viral infection)³⁹ or pathogen detection (e.g., NMR-based detection of candidemia, as discussed below)⁴⁰. A review of these methods and their application is beyond the scope of this review but have been published.⁴¹

SECTION 2. PATHOGEN-FOCUSED RAPID MOLECULAR DIAGNOSTICS

Goals of rapid molecular diagnostics (RMDs): focus on pathogen

Current challenges in the clinical microbiology laboratory—In the contemporary clinical microbiology laboratory, novel RMDs are advancing our approach to the diagnosis and treatment of infectious diseases. Although promising, the uniform implementation of these new technologies remains a challenge. As we begin to acquire experience with each new platform, it is increasingly clear that a combination of new technologies will be required to successfully and accurately identify all pathogens of interest.^{42–44} Moreover, laboratories must also show value before adopting a new test.^{45,46}

Ideal RMDs, point of care testing, and time to result—The ideal features of a RMD platform are to: *i*) identify the pathogen and mechanism of resistance quickly (presently considered within one hour, the ideal “time to result”); and *ii*) provide this data at the patient’s location (point of care, POC) to have the most impact.⁴⁴ The underlying approach that achieves this is nucleic acid–based amplification technologies (NAATs) using polymerase chain reaction (PCR) and other molecular modalities that selectively identify microbial genetic sequences with great precision, sensitivity, and specificity.⁴⁷ Furthermore, we are now witnessing the introduction of culture independent testing directly on biological samples and next-generation sequencing (NGS), permitting the analysis of complex, polymicrobial specimens in ways never imagined two decades ago.⁴⁸

Clinical applications: resistance gene identification (*mec* gene, β -lactams, and β -lactamases)—In some organisms, pathogen and resistance mechanism identification are straightforward. A clear example is the *mec* gene, which identifies an organism such as *Staphylococcus aureus* along with resistance to the β -lactam oxacillin (or methicillin). Identifying resistance to β -lactams in Gram-negative organisms is more challenging. To illustrate, there are greater than 2,000 β -lactamase genes responsible for resistance to β -lactam antibiotics in Gram-negative bacteria. Moreover, there are almost equal numbers of aminoglycoside modifying enzymes (AMEs). In contrast to *S. aureus* and *mec*, β -lactamase and AMEs are found in a variety of genera. Because of the importance of β -lactam antibiotics in our antibiotic armamentarium, many RMD platforms have focused on β -lactamase detection in Gram-negative bacteria, especially β -lactamases that confer resistance to carbapenems.

The most prevalent β -lactamase resistance determinants worldwide conferring resistance to our “last line” β -lactams, the carbapenemases, are *bla*_{KPC}, *bla*_{OXA-48}, *bla*_{OXA-23}, *bla*_{OXA24/40}, *bla*_{NDM}, *bla*_{VIM}, and *bla*_{IMP}. In principle, detecting resistance genes can significantly impact antibiotic choices when implemented in the clinical setting. However, the same approach cannot be easily applied in determining β -lactam/ β -lactamase inhibitor resistance (e.g., piperacillin/tazobactam). This is due to considerations such as plasmid and intrinsic chromosomal location, allele diversity, gene regulation, and β -lactamase expression.

As an illustration, Evans et al. performed the PRIMERS I and II studies (PRIMERS = Platforms for Rapid Identification of MDR Gram-negative bacteria and Evaluation of Resistance Studies) to define the relationship between genotype and phenotype in multidrug resistance Gram-negative infections. Specifically, they evaluated the performance characteristics of four molecular diagnostic platforms to identify genes conferring resistance to β -lactam antibiotics in *Enterobacteriaceae*.⁴⁹ β -Lactamase genes encoding SHV/TEM extended-spectrum β -lactamases (ESBLs) and non-ESBLs, as well as OXA, KPC, CMY, CTX-M, ACT, MIR, DHA, NDM, VIM, and IMP β -lactamases were queried to analyze the β -lactamase background of 196 resistant and susceptible *Escherichia coli* and *Klebsiella pneumoniae* isolates. Each gene was correlated to a specific resistance phenotype (e.g., ampicillin, ceftazidime, imipenem resistance). Imipenem and cephalosporin resistance were correctly identified in >98% of cases while resistance to piperacillin/tazobactam was <80% across all four platforms. Unlike other studies that solely evaluated performance

characteristics (sensitivity, specificity, positive and negative predictive values) of a diagnostic platform to detect resistance genes, PRIMERS I and II were the first to also incorporate the prevalence of resistance in empiric decision making.

Commercial platforms: LAMP, TMA, HDA, and SeptiFast—Other techniques and approaches may provide rapid results or improved sensitivity and specificity compared with culture-based methods.⁵⁰ Examples include GeneXpert (Cepheid, Sunnyvale, CA), FilmArray (BioFire, Salt Lake City, UT), Tigris (GenProbe, San Diego, CA), Verigene (Nanosphere, Northbrook, IL), SmartCycler (Cepheid), ABI 7500FastDx (Applied Biosystems), LightCycler 2.0 (Roche), and SeptiFast (Roche Diagnostics). There are also automated or “sample-to-result” open platforms available, including the BD Max (BD, Sparks, MD) and Abbott m2000 (Abbott, North Chicago, IL). Table 1 summarizes representative platforms. Select novel platforms will be discussed.

Advances in traditional and real-time PCR have led to the use of loop-mediated amplification (LAMP), transcription-mediated amplification (TMA), and helicase dependent amplification (HDA). LAMP and HDA in particular are very economical as they do not require the use of expensive thermocyclers. LAMP is based upon conventional PCR where several oligonucleotides that bind to the target gene are incubated at the same temperature with the DNA polymerase. As DNA polymerizes, there is a release of pyrophosphate that can be detected with a fluorescent dye or a compound that will increase the turbidity of the solution.^{50,52} LAMP provides increased sensitivity with low DNA concentrations compared to PCR and is of particular interest for the detection of carbapenemase genes. In one example, LAMP detected 10.70 pg/μl of genomic *bla*_{NDM} DNA (equivalent to 1×10^3 CFU) while endpoint PCR had a detection threshold of 1,070 pg/μl (or 1×10^5 CFU). In a recent study, Yamamoto et al. described a LAMP method for detecting *bla*_{OXA-23}-positive carbapenem resistant *Acinetobacter baumannii*.⁵² The sensitivity of LAMP was 88.6% (39/44) and its specificity was 92.1% (70/76) using culture as the gold standard. Sensitivity approached 100% upon the inclusion of clinical samples obtained for surveillance purposes. This method provided results within 40 minutes of sample collection.

Transcription-mediated amplification (TMA) of a nucleic acid target differs from PCR in that the target sequence is typically an RNA molecule (mRNA or rRNA), which may be present in high copy number. This approach has been successfully used in the diagnosis of oropharyngeal gonorrhea.⁵³

HDA is an isothermal amplification method that uses UvrD (DNA helicase) and MutL enzymes isolated from *E. coli* and single-strand binding proteins to create and maintain a single-stranded template for primer annealing and subsequent rounds of amplification. HDA has been applied to the identification of *Clostridium difficile* and *S. aureus*.⁵⁴ An advantage of HDA is that target detection can be achieved by incorporation of fluorescein or digoxigenin into the amplicon, followed by capture and visualization of the amplicon as a colored line on an enzyme immunoassay (EIA) lateral-flow strip. This obviates the need for sophisticated instrumentation while allowing for multiple target detection in a single reaction.

SeptiFast is a multipathogen, real-time PCR, probe-based system targeting ribosomal DNA sequences of bacteria and fungi. This platform detects and identifies the most frequent pathogens causing bloodstream infection. A large clinical trial involving more than 1,000 patients suspected of having bloodstream infection was recently conducted in England.⁴⁸ SeptiFast had better specificity (86%) than sensitivity (50%). This platform demonstrates a “rapid rule-in utility” for the diagnosis of bloodstream infection but low sensitivity is a significantly limiting factor. Improvement in the efficiency of pathogen DNA extraction and the capacity to detect a much broader range of pathogens and drug resistance genes is required before more widespread use is possible.

Microarrays

Currently available microarrays for detection of ESBLs and carbapenemases are broadly based on DNA amplification followed by amplicon detection either on a tube microarray (Check-Points assays, Check-Points Health, Wageningen, The Netherlands; and Identibac AMR-ve assays, Alere GmbH, Cologne, Germany) or by enzyme-linked immunosorbent assay (ELISA) (Hyplex assays, Amplex Diagnostics, Gars, Germany). Microarray technologies possess a high multiplexing capacity and can be used for detecting different β -lactamase genes present in a single strain. This ability can assist clinicians in directing antimicrobial therapy. In a study performed by Endimiani *et al.*, a total of 106 Gram-negative strains were tested.⁵⁵ Sensitivity and specificity ranged from 96–100% for *bla*_{SHV}, *bla*_{TEM}, *bla*_{CTX-M}, and *bla*_{KPC}. In a follow-up study conducted by Fishbain *et al.*, this platform, when used with a water lysis step, had a 94.4% sensitivity and 100% specificity in blood culture samples containing ESBLs and KPC producing pathogens.⁵⁶

The Hyplex assays involve multiplex PCR amplification followed by amplicon hybridization to reverse probes that are immobilized on ELISA-microwell plates, and hybridization complexes are visualized through peroxidase-conjugated antibodies. Hyplex assays can be utilized directly on patient samples; however, the specific detection of *bla* genes is time-consuming. The HyplexMBLID multiplex PCR-ELISA detects metallo- β -lactamases *bla*_{VIM} and *bla*_{IMP} and was tested with clinical samples (urine, swabs, respiratory samples, and positive blood cultures) resulting in 98% sensitivity and 99% specificity.⁵¹

Pyrosequencing and next-generation sequencing (NGS)

Whole genome sequencing and NGS technologies have advanced significantly since their first applications in the clinical microbiology laboratory. The first technologies (e.g., “454” by Roche or pyrosequencing) were utilized primarily for antibiotic resistance gene characterization and epidemiological investigations. In addition, pyrosequencing has been used to investigate novel β -lactamase variants and to discriminate chromosomal versus plasmid genes.⁵¹

NGS techniques allows deep sequencing of biological samples, and non-targeted identification of microbes. Time to result for NGS is 2–3 days. Ion torrent sequencing is a very powerful NGS approach. This platform is ideally suited to become a benchtop application and has been applied to outbreak investigations and resistance gene mapping.

Wright *et al.*, evaluated the performance of SISPA (Sequence-Independent, Single-Primer Amplification) combined with NGS (SISPA-Seq) of 75 clinical isolates of *A. baumannii* to establish whether SISPA-Seq resulted in sufficient coverage and quality to *i*) determine strain phylogenetic placement and *ii*) carriage of known antibiotic resistance genes.⁵⁷ Coverage and quality were sufficient for detection of antibiotic resistance genes by comparison to the ARG-ANNOT database and were often sufficient to distinguish between allelic variants of the same gene.

Mass spectrometry

Mass spectrometry (MS) has increasingly been used as an identification and characterization method in the clinical microbiology laboratory. Three MS-based diagnostic platforms are in common use: matrix assisted laser desorption ionization–time of flight (MALDI-TOF) MS, electrospray ionization (ESI)-MS, and ion trap-based identification. Among the MALDI-TOF platforms, two are most frequently used. The Bruker Biotyper (Bruker Daltonik, Bremen, Germany) is better at identifying Gram-negative non-fermenting bacteria and the VitekMS (bioMérieux, Marcy l’Etoile, France) is superior for the identification of anaerobes and the majority of streptococcal species. MALDI-TOF MS has been used in clinical laboratories in Europe for almost ten years and has been recently adopted in the United States. Ease of use, low cost, and simplicity of sample preparation are major benefits, whereas inability to identify pathogens directly from specimens remains a significant liability.⁵⁸ MALDI-TOF MS has been used to detect the presence of carbapenemases based upon hydrolysis of a carbapenem molecule, and its ability to detect ESBL and AME has also been recently evaluated.⁵⁸

T2 Magnetic resonance (T2MR)

A new technology combining PCR and T2 magnetic resonance has been developed to detect *Candida* bloodstream infections. With a limit of detection of 1–3 colony-forming units/mL (CFU/mL), T2MR can be used directly on blood without waiting for blood culture bottle positivity. This high sensitivity reduces detection time from days to hours.⁵⁹ T2MR recently demonstrated a 99.4% specificity with a mean time to negative result of 4.2 hours, while sensitivity was 91.1% with a mean time of 4.4 hours to detect and identify the species.⁵⁹

Polymerase chain reaction electrospray ionization mass spectrometry PCR/ESI-MS

The analysis of multilocus PCR amplicons by electrospray ionization mass spectrometry, ESI-MS, on a platform in which PCR is coupled to ESI-MS is a technique that has demonstrated the capacity to detect microbes from both environmental samples and clinical patient specimens.⁶⁰ Detection does not require *a priori* knowledge of the organisms; the technology detects unknown and unculturable organisms, and is particularly useful when multiple microbes may be present. The clinical specimen can be processed and used as the template for PCR reactions, which target a specific group of agents (e.g., food-borne pathogens, biothreat agents, etc.). The amplicon from each PCR is subjected to ESI-MS, and the exact molecular weight of the PCR product is determined. The clinical performance of ESI-MS is dependent on a robust reference library. Closely related species or complexes of organisms can be easily separated based on discrimination of single nucleotide substitutions. This platform was clinically tested in the RADICAL trial.⁶¹ Here, 616 bloodstream

infections, 185 pneumonia cases, and 110 sterile fluid and tissue specimens were analyzed. PCR/ESI-MS identified a pathogen in 228 of the bloodstream infection cases (37%) compared to 68 (11%) for conventional blood culture. Culture was positive and PCR/ESI-MS negative in 13 cases, and both were negative in 384 cases giving PCR/ESI-MS 81% sensitivity, 69% specificity, and 97% negative predictive value at 6 hours from sample acquisition. The distribution of organisms was similar with both techniques. Similar observations were made for pneumonia and sterile fluid and tissue specimens. Independent clinical analysis of results suggested that PCR/ESI-MS technology could have potentially altered treatment in up to 57% of patients.

SECTION 3. CHALLENGES TO IMPLEMENTATION

Technical

Before advances in infectious diseases diagnosis impact clinical practice, there are a number of obstacles to overcome. Many of these are practical. Whether host-based or pathogen-based, results from RMDs need to be communicated to the healthcare team from the clinical microbiology laboratory in an “actionable manner”. Tests need to be simple to conduct and interpret, available every shift, inexpensive, and integrate into a patient care model that improves decision-making for empiric and definitive therapy. More significantly, the diagnostic platform needs to provide information that can be interpreted within the clinical syndrome being examined (e.g., distinguishing bloodstream infection from colonization). Lastly, the ideal platform should also handle a variety of specimen types including blood, respiratory secretions, urine, clinical swabs, cerebrospinal fluid, and even stool samples.^{50,62} Current platforms need to work with very small samples of material and be available for inpatient as well as outpatient care. Development of host-based diagnostics is currently limited by the lack of a commercially available platform that easily measures gene expression in the time frame required for point-of-need decision making. Among the greatest barriers to innovation in diagnostics is the low reimbursement rate particularly when compared to drug reimbursement.

Clinical Implementation

When a new diagnostic test overcomes all of these barriers and enters the clinical environment, there are still substantial challenges. Clinicians, who would order the test, may not know about its availability. Therefore, implementation requires extensive education to make the user aware of the test’s availability. Given the required herculean educational effort, emphasis has instead shifted elsewhere. Most innovations for pathogen diagnosis occur within the clinical laboratory environment. Consequently, the clinical laboratory director may best guide the appropriate diagnostic testing strategy rather than the clinician who placed the order.

Even if the burden of knowing about the new test shifts from the clinician to the laboratory, it is still critically important for the clinician to know how to act on the result. This concept was demonstrated in the BCID study, which introduced a new diagnostic test to clinical practice with and without decision support.⁶³ Specifically, the BioFire Blood Culture Identification (BCID) Panel detects 27 targets in a positive blood culture, taking about one

hour to result. This new molecular assay was deployed in a three-arm, single-center, prospective, randomized trial. The trial compared standard-of-care testing to the new BCID test with electronically-delivered treatment guidance alone or with active oversight by an antimicrobial stewardship team (Figure 2). Both BCID arms led to faster pathogen identification than conventional methodologies (21 hours faster), which led to more focused antibiotic use, less treatment of contaminants, and faster antibiotic escalation where appropriate. However, BCID with stewardship led to faster antibiotic de-escalation. This trial demonstrated that automated decision support and more intensive support in the form of stewardship are needed in conjunction with test development in order to maximize clinical impact.

CONCLUSIONS

Recent advances in technology have given rise to a number of host- and pathogen-centered diagnostic approaches. Rapid diagnostics promise to improve clinical care in acute infectious syndromes by ensuring that patients quickly receive effective treatment, while promoting public health by reducing unnecessary antibiotics when they are not required. While this progress is encouraging, it is unlikely that any single diagnostic platform will fully address the clinician's need for actionable data with short turn-around times in all settings. Thus, a variety of diagnostic tests will ultimately be employed in clinical microbiology laboratories to fully capitalize upon the promise of novel diagnostic approaches.

ACKNOWLEDGEMENTS

This article was supported by the National Institute of Allergy and Infectious Diseases of the NIH (award number UM1AI104681). RAB was also supported by the Department of Veterans Affairs Research and Development under Award Number I01BX001974, VISN 10 Geriatrics Research, Education and Clinical Center, by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) under Award Numbers R01AI072219, R01AI063517, R01AI100560, and by the National Center for Research Resources under Award Number U1TR000439. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans Affairs or the National Institutes of Health.

DISCLOSURES

ELT has consulted for Immunexpress and bioMerieux; has received grant support from DARPA, NIH, bioMerieux, Novartis Vaccines and Diagnostics Inc., Henry M. Jackson Foundation; holds equity in Host Response, Inc.; and has patents pending for Biomarkers for the Molecular Classification of Bacterial Infection and Methods to Diagnose and Treat Acute Respiratory Infections.

RAB was supported by Research grants from Allergan (Pfizer), Merck, Wockhardt, Entasis, and Roche.

VGF served as Chair of V710 Scientific Advisory Committee (Merck); has received grant support from Cerexa/Actavis, Pfizer, Advanced Liquid Logics, NIH, MedImmune, Cubist/Merck, Karius, Contrafect, and Genentech. NIH STTR/SBIR grants pending: Affinergy, Locus, Medical Surface, Inc.; has been a paid consultant for Achaogen, Astellas, Arsanis, Affinergy, Basilea, Bayer, Cerexa, Contrafect, Cubist, Debiopharm, Durata, Grifols, Genentech, MedImmune, Merck, Medicines Co., Pfizer, Novartis, Novadigm, Theravance, xBiotech, and has received honoraria from Theravance, Green Cross, and has a patent pending in sepsis diagnostics.

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Figure 1.

Overview of the development process for a host diagnostic biomarker. Begin with a population that is dichotomized by susceptibility, diagnosis, or prognosis. Omic measurements are run on acquired samples, which generates large quantities of data. Dimension reduction and statistical analyses generate a classifier or signature that distinguishes the desired characteristic from the original population. The classifier is then validated against a different population to test generalizability. (Used with permission from Yang WE, et al. Host-based diagnostics for detection and prognosis of infectious diseases. In: Sails A, Tang YW, eds. *Methods in Microbiology*. Elsevier Ltd, 2015;42[13]:465–500.)

Figure 2.

Comparison of time to organism identification, availability of phenotypic antimicrobial susceptibility results, and first appropriate modification of antimicrobial therapy for the subset of study subjects with organisms represented on the rapid multiplex polymerase chain reaction (rmPCR) panel (n = 481). Time 0 is when the positive Gram stain result was reported. Median time in hours (interquartile range [IQR]) to organism identification: control 22.3 (17–28), both rmPCR and rmPCR + stewardship 1.3 (0.9–1.6); de-escalation: control 39 (19–56), rmPCR 36 (22–61), rmPCR + stewardship 20 (6–36); escalation: control 18 (2–63), rmPCR 4 (1.5–24), rmPCR + stewardship 4 (1.8–9). *P < .05 vs control; †P < .05 vs control and rmPCR groups. (Used with permission from Banerjee R, et al. Randomized Trial of Rapid Multiplex Polymerase Chain Reaction-Based Blood Culture Identification and Susceptibility Testing. Clin Infect Dis. 2015 Oct 1;61(7):1071–80.)

Table.

Representative NAAT platforms that detect pathogens from blood samples and positive blood cultures.

	Time to Result	Availability	Target Profiles
FilmArray® Blood Culture Identification Panel	1–1.5 h	Commercially available	<p><u>Gram (+)</u> <i>Enterococcus</i> spp., <i>L. monocytogenes</i>, <i>Staphylococcus</i> spp., <i>S. aureus</i>, <i>Streptococcus</i> spp., <i>S. agalactiae</i>, <i>S. pneumoniae</i>, <i>S. pyogenes</i>, <u>Gram (–)</u> <i>A. baumannii</i>, <i>H. influenzae</i>, <i>Neisseria meningitidis</i>, <i>P. aeruginosa</i>, <i>E. cloacae</i> complex, <i>E. coli</i>, <i>K. oxytoca</i>, <i>K. pneumoniae</i>, <i>Proteus</i> spp., <i>S. marcescens</i>, <u>Fungi</u> <i>C. albicans</i>, <i>C. glabrata</i>, <i>Candida krusei</i>, <i>Candida parapsilosis</i>, <i>Candida tropicalis</i>) <u>Resistance genes</u> <i>mecA</i>, <i>vanA/B</i> for vancomycin and <i>KPC</i></p>
Nanosphere Verigene	2.5 h for Gram + and 2 h for Gram -	Commercially available	<p><u>Gram (+)</u> <i>S. aureus</i>, <i>S. epidermidis</i>, <i>S. lugdunensis</i>, <i>S. anginosus</i> group, <i>S. agalactiae</i>, <i>S. pneumoniae</i>, <i>S. pyogenes</i>, <i>E. faecalis</i>, <i>E. faecium</i>) and four genera (<i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., <i>Micrococcus</i> spp., <i>Listeria</i> spp.), <u>Gram + resistance genes</u> <i>mecA</i>, <i>vanA</i> and <i>vanB</i> <u>Gram-negative</u> <i>Escherichia coli</i>, <i>Klebsiella pneumoniae</i>, <i>Klebsiella oxytoca</i>, <i>Pseudomonas aeruginosa</i>, <i>Serratia marcescens</i>) and four genera <i>Acinetobacter</i> spp., <i>Citrobacter</i> spp., <i>Enterobacter</i> sp. <u>Gram (–) resistance genes</u> <i>bla</i> CTX-M⁻ IMP, -KPC, -NDM, -OXA and -VIM</p>
SeptiFast	<6h	limited	<p><u>Gram +</u> <i>S. aureus</i>, <i>Coagulase negative staphylococci</i>, <i>S. pneumoniae</i>, <i>Streptococcus</i> spp., <i>E. faecium</i>, <i>Enterococcus faecalis</i> <u>Gram (–)</u> <i>E. coli</i> <i>K. (pneumoniae/oxytoca)</i>, <i>Serratia marcescens</i>, <i>Enterobacter (cloacae/aerogenes)</i>, <i>P. mirabilis</i>, <i>P. aeruginosa</i> <i>A. baumannii</i>, <i>S. maltophilia</i> <u>Fungi</u> <i>C. albicans</i> <i>C. tropicalis</i> <i>C. parapsilosis</i> <i>C. krusei</i> <i>C. glabrata</i> <i>A. fumigatus</i></p>
BD Max	6 h	Not commercial in US	<p><u>Gastrointestinal</u> <i>C. difficile</i> <i>Campylobacter</i>, Shiga toxin-producing <i>E. coli</i>, <i>Salmonella</i>, and <i>Shigella</i> <u>Vaginitis</u> <i>Trichomonas vaginalis</i> <i>Candida krusei</i> and <i>C. glabrata</i></p>
VAPChip	4 h	Not commercially available	<p><u>Gram (+)</u> <i>S. aureus</i>, <i>S. pneumoniae</i> <i>Legionella pneumoniae</i>, <u>Gram (–)</u> <i>E. coli</i>, <i>K. pneumoniae</i>, <i>E. aerogenes</i>, <i>K. oxytoca</i>, <i>S. marcescens</i>, <i>A. baumannii</i>, <i>P. aeruginosa</i>, <i>Stenotrophomonas maltophilia</i>, <u>Resistance Genes</u> <i>bla</i>_{TEM} ESBLs, -SHV ESBLs, -CTX-M, VIM, -IMP, -NDM, -KPC</p>
Hyplex (Amplex) Bloodscreen, Hyplex ESBL,	2.5–4 h	Commercially available	<p><u>Gram (+)</u> <i>S. aureus</i>, <i>S. epidermidis</i>, <i>S. pyogenes</i>, <i>S. pneumoniae</i>, and <i>E. faecalis/E. faecium</i>.</p>

	Time to Result	Availability	Target Profiles
Hyplex Superbug ID, Hyplex MBL, Hyplex CarbOX ID			Gram (–) <i>E.coli</i> , <i>P.aeruginosa</i> , <i>E. aerogenes</i> , <i>Klebsiella</i> spp., Resistance genes <i>van A</i> , <i>vanB</i>
GeneXpert GeneXpert MRSA	1 h	Commercially available	<i>S. aureus</i> (MRSA and MSSA)