# Better Tests, Better Care: Improved Diagnostics for Infectious Diseases

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**In this IDSA policy paper, we review the current diagnostic landscape, including unmet needs and emerging technologies, and assess the challenges to the development and clinical integration of improved tests. To fulfill the promise of emerging diagnostics, IDSA presents recommendations that address a host of identified barriers. Achieving these goals will require the engagement and coordination of a number of stakeholders, including Congress, funding and regulatory bodies, public health agencies, the diagnostics industry, healthcare systems, professional societies, and individual clinicians.**

***Keywords.*** diagnostics; rapid diagnostics; point-of-care testing; molecular diagnostics; clinical microbiology; infectious diseases



## EXECUTIVE SUMMARY

Whether caring for an individual patient with an infectious disease or responding to a worldwide pandemic, the rapid and accurate establishment of a microbial cause is fundamental to quality care. Despite dramatic advances in diagnostic technologies, many patients with suspected infections receive empiric antimicrobial therapy rather than appropriate therapy dictated by the rapid identification of the infectious agent. The result is overuse of our small inventory of effective antimicrobials,

The Infectious Diseases Society of America (IDSA) is a national medical society representing infectious diseases physicians, scientists, and other healthcare professionals dedicated to promoting health through excellence in infectious diseases research, education, prevention, and patient care. The Society, which has >10 000 members, was founded in 1963 and is based in Arlington, Virginia. (For more information, visit www.idsociety.org.) This policy paper was developed for and approved by the IDSA Board of Directors on 20 August 2013.

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whose numbers continue to dwindle due to increasing levels of antimicrobial resistance.

New tests are needed that can identify a specific pathogen or at a minimum, distinguish between bacterial and viral infections, and also provide information on susceptibility to antimicrobial agents. Tests should be easy to use and provide a rapid result (ideally within an hour) to have a positive impact on care. Results must be effectively communicated to the healthcare provider or public health practitioner and may require the interpretive expertise of an infectious diseases physician or clinical microbiologist. The infectious diseases physician can serve as a bridge between the laboratory and the healthcare provider to ensure the proper use and interpretation of diagnostic testing. The expertise of the infectious diseases clinician becomes more important with the advent of newer, more complex tests. The availability of needed tests will lead to improvements in clinical outcomes for patients, antimicrobial stewardship, detection and tracking of disease outbreaks, and investigation of unknown pathogens, both in the United States and globally.

Emerging technologies enable the detection and quantification of pathogen burden with new speed, sensitivity, and simplicity of use. However, there are significant challenges to the development, regulatory approval, and clinical integration of diagnostic tests that use these new technologies. The need for diagnostics that advance clinical care and public health has never been greater, and there is a critical window of opportunity to harness new technologies to address the greatest unmet needs.

In this Infectious Diseases Society of America (IDSA) policy paper, we review the current diagnostic landscape, including unmet needs and emerging technologies, and assess the challenges to the development and clinical integration of improved tests. To fulfill the promise of emerging diagnostics, IDSA presents recommendations that address a host of identified barriers. Achieving these goals will require the engagement and coordination of a number of stakeholders, including Congress, funding and regulatory bodies, public health agencies, the diagnostics industry, healthcare systems, professional societies, and individual clinicians.

## RECOMMENDATIONS

### Stimulate Diagnostics Research and Development

1. Because of the need for research and development (R&D) to translate new technologies into practical tests, federal funding agencies should prioritize diagnostics R&D through innovative funding mechanisms and clinical research infrastructure.

   (a) The National Institutes of Health (NIH) should increase funding of diagnostics research, especially through the Small Business Innovation Research (SBIR) program and the U01 funding mechanism.

   (b) NIH should ensure that the peer review process for diagnostics grant submissions includes study sections with appropriate expertise to evaluate feasibility and clinical applicability, as well as scientific merit.

   (c) Biorepositories or other infrastructure to facilitate the procurement of critical clinical specimens should be developed and maintained to aid in the validation and verification of novel diagnostic methods (see Appendix B for a model of how a repository could work).

2. Because of the impact on patient care, public health surveillance, and biodefense, Congress should support increased appropriations for diagnostics activities, including to:

   (a) NIH, including the SBIR diagnostics program at the National Institute of Allergy and Infectious Diseases (NIAID) and the Point-of-Care Technologies Research Network at the National Institute of Biomedical Imaging and Bioengineering;

   (b) The Biomedical Advanced Research and Development Authority for the advanced development of innovative infectious diseases diagnostics; and

   (c) The Centers for Disease Control and Prevention (CDC) for the Advanced Molecular Detection (AMD) initiative, designed to acquire updated technologies for public health surveillance.

3. Because of the need to accelerate specific areas of diagnostics development, such as rapid diagnostics and diagnostics for resource-limited settings, Congress should enact:

   (a) Legislation in support of a tax credit to cover 50% of clinical research costs for qualifying rapid diagnostics; and

   (b) The 21st Century Global Health Technology Act (H. R. 1515), which will strengthen health R&D programs at the United States Agency for International Development (USAID) and require no new funding.

4. To address the highest priority unmet needs, federal incentives to stimulate diagnostics R&D should focus on tests with the following characteristics:

   (a) Performed directly from accessible, minimally invasive clinical specimens, such as blood, respiratory samples, urine, and stool;

   (b) Able to rule out infection with high certainty (eg, ≥98% negative predictive value) as a first step for a variety of clinical syndromes;

   (c) Incorporating biomarkers that are either pathogen- or host-derived and capable of indicating host response to a pathogen or further classifying clinically significant infectious processes into relevant categories (eg, bacterial, fungal, viral, or parasitic);

(d) Panels targeting the most important clinical syndromes, including central nervous system (CNS) infections, sepsis and bloodstream infections, respiratory tract infections, and most important pathogens, including fungal agents;

(e) Special considerations for pediatric use, especially for tests with biomarkers and syndromic panels;

(f) Pathogen-specific diagnostics, linked to drug resistance information;

(g) Rapid diagnostics that substantially improve upon the "time to result" metric of currently approved tests;

(h) Point-of-care diagnostic testing that allows for usage in many clinical settings, including physician offices; and

(i) Improve outbreak detection and maintain public health surveillance capability.

### Expedite Integration of Improved Diagnostic Tests Into Patient Care

1. To ensure that diagnostic tests can be conducted, and results communicated and acted upon rapidly around the clock, appropriate infrastructure is needed at healthcare institutions, including personnel and information technology.

(a) The Centers for Medicare and Medicaid Services (CMS), in coordination with the Office of the National Coordinator for Health Information Technology, should encourage healthcare systems to improve electronic medical record systems, including the establishment of electronic reporting of laboratory results to health departments.

(b) CMS should provide incentives for healthcare facilities to form dedicated multidisciplinary teams (including infectious diseases consultants, other physicians, nurses, pharmacists, clinical microbiologists, infection preventionists, and the antimicrobial stewardship team) to develop protocols for responding to clinically significant test results.

(c) Congress and the Administration should fund information technology solutions for data integration and dissemination, to be developed locally within healthcare institutions.

(d) Diagnostic companies should work with healthcare systems to ensure that new diagnostics are integrated into current laboratory workflow practices.

2. Healthcare systems should use clinical guidelines from IDSA and other professional societies to guide patient management decisions regarding the use of diagnostics.

3. Outcomes research should be supported that addresses the need for data on diagnostics use in varied clinical settings and data to document the effect of diagnostic testing on the individual patient and the healthcare system.

(a) Funding bodies (including the NIH, the Agency for Healthcare Research and Quality [AHRQ], and the Patient-Centered Outcomes Research Institute) and industry should fund outcomes research to determine whether use of specific tests improves patient outcomes (eg, morbidity and mortality) and/or resource utilization.

(b) Healthcare systems should be encouraged to develop cost-effectiveness models that assess the impact of diagnostics on all facets of patient care, eg, mortality, length of stay, use of antimicrobials, and infection control practices.

### Address Regulatory Challenges to Diagnostics R&D

1. There is an urgent need for Congress and the NIH to clarify and revise conflict of interest policies to allow collaboration between diagnostic companies, diagnostic laboratories, and key opinion leaders. It takes the coordinated expertise of sponsors, academicians, and laboratory personnel to conduct high-quality clinical trials that meet US Food and Drug Administration (FDA) regulatory requirements.

2. Because it would severely limit the conduct of diagnostics research, the Department of Health and Human Services should withdraw the draft proposal to institute a new informed consent requirement for research with de-identified residual clinical samples, outlined in the 2011 Advanced Notice of Proposed Rulemaking for human subjects research protections (ie, the Common Rule).

3. Congress should provide incentives and support for institutions to save de-identified specimens when possible for the purposes of new test development, FDA clinical evaluations, and assay verification and validation.

4. In the interest of addressing unmet patient need, the FDA Center for Devices and Radiological Health (CDRH) should revise the guidance for research use only/investigational use only (RUO/IUO) devices and permit use in cases where there are no other diagnostic options.

5. FDA CDRH should exercise its flexibility and exempt companies from redemonstrating the clinical validity of a novel diagnostic product after multiple studies for similar products have been conducted.

6. FDA CDRH and the FDA Center for Drug Evaluation and Research should provide greater clarity in guidance for the codevelopment of drugs and diagnostics.

7. FDA should assist in the development of strategies to preserve specimens for public health surveillance purposes, for example, by asking developers of new technologies to include a "public health plan" with their submission.

### Ensure Appropriate Levels of Reimbursement for Diagnostic Testing

1. CMS should eliminate the wide regional variations in reimbursement for diagnostic testing and ensure that reimbursement covers the cost of testing at a minimum.

2. To improve the adoption of new tests, CMS should simplify, expedite, and increase the transparency of the process for assigning new Current Procedural Terminology (CPT) codes

and subsequent incorporation of new codes for laboratory tests into the Clinical Laboratory Fee Schedule.

3. To improve the process of Medicare payment rate determination for diagnostic testing, Congress should enact the Diagnostic Innovation Testing and Knowledge Advancement Act of 2013 (H.R. 2085 in the 113th Congress).

### Encourage Adoption of New Tests

1. To provide laboratories with greater clarity about the processes for clinical validation or verification for new diagnostic assays, CMS should work to harmonize recommendations under the Clinical Laboratory Improvement Amendments (CLIA) with those from various professional societies and organizations (eg, Clinical and Laboratory Standards Institute [CLSI], College of American Pathologists).
2. There is an urgent need for all stakeholders to work together to develop guidelines on how to establish reference methods for new technologies that are more sensitive and specific than the existing "gold standard."
3. CMS should discourage facilities that do not receive enough specimens to maintain competency and accuracy from conducting highly complex diagnostic testing.
4. Diagnostic companies should convert highly complex assays to moderately complex tests that can be performed in a variety of clinical settings using "walk-away technology."
5. To ensure an adequate pool of well-qualified professionals, Congress and the Health Resources and Services Administration (HRSA) should work with professional societies to support the recruitment and retention of clinical microbiologists and medical technologists.
6. Diagnostic companies should promote training in new technologies for the laboratory workforce.

### Educate Healthcare Providers on the Use of Diagnostics

1. AHRQ and HRSA should fund and encourage healthcare institutions and professional societies to strengthen educational programs that disseminate the results of diagnostics-focused health sciences research and that inform physicians about the utility of available tests.
2. Professional societies, educational institutions, and other entities involved in the education of clinicians, including graduate medical education, continuing medical education, and maintenance of certification, should ensure that education includes the performance of diagnostic tests, interpretation of test results in individual clinical settings with varied patient populations, available guidelines, and cost of testing.
3. Professional societies and other organizations should include clinical microbiology experts in the development of clinical practice guidelines that make recommendations on the use of diagnostic tests.

## INTRODUCTION

Diagnostic tests play a major role in the clinical care of patients with infectious diseases, including detection of specific pathogens, discovery of new pathogens, determining appropriate therapy, monitoring response to therapy, assessing prognosis, and disease surveillance. Despite the increased use of rapid tests and the availability of molecular and proteomics-based tests, diagnostics are not being integrated into clinical care optimally. Integration is influenced by the clinical syndrome, the availability of and access to appropriate diagnostics, the place of service, and the experience and knowledge of the healthcare provider. The goal of this paper is to increase awareness of the current and potential value of infectious diseases diagnostics for patient care and public health, and to promote further development of needed diagnostics.

Antonie van Leeuwenhoek (1632–1723), the "father of the microscope," changed the course of infectious diseases when he enabled the visualization of the microbial world, a world no one had imagined. Since then, the goal of infectious diseases diagnostics has been to detect the source of the infection and enable an appropriate response. Although we have better stains, better microscopes, and novel agar media types, standard microscopy and culture methods have not changed dramatically in more than a century. Recently, new technologies have brought great advances in infectious diseases diagnostics. The field of clinical microbiology is currently in transition and standard-of-care testing is now a hybrid of old and new methodologies.

The evolution of infectious diseases diagnostics has resulted from advances in chemistry, immunology, molecular biology, engineering, automation, and nucleic acid amplification. It is now possible to determine the specific etiology of a patient's infectious disease in the hospital, clinic, office, remote village, or even a patient's home. With automation and highly multiplexed assays, individual pathogens can be readily identified in a wide variety of specimen types including blood, urine, tissue, mucosal swabs, cerebrospinal fluid (CSF), respiratory secretions, and stool samples.

### Evolution of Diagnostic Assays Through Present Day

The Gram stain was, and still is, often the first line of diagnosis for the infectious diseases consultant. Microscopy, with or without staining, permits the diagnosis of many infectious diseases, for example, acute gonococcal urethritis, primary syphilis, differentiation of gram-positive or gram-negative pathogens on sputum smears, and detection of *Mycobacterium tuberculosis* and similar pathogens. Monoclonal antibodies, tagged with fluorescein, enable microbiologists to visualize organisms such as *Legionella pneumophila* that do not stain well with conventional stains, or to enhance detection of pathogens such as *M. tuberculosis* or *Pneumocystis jirovecii*.

**Table 1. Historical Evolution of Diagnostic Methods and the Associated Time Required for Pathogen Identification**

| Diagnostic Method | Time for Pathogen Identification |
|---|---|
| Microscopy | Morphology in minutes |
| Gram stain | General category in minutes |
| Culture and phenotypic biochemistry on/in artificial media (bacterial, mycobacterial, fungal) | Days to weeks |
| In vitro antimicrobial susceptibility | Days to weeks |
| Acute and convalescent antibody | Weeks |
| Monoclonal antibodies | Hours |
| Antigen detection | Minutes to hours |
| Real-time polymerase chain reaction for microorganisms and drug resistance genes | One to several hours |
| Mass spectrometry | Seconds to minutes, after growth on/in media |

Evolving bacterial culture methods and eventually viral tissue culture further amplified the ability to detect specific pathogens, enabled recovery of the pathogens in pure culture, and allowed for susceptibility testing of bacteria against specific antimicrobial agents. However, a major disadvantage of culture-based methods is the time needed for culture growth. Table 1 provides a snapshot of the time required for pathogen identification using various diagnostic methods.

Developments in biochemistry enabled the detection of a specific metabolic product; for example, the time required for detection and identification of the slow-growing *M. tuberculosis* is substantially reduced by the radiometric detection of $C^{14}$-labeled carbon dioxide produced by the metabolism of palmitic acid. Antibody detection by enzyme immunoassay (EIA) and enzyme-linked immunosorbent assays is enhanced by the use of analytic detectors, such as the spectrophotometer, fluorometer, luminometer, and radioactive counter. The specificity of antigen and antibody detection is increased by the use of monoclonal antibodies and recombinant antigens. Modern antibody panels can detect multiple antigens and/or antibodies or the presence of IgM or IgA antibodies within hours of specimen submission. Examples of antigen detection with specific antibody include rapid testing for *Streptococcus pyogenes* in the throat; cryptococcal antigen in blood and CSF; and detection of the antigens of *Streptococcus pneumoniae*, *Legionella pneumophila* serogroup 1, and *Histoplasma capsulatum* in urine. These assays are more rapid than culture-based tests and do not require cultivation of viable organisms. However, they do not increase sensitivity over that of culture, nor do they provide information on susceptibility of microorganisms to antimicrobial drugs.

The last 2 decades have witnessed the development of polymerase chain reaction (PCR) and other nucleic acid–based amplification

technologies (NAATs) that detect microbial and host genetic sequences with great sensitivity and specificity. Nucleic acid amplification methods are increasingly employed to detect and often quantitate an ever-increasing number of pathogens, for example, human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), and BK virus. The use of molecular diagnostics for quantifying HIV type 1 (HIV-1), HBV, and HCV revolutionized the development of antiretroviral drugs, which could be utilized in the management and treatment of viral infection. We are at the beginning of a significant transformation in diagnostics and it is critical to capitalize on the current opportunity to invest in the most needed diagnostics and enable the utilization of improved diagnostics for both clinical management and public health surveillance.

## SECTION I: VALUE OF INFECTIOUS DISEASES DIAGNOSTICS

Although an economic value of infectious diseases diagnostics is not always easily quantifiable, it is clear that diagnostics play a valuable and critical role in the care of patients with and those at risk of developing an infectious disease. Diagnostics clarify the etiology of the patient's illness, influence treatment modalities, and enable public health surveillance. Diagnostics are applied to different patient populations in settings ranging from outpatient clinics and hospital intensive care units (ICUs) to point-of-care (POC) tests on the battlefield. Advances in POC testing have demonstrated that it is possible for sample collection and testing to be done in remote settings, away from the standard hospital and laboratory healthcare settings. If the tests are simple enough, collection and testing can be conducted by minimally trained personnel without extensive technical skills, or even at home by the patient. POC testing may also be of value in the determination of whether a higher level of care (eg, outpatient to inpatient) is indicated. Interpretation of test results, however, requires putting the data into the appropriate clinical context by a healthcare provider. Increasingly, this is best done by an infectious diseases consultant, who may or may not be on site.

The characteristics of an ideal diagnostic test include accuracy wherever used; heat-stable reagents with an extended shelf life; portability; minimal technical skills for operation; rapid, sensitive, and specific results; on-demand testing capability or minimal batch sizes; low-cost and/or cost-effective for patient care; and suitable for a broad range of clinical samples. Current antigen and nucleic acid detection tests meet some, but not all, of these idealized criteria.

### Diagnosis and Patient Management

A primary role for diagnostics is to identify disease and enable management of the individual patient. Nucleic acid–based technologies have enhanced the diagnosis of bacterial and viral infections as the result of increased test sensitivity and rapid turnaround time. In addition, knowledge of sequences that underlie drug resistance allows detection of microorganisms carrying drug resistance genes. For example, early detection of HIV-1 infection and strain resistance to selected antiretroviral drugs has enabled physicians around the world to more accurately diagnose and treat HIV-1 infection with the appropriate antiretrovirals. Newer automated tests using nanotechnology that no longer require addition of multiple reagents are being used for point-of-care diagnosis. The number of commercially available tests and their uses continue to increase logarithmically, and the cost of instruments and their assays continue to decrease. In the last 2 years, the FDA has cleared (through the 510 [k] pathway) or approved (through the premarket approval pathway) numerous molecular diagnostic tests (Table 2).

Nucleic acid amplification methods for viral targets are on the whole faster, more sensitive, and more cost-effective than traditional culture methods. The diagnosis of enteroviral meningitis, herpes simplex virus (HSV) encephalitis, and CMV infections in immunocompromised patients are examples of clinically relevant and cost-effective applications of nucleic acid–based tests. Conventional methods are more readily replaced in virology than in bacteriology because the tissue culture–based virology methods are costly and generally less

**Table 2. Food and Drug Administration Microbiology Devices Cleared Through the 510(k) Pathway or Approved Through the Premarket Approval Pathway[a]**

| | FDA Center for Devices and Radiological Health Division of Microbiology Devices: Devices Cleared/Approved January 2011–February 2013 | | | | |
|---|---|---|---|---|---|
| Year | 510(k) Molecular | 510(k) Serology/ Other | CLIA Waived[b] | PMA Molecular | PMA Serology/ Other |
| 2013 | 7 | 2 | 0 | 0 | 0 |
| 2012 | 16 | 33 | 3 | 2 | 2 |
| 2011 | 17 | 25 | 6 | 3 | 9 |
| Total | 40 | 60 | 9 | 5 | 11 |

Abbreviations: 510(k), Section 510(k) of the Food, Drug, and Cosmetic Act; CLIA, Clinical Laboratory Improvement Amendments; FDA, Food and Drug Administration; PMA, premarket approval.

[a] FDA regulatory pathways for in vitro diagnostics (IVDs) are based on the risk of a false-negative or false-positive result for a particular microorganism on subsequent patient management. IVDs are grouped into 3 categories depending on level of risk and knowledge of a particular disease state: Class I and II analytes are regarded as having a low (I) or moderate (II) risk of harm and require a 510(k) submission to be sent to the FDA for premarket clearance. Class III analytes are considered to have a high or unknown risk and require more detailed information to be submitted to the FDA in the form of a PMA submission to determine if they are safe and effective.

[b] CLIA-waived assays are those that demonstrate that they are simple enough to be run by untrained personnel in settings such as physicians' offices and that results obtained are similar to those generated by a high/moderate complexity laboratory with trained technical staff.

sensitive than newer molecular methods. Some viruses are highly labile (eg, respiratory syncytial virus [RSV]), cannot be routinely cultured (eg, coronaviruses), or are dangerous to culture (eg, variola). Antiviral susceptibility testing is not routinely performed, but when it is needed, such as for the identification of ganciclovir-resistant CMV, mutations can be rapidly identified using nucleic acid amplification coupled with sequencing. In addition, FDA-cleared multiplex assays utilizing real-time amplification methods enable the simultaneous detection of multiple respiratory viruses, and research assays can detect the nucleic acid of viruses heretofore unrecognized and/or not cultivatable in vitro.

Beyond pathogen identification, molecular methods guide patient management. Quantification of viral nucleic acid is used to stage disease activity, prognosticate disease progression, and monitor efficacy of therapy. For example, the risk of progression to AIDS and ultimately death is directly related to the magnitude of the HIV-1 RNA level in plasma. Similarly, viral load determines the risk of perinatal and sexual transmission. Reduction in viral load levels is associated with increased patient survival and decreased viral transmission. Genotyping of selected pathogens is important for specific therapeutic protocols, especially for HCV and HIV-1. Antiviral susceptibility testing also guides specific therapy.

Genotyping by molecular methods also has prognostic value. For example, human papillomavirus (HPV) types 16 and 18 are associated with high-risk progression to neoplasia, and types 6 and 11 are associated with venereal warts and a low risk of neoplasia. Testing for high-risk HPV DNA is an established method for managing women with the cervical cytologic diagnosis of atypical squamous cells of undetermined significance. Patients are referred for colposcopy based on the detection of high-risk types of HPV DNA. Similarly, CMV load testing can help distinguish between active disease and asymptomatic infection, as higher levels of CMV DNA increase the risk of active disease. Thus, CMV load measurement is useful for deciding when to initiate preemptive therapy in organ transplant recipients. It is likely that quantitative assays will also help distinguish disease from asymptomatic infection with other herpesviruses such as Epstein-Barr virus and human herpesvirus 6.

Molecular methods have had a particularly significant impact on the discovery of previously unrecognized or uncultivable pathogens. HCV and *Tropheryma whipplei* are examples of uncultivable microorganisms first detected through molecular methods. Nucleotide sequencing analysis of the 16S bacterial RNA gene is expanding our knowledge of the phylogenetic relationships among bacteria and is frequently used for bacterial identification, for assessing nosocomial and community outbreaks, and for epidemiological surveillance. Pyrosequencing or ultradeep sequencing enables the detection of multiple strains of HIV-1 within a single individual and identification of minority mutants in the quasispecies. Similarly, pyrosequencing is used to classify mycobacteria and nocardia into clinically important groups and to identify yeast and filamentous fungi.

### Use of Diagnostics Beyond Individual Patient Management

Infectious diseases are closely dependent on the nature and complexity of human behavior, as they reflect who we are, what we do, and how we interact with other people, animals, and the environment. More recent changes in the global climate and environmental changes from hurricanes, flooding, and earthquakes have dramatic influence on the frequency of certain diseases in new locations such as postearthquake cholera in Haiti, the expansion of dengue into North America, or dysentery in Pakistan after floods. Minute or dramatic changes in the environment can have significant impact on the spread of mosquitoes and other disease vectors, resulting in resurgence of diseases such as malaria and dengue. Diagnostics are critical tools in keeping up with continuously changing disease dynamics, and have many applications beyond individual patient management.

The use of infectious diseases diagnostics includes epidemiologic surveillance, infection control efforts, antimicrobial stewardship, facilitation of clinical trial enrollment, companion diagnostics, and other codevelopment of drugs and diagnostic tests. In the case of epidemiologic surveillance, genetic detection of rifampin resistance in *M. tuberculosis* is used as a marker for multidrug resistance, enabling public health officials to estimate the extent of the disease within selected populations and to optimize antimycobacterial therapy.

Antimicrobial stewardship, or the optimization of antimicrobial use in clinical settings, is enhanced by assays that can detect antimicrobial resistance genes. For example, molecular assays can detect the presence of methicillin resistance genes in *Staphylococcus aureus* isolates, or extended-spectrum β-lactamase or carbapenemase resistance genes in gram-negative bacilli. Surveillance for penicillinase-producing and/or fluoroquinolone-resistant *Neisseria gonorrhoeae* can be achieved with new assays. Pulsed-field gel electrophoresis, repetitive sequence–based PCR (rep-PCR), and, increasingly, whole genome sequencing are used to characterize outbreaks of disease in multiple healthcare settings.

A new generation of antimicrobial and antiviral agents highlights the need for diagnostic tests to identify the subpopulations of patients who will benefit from therapy. A classic example is the detection of CCR5 and CXCR4 tropism of HIV-1 when using an antiretroviral drug such as maraviroc, which competitively binds to CCR5 on $CD4^+$ T cells. The presence of a CXCR4 tropic virus strain dramatically lowers the effectiveness of the drug. Hence, use of maraviroc and other entry inhibitors is encouraged by the FDA only in individuals who have been identified with the CCR5 HIV tropism.

### Increased Use of Point-of-Care and Near-Patient Tests

FDA-cleared multiplex PCR platforms are designed to probe respiratory specimens, stool samples, and positive blood culture bottles for an array of pertinent pathogens. Many of these tests are classified as "moderately complex" or "waived" under CLIA regulations. In addition to FDA approval or clearance of new diagnostic tests, CMS regulates the conduct of diagnostic testing in the United States through CLIA. Tests are categorized according to their complexity, with differing requirements for personnel expertise, documentation, and oversight. Simple, low-risk tests can be "waived" and performed in physicians' offices and other locations without routine regulatory oversight. With appropriate training, waived or moderately complex tests can be performed by laboratory assistants or other healthcare personnel (such as nurses or clinical personnel) on instruments located outside the central laboratory (eg, rapid response laboratories adjacent to emergency rooms or in large physician office buildings). Results are available within 1–2 hours and hence can inform critical patient management decisions: for example, whether to initiate antiviral therapy for influenza, begin therapy for tuberculosis, or initiate antibiotic prophylaxis for pregnant women for carriage of group B streptococci, or whether a higher level of care (ie, outpatient to inpatient settings) may be appropriate.

### Future of Infectious Diseases Diagnostics

Whereas there have been significant recent advances in the field of clinical microbiology, and increased availability of molecular and other diagnostic tests, the tests are still not optimally integrated into clinical care for the benefit of patients. Is this because available tests do not address priority clinical needs? Are healthcare providers inadequately informed about the availability and utility of many tests? Are there barriers to the research, development, and regulatory approval of the most needed diagnostics? What are the challenges to laboratory adoption and integration of diagnostics into diverse healthcare settings? In the rest of this paper, we investigate these questions and offer recommendations to address the identified challenges.

## SECTION II: UNMET DIAGNOSTIC NEEDS IN THE CLINICAL SETTING

Prompt initiation of appropriate antimicrobial therapy has led to dramatic reductions in infection-associated morbidity and mortality. However, antibiotic overuse may cause considerable harm as a result of unintended drug toxicity, the development of resistance, and *Clostridium difficile* infection. Diagnostic tests have the potential to improve patient care when the results are available to clinicians in a meaningful time frame, are reliable enough to influence pathogen-directed treatment decisions, and provide important epidemiologic information. Here we summarize the unmet diagnostic needs for infectious diseases, and describe the basic elements that are required for tests to have clinical impact using a variety of healthcare settings, disease states, public health activities, and patient populations as illustrative examples.

### The Outpatient Clinic

Patients in the outpatient clinic setting are most often not seriously ill, but may have an infection that would benefit from antimicrobial therapy. Overuse of antibiotics, however, is a significant problem among outpatients. For example, several studies document that 50% or more of adult and pediatric outpatients who present with acute upper respiratory tract infections receive antibiotics [1–3], despite the fact that the majority of these illnesses are caused by viruses. Antibiotic prescribing in these situations is partly due to the inability to exclude a bacterial infection or to identify a viral pathogen quickly.

Unmet diagnostic needs in the outpatient clinic thus include the development of tests that can accurately rule in or out a bacterial infection (eg, biomarkers that distinguish viral vs bacterial pneumonia) with sufficient certainty that antibiotics can be avoided. Application of POC testing that can reliably detect viral and/or bacterial pathogens would result in enhanced care, less antibiotic empiricism and, at least in theory, reduced patient and societal cost of illness. Accurate tests to detect infection with a single common pathogen, such as influenza, that can be used to direct downstream patient management are also useful in the outpatient setting. Pediatric studies in particular have shown decreased use of antibiotics and increased use of antivirals when influenza is diagnosed by rapid testing [4–7]. Similar results have been shown for group A streptococcus [8]. Unfortunately, the most widely utilized POC diagnostics are rapid antigen tests, which often have limitations in sensitivity [9, 10].

To impact clinical management decisions, test results are needed within roughly 1 hour. The short time frame would be met by a CLIA-waived test performed by the physician, the medical assistant, or an immediately contiguous rapid response laboratory. Few tests are currently available that meet these criteria. Fully automated molecular technologies with high sensitivity and specificity (eg, for respiratory tract infection) are currently either too expensive, time consuming, and/or not of low enough complexity for routine use in the clinic. It is anticipated, however, that the price of fully automated molecular platforms will decrease as a result of manufacturing advances and free market competition.

### The Emergency Department

Physicians in the emergency department (ED) face many of the same issues as outpatient physicians, but work within a different framework and with a different set of resources at their disposal. ED physicians are often managing patients who are sicker, and with whom they do not have an ongoing relationship or

means for follow-up. Infectious diseases decision making in the ED revolves around empiric antimicrobial prescribing, the extent of testing, and whether the patient's illness requires further care in a general hospital bed or in the ICU.

The diagnosis and management of community-acquired pneumonia (CAP) illustrates the need for rapid results (ie, test results that are available to the clinician during the ED encounter) and tests with high enough prognostic value to allow safe outpatient discharge versus hospital admission. Microbiologic testing for an etiologic diagnosis in CAP is presented as "controversial" in the 2007 IDSA/American Thoracic Society (ATS) guidelines, because at the time, the literature indicated a low diagnostic yield with traditional culture and sensitivity and hence minimal impact on clinical care [11]. The IDSA/ATS recommendation calls for improvements in diagnostic capabilities, as the symptoms of bacterial CAP overlap with viral causes of pneumonia as well as exacerbations of asthma or chronic obstructive pulmonary disease. In addition, Medicare performance measures require that appropriate empiric therapy be selected for CAP in the ED.

The FDA clearance of competing multiplex platforms signals a dramatic increase in the power to detect pathogens in the airway, with panels containing up to 17 viral analytes and 4 or more bacterial analytes. The prospect for reduced empiric antibiotic use is evolving rapidly. Literature over the last several decades indicates identification of the etiology of CAP in only 20% of patients; at least 1 Scandinavian study, using modern methods, increased that percentage to 89% [12]. The problem now is that current panels detect only atypical bacteria (eg, *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae*) and not typical bacterial pathogens such as *Haemophilus influenzae* and *S. pneumoniae*. The challenge with these typical bacteria and some viral pathogens is the need to determine if the identified pathogen is colonizing or invading. Hence, there is increased interest in biomarkers; procalcitonin (PCT) is a promising biomarker that can be used in addition to fever, leukocytosis, and clinical syndrome as a predictor of bacterial infection.

As opposed to the outpatient clinic setting, most EDs are associated with inpatient facilities. Therefore, diagnostic tests ordered from the ED can be more complex and do not necessarily require a CLIA waiver to meet the need for rapid turnaround time. Many hospitals are now developing "rapid-response" sections within the clinical laboratories that are located near the patient and staffed for selected testing 24 hours a day, 7 days a week. These laboratories can be structured to perform fully automated molecular testing [13] and/or PCT testing. In addition, many EDs have adopted CLIA-waived PCT testing, urine dipstick testing, and pregnancy testing that is performed by ancillary staff in the ED at the time of the patient visit. Turnaround time is critical for tests conducted in the ED that are used to make prescribing decisions for patients who are ultimately discharged, or to determine the need for a higher level of care (such as ICU admission, or contact or respiratory isolation), with diagnostic tests ideally providing results within an hour.

### The Hospital Ward and Intensive Care Unit

Physicians working in the inpatient setting are faced with increasingly complicated diagnostic dilemmas. Even in the most state-of-the-art hospital facilities, there are multiple high-acuity diseases for which current infectious diseases diagnostics fall short, and we examine 3 key disease areas.

#### *Central Nervous System Infection*

The diagnosis of meningoencephalitis can be challenging because the differential diagnosis is often extensive and includes infectious, postinfectious, and noninfectious causes. Additionally, routine culture methods are slow and the recovery of viable microbes may be diminished by prior receipt of antibiotics. More sensitive molecular-based assays for the detection of viral pathogens directly in CSF have been developed, but currently the only FDA-cleared tests are for enteroviruses.

The potential benefits of rapid NAAT are perhaps best exemplified by clinical studies of HSV encephalitis in adults and enterovirus meningitis in children. PCR testing on CSF is as sensitive as brain biopsy for the diagnosis of HSV encephalitis [14]. Timely access to enterovirus reverse transcription PCR (RT-PCR) results facilitates shorter hospital stays, reduces antibiotic use, and lessens ancillary laboratory testing [15].

Despite comprehensive evaluations with a variety of different laboratory tests including serology, culture-based methods, and currently available molecular assays, as many as 62% of patients with encephalitis remain undiagnosed [16]. New tests for the diagnosis of acute and chronic CNS infections are clearly needed. Given the breadth of pathogens implicated in CNS disease, more comprehensive molecular panels for the agents of meningoencephalitis, both in the immunocompetent as well as the immunocompromised host, would bring added value to commercially available tests. In addition, the impact that these assays have on patient outcomes and the overall cost of hospital care are required to justify their routine use.

#### *Sepsis*

Acute organ dysfunction secondary to infection is a medical emergency with increasing incidence. Like encephalitis, the diagnosis of sepsis may be difficult because clinical signs can overlap with noninfectious causes of systemic inflammation and blood culture results typically require 1–5 days to complete. Early fluid resuscitation combined with the initiation of appropriate antimicrobial therapy improves outcomes [17, 18], but the clinical parameters at the heart of the sepsis definition (ie,

the systemic inflammatory response syndrome [SIRS]) lack specificity toward an etiologic diagnosis. Consequently, approximately 20%–30% of patients with severe sepsis receive inadequate empiric antimicrobial therapy [19–21]. On the other hand, indiscriminate use of multiple antibiotics may have detrimental effects, such as adverse events, *C. difficile* infection, and a rise in antimicrobial resistance.

Procalcitonin has been widely studied as a surrogate marker of bacterial infection in patients with SIRS. PCT levels increase in a variety of "shock" states—for example, bacteremic shock, cardiogenic shock, neurogenic shock, and any condition that causes a low flow state and perhaps allows translocation of gut bacteria. On the other hand, 1, or preferably, 2 normal PCT levels in the hypotensive patient virtually eliminates invasive bacterial infection as an etiology; the negative predictive value is >95% [22–24]. Sequential PCT levels are an excellent guide to the duration of antibacterial therapy [25, 26], but a better understanding of how PCT functions within the broader innate immune response and correlations with microbial etiology are still needed. Furthermore, a highly sensitive test (ie, negative predictive value of >99.9%) would be required to confidently withhold broad-spectrum antibiotics from a critically ill patient with systemic signs of infection. Research is ongoing to identify new biomarkers or combinations of markers that distinguish SIRS related to infection. In addition, host gene expression profiling using peripheral blood mononuclear cells suggests that distinct "biosignatures" can be used to differentiate gram-positive from gram-negative infections as well as viral from bacterial or fungal processes [27]. Proteomic patterns are similarly being assessed for their diagnostic potential. The challenge now is to conduct clinical studies to identify and validate these diagnostic and/or prognostic signatures.

Ultimately, pathogen-specific tests are needed to guide antimicrobial management in septic patients. FDA-cleared multiplex PCR panels are available to identify a small number of microorganisms in positive blood culture bottles within a few hours as opposed to the several days usually required. Molecular diagnostics that detect microbial DNA directly in blood have achieved a modest level of success, but several limitations still exist. Studies of pan-bacterial PCR, for example, have been confounded by the presence of pathogen DNA contamination introduced at the time of specimen collection and/or preparation [28], and the turnaround time to results is lengthened by the need for DNA sequencing following nucleic acid amplification. A multiplex PCR panel, designed to directly detect and identify 25 of the most common bacterial and fungal causes of bloodstream infection within several hours, has been developed. Positive agreement between the commercial PCR and blood culture has ranged from 55% to 85% in nonneutropenic patients [29–35], illustrating that current molecular assays remain relatively insensitive compared to blood culture. Furthermore, the clinical significance of specimens positive only by PCR, with negative cultures for the same organism at other body sites, remains difficult to interpret, as standard NAAT testing does not differentiate viable from nonviable organisms. Multicenter clinical trials are necessary to define the diagnostic accuracy of molecular assays performed on blood, and more sensitive approaches are obviously needed. Based on available data, well-designed multiplex PCRs appear to have value as sepsis diagnostics when used in conjunction with conventional culture and routine antibiotic susceptibility testing.

Looking forward, a diagnostic strategy that incorporates sensitive biomarkers (eg, infection present yes/no) followed by pathogen-specific tests that are linked to a rapid assessment of drug resistance could revolutionize sepsis management. Timely reporting of key drug resistance determinants, such as the *mecA* gene associated with *S. aureus* methicillin resistance or the $bla_{KPC}$ carbapenemase harbored by some *Klebsiella pneumoniae* and *Escherichia coli* isolates, is essential for optimum impact on antimicrobial selection and infection control practices. Backup in vitro susceptibility testing would still be necessary to verify that the resistance gene(s) was functional.

### *Hospital-Acquired and Ventilator-Associated Pneumonia*

Healthcare-associated pneumonia (HCAP), including hospital-acquired pneumonia (HAP) and ventilator-associated pneumonia (VAP), are common healthcare-associated infections that manifest high morbidity and mortality. HCAP, HAP, and VAP are caused by a wide variety of mostly bacterial pathogens and may be polymicrobial. The frequency of specific multidrug-resistant pathogens is likely to vary across institutions and different patient populations, thus emphasizing the need for local surveillance data.

Current HCAP guidelines recommend immediate empiric therapy because treatment delays affect mortality and a definitive diagnosis can be difficult [36]. This approach likely leads to overtreatment of many noninfectious processes that mimic pneumonia. Alternatively, management strategies predicated on microbiology are associated with less antibiotic usage in ICU patients and have the potential to improve patient outcomes [37, 38]. Lower respiratory tract specimens (eg, tracheal aspirate, bronchoalveolar lavage, or protected brush) are preferred for making a microbiologic diagnosis of HAP or VAP, but invasive sampling may not always be possible. Furthermore, positive Gram stain or culture from the lower respiratory tract does not always distinguish an invasive pathogen from airway colonization. Quantitative cultures of lower respiratory tract specimens have been advocated to improve diagnostic specificity; however, a recent Cochrane review concluded that quantitation did not improve outcomes for intubated patients as compared to those managed with qualitative results only [39].

Respiratory specimens are amenable to rapid molecular testing, yet there are currently no commercially available pathogen-specific assays for HAP or VAP, although off-label studies have shown some success for detection of *S. aureus* infections [40]. Like sepsis, future assay development should also include simultaneous assessment of key drug resistance genes. To separate commensals and asymptomatic colonizers from overt pathogens in patients with fever, leukocytosis, and pulmonary infiltrates, quantitative molecular results may prove to be useful, unlike quantitative cultures. In addition, novel approaches, such as detection of specific virulence determinants, are needed.

### Infection Control and Hospital Epidemiology

The hospital epidemiologist and infection preventionist deal with issues ranging from isolation of patients with transmissible infections to outbreak investigation. Unmet diagnostic needs in this arena primarily relate to rapid identification of hospitalized patients with a contagious illness or drug-resistant pathogen, and rapid typing of organisms to determine epidemiologic linkage.

Rapid identification of antibiotic resistance is central to timely isolation of patients harboring drug-resistant organisms. Rapid tests have been FDA cleared for methicillin-resistant *Staphylococcus aureus* (MRSA) screening and *vanA*-containing vancomycin-resistant enterococci (VRE), and new assays are now available for the rapid detection of MRSA and VRE from positive blood culture bottles. Gram-negative resistance is highly complex, and tests to identify organisms that produce extended-spectrum β-lactamases and carbapenemases are recently available or on the horizon.

A recent issue in hospital epidemiology and infection control, one that has been particularly prominent in pediatrics, is isolation of patients testing positive for respiratory pathogens by PCR. The increased sensitivity of PCR and the introduction of large pathogen panels have significantly increased the number of hospitalized patients in respiratory isolation. But is this necessary, particularly in the case of rhinovirus, for which shedding can be prolonged [41] and detection is common among healthy persons [42]? Understanding which children require isolation is an important unanswered question. Additionally, determining whether PCR-based detection indicates active infection and transmissibility, when postexposure prophylaxis is necessary, and which pathogen is primary in a dual detection is important both clinically and for cohorting purposes. *Bordetella pertussis* detection by PCR has raised all of these issues, and recent outbreaks have highlighted the need for a solution. Quantitative PCR may be helpful in this regard but will need additional study.

Prompt outbreak identification is central to controlling the spread of disease, but recognizing that an outbreak has occurred can be difficult. Most typing methods to detect organism relatedness require that specimens be sent to reference laboratories, with confirmation of an outbreak possibly requiring days to weeks. Rapid methods to determine relatedness between organism isolates that could be performed by the hospital microbiology laboratory would improve outbreak detection. One commercial system, based on analysis of repetitive elements, is available for bacterial strain typing, but it has a number of limitations and is not widely used.

### Public Health Surveillance

Public health surveillance monitors infectious diseases in the community in order to identify their occurrence and trends so that measures can be identified to control them and prevent them from reappearing. Examples include identification of resurgence in vaccine-preventable diseases, detection of foodborne illness outbreaks, and monitoring the emergence of antimicrobial-resistant organisms. Testing to support surveillance activities may occur in clinical laboratories but is often conducted and/or confirmed in public health laboratories and the CDC.

For public health surveillance, tests must provide sufficient information to distinguish among strains or serotypes and perhaps identify virulence characteristics and susceptibility to antimicrobial agents, a more detailed characterization than clinical laboratories may require. Although public health laboratories are preparing for a transition to novel technologies, currently they rely on cultured organisms to support such characterization. For example, PulseNet enables the detection of foodborne outbreaks through the characterization of DNA fingerprints using pulsed-field gel electrophoresis. Isolates with indistinguishable DNA patterns can be identified, thus linking patients and, ultimately, the source of the infection.

As culture-independent technologies become predominant on the clinical front lines, strategies for specimen preservation and partnerships with clinical laboratories for specimen collection will be critical. One approach may be to ask developers of new technologies to propose a "public health plan" at the time of their submission for product approval. Whereas preserving organism-based surveillance capabilities is an important short- to medium-term necessity for public health, development of alternative surveillance strategies is a crucial long-term goal. Molecular strategies such as whole genome sequencing are under consideration, but will take significant bioinformatics capabilities, and time to build databases that can replace those built up over many years using older technologies. It will also be essential to ensure that laboratories are providing standardized information for surveillance purposes. The CDC has recently proposed a new initiative, the AMD initiative, designed to improve its genomic sequencing and bioinformatics capabilities, which will be important for public health surveillance using these new technologies.

### Biodefense and Emerging Infectious Diseases

Diagnostics for biodefense-related pathogens offer unique challenges in both their development and utilization. Perhaps the greatest impediment to development lies in the limited availability of clinical specimens that are typically required as part of the traditional regulatory submission process. Insufficient quantities of appropriate samples have been partially addressed by allowing an alternative methodology, the use of spiked specimens that mimic the clinical specimens that are expected to be encountered. This paradigm may also prove valuable in the setting of an emerging infectious disease for which a new assay must be rapidly developed, as in the recent case of the novel Middle East respiratory syndrome coronavirus (MERS-CoV). Other relevant issues concern prompt diagnostics implementation, likely to be required in mass casualty situations. Thus, high throughput, field portability, and minimal training, both in operation and interpretation, will be paramount features of such novel assays. Finally, because these assays will not be in routine use, the development of stand-alone assays, with necessary long-term storage and continual replenishment of equipment and reagents, would be costly. As a result, the development and adaptation of existing multiplexed platforms in routine use for more common infectious diseases is likely to be pursued to provide a robust capability if and when a need should arise.

Current regulations allow for the development and deployment of diagnostic assays in the case of a public health emergency through an Emergency Use Authorization (EUA). Use of the EUA had its first real test during the 2009 influenza A(H1N1) pandemic (A[H1N1]pdm09). The CDC developed a PCR assay and the FDA cleared its use under the EUA, allowing for rapid development and deployment of an assay to detect the A(H1N1)pdm09 strain to the public health laboratories. This was followed by the development of a number of commercial assays that were also cleared through the EUA for use in routine clinical laboratories. The EUA mechanism was utilized again in April 2013, as the CDC developed and the FDA cleared a diagnostic test for the emerging influenza A (H7N9) strain. Similarly in June 2013, the FDA worked with the CDC to authorize its assay for MERS-CoV, which was declared a potential threat to public health at the end of May. The regulatory hurdles that might normally hinder such rapid development can be overcome through the EUA mechanism during a declared emergency or declared potential high risk for an emergency.

### Resource-Constrained Settings

Diagnostic implementation and utilization in resource limiting and/or constrained settings present unique challenges relative to traditional secondary and tertiary care settings. Although sensitivity and specificity are considerations regardless of setting, differences in prevalence of disease and the potential for a larger differential diagnosis may render predictive values less clinically useful in particular applications. In terms of significant resource limitations, desirable diagnostic assays should require little to no power input (such as electricity) as well as minimal technical skill and training in both the operation and interpretation of the test. Portability should be high and storage requirements for specimens and reagents (such as cold chain) should be minimal. The instruments should be able to withstand temperature extremes and power surges (if electricity is required). Finally, the interval from sample collection to a clinical decision-making result must occur during a single visit. A novel rapid test for diagnosis of drug-resistant tuberculosis, rapid antigen testing for malaria, and a novel rapid test for early diagnosis of leprosy are examples of emerging technologies with applications in the developing world. It should be noted that resource-constrained settings can occur within developed countries, such as underserved inner-city and rural areas. While the issues described here are relevant for less developed environments, all of these attributes are also appropriate for medical office practices, public health clinics, and field operations. In fact, the development of technology suitable for diagnostic assays for resource-limited countries, as in the case of tuberculosis or malaria, may create opportunities for novel applications in countries with more abundant resources.

### Special Patient Populations

#### *Immunocompromised Hosts*

The diagnosis of severe infection in immunocompromised patients also presents unique diagnostic challenges. Immunosuppressed patients may have minimal or atypical signs of infection, and invasive testing may not be possible due to coagulopathy or severe illness. Minimally invasive approaches that are predicated on the host response to infection, however, are unlikely to be effective in the face of neutropenia and/or immunosuppressive therapy. In addition, the breadth of potential pathogens affecting the immunocompromised greatly exceeds that of other patient populations. The creation of diagnostic panels that target an even broader range of organisms is required for optimal patient care.

Diagnosing invasive fungal infection is particularly problematic due to the insensitivity of classical culture and microscopy combined with the moderate predictive value of current fungal biomarkers. Diagnostic uncertainty unnecessarily delays initiation of appropriate antifungal therapy, which is in turn linked to poor outcomes for a variety of invasive fungal infections. Different amplification-based strategies have been evaluated for the diagnosis of invasive *Candida* and *Aspergillus* infections in high-risk hematology patients with varying results. Recent meta-analyses highlight the lack of assay standardization combined with inconsistent testing algorithms as sources of heterogeneity across studies [43, 44]. New options for the diagnosis of

a variety of emerging fungal diseases in addition to *Candida* and *Aspergillus* are urgently needed, and test development should be paired with robust laboratory standardization efforts akin to the European *Aspergillus* PCR Initiative [45].

### *Children and Adolescents*

Pediatric patients are seen in all of the clinical settings previously discussed, and their general diagnostic needs are similar. However, pediatric patients have several unique needs that should be specifically addressed. An overarching unmet need is validation of tests within pediatric populations. Due to the enhanced costs associated with obtaining FDA clearance or approval for a new diagnostic product specifically in children—because of the large numbers of children in various age groups that need to be enrolled, difficulty in obtaining informed consent, and inability to get adequate amounts of specimens for testing—most diagnostics are vetted only for adult patients, with results assumed to be similar for children, which is not always true. For example, biomarker-based testing such as PCT needs specific reference ranges for young infants [46]. Pathogen-based testing also needs to take into account colonization rates in children; this is true for urinary antigen testing for pneumococcal disease, a test that has a poor positive predictive value in children due to their high pneumococcal colonization rates [47]. Finally, *C. difficile* testing is of questionable utility in children <2 years of age as asymptomatic carriage of toxigenic strains is common [48].

Additional needs unique to pediatrics include the need for smaller sample volumes, as blood volumes required for testing often cannot be obtained safely from young or premature infants. A term newborn has approximately 300 mL of total blood volume and premature infants can have significantly less. Testing validated for less invasive clinical samples is also desired, although this is not exclusive to pediatrics. Invasive procedures can be more difficult to perform in children and there is a reluctance to put children through an invasive test. Last, pediatrics is almost always a smaller diagnostic market than that for adult testing and there is often little incentive for test validation in children. As with therapeutics, additional incentives should be provided for pediatric diagnostic validations and FDA clearance.

## Communication and Utilization of Results

As new testing methodologies are introduced into the clinical laboratory, particularly assays for which timeliness is essential to changing clinical practice, an important hurdle to address is how to quickly communicate the rapid results to the appropriate care provider, and when appropriate, to public health authorities. In studies investigating the utility of a rapid test to identify MRSA versus methicillin-susceptible *S. aureus* (MSSA) and coagulase-negative staphylococci from positive blood cultures, improvements in antibiotic management and mortality were contingent upon rapid result communication directly to the treating physician or a designee such as an infectious diseases–trained pharmacist or member of the antibiotic stewardship team [49]. Given the financial constraints and staffing limitations faced by many laboratories, it is essential that an efficient means of communication be developed that does not involve manual phone calls. Communication through an intermediary such as a pharmacist, infectious diseases consultant, or the antibiotic stewardship team may be ideal because guidance about appropriate therapy and infection control measures can also be provided on a case-by-case basis. Information technology services, such as delivery of results to mobile devices, are also required to provide electronic communications support for the effective, and preferably automated, transmission of laboratory results such that the benefits of rapid testing can be fully realized.

# SECTION III: NEW AND DEVELOPING TECHNOLOGIES: IMPACT ON UNMET CLINICAL NEEDS

During the last decade, new diagnostic technologies and testing platforms revolutionized the way in which laboratories identify the agents of a wide range of infectious diseases and genetic markers related to antimicrobial resistance. The advances will continue to accelerate in the near future. These technologies rely on a variety of established and novel applications to detect nucleic acids and/or proteins, from the pathogen and/or the host. These advances provide laboratories with the ability to greatly improve testing services through methods that are often more sensitive, more specific, faster, and, depending on the target, can offer a broader range of pathogen detection as compared to most traditional methods. Automation, including platforms that provide simple sample-in-result-out on-demand testing, allows laboratories of every size to implement "cutting-edge" technology. Importantly, these technological advances are demonstrating significant impact on the practice of medicine, including not only a rapid diagnosis, but decreased length of stay, optimization of treatment selection and antimicrobial stewardship, enhanced infectious disease surveillance, and initiation of infection control practices [50–57]. Many of these emerging technologies have potential to address current unmet clinical needs, as outlined in Table 3 and further illustrated in the rest of the section.

## Evolving Changes in Test Services and Methods

### *Shift From Centralized to Decentralized Point-of-Care Testing*

Although the majority of testing for infectious diseases is laboratory based, POC rapid antigen-based tests have been widely used. However, FDA-approved rapid antigen tests are only available for select pathogens (eg, adenovirus, rotavirus, influenza A, influenza B, RSV, *L. pneumophila* serotype 1, *S. pneumoniae*,

**Table 3. Potential of New Technologies to Address Unmet Clinical Needs**

| Unmet Need | Example of Pathogen/Syndrome | Potential Technologies |
|---|---|---|
| Rapid testing from clinical specimen (≤60 minutes) | HSV-1/2, VZV, enterovirus, parechovirus, influenza, RSV, bacterial resistance (KPC, NDM-1) | Single-step molecular cartridge-based tests |
| Rapid testing from clinical isolate (≤60 minutes) | Bacterial, fungal, or mycobacterial isolate | MALDI-TOF MS, single-step molecular cartridge-based tests |
| POC or near-patient testing (≤60 minutes) | Respiratory infections (viral and bacterial), meningitis | Single-step molecular cartridge-based tests, handheld devices for molecular testing, LAMP coupled with Biosensors |
| Simplicity (CLIA waived) | Influenza, tuberculosis, malaria | Handheld devices for molecular testing, single-step molecular cartridge-based tests |
| Syndromic testing | Sepsis, pneumonia (HAP, VAP, CAP), meningitis, diarrheal diseases | Highly multiplexed single-step molecular cartridge-based tests, PCR coupled with T2 magnetic resonance |
| Screening for infection | Biomarkers to distinguish infection from no infection, bacterial from viral infection | Biosensors, biomarkers |
| Resource-constrained settings | HIV-1, tuberculosis, malaria | Handheld devices for molecular testing, single-step molecular cartridge-based tests |
| Infection control/hospital epidemiology | Outbreak evaluations of multidrug-resistant organism, rapid strain typing | Next-generation sequencing |
| Discovery of emerging pathogens | Influenza A H5 and H7, MERS-CoV | PCR coupled with ESI-TOF, next-generation sequencing |

Abbreviations: CAP, community-acquired pneumonia; CLIA, Clinical Laboratory Improvement Amendments; ESI-TOF, electrospray ionization time-of-flight; HAP, hospital-associated pneumonia; HIV-1, human immunodeficiency virus type 1; HSV-1/2, herpes simplex viruses 1 and 2; KPC, *Klebsiella pneumoniae* carbapenemase; LAMP, loop-mediated amplification; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MERS-CoV, Middle Eastern respiratory syndrome coronavirus; NDM-1, New Delhi metallo-β-lactamase 1; PCR, polymerase chain reaction; POC, point-of-care; RSV, respiratory syncytial virus; VAP, ventilator-associated pneumonia; VZV, varicella zoster virus.

*S. pyogenes*, *Plasmodium* species, *C. difficile*, *Cryptosporidium*, *Giardia*, *Cryptococcus neoformans*, *H. capsulatum*, and other fungi). The performance of such devices ranges from good (eg, 80%–85% sensitive for RSV in children <5 years of age) to poor (eg, 30%–50% sensitive for influenza B in geriatric patients). Results depend on the analyte, sensitivity of the test, type of sample collected, age of the patient, and time of testing after onset of clinical symptoms. Clinicians are not always aware of the performance limitations of each test, and, when clinically indicated, the need to perform additional testing.

As a result of reduced sensitivity, specificity, and limited number of analytes detected, the current evolution is to replace rapid antigen POC tests with commercial platforms that perform real-time amplification and detection assays for numerous pathogens. Single-step, cartridge-based molecular test devices target pathogens pertinent to clinical syndromes (eg, respiratory tract infections, CNS infections, gastroenteritis, and sepsis). These assays can be performed on-demand, require minimal hands-on time (1–2 minutes), require minimal technical skills to operate, and provide rapid results (20 minutes to 5 hours). However, a limitation of some cartridge-based platforms is throughput capability. Some systems only allow 1 test to be performed at a time, often requiring multiple expensive separate platforms or additional modules. Until there is an FDA CLIA-waived molecular POC bedside test, self-contained multiplex amplification platforms can be located in central/referral clinical laboratories, rapid response laboratories, and CLIA-certified mini-laboratories in clinic and public health facilities, while still providing a result within the needed time frame. Ideally, future POC tests will be designed such that testing is performed with portable handheld, rechargeable or battery operated, rapid cycling devices.

### *Shift From Single-Analyte Testing to Multiplex Testing*

With the exception of the tests for the detection of *Chlamydia trachomatis* and *N. gonorrhoeae*, the majority of the older molecular diagnostic assays were developed as single-analyte tests. Today, molecular diagnostic testing has moved toward a more broad syndromic screening approach. This trend is the result of sophisticated technologies that enable the detection of >1 analyte at a time. Tests/devices vary from low multiplex (2–5 analytes) to 25 or more different analytes, often now combining a mixture of pathogen types, (eg, bacteria, viruses, and parasites), and also antibiotic resistance markers. New highly multiplexed cartridge-based systems can detect the majority of bacterial pathogens that cause community, ventilator-associated, and hospital-acquired pneumonia within 1–2 hours. In addition, these systems can detect >20 antibiotic resistance genetic markers. The intent is not to replace culture, as additional characterization of isolates still needs to be performed on cultured samples, but to provide a POC early diagnosis that can lead to more focused appropriate antimicrobial therapy. Other

multiplex panels focus on pathogens found in patients with a clinical picture of septic shock or diarrhea caused by a gastrointestinal pathogen. Multiplexed enteric disease panels target common bacterial pathogens such as *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, and *Campylobacter* species, and in addition, bacterial toxins (eg, Shiga toxin), parasites (eg, *Giardia*), and viruses (eg, norovirus). The selection of assay analytes must be clinically relevant; more analytes is not always better and the testing expense may not be reimbursed. At least in the near term, multiplex amplification platforms will replace some activities (viral culture) but will supplement other testing (antibiotic in vitro susceptibility testing).

Molecular platforms need to adapt quickly to changing clinical environments. New pathogens are identified (eg, MERS-CoV), variants of old pathogens emerge (eg, influenza A[H1N1]pdm09 and H7N9), and new antibiotic resistance mechanisms evolve (eg, *K. pneumoniae* carbapenemases and New Delhi metallo-β-lactamase-1). Hence, there is a strong indication for flexibility in test or instrument design. For example, multiplex systems involve complex primer and probe interactions during the initial or subsequent amplification and detection steps. The addition of new primers and probes to detect an additional target will require validation of the newly added target and demonstration that the performance for existing targets is not affected. The revalidation of all existing targets does not need to be undertaken again, and the experimental evidence required demonstrating performance is limited to key points that can be addressed through in-house studies. Furthermore, FDA encourages assay developers to archive specimens used during the clinical evaluation for future use, especially in cases where the multiplex panel is being expanded. These ideas can and have been applied to existing cleared multiplex devices to address detection of emerging and evolving pathogens; however, it is important to note that these mechanisms should not be used to inflate the assay menu of a device to increase marketability, but rather should be used to address true public health needs.

## Nucleic Acid–Based Testing Options

### *Amplification-Based Technologies*

To test for the presence of nucleic acid targets in clinical samples, it is necessary to extract the nucleic acids from the pathogen, amplify the genetic target, and then detect the amplified sequences. Traditional labor intensive nucleic acid isolation and purification methods are now replaced by automated platforms or incorporated directly into 1-step cartridge-based devices. Nucleic acid amplification technologies have evolved from traditional and real-time PCR, to newer methods such as loop-mediated amplification (LAMP), transcription-mediated amplification, nucleic acid sequence-based amplification (NASBA), and strand displacement amplification (SDA). Technologies such as LAMP, NASBA, and SDA are isothermal and do not require expensive thermocyclers.

Real-time nucleic acid detection methods rely on fluorometric probes, of which there are many varieties (eg, molecular beacons, fluorescent energy transfer, TaqMan, scorpions, and locked nucleic acids). Amplified targets are detected by turbidity, chemiluminescence, or either solid-based or liquid bead-based microarrays. In lieu of target amplification, nucleic acids are detected directly using sensitive signal amplification methods such as branched DNA and probe amplification methods.

### *Electrospray Ionization Time-of-Flight Mass Spectroscopy*

For electrospray ionization time-of-flight (ESI-TOF) mass spectroscopy, nucleic acids isolated from clinical samples are amplified with broad-range primers targeting highly conserved genomic regions that flank sequences with variable genetic content. Amplified nucleic acid fragments enter the ionization chamber of an ESI-TOF mass spectrometer. Charged strands leave the electrospray phase and are pulsed under high vacuum. The nucleic acid strands move through the flight tube; lower mass amplicons travel faster and reach the detector before higher mass amplicons. Each time an amplicon is detected, the mass spectrum increases. In general, the ESI-TOF interrogates the amplicon weight to determine the base compositions of complementary DNA strands from multiple sequences. A small number of possible base compositions are consistent with each measured mass. Base compositions of forward and reverse amplicon strands must be complementary, reducing possibilities to a single, unique base composition, allowing for accurate identification of the target.

This technology has been used to identify viral respiratory and biothreat pathogens, including novel variants of influenza. Resistance genes and specific toxins have also been detected. A major benefit is the ability to identify many diverse pathogens without having to target each analyte specifically. As with all mass spectrometry systems, the scope of pathogen detection is dependent on the accuracy of the ESI-TOF database. Although this technology has great promise, there are several drawbacks with the current instruments. The process is slow, labor intensive, and, for direct sample testing, would require nucleic acid isolation and amplification to produce sufficient concentrations of nucleic acids for detection. The current platforms are too large and costly to be practical in a clinical laboratory. Future systems need enhanced sensitivity so as to allow direct detection of pathogens from clinical samples. New instruments will be significantly reduced in size, cost, and complexity.

### *Sequencing*

For many years NAATs have been combined with traditional capillary electrophoresis sequencing or pyrosequencing applications. Sequencing has been used for pathogen

identification from isolates or directly from clinical specimens such as fresh-frozen or paraffin-embedded tissue. Sequencing can establish a microbial etiology in cases where no pathogen was identified by traditional culture methods or due to previous antimicrobial therapy (eg, culture-negative endocarditis). Sequencing can provide a genus identification for generally >90% of the strains, and species-level identification in 65%–85% of the isolates tested. Some isolates are either misidentified due to high sequence similarities to other pathogens (eg, *N. gonorrhoeae* and *Neisseria meningitidis* using 16S RNA sequences) or are never identified. Other concerns include incorrect sequences present in public databases and the high cost of access to commercially available vetted databases. Sequencing is also used to identify known and novel mutations relating to drug resistance. Due to complexity and cost, this approach is generally restricted to large university laboratories and reference laboratories.

Fueled by the human genome project and market incentives such as XPRIZE, "next-generation sequencing" (NGS) systems were developed and demonstrate better performance, longer read lengths, and increased accuracy, utilizing applications that require fewer consumables and are less labor-intensive than traditional sequencing methods. Conversely, NGS requires extensive bioinformatics for data interpretation [58]. To meet the needs for routine clinical testing, compact NGS systems have been developed with a small footprint and fast turnaround time. Using these systems, sequencing could replace complex multifaceted traditional microbiological identification procedures such as biochemical testing, and sequencing could detect virulence determinants and the genetic markers of antimicrobial resistance. Sequencing, however, may miss new and uncharacterized genetic elements responsible for phenotypic resistance mechanisms. This will require companies to perform extensive phenotypic/genotypic comparisons and may in addition require gene expression analysis for detecting complex phenotypes (eg, changes in porin expression related to carbapenem resistance). Sequence data has already proved its value in tracking outbreaks of infection due to resistant bacteria (eg, the NIH outbreak of multidrug-resistant gram-negative bacteria [55] and the cholera outbreak in Haiti [50]). Future studies need to address the clinical relevance of finding a fragment of nucleic acid that may not correlate with the patient's clinical syndrome. Finally, NGS systems need easy, concise, low-cost data management and interpretation software and access to well-vetted databases. Only then will NGS systems become a primary diagnostics approach for infectious diseases. The development and implementation of standardized methods that allow for data sharing across institutions will be critical to the success of NGS as a public health tool for outbreak detection and response and surveillance.

## Proteomics

### *Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry*

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is used for many applications in biochemistry, polymer chemistry, and proteomics. New MALDI-TOF MS platforms can be used routinely in clinical microbiology laboratories for the rapid identification of bacteria, fungi, mycobacteria, and parasites [59, 60]. MALDI-TOF MS testing is relatively simple and, for most bacteria, only requires transferring a portion of an isolated colony onto a well in a designated disposable or reusable target slide. Organisms such as mycobacteria and fungi usually require an additional pretreatment step to ensure nonviability of the organism before manipulation and extraction of the proteins of interest. The inoculated well is covered with a chemical matrix, generally α-cyano-4-hydroxycinnamic acid, dried, and the target plate is loaded into the instrument. Vacuum is applied to the chamber and the target slide wells are pulsed by a laser, converting the sample into an ionic gas composed of small molecules, peptides, and small proteins. In the ionization chamber, positively charged molecules are accelerated through an electric field and then enter the time-of-flight mass analyzer. Smaller particles travel faster through the tube to the particle detector. The particle detector measures the flight times, which are converted into mass/velocity values and plotted on a mass spectrogram. The spectrogram is then compared to a validated library of spectrograms using proprietary algorithms specific to each manufacturer. Testing of calibrators and controls ensure the appropriate performance of the MALDI-TOF MS. The time to first result is approximately 10–20 minutes and each new subsequent result is available within 1 minute.

The MALDI-TOF MS systems have the essential characteristics for success: rapid and accurate results, minimal technical time, and simple sample preparation. These systems are also amenable to integration into automated platforms that can inoculate the MALDI-TOF MS slide and collate the MALDI-TOF MS identification with antimicrobial susceptibility data. The initial cost for the MALDI-TOF MS unit ranges from approximately $160 000 to $250 000, and the yearly maintenance costs roughly $35 000 a year. However, these expenses are offset by the low reagent cost per identification ($.10–$.50) as compared to traditional microbiologic systems (approximately $2.50–$5.00 per identification) plus savings in technical time (about 1 minute per identification) and the clinical benefit of providing an organism identification in about 20 minutes [57]. Currently, when a blood culture is flagged as positive for organism growth, laboratories generally report the results of a Gram stain and need an additional 24–48 hours or more for organism identification and susceptibility test results. Alternative technologies

such as peptide nucleic acid fluorescence in situ hybridization and NAAT systems can provide identification of an organism and some susceptibility data within hours. However, these tests are currently limited by the number of pathogens detected, cost per test (on average $25–$75) and, for some methods, technical time required to perform the testing. With MALDI-TOF MS, direct identification of virtually any organism from a positive blood culture broth is possible within 20 minutes at a cost of a few cents. Rapid pathogen identification has a major impact on the selection of antimicrobial therapy [61]. Preliminary studies demonstrate that MALDI-TOF MS can also detect certain types of antibiotic resistance mechanisms that result in structural modifications to the antibiotic [62]. For example, the hydrolysis of β-lactam drugs by bacterial β -lactamase enzymes can be detected due to a mass shift in the size of the original compound upon hydrolysis [62]. This shift occurs within hours of exposure of the drug to the organism of interest. Resistance detection applications of MALDI-TOF MS are in the early stages of development. The ultimate goal is to couple rapid susceptibility testing with MALDI-TOF MS identification within 24–36 hours of receipt in the laboratory.

Recently, a new technology that combines PCR and T2 magnetic resonance has demonstrated that *Candida* species can be detected directly from as little as 1 mL of whole blood with a sensitivity ranging from 1 to 3 colony-forming units/mL [63]. This technology may overcome the major limitation of the current detection assays that can only be performed once a blood culture bottle is flagged as positive, thereby reducing detection time from days to hours.

### *Biosensors*

A biosensor is an analytical device comprised of 2 elements in physical proximity: a biological recognition element able to interact specifically with a target (eg, nucleic acid or protein of a pathogen) and a transducer that is able to convert the recognition of the pathogen into a measurable signal. The biological recognition element may be naturally occurring (eg, microorganism, enzyme, antibody, antigen, nucleic acid sequence, cell receptor), a biologically derived material (eg, recombinant antibodies and antigens, aptamers, or engineered proteins), or a biomimic (eg, synthetic catalysts, combinatorial ligands, or imprinted polymers). The physical transducer can be optical, electrochemical, thermometric, piezoelectric, magnetic, or micromechanical. This interaction generates a signal (electrochemical, optical, acoustical, mechanical, calorimetric, or electronic) that is amplified and proportional to the concentration of the target analytes.

Successful POC biosensor diagnostic devices will need to combine miniaturization using, for example, microfluidics, with simple isothermal amplification technologies such as LAMP and robust sensing techniques. Preliminary feasibility studies have demonstrated that integrated nucleic acid and protein detection on an electrochemical biosensor array can be used to detect monomicrobial or polymicrobial urinary tract infections [64, 65]. The electrochemical-based sensing technique provided sufficient selectivity, sensitivity, and low-cost detection of nucleic acid sequences amplified from urine. To complement bacterial identification, a direct biosensor quantification of bacterial 16S RNA was developed to monitor bacterial growth for antibiotic susceptibility testing [66]. Additionally, differential impedance has been used for the direct detection of bacterial stress responses in contaminated platelet samples [67]. Low-cost power requirements, compatibility with different matrices, ease of use, and interpretation are essential for clinical applications. Ideally, biosensors would be able to detect the target of interest without an amplification step. This would require a means to enhance the capture of the target nucleic acids and/or proteins with sufficient efficiency for direct detection. Alternatively, a combination of direct nucleic acid and protein capture (pathogen or biomarker) might have the potential to achieve this goal. Essentially, POC biosensor properties proven relevant for such things as plant disease management are highly applicable to early detection, identification, and quantification of human infectious agents. Detection of patient colonization with certain pathogens allows for the initiation of early treatment or infection control strategies before a significant outbreak can occur.

## Clinical Relevance

The sensitivity and timeliness of culture results are influenced by many factors, especially for hospitalized patients with previously administered and/or concurrent antimicrobial treatment. As more technologies are introduced, significant challenges will be faced in interpreting the data. Among approaches that rely upon the detection of nucleic acids directly from clinical samples, there is sometimes uncertainty regarding the clinical significance of the results. The nucleic acids detected may be from nonviable organisms or from commensal (nonpathogenic) or colonizing bacteria or viruses that are noncontributory to the disease. Therefore, nucleic acid–based assays must provide a "clinically" relevant level of sensitivity and be of sufficient scope to detect all relevant pathogens, or in the case of many viruses, all genotypes. An incorrect diagnosis based on the presence or absence of nucleic acids could actually obscure the determination of the real pathogen.

Clinical studies that evaluated the presence of respiratory viruses in asymptomatic patients indicate that the old doctrine, which considered the presence of any respiratory virus clinically significant, is no longer true [68, 69]. Individuals can shed respiratory virus without any evidence of disease (eg, adenovirus). Additionally, the clinical significance of mixed viral infections is not always clear (eg, bocavirus as part of a mixed viral

infection). In these instances, clinicians are faced with the challenge of asking "is this colonization versus infection" or "what pathogen(s) is responsible for the disease?" To distinguish colonizers and contaminants from true pathogens, and interpret test results, close collaboration with the laboratory is critical, including adherence to specimen collection and transport guidelines, an understanding of test performance, pretest probability of disease, and reliance on clinical judgment, including consultation with infectious disease physicians. For example, in the diagnosis of bloodstream infections, the detection of the nucleic acids of a coagulase-negative *Staphylococcus* species in the blood of a person with a prosthetic valve and possible endocarditis is very different than detection in an immunocompetent 30-year-old with community-acquired pneumonia. When both *S. aureus* and *mecA* gene nucleic acids are detected in a febrile patient with appropriate risk factors, a diagnosis of MRSA bacteremia is clear. The answer is not so straightforward when CMV viremia is detected in a patient with fever in the ICU whose blood cultures are negative for bacteria.

Lastly, the detection of antibiotic resistance genes directly from clinical samples is challenging, as mere presence may not always correlate with gene expression and clinical resistance to therapy. In addition, linkage of a resistance marker to a specific organism is necessary to determine the relevance of a positive result. For example, the detection of the *mecA* gene from a skin infection does not necessarily mean that the patient has MRSA, as the majority of nonpathogenic coagulase-negative *Staphylococcus* species that colonize the skin also contain the *mecA* gene. Detection of the *vanB* gene in rectal swabs does not necessarily indicate that a patient is colonized with a vancomycin-resistant *Enterococcus* species as some anaerobes, which are part of the normal stool flora, contain the *vanB* gene.

Despite these limitations, molecular methods that rely on nucleic acid amplification offer a unique advantage in the detection of pathogens in specimens collected after initiation of antimicrobial treatment; hence, there is an opportunity to deescalate empiric therapy (or increase the specificity of antimicrobial therapy). Furthermore, the disappearance of pathogen nucleic acids can suggest that the organism is "eradicated" and might justify shorter courses of treatment. Last, these novel molecular technologies may lead to new insights into disease pathogenesis by revealing previously unknown information.

### Analysis and Interpretation of Novel Data

Information generated by sequencing, mass spectrometry, and other complex tests bring their own challenges. Sequencing, for example, provides a significant amount of data, but how the data are interpreted, what part of the data are most important, and how the data relate to the infectious organisms detected all need study and clarification. How genotypic data relate to phenotypic data may also be unknown. Complex tests may require complex data analysis, and algorithms for analysis are incomplete.

Data from whole-genome sequencing, arrays, and other technologies are not consistent with the data received from older technologies. For example, whereas previously a test result may have simply been reported as "reactive," advanced technologies provide much more information, which in turn necessitates decisions about data interpretation, priorities, and reporting formats. Links between the laboratory equipment and the laboratory information management system must be designed so that the results are clearly presented and easily understood by the clinician.

## SECTION IV: CHALLENGES TO DIAGNOSTICS RESEARCH AND DEVELOPMENT

Many diagnostic companies have been successful recently in developing and launching novel products for rapid detection and identification of infectious agents. These products are formatted either for culture confirmation (eg, identification of gram-positive cocci in positive blood culture bottles) or for the detection of organisms in clinical samples (eg, viruses from respiratory samples or staphylococci from wounds). However, investigators and developers face several challenges that can impede the research, development, or approval of a new product. These may occur anywhere along the pathway from initial concept to final licensure for commercial use in the United States or globally.

### Challenges in Product Development

In the concept phase of a potential new diagnostic product, information is collected from healthcare institutions, scientific advisory boards, and marketing data. Then, the potential market for the product is estimated, research and development costs are anticipated, the costs of clinical trials and regulatory requirements are calculated, and the potential return on investment is determined. If the return on investment is favorable, the project proceeds to concept phase. Because the number of target organisms in a new diagnostic product may vary from 1 to 2 dozen or more (depending on the intended use of the product), with each additional target increasing the cost of the assay incrementally, input from laboratory directors and clinicians is critical to balance clinical need versus cost and efficacy.

Recent changes in ethics guidelines and conflict of interest rules in many academic centers and healthcare systems now make it difficult for key opinion leaders, and those with specific expertise in infectious diseases, to participate in company advisory boards or expert panels. Service is forbidden even if the consultant serves on his/her own time or without compensation. Thus, it is difficult for industry to gauge unmet needs in

the laboratory and ultimately at the bedside. Although it is appropriate to avoid conflicts of interest, the loss of access to key experts is detrimental to product design and development.

The next phase is technical feasibility, which progresses as a function of multiple factors, including creativity, availability of materials, and freedom to operate. Creativity is not in short supply, but intellectual property (IP) and freedom to operate (ie, the absence of IP barriers) can limit the direction a project may take. Exploring IP issues is beyond the scope of this paper, but we note that IP can be a major barrier to product development. While companies have the option to license technology owned by others, the high cost of royalties and licensing fees, particularly on some infectious disease targets, is cost prohibitive.

### Challenges in Clinical Trials

The next step is beta trials and test method validation. Here again, conflict of interest rules impact the ability of a company to get independent validation of novel test methods in a real-world laboratory setting. Even clinical trials to evaluate new products under the auspices of an institutional review board (IRB) may now be considered conflicts of interest if the laboratory is compensated for the work provided. When the laboratories of experts become inaccessible, companies are forced to engage laboratories lacking the specific expertise (eg, viral culture, or extraction of RNA from clinical samples) needed for evaluation of a new product, and must provide additional training. Finding or developing the necessary number of laboratories with the appropriate expertise to process the large number of samples needed for a clinical trial is a costly challenge, even if samples are sent to a central laboratory, which in some cases is not feasible. In one recent study, up to 12% of samples were either lost or destroyed during shipment to a central laboratory. In the 1980s, many clinical microbiology laboratories had research technologists whose job it was to oversee clinical trials of new diagnostic testing methods. Those research positions have long been eliminated due to financial constraints, thereby making clinical trials a burden to the existing laboratory staff. Thus, the number of microbiology laboratories willing to take part in clinical trials of new products has shrunk over the last decade, limited even further by conflict of interest rules, institutional regulations, and overhead costs, which now exceed 50% in some institutions, making contracts with those places too expensive to pursue.

After identification of appropriate laboratories, companies must then validate the accuracy of a new diagnostic test against a gold standard or reference method that is often less sensitive (sometimes considerably so) and in some instances less specific than the new method. How this conflict is managed varies from product to product. By this stage, the company has likely had multiple interactions with the FDA, resulting in agreement on reference methods to be used in clinical trial design. Resolution of "truth" (ie, what constitutes a true positive result and a true negative result) may require the participating laboratories to run multiple commercial assays to establish a "patient infected status," as has been done for evaluation of NAATs for chlamydia and gonorrhea assays [70]. This strategy has been successful in multiple product evaluations but is also very expensive.

Increasingly, bidirectional sequencing of target nucleic acids from organisms or directly from clinical samples is used as the ultimate arbitrator of positivity or negativity [71]. Bidirectional sequencing requires the development and validation of additional sequencing primers and test protocols. While the cost of nucleic acid sequencing has decreased dramatically for routine laboratory use, the cost of "good laboratory practice certified" (or GLP) sequencing from reputable laboratories (with Phred scores for the sequencing data of $\geq$20) has almost doubled in the last 5 years. The cost of nucleic acid sequence analysis can add >$100 000 to the cost of a clinical trial, which may be prohibitive for smaller companies.

Another challenge is access to clinical samples containing rarely encountered pathogens. As many clinical laboratories no longer freeze specimens containing novel or unusual organisms for further workup, it is becoming more difficult to find, for example, CSF samples containing the agents of viral or bacterial meningitis, or nasal washes with novel respiratory viruses. Even when such critical clinical samples are available, the cost of accessing the samples has, in many cases, become prohibitive, and some institutions may have strict IRB policies regarding provision of samples. The availability of biorepositories of prospectively collected validated clinical samples would directly address this problem (see Appendix B).

Such prospectively archived sample repositories have been recommended in recent reports from the Transatlantic Task Force on Antimicrobial Resistance (TATFAR) [72] and the Center for Health Security (formerly Center for Biosecurity) of the University of Pittsburgh Medical Center [73] as holding potential to address the challenges to diagnostics development. However, there are challenges to successful implementation of repositories, as acknowledged by TATFAR, including expense, difficulty in anticipating exactly what types of specimens to collect, and informed consent. The key issues are the costs of maintaining thousands of samples that may or may not be used, and that may or may not be the appropriate specimens for a given assay in development. Variables, such as how the samples were collected and stored, are critical factors. It will be important to develop standardized protocols for collection and storage in consultation with regulators.

An example of a current infectious diseases biorepository is a contract resource from the NIAID, run by the Aspergillus Technology Consortium, which contains prospectively collected and archived samples from at-risk subjects who develop proven or probable invasive aspergillosis [74]. The repository can provide companies that are developing diagnostics for

aspergillosis with a source of positive and negative clinical samples. Although thousands of specimens have been collected, to date, no new diagnostic test has been developed. A second approach is an on-demand model where access to prospectively collected samples is made available to companies with a demonstrated product. This approach to specimen collection is a requirement of the new NIAID Vaccine and Treatment Evaluation Units, designed to facilitate early-stage development of drugs, vaccines, and diagnostics. This model may reduce some of the risks associated with developing a new assay.

Companies seeking regulatory approval for some novel diagnostic products must demonstrate, or redemonstrate, the clinical utility of the product for regulatory approval. Because of the additional clinical trials, the PMA pathway (for a new unique product) is 10-fold more expensive than the 510(k) clearance pathway (for a modification of a previously approved test). This difference may exceed the presumed return on investment for a product. Fortunately, this is primarily an issue for molecular assays targeting viral pathogens such as HBV, HCV, HIV-1, HPV, CMV, EBV, and the coronavirus causing severe acute respiratory syndrome (SARS). Unfortunately, the extended clinical trials may be cost prohibitive, even though these are commercially viable markets. Even the commercialization of well-established PCR assays, such as an assay for HSV to diagnose meningoencephalitis from CSF, is quite difficult in the current regulatory environment, given the paucity of samples available, the lack of a predicate device, and the need to demonstrate positive outcomes for the testing algorithm.

Following FDA approval or clearance of a new assay, companies must be able to manufacture all the components of the assay (including collection and transport devices, instruments, reagents, and disposables) under good manufacturing practices in a consistent and dependable fashion. Gearing up manufacturing facilities for large-scale production of an assay can be a challenge, particularly for smaller companies, who often rely on multiple manufacturers to provide the requisite components of an assay. Furthermore, small companies often underestimate the effort and expense required to coordinate the manufacture of parts, kit production, shipping of product, and subsequent technical support, including documenting every aspect of each process.

### Laboratory-Developed Tests

Laboratory-developed tests (LDTs) are in vitro diagnostic tests that are developed, validated, and used primarily for in-house pathology and diagnostic testing. They are intended for use by the laboratory that develops them. However, some larger reference laboratories do offer the tests more broadly on a commercial basis. These tests are not currently required to go through FDA approval or clearance pathways, like commercially developed diagnostics tests. FDA has generally chosen to exercise "enforcement discretion" but this may change in the near future.

Analyte-specific reagents and LDT components are also regulated by the FDA. For the use of RUO/IUO devices for clinical diagnostics, FDA issued a guidance recommending that they carry a label signaling use limited to investigational purposes. The manufacturer of an investigational product "may legally distribute the product commercially without FDA premarket review, as long as the marketing is only for investigational use" [75].

Many LDTs are diagnostic molecular tests that are not available commercially and therefore are being used at geographically distant states from where the clinical specimen was obtained. LDTs present some unique regulatory issues. Although they are vital for healthcare, there are concerns that high-risk LDTs be assured to provide clinically relevant information to clinicians and their patients. Whereas many laboratories go to considerable lengths to validate the assays prior to making them available commercially and have developed in-house quality assurance programs to monitor their performance, quality assurance data on other assays are often not readily available and their accuracy may be difficult to assess. It is incumbent upon the laboratory that is submitting samples for testing using an LDT to substantiate the accuracy and performance of the test prior to sending samples. It is important that the need for safety and efficacy be balanced with the need for flexibility and availability of customized tests for a local population.

### The Global Regulatory Environment

The United States is not the only country that has regulations for the marketing of in vitro diagnostic products. In Europe, the Conformité Européenne In Vitro Diagnostic (CE-IVD) mark, indicating clearance for use, comes with its own set of regulations and requirements. Clearance for many products is "self-declared" and is based on "data on file"; diagnostic tests for some sexually transmitted infections, HIV-1, and other targets have more rigorous clearance pathways. Companies planning to market diagnostic products in countries such as Australia, Canada, China, India, Japan, Mexico, and other countries in South and Central America will find additional regulatory hurdles, some of which are quite complicated and involve multiple clinical trials run exclusively in that country before permission to market can be sought. Although there have been a number of multilateral discussions among various regulatory bodies focused on harmonizing regulatory requirements globally, this is likely several years away. In the meantime, companies must often undertake multiple clinical trials, each with its own set of guidelines, to gain the necessary approvals to market their products in multiple countries around the world.

### Basic Research and Early-Stage Development

Diagnostic companies involved in the development of novel tests for infectious agents range in size from a few employees to

thousands of employees with research and development budgets that range from thousands to billions of dollars. Many smaller companies seek funding from a variety of sources, including government grants, public–private partnerships, venture capital companies, and pharmaceutical companies to finance their research and development efforts. Academic partnerships are often critical to smaller firms, many of which integrate product development with other lines of research. In some cases, small companies evolved from successful academic programs on university campuses. Many NIH study sections have not given diagnostic grants high priority. The SBIR program and public–private partnerships between industry and the federal government have been more fruitful, yielding a number of successful products.

### Diagnostics for Pharmaceutical Company Use

There is considerable interest in exploring the role of molecular diagnostics in clinical trials of novel antimicrobial agents. Potential uses of rapid diagnostic tests include (1) screening of patients with specific clinical syndromes for targeted microorganisms prior to, or concomitant with, study enrollment; (2) enrichment of study population with patients with less common infections; (3) smaller clinical trials in that more patients enrolled would have a "proven" microbial etiology of their illness; or (4) as companion diagnostic tests, where the test and the drug are linked together as part of the clinical trial and ultimately for prescribing. Evolving regulatory guidance for the codevelopment of drugs and diagnostics currently make this a challenging area.

Many pharmaceutical companies are searching for diagnostic partners whose assays can be implemented to make clinical trials more cost effective by preenriching for evaluable patients. For example, a clinical trial of a novel antimicrobial agent with activity against both MRSA and MSSA in skin and skin structure infections may want a rapid test (1 hour or less) to screen wound specimens from potential patients to identify those specifically with MRSA infections. Such a strategy usually goes smoothly when the diagnostic test already exists. However, pharmaceutical companies may seek a diagnostic partner to develop a test for a single pathogen in a body site or organ (for example, in the lung), where multiple pathogens may be the etiologic agent of disease. Here a conflict begins to emerge between the diagnostic company that wants to develop a product with broad clinical utility and marketability (ie, one that detects multiple infectious agents), and the pharmaceutical company that is interested in both a limited panel of infectious agents (often a single organism) and a limited financial commitment. Although it would appear that there would be considerable synergy between pharmaceutical and diagnostics companies that could share the costs of both development and clinical trials, such synergies have been few and far between.

## SECTION V: CHALLENGES TO ADOPTION OF DIAGNOSTIC TESTS

### Clinical Challenges to Adoption

A major barrier to adoption of diagnostic tests is that tests may not address the needs of a given clinical setting, as outlined in Section II. Here we present additional clinical challenges to adoption of diagnostics and potential solutions (summarized in Table 4).

#### *Test Availability, Performance, and Applicable Data*

For some clinical conditions requiring antimicrobial therapy, either no optimal diagnostic assay is available or poor diagnostic test performance in the clinical setting or in the clinical population being tested limits the utility for patient management. Because many FDA-approved or -cleared tests and LDTs have been validated using ideal specimens from a very specific, well-defined population, interpretation of results in real-world clinical practice (eg, where patients may be on antibiotics at the time of diagnostic testing) remains unsatisfactory for widespread uptake by practitioners. In fact, acceptable standards for positive and negative predictive values that would translate to changes in patient management are lacking and will vary based on the disease under consideration.

Turnaround time will also determine the utility and uptake of diagnostic tests. Some "time-sensitive" diagnoses require immediate specific targeted therapy to avoid sequelae of the diseases or relative toxicity of empiric treatment. These tests are best performed near the patient as either POC tests or in rapid response laboratories. Conversely, for diagnoses of diseases that progress at a much slower rate, the turnaround time is less urgent, permitting the use of tests performed in centralized laboratories.

Another significant barrier to widespread uptake and use of many diagnostic tests is the paucity of clinically applicable outcomes data to show that use of the test in making treatment decisions is superior in terms of morbidity, mortality, or cost compared to empiric therapy. Outcomes data with clinically relevant parameters (eg, clinical outcomes, complications, and mortality) are critical for providers to effectively use any laboratory assay.

#### *Clinical Guidelines and Clinician Education*

Consensus or professional society guidelines influence how diagnostic tests are incorporated into clinical practice for infectious diseases, but guidelines do not always explicitly include the use of diagnostic tests. Consensus guidelines are usually created by a panel of clinical experts to evaluate the performance of a relevant diagnostic assay based on available literature as well as expert opinion; however, often there is no clinical microbiology expert in the use of that test at the table. If the use of a relevant assay is included into clinical practice

**Table 4. Examples of Clinical Challenges to Use of Diagnostic Tests**

| Challenge | Example | Explanation | Solution |
|---|---|---|---|
| Limited availability | Varicella, measles | Diagnosis based on clinical suspicion; antibody detection tests are inaccurate, especially early in the course of illness | Consensus guidelines |
| Poor performance in clinical setting | Rapid influenza antigen detection tests | Pooled sensitivity only 62% with greater sensitivity for influenza in children | Rapid molecular diagnostic assays |
| Turnaround time | Bacterial culture and antimicrobial susceptibility testing | Typically ranges from 24 to 72 hours | Rapid testing, eg, molecular diagnostics, MALDI-TOF MS |
| Lack of outcomes data | Rapid molecular testing | Limited data on morbidity and mortality benefit of molecular panels for respiratory pathogens | Additional outcomes research |
| Guidelines | Community-acquired pneumonia | 2007 IDSA/ATS guidelines do not recommend routine use of sputum culture and diagnostic tests due to poor performance | Inclusion of clinical microbiology experts in development of guidelines; additional research on alternative diagnostic methods directly from clinical specimens including molecular diagnostics |
| Clinical education | Acceptability and interpretation of molecular tests | Interpretation of test results requires knowledge of principles of PCR | Collaboration between clinical microbiology laboratory and clinical specialties; additional training of healthcare providers |

Abbreviations: IDSA/ATS, Infectious Diseases Society of America/American Thoracic Society; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PCR, polymerase chain reaction.

guidelines, there is an implied "standard of care," as well as "standard of endorsement." With few exceptions, when published professional society guidelines recommend the use of a diagnostic assay, it makes it easier for a clinician to use and argue for the test to be covered by reimbursement.

Beyond guidelines, clinician education is a key requirement for successful uptake and appropriate use of new diagnostic tests; without this knowledge, clinicians will not understand the potential value and appropriate use of new tests. Whereas graduate medical education plays an important role in teaching new healthcare providers how to use existing diagnostic tests, continuing education programs and regular interactions between clinicians and clinical microbiologists are also vital in facilitating appropriate use of new tests and interpretation of results. Examples of recent challenges include molecular diagnostic testing for viral respiratory pathogens and the importance of appropriate testing practices for *C. difficile* testing that exclude formed stool specimens to avoid overtreatment of colonization [76]. Recent changes and developments in rapid molecular tests have in many cases outpaced clinician awareness and uptake. Laboratorians can facilitate education of healthcare personnel in the scope of use of a diagnostic test, interpretation, and recommendations for repeated or follow-up testing through, for example, blogs of new assays, updated specimen collection guidelines, and presentations to key stakeholders. Infectious diseases clinicians serve as a critical liaison between healthcare providers and the clinical microbiology lab, and engaging their expertise will assist in determination of the best diagnostic test and interpretation of laboratory results, leading to improved patient care.

### Challenges in Resource-Limited Settings

Limited resources and access may impact the availability of diagnostic tests in both resource-limited settings around the world as well as rural and other resource-limited settings in the United States. In many cases, for example, the newly available rapid molecular tests, cost is high and thus the tests are not available as standard of care, or they may not be billed as a reimbursable test. Some new technologies may be too complex for all levels of healthcare. For complex testing, volume of testing at a particular site is sometimes too low to ensure competency of testing by laboratory personnel. However, this may be less of an issue for newer technologies that are more user-friendly. In addition, the robustness of technology breakage and the needs for maintenance are too onerous for many settings.

Technological needs for specimen preparation and transport, such as the need for high-speed centrifuge, refrigeration of specimens during transport, or the inability to batch specimens may also affect the feasibility of their use. Distance of reference laboratories and specimen transport remain a significant barrier for resource-limited settings, although creative approaches for transport (such as using public buses and motorbikes and text messaging for result transmission) and sample preparation (such as dried blood spots) are starting to have an impact. Nonetheless, even where these opportunities currently exist,

efficiency of specimen transport to a centralized lab is sometimes lacking. Furthermore, there remains variability in the robustness of the information technology infrastructure for results reporting, including interfacing of diagnostic instruments and rapid delivery of results via the laboratory information system and electronic health records.

## Financial Challenges to Use of Diagnostic Tests

### *Reimbursement for Diagnostic Device Use*

Difficulties and concerns regarding reimbursement for new or expensive diagnostic tests may hamper innovation and are a significant challenge to the widespread use of these technologies. Reimbursement in the United States involves (1) coding of health services or conditions to determine payment, (2) coverage by third-party payers, and (3) determination of level of payment. Codes for diagnostic tests are developed under the CPT system maintained by the American Medical Association (AMA). The International *Classification of Diseases, Ninth Revision, Clinical Modification—ICD-9-CM* (or the soon to be implemented *ICD-10*)—codes for patient diagnoses are used in combination with CPT codes in billing.

Obtaining new CPT codes or revising existing ones to accommodate emerging diagnostic tests is a complex and often lengthy process that can take up to 2 years. Applications for new CPT codes must be submitted to the AMA CPT Editorial Panel according to a specific schedule 3 times per year, and must be supported by the CPT Advisory Committee and other stakeholders. Furthermore, once obtained, coverage of these tests may vary by insurer, and current coding and payment mechanisms often do not reflect the value of new diagnostic tests.

Payment for laboratory tests performed on inpatients depends on the type of insurance covering the patient (eg, HMO or other managed care organizations, Medicare, Medicaid, indemnity, and self-pay) and the specifics of the payment contract between the healthcare provider and the insurer. Laboratory tests performed for Medicare inpatients are part of the Diagnosis Related Group payment method, and separate payment for individual laboratory tests is generally not available [77]. Similar bundled payment is mandated for many non-Medicare patients by insurance and hospital contracts.

For outpatient tests with CPT codes, the Medicare Coverage Advisory Committee advises CMS regarding diagnostic test coverage, including determination of sufficient evidence, as well as health benefit. Medicare pays for outpatient laboratory tests according to fee schedules. However, most Medicare reimbursement decisions are made locally rather than at the national level. Coverage for diagnostic tests varies regionally, and given the lack of standards in determining coverage, this can pose challenges for development and dissemination of a new diagnostic product. CMS in 2001 created policies to address inconsistencies in coverage for 23 tests, including hepatitis, HIV, and bacterial urine culture.

Payments for new diagnostic tests are often assigned by *cross-walking* (when CMS decides a new test is similar to an existing clinical test) or *gap filling* (the use of local payment data to assign a new test code payment); novel diagnostics are a challenge given that CMS may not easily be able to determine whether to use cross-walking or gap filling to set reimbursement. Collection of gap filling data is time consuming, and therefore most new test code prices are assigned using cross-walked codes, which frequently receive lower payment rates.

Compliance and billing remain challenges for clinical laboratories and may also limit the availability of testing. In some cases, the reimbursement does not cover the cost of the test, which limits laboratory interest in offering the test and may limit test availability and use by clinicians. In other cases the charge may be high but leave considerable cost to the patient, thus limiting clinicians from ordering the test routinely. Reimbursement is essentially nonexistent for tests in development. Until recently, MALDI-TOF instruments and databases were available as research use only (RUO), limiting billing for public and private payers. At the federal and state level, laboratories typically cannot bill for RUO tests and current guidelines do not allow for the use of RUO instruments and reagents in the clinical laboratory.

### *Cost-effectiveness*

Cost-effectiveness is another important consideration for use of a diagnostic test, as decision makers in healthcare systems consider the relative costs and outcomes of diagnostic testing. Hospitals and clinicians are now facing scrutiny of costs of therapy, including the evaluation of physician-specific management costs. Clinicians and administrators are faced with decisions to determine when a test is indeed worth the cost. When assessing the cost-effectiveness of laboratory tests, it is important to evaluate the cost savings to the healthcare system, as the newer molecular tests may increase the cost to the laboratory while leading to decreased length of stay, reduced use of antibiotics, or other savings that more than offset the additional cost of the test. Unfortunately many institutions have a "siloed" approach to budgeting that leads them to consider only laboratory costs and see the novel tests as expensive compared to traditional methods.

Studies establishing the cost-effectiveness of traditional diagnostic tests are rather limited; the widespread use of molecular methods has led to an increased number of studies examining the cost savings associated with these tests. Molecular tests with increased sensitivity compared to an EIA led to better detection of *C. trachomatis* infections in women, resulting in the reduction of complications such as pelvic inflammatory disease and ectopic pregnancy and an overall cost savings, even though the

cost of the molecular test was significantly higher than the cost of the EIA [78, 79]. These findings were followed by studies assessing the clinical impact of RT-PCR for the detection of enterovirus in the CSF of pediatric patients with aseptic meningitis. Multiple studies have shown a reduction in antibiotic use, hospital costs, ancillary tests, and/or length of hospital stay when the enterovirus testing is performed within 24 hours of specimen collection [15, 54, 56].

A 2010 historically controlled study illustrated the importance of ensuring clinical response to rapid testing. A rapid (1 hour) PCR assay for *S. aureus* and MRSA was performed on blood cultures with Gram stain results showing gram-positive cocci in clusters. The results were immediately paged to both the responsible physician and an infectious diseases pharmacist. Compared to controls from a time period before the availability of PCR, the mean time to switch from empiric vancomycin to either nafcillin or cefazolin for MSSA bacteremia decreased by 1.7 days. After implementation of *S. aureus* PCR testing, the mean length of hospital stay decreased by 6.2 days and the mean per patient hospital cost decreased by $21 387 [80]. In contrast, another study with a similar design failed to show a reduction in time to optimal antibiotic therapy. Benefits were not achieved due to failure to facilitate actions on the part of physician providers [81]. Similar to the first study, the rapid detection of *Candida* species directly from positive blood cultures *and* rapid reporting to a healthcare provider or pharmacist allowed the targeted use of antifungal therapy resulting in a reduction in the use of echinocandin therapy and an overall cost savings [51, 52, 82].

MALDI-TOF MS instruments are recently available for use in clinical microbiology laboratories. Several studies have shown that incorporating the use of MALDI-TOF MS for the identification of bacteria and yeast provided results several days earlier compared to standard identification methods with a substantial reduction in reagent and labor costs [53, 57]. Combined with antimicrobial stewardship interventions, MALDI-TOF MS can reduce unnecessary days of antibiotic treatment [83].

More work needs to be done to assess the cost-effectiveness of diagnostic tests, and as the availability of rapid tests increases, it is imperative to determine which tests will impact clinical decisions in a meaningful way. The success of diagnostic tests requires that, in addition to being accurate and clinically relevant, the test be performed in a timely manner with rapid communication of results to the team providing care to the patient.

### Operational Challenges to Use of Diagnostic Tests

Transportation and specimen handling requirements can be additional barriers to use of diagnostic tests. In contrast to other areas of clinical pathology, diagnostic microbiology laboratories are unique in the variety of specimens that are submitted and processed, and in the lack of highly automated instruments to test primary specimens directly for the most common pathogens. Microbiology laboratories must have detailed procedures for specimen collection and ensure prompt and appropriate transport, particularly if testing is not performed on-site. For preanalytical specimen processing, "simplicity is usability." The more difficult it is to collect, process, and transport a specimen, the lower the likelihood it is to be done correctly. Here, infectious diseases clinicians can serve an important role by advising other healthcare providers on obtaining optimal samples and diagnostic testing. The ideal specimen would be collected at room temperature and in such a vessel that permitted room temperature transportation without time limitations or processing before transporting. In addition, laboratories must develop and/or verify preanalytical and analytical procedures that do not compromise assay performance for each matrix type tested.

Currently, diagnostic testing in clinical microbiology laboratories is evolving and microbiologists are faced with supporting classical culture and other methods with less than ideal performance characteristics while embracing and verifying novel technologies that are in some cases more complex and usually more expensive. Some of the tests on current molecular platforms take an entire day to perform and require technologists who are highly trained in molecular techniques. Depending on the assay, laboratory space may need to be reconfigured to support the platform and to prevent laboratory contamination. If the volume of testing is low, then practical issues such as cost and employee proficiency must be considered for the larger platforms. Historically, single test instruments have been costly both in terms of initial capital and cost per reportable result, and many smaller laboratories therefore did not embrace them. However, the target is now moving, and the value of these instruments is being increasingly recognized.

Microbes are evolving and the clinical microbiologist must constantly be on the alert for unusual pathogens. The crisis in medical technology education, an aging workforce, and the difficulty in recruiting and retaining technologists have resulted in fewer technical specialists in anaerobic bacteriology, mycology, mycobacteriology, and parasitology—subspecialties that still rely upon interpretation of culture-based and microscopic methods. As a result, many facilities outsource this work to large reference laboratories, compromising specimen integrity and potential recovery, and/or delaying identification that can substantially impact patient care. Some tests may be sent to public health laboratories, where there is expertise.

Although many of the newer technologies are simpler and can be operated by less skilled personnel, the consequence is that less knowledge about the biology of infectious diseases is found in the laboratory. Ensuring proper quality assurance is instituted, knowing when the results are questionable, and troubleshooting abilities can be compromised when less technically

challenging tests are used by less technically savvy personnel. Advancement of the status, perception, and financial compensation for persons entering the field of laboratory medicine will be essential to ensure a continuance of highly trained laboratorians to replace the aging workforce.

### *Assay Verification and Validation*

As laboratories embrace new technologies, under CLIA, verification of accuracy and performance is required. The process follows a simpler pathway for qualitative assays than for quantitative assays, yet the exact requirements for successful verification of a commercial product can be elusive. A number of professional societies have weighed in on what constitutes an acceptable verification or validation study, but those guidelines are often conflicting. Verification studies are not difficult for organisms that are prevalent, but for the rare pathogen or disease, finding clinical material may be difficult. In addition, often laboratories are faced with requests to test a matrix for which the assay does not have an FDA indication, yet may be a common specimen received in the laboratory for diagnosis of that infection. For example, given the variability in samples obtained by bronchoscopy, diagnostics companies may not include those specimens among the approved sources for respiratory virus detection when submitting their applications to the FDA. Yet, this may be the best specimen for some of our sickest patients or for certain syndromes. Either the laboratory cannot offer the test (or report the specific analyte) for an alternative sample type or must go through great expense and effort to validate the specimen type.

For the verification of multiplex panels, the laboratory may need to find enough specimens containing 12–22 different pathogens (depending upon the assay). This requires tremendous resources and can significantly delay assay implementation. The laboratory faces a dilemma when a target on a panel (such as a novel virus in a respiratory panel), which has yet to be verified, becomes positive. What the laboratory should do in this instance is hotly debated and depends in many instances on the clinical, social, or public health impact of a correct or an incorrect result (eg, the reporting of a false-positive test for influenza A[H7N9]). As with alternative sample types, either the laboratory cannot offer the test (or report the specific analyte), or must go through great expense and effort to validate the analyte. Moreover, for the verification/validation of rare analytes, materials may not even be available for such studies.

Another challenge to implementing newer assays is that often the new assay is more sensitive than the existing reference method. The problem then becomes how to resolve results that are positive by the new method, but negative by the "gold standard." In some cases the old gold standard (eg, *C. trachomatis* culture or *C. difficile* EIA testing) is called into question. Ideally, the laboratory would have access to the clinical presentation of the patient as part of the verification or validation process, but there are major barriers in most cases to obtaining this information. In the case of FDA clinical trials, providing clinical data may require obtaining informed consent, which adds expense to the trial. Additionally, many laboratories with extensive experience in conducting FDA trials may not have the infrastructure to obtain consent and therefore would not be able to participate, access to the patients' records may not be possible as they may be in a facility some distance from the trial laboratory, and other institutional barriers may exist. Laboratories wishing to evaluate new technologies with a clinical partner outside of the FDA approval process often find it increasingly difficult to find funding sources to support the evaluation, given the cost of testing and the lack of reimbursement.

## RECOMMENDATIONS AND CONCLUSIONS

Diagnostics have had a tremendous impact on the management of patients with infectious diseases and are essential for outbreak detection and response, and public health surveillance. As we transition from conventional culture and antigen detection methods to newer molecular methods, the ability to provide accurate results in a clinically meaningful time frame, near the point of care, has never been greater. Diagnosing HSV encephalitis without molecular tests or managing patients without viral load values are distant memories, as the molecular revolution of the past 20 years dramatically changed the management of patients with viral infections and now expands to bacterial and fungal infections. One technology, MALDI-TOF MS, is poised to completely change the identification of bacterial and fungal pathogens, allowing for identification within a few minutes after growth on media.

Yet despite these extraordinary technological advances, challenges remain. FDA approved or cleared molecular tests are available for a remarkably limited number of pathogens and generally diagnostics are notably underutilized. Some of the major issues that prevent broad adoption of diagnostic tests include slow turnaround time, poor test performance characteristics, high complexity testing that cannot be easily adopted in many clinical settings, lack of understanding of the value of diagnostics, limited access to testing, and high cost. Clinicians need access to rapid, simple tests that can rule in or out infection, diagnose a syndrome (sepsis, pneumonia), or identify a specific pathogen or resistance determinant. When such a test is available, it can dramatically improve patient care and reduce healthcare costs, as has been shown for molecular testing for the diagnosis of enteroviral meningitis. More tests that meet these standards are desperately needed.

In this policy paper, IDSA reviews the current state of diagnostics and the unmet clinical needs, assesses new technologies that could address these needs, and investigates barriers to the

**Table 5. Recommendations to Accelerate Development of Improved Infectious Diseases Diagnostics and Integration Into Clinical Care**

| Primary Actors | Recommended Action | Section |
|---|---|---|
| **Stimulate diagnostics research and development** | | |
| Federal funding bodies | Innovative funding mechanisms and clinical research infrastructure<br>• Increased NIH funding through SBIR program and U01 funding mechanism<br>• NIH study sections should have appropriate expertise to evaluate feasibility and clinical applicability along with scientific merit<br>• Development of biorepositories or other infrastructure to facilitate the procurement of critical clinical specimens (see Appendix B) | R&D Challenges (Section IV) |
| Congress | Increased funding to agencies and programs that address unmet diagnostic needs.<br>• To NIH for diagnostics research, including the SBIR diagnostics program at NIAID and the Point-of-Care Technologies Research Network at NIBIB<br>• To BARDA for the advanced development of innovative infectious diseases diagnostics<br>• To the CDC AMD initiative | Unmet Needs, R&D Challenges (Sections II, IV) |
| Congress | • Enact legislation to support a tax credit to cover 50% of clinical research costs for qualifying rapid diagnostics<br>• Enacting the 21st Century Global Health Technology Act (H.R. 1515), which will strengthen health R&D programs at USAID and require no new funding | Unmet Needs (Section II) |
| Congress, Administration, and federal funding bodies | Prioritize funding and incentives for diagnostics with the following characteristics:<br>• Test directly from accessible, minimally invasive clinical specimens<br>• Able to rule out infection with high certainty (eg, ≥98% negative predictive value)<br>• Incorporate biomarkers that can indicate host response to pathogen or further classify infectious processes into categories (eg, bacterial, fungal, viral, or parasitic)<br>• Panels targeting CNS infections, sepsis and bloodstream infections, respiratory tract infections, and fungal pathogens<br>• Special considerations for pediatric use, especially for biomarkers and syndromic panels<br>• Pathogen-specific diagnostics linked to drug resistance information<br>• Rapid diagnostics that substantially improve upon the "time to result" metric<br>• Point-of-care diagnostic testing that allows for usage in varied clinical settings | Unmet Needs, New Technologies (Sections II, III) |
| **Expedite integration of improved diagnostic tests into patient care** | | |
| CMS, in coordination with the Office of the National Coordinator for Health IT, and in collaboration with healthcare systems and diagnostic companies | • Encourage healthcare systems to improve electronic medical record systems, including reporting of lab results to health departments<br>• Provide incentives for healthcare facilities to form multidisciplinary teams to develop protocols for responding to clinically significant test results<br>• Healthcare systems should use clinical guidelines from IDSA and other professional societies to guide patient management decisions regarding diagnostics use<br>• Healthcare systems should be encouraged to develop cost-effectiveness models that assess the impact of diagnostics on all facets of patient care, eg, mortality, length of stay, use of antimicrobials, and isolation procedures<br>• Diagnostic companies should work with healthcare systems to ensure that new diagnostics integrate into laboratory workflow practices | Unmet Needs, Adoption Challenges (Section II, V) |
| Congress and the Administration | • Fund information technology solutions for data integration and dissemination, to be developed locally within healthcare institutions | Unmet Needs, Adoption Challenges (Sections II, V) |
| Federal funding bodies, in collaboration with industry | • Outcomes research to determine whether use of specific tests improves patient outcomes and/or resource utilization | Unmet Needs, Adoption Challenges (Sections II, V) |
| **Address regulatory challenges to diagnostics R&D** | | |
| Congress and NIH, working with other stakeholders | • Clarify and revise conflict of interest policies to allow collaboration between diagnostics companies, diagnostic laboratories, and key opinion leaders, as their expertise is necessary to conduct FDA licensing trials | R&D Challenges (Section IV) |

*Table 5 continued.*

| Primary Actors | Recommended Action | Section |
|---|---|---|
| Department of Health and Human Services | • Withdraw the draft proposal to institute a new informed consent requirement for research with de-identified residual clinical samples, outlined in the 2011 Advanced Notice of Proposed Rulemaking for human subjects research protections (ie, the Common Rule), as it would severely limit the conduct of diagnostics research | R&D Challenges, Adoption Challenges (Sections IV, V) |
| Congress | • Provide incentives and support for institutions to save de-identified specimens when possible for the purposes of new test development, FDA licensing trials, and assay verification and validation | R&D Challenges, Adoption Challenges (Sections IV, V) |
| FDA | • CDRH should revise the guidance for RUO/IUO devices and permit use in cases where there are no other diagnostic options<br>• CDRH should exercise its flexibility and exempt companies from redemonstrating the clinical validity of a novel diagnostic product after multiple studies have been conducted for similar products<br>• CDRH and CDER should provide greater clarity in guidance for the codevelopment of drugs and diagnostics<br>• Assist in the development of strategies to preserve specimens for public health surveillance purposes, eg, by asking developers of new technologies to include a public health "plan" with their submission | R&D Challenges, Adoption Challenges (Sections IV, V) |
| **Ensure appropriate levels of reimbursement for diagnostics testing** | | |
| CMS | • Eliminate the wide regional variations in the reimbursement of tests and ensure that reimbursement covers the cost of testing<br>• Simplify, expedite, and increase the transparency of the process for assigning new CPT codes and subsequent incorporation of new codes for laboratory tests into CLFS | Adoption Challenges (Section V) |
| Congress | • Enact the Diagnostic Innovation Testing and Knowledge Advancement Act of 2013 (H.R. 2085 in the 113th Congress) to improve the process of Medicare payment rate determination for diagnostic testing | Adoption Challenges (Section V) |
| **Encourage adoption of new tests** | | |
| CMS, in concert with professional societies and labs | • Harmonize recommendations from CMS through the CLIA regulations and from various professional societies and organizations (eg, CLSI, College of American Pathologists) to provide laboratories with greater clarity of the processes for clinical validation or verification for new assays<br>• Stakeholders should collaboratively develop guidelines on how to establish reference methods for new technologies that are more sensitive and specific than the existing "gold standard"<br>• CMS should discourage facilities that do not receive enough specimens to maintain competency and accuracy from conducting highly complex diagnostic testing technology | Adoption Challenges (Section V) |
| Diagnostic companies | • Convert highly complex assays to moderately complex tests that can be performed in a variety of clinical settings using "walk-away technology"<br>• Promote industry training in new technologies for the laboratory workforce | Adoption Challenges (Section V) |
| Congress and HRSA, with professional societies | • Support the recruitment and retention of clinical microbiologists and medical technologists | Adoption Challenges (Section V) |
| **Educate healthcare providers on the use of diagnostics** | | |
| AHRQ and HRSA, working with healthcare institutions and professional societies | • Fund and encourage strengthened educational programs to disseminate the results of diagnostics-focused health sciences research and to inform physicians about the utility of available tests<br>• Professional societies, educational institutions, and other entities involved in the education of clinicians should ensure that education includes the performance of diagnostic tests, interpretation of test results in individual clinical settings with varied patient populations, available guidelines, and cost of testing<br>• Professional societies and other organizations should include clinical microbiology experts in the development of clinical practice guidelines that make recommendations on the use of diagnostic tests | Adoption Challenges (Section V) |

Abbreviations: AHRQ, Agency for Healthcare Research and Quality; AMD, Advanced Molecular Detection; BARDA, Biomedical Advanced Research and Development Authority; CDC, Centers for Disease Control and Prevention; CDER, Center for Drug Evaluation and Research; CDRH, Center for Devices and Radiological Health; CLFS, Clinical Laboratory Fee Schedule; CLIA, Clinical Laboratory Improvement Amendments; CLSI, Clinical and Laboratory Standards Institute; CMS, Centers for Medicare and Medicaid Services; CNS, central nervous system; CPT, Current Procedural Terminology; FDA, Food and Drug Administration; HRSA, Health Resources and Services Administration; IDSA, Infectious Diseases Society of America; IT, information technology; NIAID, National Institute of Allergy and Infectious Diseases; NIBIB, National Institute of Biomedical Imaging and Bioengineering; NIH, National Institutes of Health; R&D, research and development; RUO/IUO, research use only/investigational use only; SBIR, Small Business Innovation Research; USAID, US Agency for International Development.

development and adoption of new diagnostics. A broad range of recommendations are provided to address the identified challenges (Table 5). There needs to be a comprehensive commitment to developing technologies that will allow testing to move from highly complex laboratories to all laboratories or to the bedside, creating programs to educate clinicians on the proper use and interpretation of tests, investing in cost-effectiveness and clinical outcomes studies, and further advancing information systems to allow not only for the rapid communication of test results to healthcare providers and public health agencies, but also for a rapid action in response to the test result. It is important to consider the public health implications of these innovations as they are developed to ensure that there are no unintended negative effects on our capacity to identify, respond to, and track infectious public health threats.

Progress will require the engagement and coordination of a number of stakeholders including funding agencies, regulatory agencies, public health agencies, diagnostic companies, healthcare systems, clinical microbiology laboratory professionals, clinicians, and professional societies. Congress must play a role in increasing funding for diagnostics research and enacting legislation that addresses identified challenges. Successful implementation of these recommendations will require a commitment from all stakeholders, and will open the door for remarkable progress in the field of diagnostics and resulting impact on many infectious diseases. As technologies advance, there is a critical window of time to harness and direct development of new diagnostics to benefit patients. The goal is not just to create more tests, but to develop rapid, reliable, accurate, simple tests that will reduce time to a diagnosis and truly improve the quality of care and patient outcomes while reducing healthcare costs.

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## Appendix A: LIST OF ABBREVIATIONS

AHRQ – (HHS) Agency for Healthcare Research and Quality
AMA – American Medical Association
AMD – (CDC) Advanced Molecular Detection
ASR – analyte-specific reagents
AsTeC – Aspergillus Technology Consortium
BAL – bronchoalveolar lavage
BARDA – (HHS) Biomedical Advanced Research and Development Authority
CAP – community-acquired pneumonia
CDC – (HHS) Centers for Disease Control and Prevention
CDER – FDA Center for Drug Evaluation and Research
CDRH – FDA Center for Devices and Radiological Health
CLFS – Clinical Laboratory Fee Schedule
CLIA – Clinical Laboratory Improvement Amendments
CLSI – Clinical and Laboratory Standards Institute
CME – continuing medical education
CMS – (HHS) Centers for Medicare and Medicaid Services
CMV – cytomegalovirus
CNS – central nervous system
CoNS – coagulase-negative *Staphylococcus*
CPT – Current Procedural Terminology
CSF – cerebrospinal fluid
DRG – diagnosis related groups
ED – emergency department
EIA – enzyme immunoassay
ELISA – enzyme-linked immunosorbent assay
ESI-TOF – electrospray ionization time of flight
EUA – Emergency Use Authorization
FDA – (HHS) Food and Drug Administration
GME – graduate medical education
HAP – hospital acquired pneumonia
HBV – hepatitis B virus
HCAP – healthcare-associated pneumonia
HCV – hepatitis C virus
HHS – Department of Health and Human Services
HIV – human immunodeficiency virus
HPV – human papilloma virus
HSV – herpes simplex virus
HRSA – (HHS) Health Resources and Services Administration
ICD – International Classification of Diseases
ICU – intensive care unit
IDSA – Infectious Diseases Society of America
IFI – invasive fungal infection
IP – intellectual property
IRB – institutional review board
IVD – in vitro diagnostic
KPCs – *Klebsiella pneumoniae* carbapenemases
LAMP – loop mediated amplification
LDT – laboratory developed test
MALDI-TOF MS – matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MERS-CoV – Middle East respiratory syndrome coronavirus
MOC – maintenance of certification

*Appendix A continued.*

MRSA – methicillin-resistant *Staphylococcus aureus*
MSSA – methicillin-susceptible *Staphylococcus aureus*
NAAT – nucleic acid-based amplification technology
NASBA – nucleic acid sequence based amplification
NDM-1 – New Delhi metallo-beta-lactamase-1
NGS – next generation sequencing
NIAID – (NIH) National Institute of Allergy and Infectious Diseases
NIBIB – (NIH) National Institute of Biomedical Imaging and Bioengineering
NIH – (HHS) National Institutes of Health
ONC – (HHS) Office of the National Coordinator for Health Information Technology
PCORI – Patient-Centered Outcomes Research Institute
PCR – polymerase chain reaction
PCT – procalcitonin
POC – point-of-care
PMA – pre-market approval
PFGE – pulsed field gel electrophoresis
R&D – research and development
RSV – respiratory syncytial virus
RT-PCR – reverse transcriptase polymerase chain reaction
RUO/IUO – research use only/investigational use only
SBIR – Small Business Innovation Research
SDA – strand displacement amplification
SIRS – systemic inflammatory response syndrome
TATFAR – Transatlantic Task Force on Antimicrobial Resistance
TMA – transcription mediated amplification
USAID – United States Agency for International Development
VAP – ventilator-associated pneumonia
VRE – vancomycin-resistant enterococci

## APPENDIX B: PROTOTYPE FOR ESTABLISHMENT OF AN INFECTIOUS DISEASES CLINICAL SPECIMEN REPOSITORY

HSV encephalitis will serve as an example of how a targeted specimen repository may assist in the FDA clearance of a diagnostic test. It has been known since the landmark study by Lakeman et al in 1995 [14] that PCR testing of CSF is a very sensitive and specific diagnostic tool for HSV encephalitis, essentially eliminating the need for brain biopsy. Yet, nearly 20 years later, there is not an FDA-cleared test for this indication, even though molecular testing is considered the standard of care for the diagnosis of HSV encephalitis. In the absence of an FDA-cleared test, clinicians rely on LDTs. One of the challenges of the approval process is that HSV encephalitis is a rare disease, with any given large medical center treating a few cases annually. The development of a specimen repository could be very useful for companies interested in developing such a test. In fact, there are several molecular tests for detecting genital tract HSV that have been FDA cleared. If FDA-defined characterized

CSF specimens were available, these tests could be evaluated to determine if the proposed diagnostic assays have adequate sensitivity and specificity for the diagnosis of HSV encephalitis. A process for establishing such a repository is outlined below and would be done in collaboration with the NIH, FDA, and/or IDSA.

- Establish a group of collaborators willing to provide clinical data and specimens to the repository. This could be done by collaborating with IDSA and the Pan American Society for Clinical Virology to identify participating sites. Given that the number of cases at any given site is low (approximately 1–5 annually), it is likely that as many as 40–50 sites will need to be identified to complete the data and specimen collection within 12–18 months. However, given the low volume, the collection of clinical information and specimens would not be burdensome to any collaborator.
- Identify a site where the repository will be housed, likely one of the clinical sites, and establish specific criteria for maintaining the repository. Preferably, the repository site would have experience in document control of samples and the maintenance of repositories to ensure integrity of the samples and clinical data. If not, criteria will be established by which the selected repository site must be compliant.
- Create a clinical case form defining patient demographics, clinical symptoms, laboratory and radiographic data, and treatment. This would be done in collaboration with the FDA, so that completed case forms contain all the needed information for the FDA clearance process. This process would also include a standardized clinical case definition of HSV encephalitis, relying on the above criteria as well as the laboratory-developed HSV PCR results used in real time for clinical decisions.
- Ideally, testing will be done on specimen volume remaining after routine clinical testing, avoiding the need for a second lumbar puncture. Specific criteria will need to be defined regarding the volume of CSF needed, conditions for local storage, and shipping to the repository site.
- Once an adequate number (eg, 50–100) of positive specimens are collected and approved in collaboration with the FDA, companies will be notified of specimen availability. Specimen collection can continue for several years to ensure that multiple companies can conduct a study.
- There may be a need to establish a review committee, comprised of collaborators from clinical sites, to assess company requests for specimens and assure that tests have been adequately validated prior to releasing specimens and clinical case forms. Alternatively, the FDA could determine that the analytical performance of the test meets specific criteria prior to the release of specimens. The goal is to avoid using the specimens on tests that have not been appropriately validated, so that a test(s) is cleared within a few years.
- The financial support for the project would include clerical and operational support for the center maintaining the repository (less than a full-time equivalent); coordinator support for IRB submission, maintenance, and fees; and salary support (20%) for the individual coordinating the study, organizing the meetings with the FDA, and responding to requests for specimens. Minimal support would be needed by the individual sites (IRB submission and fees), as they would be expected to collect just a handful of specimens annually, and provide case report forms for each sample submitted. Initial startup costs for establishing the repository would be solicited by grants from the NIH, CDC, and companies with interest in obtaining FDA clearance for in vitro diagnostic tests. Thereafter, companies will be charged an amount for the specimens to defray the total costs for supporting the repository. The final goal would be to have a financially self-sustaining repository.