Rapid Diagnostic Testing in Antimicrobial Stewardship

Effective antimicrobial stewardship is closely linked with the ability to make correct diagnoses. Many types of viral respiratory infections, for example, are clinically indistinguishable from bacterial respiratory infections (Zumla 2014). And fungal infections, such as pulmonary aspergillosis or Candida-associated sepsis, can mimic the clinical presentation of bacterial infections (Denning 2017). Incorrect diagnoses can lead not only to overuse or misuse of antibiotics, particularly the critical broad-spectrum antibiotics, but also to poor outcomes for patients resulting from failure to treat the actual disease present (Gaffin 2017; Benedict 2017).

Speed of diagnostic testing is also a key factor in effective antimicrobial stewardship. Typical turnaround time using traditional microbiological testing methods is 48 to 96 hours for pathogen identification, followed by an additional 48 to 72 hours for antimicrobial drug-susceptibility testing (Doernberg 2017; Bauer 2014). Initial treatment decisions may be made empirically (based on clinical judgment and experience) before diagnostic testing results are available, as in medical emergencies such as sepsis, or without any diagnostic testing information, as is often the case with common conditions such as pharyngitis (Review on Antimicrobial Resistance 2015). Key goals of antimicrobial stewardship can be achieved through faster and more accurate diagnostic testing—reducing time to appropriate antibiotics, reducing unnecessary use of antibiotics, and informing decisions regarding antibiotic de-escalation or discontinuation (Goff 2017; Markley 2017; Review on Antimicrobial Resistance 2015).

Accordingly, the need to speed up diagnostic testing is a central theme in recent policy initiatives to combat antimicrobial resistance (AMR). One of the five major goals of the 2015 US National Action Plan for Combating Antibiotic-Resistant Bacteria (White House 2015) is to advance the development and use of rapid and innovative diagnostic tests for identifying and characterizing resistant bacteria, including:

- Developing and validating new diagnostics that can be implemented in a wide variety of settings to detect AMR and rapidly distinguish between bacterial and viral pathogens
- Increasing the availability and use of diagnostics to improve treatment of antibiotic-resistant bacteria, enhance infection control, and facilitate outbreak detection and response in healthcare and community settings

Expanding to a global One Health perspective, the World Health Organization (WHO) 2015 Global Action Plan on Antimicrobial Resistance (WHO 2015) highlighted the need for “effective, rapid, low-cost diagnostic tools...for guiding optimal use of antibiotics in human and animal medicine.” The WHO plan calls for rapid diagnostics to be “easily integrated into clinical, pharmacy and veterinary practices” and for “affordable, point-of-care diagnostic tools to inform health practitioners and veterinarians of the susceptibility of the pathogens to available antibiotics.”
The Promise and Potential of Rapid Diagnostics

Novel, rapid diagnostics for bacterial, viral, and fungal infections is an active area of research and development. Its outcomes have already begun to transform infectious disease diagnostics into a more precise, timely, and flexible process. Rapid diagnostic testing has the potential not only for enhancing patient care and helping to preserve the effectiveness of current antibiotic agents, but also boosting capabilities for AMR surveillance and new antibiotic development.

In practice, the newer methods, including molecular and proteomic technologies, have not yet replaced standard phenotypic microbiological methods. Current diagnostic capabilities typically include a combination of established methods and a broad range of new diagnostic technologies, which are in various stages of transition from early development to regulatory clearance to validation and standardization for routine use (van Belkum 2017; Arroyo 2017).

Established methodologies for diagnosing infectious diseases are summarized below, along with a brief overview of remaining unmet needs and the integration of rapid diagnostic methodologies with antimicrobial stewardship interventions. In a second CIDRAP-ASP policy update to be posted in 2018, we will address priorities for the development of novel diagnostic approaches in low-resource countries, in addition to recent initiatives aimed at stimulating the development and implementation of rapid diagnostic methodologies.

Roles of Diagnostic Testing

The Diagnostics and Devices Committee of the Antibacterial Resistance Leadership Group observed that, in most other medical domains, “diagnostics focus on narrowly defined questions, provide readily interpretable answers, and use true gold standards for development. In contrast, infectious diseases diagnostics must contend with scores of potential pathogens, dozens of clinical syndromes, emerging pathogens, rapid evolution of existing pathogens and their associated resistance mechanisms, and the absence of gold standards in many situations” (Tsalik 2017). Furthermore, infectious disease diagnostic testing needs to fulfill a variety of different roles in clinical practice and research (Wellcome Trust 2016), including:

- **Identifying pathogens** to guide selection of antimicrobial agents, de-escalation from broad-spectrum to narrow-spectrum agents, and/or discontinuation of antimicrobial treatment
- **Identifying resistance genes or markers** that may predict antibiotic treatment failure
- **Determining virulence factors** that modify the expression of resistance to antimicrobial agents in vivo
- **Identifying antimicrobial susceptibility** to available antibiotic agents (inhibition of in vitro pathogen growth) to help predict which antimicrobial agents are likely to effectively treat the infection
- **Measuring biomarkers** as indicators of host response to infection or high-risk conditions.
- **Distinguishing viral from bacterial causes of infection** to avoid or discontinue antibiotics for nonbacterial (viral, fungal, or parasitic) infections
- **Providing point-of-care testing**, eg, at the patient’s bedside or in outpatient clinics, in addition to traditional skilled laboratory-based services
- **Supporting clinical trials** to improve the efficiency of studies evaluating new antibiotics

Optimal testing methodologies for pathogen identification are those with high sensitivity (ability to correctly identify the presence of a pathogen) and high specificity (ability to correctly identify the absence of a pathogen). False-negative diagnostic results can lead to discontinuing needed antimicrobial
treatment and failing to provide sufficient infection control measures, while false-positive or misidentified results can lead to inappropriate administration of antimicrobial agents, delays in making correct diagnoses, and unnecessary infection control measures such as patient isolation. Other relevant considerations include **positive and negative predictive value**, which depend not only on sensitivity and specificity, but also on the prior likelihood of infection (eg, positive predictive value refers to the probability that those testing positive are truly infected). Without a broadly applicable gold standard for pathogen detection, specific diagnostic tests may play complementary rather than definitive roles in patient care.

**Established Diagnostic Methodologies**

**Pathogen identification** has relied on two types of established methodologies performed in clinical laboratories, using specimen samples (eg, blood, sputum, cerebrospinal fluid, or urine) collected from patients, preferably before antibiotics are administered (Caliendo 2013):

- **Phenotypic microbiological methods** based on bacterial growth in culture media (eg, agar plates or liquid culture bottles), Gram staining under light microscopy to visually identify bacterial shapes and staining patterns, and the production of microbial isolates that can be stored for reference and further analysis (including drug susceptibility testing; see below)
- **Biochemical methods**, such as antigen and antibody detection, eg, using monoclonal antibodies tagged with fluorescein for visualization under light microscopy

Key limitations to these methods include the following: (1) not all pathogens can be grown in culture, (2) some pathogens require special media to enable growth in culture, and (3) for those pathogens that can be grown in culture, identification requires relatively long turnaround times, ranging from a few days to several weeks (Goldenberg 2017).

In addition, traditional microbiological methods, such as histopathology and culture, generally have low sensitivity for detecting fungal infections such candidemia, a common form of nosocomial bloodstream infection (Hamdy 2016; Arvanitis 2014). There is currently no gold-standard test available for diagnosing invasive fungal infections, although several new diagnostic testing methodologies, such as the nuclear magnetic resonance spectroscopy–based T2Candida panel (T2 Biosystems) for the direct detection of *Candida* species in whole blood, have been developed for routine clinical use and have the potential to significantly improve the detection of fungal infections (Zervou 2017).

**Antimicrobial susceptibility testing (AST)** requires an additional 1 to 2 days beyond pathogen identification. Traditional AST methods are based on continuous exposure of bacterial isolates to a set of antimicrobial agents, followed by visual detection of growth (resistance) or inhibition (susceptibility). Results can be provided qualitatively (as susceptible, intermediate, or resistant) and quantitatively (as minimum inhibitory concentrations) (Leekha 2011; Maurer 2017). AST methods include broth microdilution testing (the current reference standard), agar dilution, disc diffusion, antimicrobial gradient methods, and breakpoint testing (Schumacher 2017). Faster, automated AST systems are also available—for example, based on isothermal microcalorimetry, microfluidics, and automated time-lapse microscopy on individual bacterial cells directly from positive blood cultures (Maurer 2017; Forbes 2017; Humphries 2016). Novel rapid methodologies, such as using mass spectrometry (see below), can also be applied in specific situations, such as documenting activity of antibiotic-inactivating enzymes, confirming the presence of genetic resistance markers, or measuring changes in proteins that correlate with antimicrobial susceptibility (van Belkum 2013).

AST results provide information relevant to the patient’s response to specific antimicrobial agents, although because in vitro testing does not take into account variables in individual response to
antimicrobials in vivo, AST may fail to identify drugs that would be effective in the patient (Dunne 2017). Ersoy and colleagues suggest that AST may need to be enhanced to account for pathogen conditions in the host (eg, through host-mimicking media to predict antimicrobial efficacy in vivo) (Ersoy 2017). Other limitations with AST may include the need for relatively large numbers of viable cultured organisms, complicated pre-analytical processing, limited organism spectrum, analytical variability, and long turnaround times (van Belkum 2013; Jorgensen 2009).

**Unmet Needs**

Various professional groups have reviewed the established infectious disease diagnostic methodologies and issued recommendations for developing new methodologies to address the existing limitations. The Infectious Diseases Society of America (IDSA) Diagnostics Task Force, for example, recommended the development of the following diagnostic capabilities to meet high-priority unmet needs (Caliendo 2013):

- Enable testing directly from accessible, minimally invasive clinical specimens (eg, blood, respiratory samples, urine, or stool).
- Rule out infection with high certainty (high negative-predictive value).
- Incorporate relevant biomarker analyses (eg, using procalcitonin and C-reactive protein testing) to determine host response to infection or distinguish bacterial, fungal, viral, and parasitic infections.
- Provide testing panels (eg, for identifying sepsis, bloodstream infections, respiratory tract infections, central nervous system infections, and groups of key pathogens).
- Identify key pathogens and their drug susceptibilities.
- Provide faster turnaround time compared with established culture methods.
- Enable point-of-care testing for use in a variety of healthcare settings.
- Improve capabilities for AMR surveillance and outbreak investigation of drug-resistant pathogens.

In addition, the US Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria recently highlighted three key unmet needs regarding diagnostic testing (PACCARB 2017):

- Rapid, affordable, approved diagnostic tests that distinguish between bacterial and viral infections.
- Better biomarker tests to guide antibiotic de-escalation, including standardized procedures for determining when it is safe and appropriate to discontinue antibiotics.
- Tests that rapidly identify pathogens and provide drug-susceptibility results directly from clinical samples (rather than from positive blood cultures).

**Rapid Diagnostic Testing Methodologies**

A wide range of rapid diagnostic testing methodologies have been developed, or are currently in development, for pathogen identification and characterization. Many of the novel methodologies offer fast and accurate results, and some also allow for rapid identification of resistance genes or markers, potentially combining pathogen identification and AMR analysis into a single test system. In practice, many are used in conjunction with, rather than in place of, culture-based methods. Diagnostic tests that integrate culture-based and rapid methodologies may be more likely to provide actionable information for clinicians than either traditional or novel testing alone (Li 2017).

Validity of the rapid methodologies is difficult to assess where reference standards are lacking. In addition, the overall effects of these technologies on patient outcomes, antibiotic use, and healthcare costs have not been fully evaluated. Traditional methods providing antimicrobial susceptibility profiles in
microbial culture are still critical components of the diagnostic process; the detection of resistance genes or markers through rapid molecular assays offers useful but incomplete and potentially even misleading information without corresponding susceptibility profiles to guide antibiotic selection (Brady 2016).

Novel diagnostic approaches currently include culture-independent rapid technologies, some of which (eg, automated platforms that integrate sample processing, fluid handling, and detection) may be developed for point-of-care use in settings such as emergency departments, intensive care units, and outpatient clinics, such as the following:

- **Nucleic acid amplification tests (NAATs)**, based on real-time polymerase chain reaction (RT-PCR) and multiplex PCR. Using genetic probes, NAATs can detect and quantify known genetic sequences to identify multiple pathogens simultaneously (including multidrug-resistant organisms) and key resistance genes or determinants, directly from patient specimens or from positive blood culture bottles. For example, for diagnosing tuberculosis (TB), the WHO recommends the use of the Xpert MTB/RIF assay (Cepheid), which integrates sputum sample processing and PCR testing into a single self-enclosed test unit that can simultaneously detect TB and rifampicin resistance within 2 hours (Kafr 2016; CDC 2015). Other types of NAATs can be used to distinguish bacterial and viral infections and can identify multiple different pathogens, providing results in an hour or less. Syndromic panels of NAATs are available, eg for respiratory, gastrointestinal, and bloodstream infections. The rapid, automated BioFire FilmArray blood culture identification panels (bioMérieux), for instance, use multiplex PCR to enable the simultaneous identification of gram-positive and gram-negative bacteria, viruses, yeast, parasites, and selected AMR genes (mecA, vanA/B, and blaKPC). As a culture-independent methodology, NAATs do not provide microbial isolates for further characterization (Caliendo 2013; Bauer 2014; Maurer 2017; ASM 2017b).

- **Mass spectrometry (MS)**, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). This methodology uses lasers to ionize and accelerate bacterial and fungal molecules, which separate according to the mass-to-charge ratio, yielding a distinct signal that can be used to identify pathogens to the species level (using a database of characterized organisms for comparison) and resistance determinants or biomarkers expressed by resistant pathogens (Maurer 2017; van Belkum 2017). Bacteria, fungi, and mycobacteria can be identified from positive blood cultures using this approach to analyze microbial proteins or glycolipids (Leung 2017). MALDI-TOF MS technology can also provide high-volume analyses. Commercial MALDI-TOF MS systems, such as Vitek MS (bioMérieux) and Biotyper (Bruker), are used for rapid identification of a broad range of bacteria from positive blood cultures. A related culture-independent technology, PCR/electrospray ionization–mass spectrometry (eg, the IRIDICA bacterial bloodstream infection assay system, Abbott Laboratories), can provide molecular detection of sepsis-related pathogens directly from patients’ blood samples in less than 8 hours (Metzgar 2016).

- **Peptide nucleic acid fluorescence in situ hybridization (PNA-FISH)**, a cytogenetic methodology that uses fluorescent probes that bind to complementary genetic sequences in bacterial pathogens, detectable via fluorescence microscopy. PNA-FISH was one of the first commercially available rapid diagnostic testing methodologies and has been modified to enable pathogen identification results in less than an hour (Bauer 2014; Davenport 2017). PNA-FISH technology can also be used in conjunction with other technologies. For example, the Accelerate Pheno
system (Accelerate Diagnostics), an automated test system for identifying gram-negative bacteria and antimicrobial susceptibility from positive blood cultures, combines PNA-FISH and gel electrofiltration with automated microscopy for analyzing bacterial growth rates and extrapolating MIC values (Marschal 2017).

- **Whole-genome sequencing (WGS)**, combined with informatics tools, can detect all potential pathogens directly from patient samples. WGS can also provide data on AMR by identifying known resistance genes, but it does not provide information on whether the genes are expressed as phenotypic resistance. It can also provide information on slow-growing or difficult-to-culture organisms (Goldberg 2015), including viruses, bacteria, yeast, fungi and parasites. WGS has been used to identify pathogens where all other diagnostic methods have failed (Wilson 2014; Relman 2015) and can be particularly useful in hospital infection control surveillance programs and community outbreak investigations (Goldberg 2015).

**Diagnostic Stewardship**

The WHO defines diagnostic stewardship as “coordinated guidance and interventions to improve appropriate use of microbiological diagnostics to guide therapeutic decisions. It should promote appropriate, timely diagnostic testing, including specimen collection, and pathogen identification and accurate, timely reporting of results to guide patient treatment” (WHO 2016). As part of a broader antibiotic stewardship program, diagnostic stewardship is aimed at reducing false-positive testing results, which can lead to overuse of antibiotics, and enhancing the detection of true-positive cases of infection for appropriate treatment. Key components include the measurement of host-response infection biomarkers, such as procalcitonin, to inform treatment decision-making (Bergin 2017; Sager 2017).

Integrating novel rapid diagnostic testing into clinical practice provides opportunities to enhance antimicrobial stewardship by examining how diagnostic testing is ordered, performed, and reported. Optimal interpretation and application of the results require combining such reporting with ASP interventions. Considerations include the following (Morgan 2017; Messacar 2017; ASM 2017a):

- **Indications**: ordering tests only when there is a high pretest probability of infection (positive predictive value, or when the patient’s symptoms suggest infection, likely disease, or relevant conditions such as pregnancy)
- **Sampling**: ensuring appropriate collection (eg, aseptic technique) and transport of samples to the laboratory to optimize successful testing and minimize risks of contamination
- **Minimizing false-positives**: distinguishing colonization from infection and conducting further diagnostic testing on potential false-positive results from syndromic testing panels, which detect many pathogens, some of which may have a low pretest probability of infection, and from WGS, which detects microbial genetic material from viable and nonviable organisms
- **Actionable communication**: reporting results to the physician and ASP in a way that clearly informs treatment decision making; NAATs may be used to confirm the absence of pathogens and allow discontinuation of empirical antibiotics
- **Evaluation**: monitor the process and outcome of testing on patient care to identify potential unintended consequences, costs, effects on AMR rates, and opportunities to enhance the availability of rapid diagnostic testing in low-resource settings

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References


American Society for Microbiology (ASM). Changing diagnostic paradigms for microbiology (report). 2017a [Full text]

American Society for Microbiology (ASM). Multiplex array white paper: clinical utility of multiplex tests for respiratory and gastrointestinal pathogens. Jul 18, 2017b [Full text]


Bergin SP, Tsalik EL. Procalcitonin: the right answer but to which question? Clin Infect Dis 2017 Jul 15;65(2):191-3 [Full text]


CDC. A new tool to diagnose tuberculosis: the Xpert MTB/RIF assay. 2015 [Full text]


Gaffin N, Spellberg B. Making the correct diagnosis: the cornerstone of antibiotic stewardship. Medscape, May 19, 2017 [Full text]


Hamdy RF, Zaoutis TE, Seo SK. Antifungal stewardship considerations for adults and pediatrics. Virulence 2016 Sep 2:658-72 [Full text]

Humphries RM, Hindler JA. Emerging resistance, new antimicrobial agents ... but no tests! The challenge of antimicrobial susceptibility testing in the current US regulatory landscape. Clin Infect Dis 2016 Jul 1;63(1):83-8 [Full text]


Li Y, Yang X, Zhao W. Emerging microtechnologies and automated systems for rapid bacterial identification and antibiotic susceptibility testing. SLAS Technol 2017 (published online Aug 29) [Abstract]


Morgan DJ, Malani P, Diekema DJ. Diagnostic stewardship — leveraging the laboratory to improve antimicrobial use. JAMA 2017 Aug 15;318(7):607-8 [Abstract]

PACCARB. Recommendations for incentivizing the development of therapeutics, diagnostics, and vaccines to combat antibiotic-resistance. Working group draft, Sep 2017 [Full text]


Wellcome Trust. Four diagnostic strategies for better-targeted antibiotic use. 2016 [Full text]


WHO. Global action plan on antimicrobial resistance. 2015 [Full text]

WHO. Global AMR Surveillance System (GLASS). Diagnostic stewardship: a guide to implementation in antimicrobial resistance surveillance sites. 2016 [Full text]
