Rapid Diagnostic Testing in Infectious Diseases

Despite myriad publications on rapid diagnostic testing (RDT) methodologies for infectious diseases, such testing has become neither commonplace nor an integral component of services offered by clinical microbiology laboratories in the United States. In the current era of managed care, the need for RDT is underscored by the emergence of virulent strains of influenza virus and novel pathogens such as the coronavirus that causes the severe acute respiratory syndrome (SARS), as well as the often grave consequences of healthcare-associated infections caused by methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus spp. (VRE), Clostridium difficile, extended spectrum beta-lactamase (ESBL)-producing Klebsiella spp., and Mycobacterium tuberculosis.

Debate on the value of RDT has broadened and now encompasses infections caused by group A streptococcus, herpes simplex virus, West Nile virus, human immunodeficiency virus (HIV), and extremely drug-resistant tuberculosis (XDR-TB). More recently, the role of RDT in the routine diagnosis and prevention of syphilis and malaria has been discussed in some circles.

Prohibitive costs and doubtful cost-effectiveness of certain rapid tests are typically blamed for the unavailability of RDT. To be cost-effective, a test must have sufficient diagnostic value, and its use must be limited to the organisms most likely to be clinically relevant, and to circumstances in which earlier diagnosis would likely have an impact on patient management. "Clinical value" encompasses many questions:

- Why was the test requested?
- Will the result aid or alter patient management?
- Would a simpler or cheaper test provide the same information?
- Will the use of a specific RDT lead to improved understanding of the medical condition?
- Could we do without RDT for the clinical situation under consideration?
- Is the specific RDT test of public health or clinical importance?
- Is the test reliable?

Even if the RDT is affordable, will it be cost-effective and sustainable in the long term? This is particularly relevant for less-developed countries, and even for countries such as the United States, where, in a recent trend, healthcare facilities (including academic centers) are purchasing microbiology services from private, rather than hospital-based, laboratories.

The Ideal RDT

In the conventional view of RDT, a clinical specimen (serum, plasma, saliva, urine, stool, tissue, or body fluids) is processed in a single step at the site where it is collected and a qualitative or quantitative result is available within 20 minutes -- the basis of point-of-care testing. However, RDT now encompasses more than just a single-step testing procedure. A specimen is often sent to a laboratory for immediate workup that might involve several steps, ending with the availability of results within 20 minutes to 2 hours, although for certain RDT, obtaining results within 18 hours instead of 4 days (eg, for MRSA) or 1 week instead of 6 weeks (eg, tuberculosis) still renders the test "rapid."

The properties of RDT kits play an enormous role in determining their utility in the diagnosis of infectious diseases. The prerequisites of the ideal RDT are:

- High sensitivity and specificity;
- Relatively high negative and positive predictive values;
Reproducible results;
- Rapid turnaround time;
- Availability and reporting of results to those who need them in a timely manner; and
- Affordable pricing.

How these requirements fit in with standard clinical practice in US hospitals is undetermined. A low sensitivity will result in patients with true infection being falsely reassured by a negative test result, whereas a low specificity will lead to a relatively high number of false-positive test results.

In the microbiology laboratory, RDT is generally grouped into the following categories:
- 1) antigen detection, such as enzyme immunoassay (EIA);
- 2) molecular detection (nucleic acid probes and nucleic acid amplification);
- 3) rapid biochemical tests, such as nitrite and leukocyte esterase performed on a urine dipstick;
- 4) direct microscopy of specimens using microbiologic stains, including Gram and calcofluor white stains; and
- 5) serologic testing.

**Rapid Microscopy Testing**

RDT for infectious diseases has been based largely on rapid microscopy or immunochromatography. In the current era of advanced technology, it is very easy to disregard the value of basic light microscopy in the rapid diagnosis of infections. A Gram stain can confirm within minutes the presence of gram-positive diplococcic (e.g., *Streptococcus pneumoniae*) in a sputum smear, gram-negative diplococcic (*Neisseria gonorrhoea*) in a urethral smear, or gram-negative rods in a spun specimen of urine.

Mycobacteria can be identified rapidly by microscopy of specimens stained with Ziehl-Neelsen, Kinyoun, or rhodamine auramine stains. *Giardia lamblia* and *Entamoeba histolytica* are readily identified by microscopy of direct fecal smears prepared with saline or lugol iodine. Microscopy of India ink (nigrosin) wet mounts prepared from cerebrospinal fluid (CSF) is a useful method for confirming the presence of encapsulated cells of *Cryptococcus* species.

Thick and thin blood smears prepared on a microscopy slide, fixed with methanol, stained with acridine orange, and examined with a fluorescence microscope reveal the presence of trypanosomes that cause sleeping sickness (*Trypanosoma brucei*) or Chagas disease (*Trypanosoma cruzi*).

The Gram stain is the preferred RDT for evaluating urethritis and is highly sensitive and specific for documenting both urethritis and the presence or absence of *N gonorrhoea* infection. A host of other specialized stains are essential elements of the infectious disease diagnostic repertoire in the clinical microbiology laboratory.

Rapid and accurate diagnosis of an infection should enhance patient outcome by enabling early initiation of appropriate therapy and implementation of relevant infection control measures, and reducing unnecessary diagnostic testing and treatment. Much current RDT involves genomic testing methodologies, such as nucleic acid hybridization with RNA or DNA probes, amplification, polymerase chain reaction (PCR) technologies, or nucleic acid sequencing. Tests used for direct detection of organisms in clinical specimens must be highly sensitive; otherwise, processing will require an amplification step.

The speed and sensitivity of real-time PCR have made it a popular method for the detection of microbiologic agents in both research and clinical specimens. Various companies have developed PCR platforms for early detection of infections caused by MRSA, *C difficile*, VRE, and *Neisseria gonorrhoea*. Although PCR is one of the best diagnostic assays now available, routine use in clinical microbiology is precluded for the following reasons:

- Identification of clinical specimens with known viral, bacteriologic, or parasitic loads remains problematic, rendering it virtually impossible to carry out studies to validate the reliability, reproducibility, and clinical utility of PCR test results;
• Maintaining a specialized laboratory with adequately skilled scientists, technicians, and supportive personnel is a costly endeavor -- the new generation of real-time PCR rapid testing is particularly expensive and labor intensive;
• Results may be delayed if specimens are sent to a reference laboratory (and therefore, by definition, not rapid);
• Nucleic acid extraction is expensive, time-consuming, and can easily be invalidated by contamination prior to the processing and analyses; and
• Specimens sent to the laboratory for RDT might not have been included in the specific specimen panel cleared by the US Food and Drug Administration (FDA) for that specific RDT.

For countries with adequate resources, relatively older RDT, such as diagnostic electron microscopy, need not be expensive or difficult to perform if executed in a diagnostic network -- eg, by recruiting and using instruments and electron microscopists working in other departments or services. Because the unusual and unexpected can be rapidly identified, electron microscopy is a major fixture in rapid diagnostic virology services, especially in the current era of vigilance for potential bioterrorist events, emerging pathogens, or new and unusual cases in which an infectious etiology is suspected.\textsuperscript{[10,11]} Moreover, to reduce costs, some facilities in the United States still use selective and differential solid media for the qualitative direct detection of VRE and MRSA. Typical of these media are specialized agar that render a pigmentation specifically to VRE and MRSA colonies, enabling them to be easily identified and isolated within 24 hours.\textsuperscript{[12]} Nonetheless, in the 21st century, genomic testing platforms are the principal technologies upon which rapid diagnoses of infectious diseases are based.

**When Should RDT Be Used?**

The rapid test methodologies used for various clinical infections, along with features such as specificity, sensitivity, predictive value, and time to results are summarized in the table.

<table>
<thead>
<tr>
<th>Table. Properties of Rapid Diagnostic Testing for Selected Infections of Public Health Significance</th>
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<td><strong>Infection</strong></td>
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<td>HIV\textsuperscript{c}</td>
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<td>C difficile</td>
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\textsuperscript{[10,11]} http://www.medscape.com/viewarticle/748139_print
<table>
<thead>
<tr>
<th>Disease</th>
<th>Test Description</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<td>Fecal toxin assay by cell cytotoxicity neutralization assay</td>
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<td>97-100</td>
<td>100</td>
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<td>Pulmonary tuberculosis: smear-positive; culture-positive</td>
<td>PCR: Nucleic acid amplification and hybridization (respiratory specimens)</td>
<td>94-100</td>
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<tr>
<td>Pulmonary tuberculosis: smear-negative; culture-positive</td>
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<td>Tuberculous meningitis</td>
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<td>PCR/microarray platform</td>
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<td><em>Bordetella pertussis</em></td>
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<td>100</td>
<td>Within hours</td>
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<td>Rapid antigen assays</td>
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<td>(respiratory specimens)</td>
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<td>95-100</td>
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<td>Real-time PCR</td>
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<td>99</td>
<td>70-92</td>
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*aPVP = positive predictive value
bNVP = negative predictive value
cHIV = human immunodeficiency virus (in regions of low HIV prevalence, the predictive value of a single rapid negative test is, generally, relatively high, whereas the predictive value of a positive test tends to be relatively low.)
dPCR = polymerase chain reaction

**Herpes simplex virus.** Extensive literature describes the application of real-time PCR for detection and quantification of viral pathogens in human specimens. For example, real-time PCR is faster and more sensitive than previous technologies, such as cell cultures or immunofluorescence microscopy, for detecting and genotyping herpes simplex virus (HSV) in clinical specimens. Currently, real-time PCR has replaced viral culture as the gold standard for the rapid and accurate detection of HSV in CSF. In the management of encephalitis,
differentiation of HSV from other viruses (eg, West Nile virus or varicella) is important since patients with HSV encephalitis have a better prognosis if therapy if instituted in a timely manner with intravenous acyclovir. However, in clinical practice, physicians are likely to initiate empirical antiviral therapy anyway after requesting testing by conventional methods, especially for patients with typical central nervous system symptoms and signs, and vesicular skin, oral or genital lesions. In this case, RDT is likely to not make a difference in clinical decision-making.

**Septicemia.** Septicemia is characterized by the presence of microorganisms or bacterial products in the bloodstream, together with clinical evidence of a systemic response to infection. A blood culture is one of the most important microbiologic investigations in suspected septicemia, and the discovery of living microorganisms in a patient's blood has great diagnostic and prognostic implications. Without an isolated microorganism, therapy is empirical and antimicrobial susceptibility testing is impossible. Molecular tests that use whole blood specimens for detection of organisms causing sepsis have been in development.\(^{[13]}\)

Of the various molecular RDT methodologies for detecting bloodstream pathogens, multiplex-PCR, a modification of PCR, is a promising molecular technique.\(^{[14,15]}\) Multiplex-PCR is designed to rapidly detect deletions or duplications in a large gene. The process consists of multiple primer sets within a single PCR mixture, enabling simultaneous amplification of many targets of interest. Real-time multiplex PCR evaluations have detected up to 25 bacterial or fungal species; however, sensitivity is relatively low (in the 50% range).

In another variation of PCR methodology, a new DNA-based microarray platform has been developed to enable rapid detection of bloodstream pathogens.\(^{[16]}\) Such platforms are based on amplification and detection of specific genes of up to 50 bacterial species. Identification of bacterial species with this microarray platform is highly sensitive (94.5%), specific (98.8%), and faster than culture-based broth methods.\(^{[16]}\) However, this DNA microarray system has drawbacks that preclude absolute replacement of the classic broth-based system; these shortcomings include difficulties in resolving the identities of various species in polymicrobial bacteremia, and inability of microarray systems to provide antimicrobial susceptibility testing information.\(^{[16]}\)

Thus, although molecular methods certainly shorten the time to pathogen identification in patients with suspected septicemia, these methods are primarily adjuncts to traditional broth-based blood cultures for the characterization of sepsis. Because the bloodstream is normally a sterile site, properly performed blood cultures have high positive predictive values for bloodstream infections and remain the gold standard in clinical practice. Molecular methods are merely adjunctive at this time.

**Tuberculosis.** Culture is the gold standard for laboratory confirmation of tuberculosis (TB) and is required for isolating the organism, drug-susceptibility testing, and genotyping.\(^{[17]}\) Mycobacteria isolated from cultures are identified using standard biochemical analyses, nucleic acid probes, or 16S rRNA gene sequencing.\(^{[17]}\) Culture and identification processes are time-consuming, labor intensive, and, depending on the laboratory and methodology used, may lack sensitivity or specificity.

Real-time PCR assays that rapidly and specifically detect *M tuberculosis* complex directly from acid-fast, smear-positive respiratory specimens and broth cultures are now routinely conducted in various reference laboratories across the United States. These assays offer the potential to detect gene mutations responsible for drug resistance directly from patient specimens and report the results within hours compared with the average of 2 weeks required for traditional susceptibility testing methods.

In the diagnostic workup of TB, direct nucleic acid amplification tests should always be performed in conjunction with microscopy and culture, and test results must be interpreted in the context of the overall clinical setting.\(^{[18]}\) Rapid tests for TB do not replace acid-fast smears or mycobacterial cultures; rather, they provide an index of the degree of contagiousness, facilitating decisions on implementation of infection control and general public health measures. To decide whether to perform RDT for tuberculosis, the following should be considered:

- Is the clinical suspicion of TB high, intermediate, or low?
- Is the acid-fast smear positive or negative?
- What additional diagnostic studies are planned?
Will RDT influence the diagnostic evaluation or the use of anti-tuberculosis therapy?

In patients for whom clinical suspicion of TB is high and the acid-fast smear is positive, the probability of TB is extremely high and the negative predictive value of RDT is likely to be low. In patients with chronic respiratory symptoms, typical chest radiograph changes, and positive results on acid-fast sputum smears, the probability of TB is high; hence, antituberculosis therapy and appropriate public health measures should be instituted, regardless of the results of RDT. Thus, RDT for TB in patients in whom the likelihood of TB is either very high or extremely low almost certainly provides no additional diagnostic information that would change the treatment decision. RDT in these instances is a gross waste of resources.

Although nucleic acid amplification tests are relatively expensive, the Centers for Disease Control and Prevention (CDC) recommends that such testing be performed on at least 1 respiratory specimen from each patient with signs and symptoms of pulmonary TB, in whom a diagnosis of TB is being considered but has not yet been established, and for whom the test result would alter case management and tuberculosis control measures. Therefore, RDT for TB should be used primarily when the test results will influence the decisions on initiation of anti-tuberculosis therapy or further diagnostic evaluation.

C difficile. Currently, the gold standard for diagnosis of C difficile disease is a toxigenic culture, whereby organisms are cultured on selective medium and tested for toxin production. Culture is the most sensitive and specific test available, but is slow and labor-intensive, and has an average a 3-day turnaround time. However, because the diagnosis of C difficile disease is usually made on the basis of clinical history and circumstances (such as recent antimicrobial use, hospital-onset diarrhea, and physical examination), the need for RDT is urgent.

Available EIA and glutamate dehydrogenase tests are easy to perform and offer results within 2 hours, but lack sensitivity. These assays fail to detect 20%-50% of cases. The FDA recently approved a molecular diagnostic test for direct detection of toxigenic C difficile strains from stool specimens. The 45-minute test targets the toxin B gene responsible for antibiotic-associated diarrhea and colitis, demonstrating a sensitivity and specificity of 93.5% and 94.0%, respectively. As such, it is the first test for C difficile infection to deliver both rapid turnaround and a high degree of accuracy. It is simple to perform and repeat testing to confirm a negative result is not indicated.

Bordetella pertussis. Although PCR testing for B pertussis has been available for nearly 20 years, no FDA-licensed PCR test kit is currently available. The analytical sensitivity, accuracy, and quality control of PCR-based B pertussis tests vary widely among laboratories. PCR assays used by most laboratories amplify a single gene sequence; both false-positive and false-negative results have been reported with these assays. Reported outbreaks of respiratory illness mistakenly attributed to pertussis have resulted in unnecessary investigation and treatment of putative cases, and unnecessary chemoprophylaxis of contacts. Thus, at this time, RDT does not play a significant role in the management of pertussis. Earlier types of RDT used latex or other particleagglutination technology. Most current tests use enzyme or optical immunoassay technologies, which provide results with more precise endpoints.

Group A streptococcus. Rapid diagnosis of pharyngitis caused by group A beta-hemolytic streptococci reduces the risk for transmission of the organism and mitigates the morbidity of the condition. Compared with blood agar plate cultures, rapid antigen detection tests for group A streptococcus have specificities ≥ 95%. However, the relatively low sensitivities (70%-90%) compared with blood agar plate cultures and limited data on its cost effectiveness preclude routine RDT for group A streptococci RDT.

Influenza. In the United States, RDT methods for influenza include rapid antigen testing, reverse transcription-PCR, and immunofluorescence assays that identify influenza A and B viral nucleoprotein antigens in respiratory specimens. Results from these tests are qualitative (eg, reported as either positive or negative). Real-time PCR is considerably more sensitive than cell culture for the detection of influenza A virus.

RDT for influenza A and B have enormous implications for infection control in healthcare facilities, from the management of sick patients with respiratory symptoms in critical care units to isolation and cohorting of patients.
with suspected influenza A infection. Rapid laboratory diagnosis is useful for diagnosing influenza A and B in the outpatient clinic or the emergency room and is critical for infection control, especially in hospital and nursing home settings.\textsuperscript{[24,25]} In addition, RDT provides the opportunity for initiating antiviral therapy during the early stages of the infection.

Commercial influenza RDT can detect influenza virus antigens within 15 minutes of testing. Kits vary: some can detect influenza A and B viruses but cannot distinguish between the 2 types, whereas others can detect both A and B viruses and also distinguish between the two. However none of the current commercial influenza RDT assays are able to identify any of the various influenza A virus subtypes.

Recent pandemic outbreaks of H1N1 influenza strains demonstrate the need for more sensitive RDTs to differentiate between influenza and other respiratory viruses. Specimens to be used with rapid tests should be collected as close as is possible to the start of symptoms and usually no more than 4-5 days later in adults. In very young children, influenza viruses can be shed for longer periods; therefore, in some instances, testing for a few days after this period may still be useful.

If performed on individuals with signs and symptoms consistent with influenza, the accuracy of influenza RDT appears to depend on when testing is performed during the influenza season, and on the endemicity of the strain. Influenza RDT, therefore, can be very useful for managing patients with suspected influenza and for detection of institutional influenza outbreaks. However, RDT testing alone should not be used to ascertain the cause of an individual's symptoms. A patient may be co-infected with another pathogen that is responsible for the underlying respiratory symptoms.

Influenza RDTs have limited sensitivity (11%-70%) in detecting influenza virus infection.\textsuperscript{[26]} If used for the management of patients with possible pandemic flu, false-negative test reports result in inappropriate exposure of susceptible persons to infected patients. Thus, negative test results must be interpreted with caution. In contrast, the specificity of RDT for influenza is generally high. For these reasons, CDC has recommended that negative RDT results should not be used to make treatment or infection control decisions, especially when influenza viruses are known to be circulating in the community.

In 2009, after effectively using a highly sensitive PCR assay to implement a successful pandemic response, CDC concluded that future planning efforts should identify ways to improve availability of reliable testing to manage patient care and approaches for optimal use of molecular testing for detecting and controlling emerging influenza virus strains.\textsuperscript{[27]}

**Malaria.** Despite the availability of RDT for the detection of malaria on the basis of lateral-flow immunochromatography, with which clinicians can detect malaria parasite antigens from finger-prick blood specimens within 10-15 minutes, microscopic examination of blood smears is the most cost-effective methodology for diagnosis of malaria, provided the results are available in a timely manner. Rapid test kits for malaria have limitations that preclude replacing microscopy of blood smears in the near future. These limitations include cost, inability to ascertain parasitemia quantitatively or to differentiate between the 4 plasmodium species, and sensitivity issues.\textsuperscript{[28]}

At present, the FDA requires that blood smears be taken concomitantly with rapid diagnostic strips. Studies comparing traditional blood smears with rapid antigen capture tests have consistently demonstrated that malaria RDT is superior to a single set of Giemsa-stained blood smears.\textsuperscript{[29]} PCR techniques, where available, have high sensitivity and specificity and have been useful as an adjunct to microscopy in areas with a low incidence of malaria, especially when the diagnosis is strongly suspected. The limitation is availability -- malaria PCR is generally limited to facilities in developed countries with the required expertise and equipment to carry out PCR and report the results in a timely manner. Although delay is common because of the time needed for formal preparation and reading, thick and thin blood smears are currently the cornerstones of laboratory diagnosis of malaria.

Malaria RDT with high sensitivity and negative predictive value for *Plasmodium falciparum* would be of particular use in acute care settings, especially in regions of low endemicity where the diagnosis is suspected but
laboratory expertise in malaria diagnosis is not available. Lastly, RDT for malaria also may benefit severely ill patients by confirming or excluding a malaria diagnosis rapidly and facilitating prompt intervention.

**HIV.** According to CDC, of the 1.1 million persons with HIV infection in the United States, 25% are unaware that they are infected. Current data suggest that RDT would likely facilitate the identification and improved management of many persons living with undiagnosed HIV infection. The FDA has approved several HIV RDT kits that are able to identify HIV-1 and HIV-2 antibodies within 10-30 minutes. The clinical specimens required for these tests include whole blood, plasma, serum, and oral fluids. Indications for HIV RDT include the following:

- Pregnant women in labor for whom no HIV test results are available, especially in endemic regions with high rates of vertical HIV transmission;
- High-risk persons (sex worker programs, halfway houses, health fairs, strip club workers, living in homeless shelters);
- Occupational exposure to HIV, especially when making a decision on postexposure prophylaxis; and
- An adjunct to diagnosis and counseling.

The positive predictive value (ie, the probability that a positive test result is real) of a single test depends on the specificity of the test and varies with prevalence of HIV infection in the population of concern. For example, positive predictive value is expected to be low in populations with low HIV prevalence. Thus, in the United States, CDC recommendations require a positive HIV RDT result to be validated by a second independent confirmatory test. However, in HIV-endemic regions of the globe, results from 2 different HIV RDT kits have yielded results comparable with routine EIA and Western blot.

Although more HIV-positive people will receive their test results in a timely manner, some people will receive a false-positive result before confirmatory testing, the main limitation of RDT for HIV. HIV RDT has played an important role in strategies for HIV prevention and control by facilitating voluntary HIV testing as routine part of medical care; by enabling straightforward diagnosis of HIV infections outside medical settings in at-risk persons identified through contact tracing; and by reducing rates of perinatal HIV transmission.1,30

**Sexually transmitted infections.** The arguments for using RDT for the diagnosis of sexually transmitted infections (STIs) are compelling for both developed and less-developed areas. Chief among these is the fact that diagnosis and treatment can be carried out in a single visit where RDT services are available.31 In STI clinics in developed countries, patients who present with symptoms or a history of exposure often fail to return for the results and appropriate therapy. The same pattern has been observed in less-developed countries, often for reasons that are the result of poverty (lack of transportation, inability to afford travel, or residence too far away from the treatment center).

Syphilis is one of many possible causes of genital ulcer disease.31 However, clinical diagnosis is of limited use because chancres may heal, be atypical, or patients may be asymptomatic. Moreover, although syphilitic genital lesions are often characteristic, differentiation from other genital infections can be inaccurate and difficult. Culture of _Treponema pallidum_ is not possible and does not represent a diagnostic alternative. At present, there are >30 rapid syphilis tests commercially available; few of these FDA-approved. Since virtually all available syphilis RDT kits use _T pallidum_ recombinant antigens to detect treponeme specific antibodies, the results closely reflect those generated by specific, confirmatory (treponemal) tests rather than nonspecific, non-treponemal screening tests, such as the rapid plasma reagin (RPR) test. Accurate detection of _T pallidum_ antibodies is carried out using lateral-flow immunoassay, a test based on specific binding of antibodies to antigens followed by labeling of the antibody/antigen complex, enabling visualization of the specific binding action. Most syphilis lateral flow tests become available within 30 minutes and do not require a laboratory or other instrumentation. Healthcare staff can easily interpret the results by simple visual examination. The main disadvantage of syphilis RDT is that once a person has acquired syphilis, the RDT remains reactive even in individuals who no longer have active infection.32

In summary, RDT kits for syphilis are treponema-specific, user-friendly, and do not require specialized equipment. Where available, such testing may be cost-effective, especially for patients in whom the diagnosis is
strongly suspected and for whom a return for follow-up in the STI outpatient clinic is not guaranteed. For these patients, RDT is indispensable, enabling the clinician to test and treat if positive. For other STIs, such as gonorrhea or chlamydia, the sensitivities of current RDT platforms are too low to enable use for screening, although a case may be made for using them in patient populations that are unlikely to return to the clinic for results.

MRSA and VRE. Infections caused by MRSA and VRE continue to increase in hospitals across the United States. The Society for Healthcare Epidemiology of America (SHEA) has established evidence-based guidelines to control the spread of MRSA and VRE in acute care settings. A key tenet of the SHEA guidelines is the identification and containment of spread through active surveillance cultures to identify the reservoir for spread. Ample evidence shows that active surveillance cultures reduce the incidence of MRSA and VRE infections and that programs described in the SHEA guideline are effective and cost-beneficial. Studies establish that identification of patients colonized with MRSA or VRE on admission to hospital for critical care enhances interventions to reduce intra-hospital transmission of these pathogens.

Currently, the standing screening method of detection of these pathogens is culture. Nearly all studies that evaluated PCR (either conventional or real-time) have shown improved sensitivities for detecting VRE from fecal specimens compared with culture. Broad-based surveillance for MRSA or VRE, using culture-based methods, may be especially demanding if not impossible for most clinical microbiology laboratories. Moreover, a final result can take several days. Real-time PCR testing methods for both MRSA and VRE show great promise for simplifying this process and providing same-day results.

Standard approaches to preventing MRSA and VRE transmission include implementing CDC guidelines that recommend isolation of patients colonized or infected with MRSA in a private or dedicated room, and use of gowns, gloves, and masks when appropriate, by all personnel entering the room (contact isolation). Many hospitals actively screen all patients when they are admitted to high-risk hospital areas (eg, critical care units, transplant units, or surgical wards), and implement appropriate contact isolation for identified carriers. This approach, called an "active screen and isolate program," minimizes the possibility of transmitting MRSA between patients through the hands or clothes of healthcare workers, and has been effective in some centers.

The major criticism of active screening and isolation programs is that it takes an average of 3 days from the time a screening swab is obtained from a patient and is logged in the microbiology laboratory, to the time the results are reported back to those who need to know in the inpatient service. MRSA or VRE transmission may occur during those 3 days. The problem is compounded by the lack of sensitivity of culture. Therefore, reducing the time it takes to identify a patient as a MRSA carrier should eliminate this delay and provide a rational strategy for reducing the transmission of MRSA in the inpatient setting.

Current evidence from the published literature suggests that PCR screening for MRSA and VRE on admission to critical care units and isolating patients colonized with either of these organisms is associated with reduced rates of intra-hospital transmission. Implementation of RDT for these 2 pathogens, therefore, becomes an issue of cost, expertise, and sustainability for a facility. More recently, it was shown that although RDT safely reduces the number of unnecessary isolation days, only screening with chromogenic agar (and not PCR-based screening) can be considered cost-effective. However, other current data suggest that in the long run the costs of RDT for MRSA and VRE will likely be offset by the savings incurred by preventing bloodstream infections and surgical wound infections associated with these microorganisms.

Proteomics and the New Technologies

The proteome is the protein content of a cell, tissue, or entire organism in a defined state. Proteomics is the study of the full array of proteins produced by an organism. For infectious diseases, proteomics profiles proteins generated by human cells in response to stimuli from infectious agents and their products of metabolism. Characterization of a proteomic profile of a microorganism is complicated, largely because various unique proteins can be produced by the same gene product and because of the chemical diversity of the organism's proteins. Technologies used in proteomics analysis are complex, labor intensive, and fraught with difficulty. The
technologies most widely used to screen and analyze the proteome are 2-dimensional gel electrophoresis and mass spectrometry.

**Laser ablation electrospray ionization mass spectrometry.** This technology enables analysis and imaging of cells and tissues, and identification of proteins, peptides, lipids, metabolites and other biomolecules directly and rapidly in any sample that contains water. Laser ablation electrospray ionization mass spectrometry (LAESI-MS) allows the direct identification of biomolecules in tissue sections and cells, so that the destruction of the source sample is minimized. LAESI-MS provides both qualitative and quantitative data with 2-dimensional and 3-dimensional spatial analysis and is able to identify biomolecules and metabolites in cell structures, tissues and fluids.

Because LAESI-MS is minimally invasive and does not destroy tissues, living cells or tissues can also be monitored temporally. LAESI-MS is extremely sensitive and ideally suited for the direct analysis of biofluids and other aqueous samples that contain peptides, proteins, metabolites, and other biomarkers for clinical, diagnostic, and discovery workflows.

**Surface-enhanced laser desorption/ionization-time of flight.** Surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) is an ionization method in mass spectrometry that is used for the analysis of protein mixtures. SELDI-TOF is typically used to detect proteins in tissue samples (blood, urine, or other clinical specimens). The proteins are ionized with lasers and separated by size. Comparison of protein levels between patients with and without an infection can be used for biomarker discovery.

An advantage of this technology is that it characterizes patterns of combinations of proteins or peptides in blood or other body tissues that uniquely define a specific infectious disease, rather than identifying only a single marker. The SELDI-TOF methodology is relatively insensitive, is restricted to cultured specimens, and requires inocula of at least $10^6$ organisms. On the other hand, fingerprints to define disease states rather than just pathogen detection require no assumptions about the nature of proteins and protein identification, so is not essential for diagnostic utility.

Current applications of SELDI-TOF include rapid diagnosis of sleeping sickness, invasive aspergillosis, tuberculosis, and Chagas' disease. Additional work is necessary, however, before implementation of this technology in the clinical microbiology laboratory becomes routine.

**The Future of RDT Technologies**

Despite the rapid progress of RDT technologies, these diagnostic modalities have not yet made many inroads toward replacing standard identification tests in medical microbiology laboratories. More often than not, RDTs based on molecular platforms have relatively lower sensitivities or predictive value positive compared with traditional methodologies for investigation or diagnosis of infectious diseases. Moreover, reliable molecular diagnostic tests for many infectious agents are not readily available.

Obstacles to institution or sustainability of RDTs include specimen transport issues, low concentrations of infectious agent, primer binding site genetic changes, final assay volume, inhibition, contamination, nonspecific amplification, and operator error. Also, genomic bacterial sequencing is subject to error because of sequence homology among different bacteria, database problems, and mutations. The consequences of all these limitations include false-negative and false-positive amplification results and misdiagnoses. Although the extent of microbial DNA in "normal" host tissues is unknown, the speed and sensitivity of methods like real-time PCR have rendered this particular RDT almost routine for the detection and ascertainment of microorganisms in both research and clinical specimens.

Apart from the diagnostic workup of influenza, HIV, and, more recently, *C difficile*, no evidence has shown that RDTs actually improve the diagnostic capabilities of laboratories or enhances patient outcomes to any significant degree. That said, RDTs will almost certainly continue to play an important role in the effort to improve rapid diagnosis of patients with HIV, tuberculosis, malaria, and bacteremia. It will become a tool in public health endeavors (eg, screening for group A streptococcus or antimicrobial resistance in hospital
Rapid Diagnostic Testing of Infectious Diseases (printer-friendly)

Rapid Diagnostic Testing of Infectious Diseases (printer-friendly)

pathogens) and in the screening of asymptomatic patients for infection where the possibility of lack of follow-up is real (e.g., patients attending STI clinics with probable HIV, syphilis, gonorrhea, or chlamydia).

RDT methods may be implemented as adjuncts to the epidemiologic investigation of infectious disease outbreaks. As sensitivities, specificities, positive predictive value, and negative predictive value of RDTs continue to improve and as RDTs become more widely appreciated through production of less expensive and more user-friendly platforms, it will become necessary to formulate responsible guidelines for the appropriate and optimal use in clinical practice in clarifying infectious disease diagnoses and improving patient outcomes.

References


