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Role of Molecular Diagnostics in the Management of Infectious Disease Emergencies

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KEYWORDS

- Real-time polymerase chain reaction • Molecular diagnosis • Infectious disease
- Microbiology • Normal vs immune compromised

KEY POINTS

- Clinical laboratories have traditionally relied on time-consuming phenotypic methods such as culture, serology, and biochemical tests for detection, identification, and characterization of microbial pathogens.
- Real-time polymerase chain reaction (PCR) technology is now available to identify many of the pathogenic organisms that constitute infectious disease emergencies in normal and immune-compromised hosts.
- Use of this molecular technology for the accurate diagnosis of infectious disease agents by clinical laboratories reduces the time to diagnosis for many pathogens.

INTRODUCTION

The role of molecular diagnostic tests in the evaluation of infectious etiology is rapidly evolving, with new tests becoming available each year.¹ This article discusses the various real-time polymerase chain reaction (PCR)-based assays and antigen detection assays currently available for use in emergency settings for making a specific microbiological diagnosis. Serologic assays, cultures, and stains are not covered in this article.

In the emergency setting, molecular diagnostic methods yield several advantages over serologic methods. Molecular methods directly assay for the presence of the microorganism at the time the specimen is obtained, which is ideal for the acutely ill patient. Serologic methods assay for an antibody response to the microorganism,

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typically requiring at least a week of symptoms even for immunoglobulin M tests. Immune-compromised and immune-suppressed patients frequently will not mount an appropriate antibody response. Thus for serologic assays, evaluation of the patient early in the course of the disease or testing a patient with immune dysregulation will often yield false-negative results. Patients who have been treated with intravenous immunoglobulin in the preceding year will typically have immunoglobulin G present against a wide array of infectious agents that they have never seen, which can lead to false-positive serologic tests. Optimal serologic evaluation typically requires retesting at 4 to 6 weeks after infection (ie, convalescent titers) to demonstrate an increase in titer associated temporally with the recent illness, further delaying a definitive diagnosis.

Molecular methods can also provide a more rapid diagnosis of etiology than is possible with culture, with results available within hours if the assay can be performed locally. Many infectious agents cannot be grown by standard culture methodologies (eg, *Mycoplasma pneumoniae*) or are fastidious (eg, *Bordetella pertussis*), leading to frequent false-negative results. Other organisms grow very slowly (eg, *Mycobacterium tuberculosis*) such that culture results are not available for weeks. Most clinical microbiology laboratories cannot afford to offer virology services owing to the high expense of performing tissue culture because it is labor intensive, requiring highly skilled laboratory personnel and the purchase of tissue culture cell lines every week that may not be used. Even when available, virus culture typically requires 3 to 10 days for results, depending on the particular virus. However, for the vast majority of bacterial pathogens culture still remains the gold standard of diagnosis, typically yielding a microbiological diagnosis in 1 to 3 days followed by antibiotic susceptibility results. Molecular methods to assay for resistance genes are being developed for some organisms, but are generally not available at this time.

The sections of this article have been organized by organ systems most likely to be affected in emergency settings, designed such that the clinician evaluating a patient with severe acute illness can reference the most relevant molecular diagnostics available pertinent to the predominant organ system involved. There is considerable overlap for the various tests among the organ systems, and some tests will be present under multiple organ systems headings. Each section is further divided between diagnostics most appropriate for normal hosts, then additional diagnostics pertinent to immune-compromised hosts. Thus, diagnostics for the normal host will usually also be relevant to immune-compromised hosts. Before addressing infections of the various organs systems, a brief overview is provided of the principle behind the most common molecular assay currently in use in diagnostic laboratories for pathogen detection, real-time PCR. Antigen tests for specific organisms are addressed as they appear.

REAL-TIME PCR

While a variety of molecular methods exist in diagnostic microbiology, PCR is by far the most universal methodology used for pathogen detection.² In recent years real-time PCR, also known as quantitative PCR (qPCR), has become the “go-to” technique in the clinical laboratory (reviewed in Ref.¹). In contrast to conventional PCR, which simply amplifies a targeted DNA molecule, real-time PCR can detect, amplify, and quantify a targeted DNA molecule in real time. In real-time PCR, fluorescent dyes are used to label PCR products during thermal cycling. Real-time PCR instruments then measure the accumulation of fluorescent signal during the exponential phase of the reaction for rapid and precise quantitation of PCR products. Quantitation can yield absolute numbers of copies of a given sequence or a relative amount when

standardized to a known amount of DNA. Clinically this is particularly useful, for example, in determining viral load from plasma or serum of an individual infected with human immunodeficiency virus (HIV).³ Fluorescence-based diagnostic real-time PCR assays are now available in-house as well as commercially (approved by the Food and Drug Administration [FDA]) for many human pathogens (reviewed in Ref.¹). By virtue of its ability to simultaneously detect and quantify in a sealed PCR plate with no post-PCR processing steps, real-time PCR lends itself to automation and rapid turnaround time while reducing human error. In addition, multiplex real-time PCR assays on the same automated instrument to simultaneously detect and screen for a variety of pathogens are currently under development and are expected in time to replace stand-alone assays for specific pathogens.⁴ Despite these exciting possibilities, the cost of such multiplex instrumentation is a significant limitation for many smaller clinical laboratories. This review focuses on the individual real-time PCR assays that are currently widely available in the diagnosis of infectious disease emergencies.

In the following sections, pathogenic organisms that affect each major organ system (Central Nervous System, Pulmonary, Cardiac, Gastrointestinal, and Bloodstream/Systematic) are accompanied by a table listing the following information: host (normal or compromised), microorganism (viral, bacterial, fungal, or parasitic), method of detection (antigen or real-time PCR), specimen type, and representative commercial test from national reference laboratories, Mayo Medical Laboratories (<http://www.mayomedicallaboratories.com>) and Quest Diagnostics (www.questdiagnostics.com). Regarding the nomenclature of the PCR methodology, PCR as denoted in the tables refers to real-time PCR. qPCR refers to real-time PCR that is truly quantitative. The authors have purposely avoided using the term RT-PCR, as this can refer to real-time PCR or reverse-transcriptase PCR in the literature (real-time, reverse-transcriptase PCR is used for viruses containing a plus-sense or minus-sense RNA genome [influenza virus, West Nile virus, and so forth]).

CENTRAL NERVOUS SYSTEM (MENINGITIS AND ENCEPHALITIS)

Clinical Perspective and Molecular Diagnosis

This section focuses on molecular diagnostics pertinent in the evaluation of infectious causes of encephalitis and meningitis (**Table 1**). Bacterial meningitis remains the most dangerous and treatable form, such that cerebral spinal fluid (CSF) should always be tested for bacterial culture.⁵ These molecular diagnostic tests will be most informative for testing CSF to identify the presence of genetic or antigenic material of microorganisms. However, if CSF cannot be obtained, many of these tests can also be performed on blood. For meningoencephalitis in the normal host, herpes simplex virus (HSV)⁶ and enterovirus⁷ testing have become routine tests on CSF in many centers. Bacterial antigen testing has been available for decades, but the accuracy of this test remains poor, resulting in limited clinical utility. Molecular diagnostic tests are unlikely to yield an origin in the setting of postinfectious encephalitis because the inciting microorganism is no longer present.

Molecular diagnostic tests likely have their greatest utility for immune-compromised hosts with central nervous system infection because of the ability to make an accurate diagnosis via lumbar puncture. This clinical setting is an exceptionally challenging one whereby there are many microbial pathogens that can cause similar-appearing disease, and the need for a rapid diagnosis is urgent. Many of these diagnostics have become available fairly recently, aiding the diagnostician in meeting these challenges.

Normal Host	Microorganism	Methods	Specimens	Diagnostic Test
	HSV	PCR	CSF, other	MAYO: VDER
	Enterovirus	PCR	CSF, blood, other	MAYO: LENT
	West Nile virus	PCR	CSF, blood	MAYO: WNVVP
	<i>Treponema pallidum</i>	PCR	CSF, blood, other	QUEST: 87798
	<i>Mycoplasma pneumoniae</i>	PCR	CSF, other	QUEST: 87581
	Bacterial meningitis	Antigens	CSF, blood, urine	Becton Dickinson: Directigen
Compromised	EBV	qPCR	CSF	MAYO: QEBV
	CMV	qPCR	CSF, other	MAYO: QCMV
	<i>Cryptococcus neoformans</i>	Antigen	CSF, blood	MAYO: CCRYR/SCRYP
	<i>Toxoplasma gondii</i>	PCR	CSF, blood	MAYO: PTOX
	JC virus	PCR	CSF, blood, urine	MAYO: LCJC
	HHV 6	PCR	CSF, blood, other	MAYO: HHV6V

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV 6, human herpesvirus 6; HSV, herpes simplex virus; MAYO, Mayo Medical laboratories test identifier; QUEST, Quest Diagnostics test identifier.

As illustrated in **Table 1**, real-time PCR tests are commercially available for many of the listed viruses and bacteria. Unfortunately, in the case of bacterial meningitis molecular techniques to identify the etiologic agent are currently lacking. In the absence of reliable CSF cultures (eg, antibiotic therapy before lumbar puncture), identification of suspected pathogens has traditionally depended on the use of assays such as the latex agglutination or related immunochromatographic membrane assays for the direct qualitative detection of antigens to common bacterial pathogens causing meningitis (eg, *Haemophilus influenzae* B, *Neisseria meningitidis*, Group B *Streptococcus*, *Escherichia coli*, and *Streptococcus pneumoniae*). In the latex agglutination assay, for example, specific antibodies are bound to the surface of the latex particles on a slide. On addition of the specimen, visible agglutination occurs when the specimen containing any of these bacterial antigens reacts with its respective antibody-coated latex bead. As this assay is completely dependent on accumulation of polysaccharide antigen in the specimen, many false negatives can occur and sensitivity remains a critical issue. In addition, antigen in the sample can come from organism colonization of mucosal surfaces, leading to false-positive results.⁸

For the immune-compromised category, a quantitative assay for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) exists and is in extensive use. The quantitative assay for these pathogens and HIV are discussed in the Bloodstream and Systemic Infections section. For *Cryptococcus neoformans*, identification in CSF relies on antigen identification through latex agglutination assay or enzyme immunosorbent assays; however, as opposed to bacterial antigen testing, the cryptococcal antigen test has proved itself to be very accurate and reliable.^{9,10} For the other pathogens listed here, qualitative real-time PCR assays exist to identify these pathogens in CSF and blood.

PULMONARY (PNEUMONIA)

Clinical Perspective and Molecular Diagnosis

The diagnostic tests in this section focus on the evaluation of pneumonia (**Table 2**). A variety of rapid influenza diagnostic tests (RIDTs) have been available for several

Normal Host	Microorganism	Methods	Specimens	Diagnostic Test
	Influenza A, B	Antigen, PCR	Swab	MAYO: 800167
	RSV	Antigen, PCR	Swab	MAYO: RSVP
	Parainfluenza 1,2,3	PCR	Swab	QUEST: 87798 (×3)
	Adenovirus	PCR	Swab, other	MAYO: LADV
	Human metapneumovirus	PCR	Swab	QUEST: 87798
	Rhinovirus	PCR	Swab	QUEST: 87798
	<i>Mycobacterium tuberculosis</i>	PCR	Sputum, BAL, other	MAYO: MTBRP
	<i>Mycoplasma pneumoniae</i>	PCR	Swab, sputum, other	QUEST: 87581
	<i>Chlamydia pneumoniae</i>	PCR	Swab, sputum, BAL	QUEST: 87486
	<i>Legionella pneumophila</i>	PCR	Swab, sputum, BAL	MAYO: LEGRP
	<i>Bordetella pertussis</i>	PCR	Swab	MAYO: BPRP
	<i>Histoplasma capsulatum</i>	Antigen, PCR	Urine, blood, BAL	MAYO: HBRPB
Compromised	<i>Pneumocystis jirovecii</i>	PCR	BAL, sputum	MAYO: PNRP
	<i>Aspergillus</i> spp	Antigen	Blood	MAYO: ASPBA
	<i>Aspergillus</i> spp	PCR	BAL, sputum, blood	QUEST: 87798 (×3)
	CMV	PCR	BAL, blood, other	MAYO: QCMV
	Adenovirus	PCR	BAL, sputum, blood	MAYO: LADV

Abbreviations: BAL, bronchoalveolar lavage; CMV, cytomegalovirus; MAYO, Mayo Medical laboratories test identifier; QUEST, Quest Diagnostics test identifier; RSV, respiratory syncytial virus.

years, providing a very useful tool in the emergency, urgent care, and outpatient settings for detection of influenza virus A and B.¹¹ The RIDTs are immunoassays that identify the presence of influenza A or B nucleoprotein antigens from clinical specimens in a qualitative fashion. These tests are popular in that they can provide a result within 15 minutes or less, and many such commercially available tests are approved for office/bedside use. Disadvantages of the test include suboptimal test sensitivity, resulting in false-negative results, especially in periods when influenza activity is high. In addition, although specificity is high, false-positive results may also occur when influenza activity, and therefore pretest probability, is low.¹² The H1N1 2009 pandemic strain was poorly detected by standard RIDT at the time, leading to development of reverse-transcription PCR technology to identify this strain.¹³ The reference standards for laboratory confirmation of influenza virus infection are currently viral culture or real-time PCR.

Rapid respiratory syncytial virus (RSV) antigen detection has become a standard diagnostic tool in the evaluation of children with bronchiolitis or pneumonia.¹⁴ The development of PCR diagnostics for fastidious organisms such as *Legionella pneumophila*¹⁵ and *B pertussis*¹⁶ should improve the ability to diagnose these pathogens. Real-time PCR technology to diagnose tuberculosis is relatively new, but may prove

useful for achieving a rapid diagnosis for this slow-growing organism.¹⁷ Acid fast bacillus culture will still need to be performed because of the growing problem of drug resistance in *M tuberculosis*. Also exciting is the development of new multiplex assays that evaluate for multiple respiratory pathogens rapidly and simultaneously from a single sample.¹⁸ These multiplex platforms are designed for use in local clinical microbiology laboratories such that results can be available in hours, as discussed later. **Table 2** lists the individual pathogens that are typically assayed by these multiplex platforms, but there is variation regarding how many and which organisms are tested depending on the particular platform.

As illustrated in **Table 2**, individual real-time PCR assays currently exist for each of the bacterial, viral, and fungal pathogens. In the case of influenza and RSV, the rapid antigen assays can be verified with real-time PCR. Given that influenza A/B and RSV may cause illness that is clinically indistinguishable, a real-time PCR panel test that includes both influenza A/B and RSV (Mayo Diagnostic Test: PROD) should be considered. As already mentioned, innovative PCR-based multiplex molecular diagnostic tests are currently under development that can screen multiple respiratory pathogens at once. For example, the eSensor instrument (GenMark Dx, Carlsbad, CA) can identify the following pathogens in its respiratory viral panel: Influenza A (generic), Influenza A (H1 Seasonal Subtype), Influenza A (H1 Mexico strain), Influenza A (H3 Seasonal Subtype) Influenza B, Respiratory Syncytial Virus subtypes A and B, Parainfluenza viruses 1, 2, 3, and 4, Human Metapneumovirus, Rhinovirus, Adenoviruses B, C, and E, and Coronaviruses NL63, 229E, OC43, and HKU1. Currently being considered for FDA approval, this technology has the ability to allow clinical laboratories to rapidly identify the causative agent in a matter of hours. The recent development of PCR assays for *Pneumocystis*¹⁹ and *Aspergillus*²⁰ in immune-compromised hosts will likely improve detection over current technologies.

CARDIAC (MYOCARDITIS AND PERICARDITIS)

Clinical Perspective and Molecular Diagnosis

This section focuses on diagnostic tests for the evaluation of infectious origins of myocarditis and pericarditis (**Table 3**). Although these are relatively rare events

Normal Host	Microorganism	Methods	Specimens	Diagnostic Test
	Enterovirus	PCR	Blood	MAYO: LENT
	Adenovirus	PCR	Blood	MAYO: LADV
	Parvovirus	PCR	Blood	MAYO: PARVP
	HIV	qPCR	Blood	MAYO: HIVQU
	<i>Mycobacterium tuberculosis</i>	PCR	Pericardial fluid, blood	MAYO: MTBRP
Compromised	<i>Toxoplasma gondii</i>	PCR	Blood, other	MAYO: PTOX
	<i>Cryptococcus neoformans</i>	Antigen	Blood, other	MAYO: SCRYR/SCRYP
	<i>Aspergillus</i> spp	Antigen, PCR	Blood, other	MAYO: ASPBA QUEST: 87798 (×3)

Abbreviations: HIV, human immunodeficiency virus; MAYO, Mayo Medical laboratories test identifier; QUEST, Quest Diagnostics test identifier.

clinically, they frequently are life-threatening emergencies for both normal and immune-compromised hosts. Enteroviruses and adenoviruses are the most common infectious causes of myocarditis²¹ and real-time PCR is the best technology to evaluate for viremia caused by these organisms. These organisms are also the major ones implicated in cardiac transplant rejection.²² In addition, *Toxoplasma* infections are associated with heart transplantation²³ as well as myocarditis in immune-compromised hosts.²⁴ Real-time PCR methodology is currently available for the various pathogens associated with cardiac infections except for *C neoformans* which, as mentioned earlier, currently relies on antigen-based tests such as latex agglutination or enzyme immunosorbent assays (see **Table 3**).

GASTROINTESTINAL (ENTERITIS, COLITIS, HEPATITIS, PANCREATITIS)

Clinical Perspective and Molecular Diagnosis

The diagnostic tests in this section focus on infectious causes of enteritis, colitis, hepatitis, and pancreatitis (**Table 4**). Real-time PCR for diagnosis of *Clostridium difficile* infection²⁵ is replacing the standard *C difficile* toxin assay in many centers. Real-time PCR assays are now available for bacterial enteric pathogens typically diagnosed for by standard stool culture.²⁶ These real-time PCR assays for bacterial enteric pathogens can be ordered as a panel (eg, Mayo Diagnostic Test: EPRP). Antibiotic resistance can occur for *Salmonella* species in particular, such that stool culture is necessary for testing antibiotic susceptibilities. Real-time PCR for shiga-toxin producing *E coli* can be used to identify O157/H7 strains that can cause hemolytic uremic syndrome.²⁷ Standard stool-culture techniques will not recover enteroinvasive *E coli*, which can now be assayed for by real-time PCR. Antigen detection for *Giardia* and *Cryptosporidium* is significantly more sensitive than assaying for these organisms by standard ova-and-parasite examination of stool.^{28,29} Adenovirus serotypes associated with enteritis (ie, 40 and 41) do not grow in standard viral culture and must be

Normal Host	Microorganism	Methods	Specimens	Diagnostic Test
	<i>Clostridium difficile</i>	PCR	Stool	FOCUS: 87493, 87798
	<i>Salmonella</i> spp	PCR	Stool	MAYO: EPRP
	<i>Shigella</i> spp	PCR	Stool	MAYO: EPRP
	<i>Campylobacter jejuni/coli</i>	PCR	Stool	MAYO: EPRP
	<i>Yersinia</i> spp	PCR	Stool	MAYO: EPRP
	<i>Escherichia coli</i> , enteroinvasive	PCR	Stool	MAYO: EPRP
	<i>Escherichia coli</i> , shiga-toxin prod.	PCR	Stool	MAYO: EPRP
	<i>Giardia lamblia</i>	Antigen	Stool	MAYO: GIAR
	<i>Cryptosporidium</i> spp	Antigen	Stool	MAYO: CRYPS
	Adenovirus, enteric	Antigen, PCR	Stool, other	FOCUS: 87798 MAYO: LADV
	Hepatitis B virus	qPCR	Blood	MAYO: HBVQU
	Hepatitis C virus	qPCR	Blood	MAYO: HCVQU
Compromised	CMV	qPCR	Blood, other	MAYO: QCMV
	HSV	PCR	Blood, other	MAYO: VDER

Abbreviations: CMV, cytomegalovirus; HSV, herpes simplex virus; MAYO, Mayo Medical laboratories test identifier; QUEST, Quest Diagnostics test identifier.

assayed by antigen detection or real-time PCR.^{30,31} Adenovirus can be grown in viral culture of stool, but these are not enteritis-causing serotypes. Real-time PCR for hepatitis B and hepatitis C are quantitative, and used in diagnosis and monitoring of patients with chronic infection with these pathogens both on and off of antiviral therapy.³² Testing for hepatitis virus is routinely used for patients presenting with or developing acute liver disease.

BLOODSTREAM AND SYSTEMIC INFECTIONS

Clinical Perspective and Molecular Diagnosis

This section focuses on molecular diagnostics that can be used in settings of likely bloodstream infection, sepsis, or systemic infection (**Table 5**). In the setting of a sepsis-like illness or severe systemic illness likely to be due to an infectious etiology, bacteremia remains the most common and important cause.³³ Thus, blood cultures remain the gold standard in the evaluation of these patients. In addition to blood culture, molecular diagnostics can be useful in the evaluation of these patients depending on the clinical scenario.³⁴ HIV is included in this section because acute HIV infection (ie, acute retroviral syndrome) can present as an acute febrile illness with multiple manifestations.³⁵ There is real-time PCR testing available for multiple tick-borne illnesses, with Rocky Mountain spotted fever (*Rickettsia rickettsii*) being potentially fatal.³⁶ EBV, CMV, HSV, varicella zoster virus (VZV), adenovirus, and parvovirus are included in the immune-compromised section because these infections are typically self-limited in the normal host, but potentially fatal in the immune-compromised host.

As shown in **Table 5**, specific real-time PCR assays are available for many bloodstream pathogens unlikely to be detected via standard blood cultures. In the case of HIV, the real-time PCR reaction involves an initial reverse transcription step because HIV is a retrovirus containing a bipolar, plus-sense RNA genome. Because of the well-studied relationship of HIV RNA copy number to the stage of HIV disease and efficacy of HIV therapy, many truly quantitative real-time RT-PCR assays exist for HIV, with analytical sensitivity commonly as low as 20 copies/mL.³⁷

In the United States ticks are a significant vector of infectious diseases, and rank second only to mosquitoes in disease transmission across the world.³⁸ Given the many different agents associated with tick-borne disease (eg, *Rickettsia rickettsii*, *Borrelia burgdorferi*, *Ehrlichia* spp, and *Babesia microti*), commercial real-time PCR tick-borne panels are currently available to differentially identify the causative agent. In

Table 5
Molecular diagnostics for bloodstream and systemic infections

Normal Host	Microorganism	Methods	Specimens	Diagnostic Test
	HIV	qPCR	Blood	MAYO: HIVQU
	<i>Rickettsia rickettsii</i>	PCR	Blood, skin	MAYO: PTICK EHRL
	<i>Borrelia burgdorferi</i>	PCR	Blood, other	MAYO: PTICK EHRL
	<i>Ehrlichia</i> spp	PCR	Blood	MAYO: PTICK EHRL
	<i>Babesia microti</i>	PCR	Blood	MAYO: PTICK EHRL
Compromised	EBV	qPCR	Blood	MAYO: QEBV
	CMV	qPCR	Blood, other	MAYO: QCMV
	HSV	PCR	Blood, swab, other	MAYO: VDER
	VZV	PCR	Blood, swab, other	MAYO: VDER
	Adenovirus	PCR	Blood, other	MAYO: LADV
	Parvovirus	PCR	Blood, marrow	MAYO: PARVP

Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; MAYO, Mayo Medical laboratories test identifier; VZV, varicella zoster virus.

most cases this is a qualitative assay, and results are reported as either negative or positive for the targeted organisms. In the case of Rocky Mountain spotted fever, real-time PCR in a skin biopsy can be more useful for detecting the etiologic agent than an acute blood sample, owing to the low numbers of rickettsiae circulating in the blood in the absence of advanced disease or fulminant infection.³⁹

In the immune-compromised category, the herpesviruses EBV and CMV are of significant concern in transplantation recipients.^{40–42} Quantitative real-time PCR assays exist for both these pathogens, which can reliably detect between 2000 and 200 million copies per milliliter. It is important to recognize that there may be variation between results obtained from one laboratory and another laboratory. Clinically this can cause difficulty in interpreting whether a significant change in viral replication has occurred. A common example is when an EBV or CMV copy number is reported from one institution, after which the patient is transferred to another institution and a second quantitative PCR is performed. As these tests become more standardized over time, this should be less problematic. Qualitative real-time PCR assays are also available for herpesviruses HSV and VZV as well as adenovirus and parvovirus.^{43,44}

SUMMARY

In the setting of infectious disease emergencies, rapid and accurate identification of the causative agent is critical to optimizing antimicrobial therapy in a timely manner. It is clearly evident that the age of molecular diagnostics is now upon us, with real-time PCR becoming the standard of diagnosis for many infectious disease emergencies in either monoplex or multiplex format. Other molecular techniques such as whole or partial genome sequencing, microarrays, broad-range PCR, restriction fragment length polymorphisms, and molecular typing are also being used. However, for most small clinical laboratories, implementation of these advanced molecular techniques is not feasible owing to the high cost of instrumentation and reagents. If these tests are not available in-house, samples can be sent to national reference laboratories (eg, Mayo Medical Laboratories and Quest Diagnostics) for real-time PCR assays that can be completed in 1 day. It is anticipated that over time commercial real-time PCR tests and instrumentation will become more standardized and affordable, allowing individual laboratories to conduct tests locally, thus further reducing turnaround time. Although real-time PCR has been proved to expand our diagnostic capability, it must be stressed that such molecular methodology constitutes only an additional tool in the diagnosis of infectious diseases in emergency situations. Phenotypic methodologies (staining, cultures, biochemical tests, and serology) still play a critical role in identifying, confirming, and providing antibiotic susceptibility testing for many microbial pathogens. As multiplex assays become increasingly available, there will be even greater temptation for taking a “shotgun” approach to diagnostic testing. These new technologies will not substitute for a proper history and physical examination leading to a thoughtful differential diagnosis. None the less, these new molecular tests increase the capability of the diagnostician to rapidly identify the microbiological etiology of an infection. An added advantage of rapid diagnostic tests often not emphasized is the capability to rule out certain diagnoses for which unnecessary antimicrobial therapy may otherwise be instituted and/or continued.

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