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Practice Forum

The new frontier of diagnostics: Molecular assays and their role in infection prevention and control

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Recent advances in technology over the last decade have propelled the microbiology laboratory into a pivotal role in infection prevention and control. The rapid adaptation of molecular technologies to the field of clinical microbiology now greatly influences infectious disease management and significantly impacts infection control practices. This review discusses recent developments in molecular techniques in the diagnosis of infectious diseases. It describes the basic concepts of molecular assays, discusses their advantages and limitations, and characterizes currently available commercial assays with respect to cost, interpretive requirements, and clinical utility.

The microbiology laboratory has always played an important role in public health. However, recent advances in technology over the last decade have propelled the laboratory into a pivotal role in infection prevention and control in both health care and community settings. The rapid adaptation of molecular technologies to the field of clinical microbiology now greatly influences infectious disease management and significantly impacts infection control practices. Molecular assays have, in some instances, replaced the need for traditional testing methods, such as viral cultures for herpesviruses and enteroviruses.[1] Concurrently, advances in automation and information technology have made it possible for critical diagnostic information to have the greatest impact on decisions for isolation, treatment, and care. To effectively use molecular diagnostic technology for enhancement of infection control practices, it is important to understand the scope of these rapid diagnostic tests and to appreciate the need for judicious interpretation in collaboration with the clinical microbiologist. This review discusses recent developments in molecular techniques in the diagnosis of infectious diseases. We begin by describing the basic concepts of molecular assays, discuss their advantages and limitations, and conclude by characterizing currently available commercial assays with respect to cost, interpretive requirements, and clinical utility.

Molecular assays currently in use are based on detection of the nucleic acid of the targeted pathogen by one of the following procedures: (1) target amplification, (2) signal amplification, and (3) nonamplification methods.

TARGET AMPLIFICATION OR NUCLEIC ACID AMPLIFICATION TESTS

Many molecular assays used in the clinical laboratory are based on polymerase chain reaction (PCR) amplification of DNA or RNA of the pathogen or organism of interest.[2] PCR can be performed directly from patient specimens, or alternatively, from organism isolates. The purpose of PCR is to amplify the nucleic acid of interest to a detectable limit. In its most basic format, this in vitro reaction uses a set of reagents and a thermostable polymerase enzyme to synthesize millions of copies of the target nucleic acid, also known as amplicons, by a process of thermal cycling, whereby a strand of DNA or RNA is duplicated multiple times by repeated cycles. An illustration of the PCR method is available online (http://www.sumanasinc.com/webcontent/animations/content/pcr.html). A primer pair (forward and reverse) that is specific to the target DNA sequence is used to create an identical strand which varies in length based on the design of the primers and the requirements of the assay. Repeated thermal cycling (chain reaction) is then used to generate exponential amounts of DNA, potentially increasing the sensitivity several hundred–fold. To amplify RNA targets, such as influenza, respiratory syncytial virus (RSV), human immunodeficiency virus (HIV), or hepatitis C virus (HCV), reverse-transcription PCR is used.

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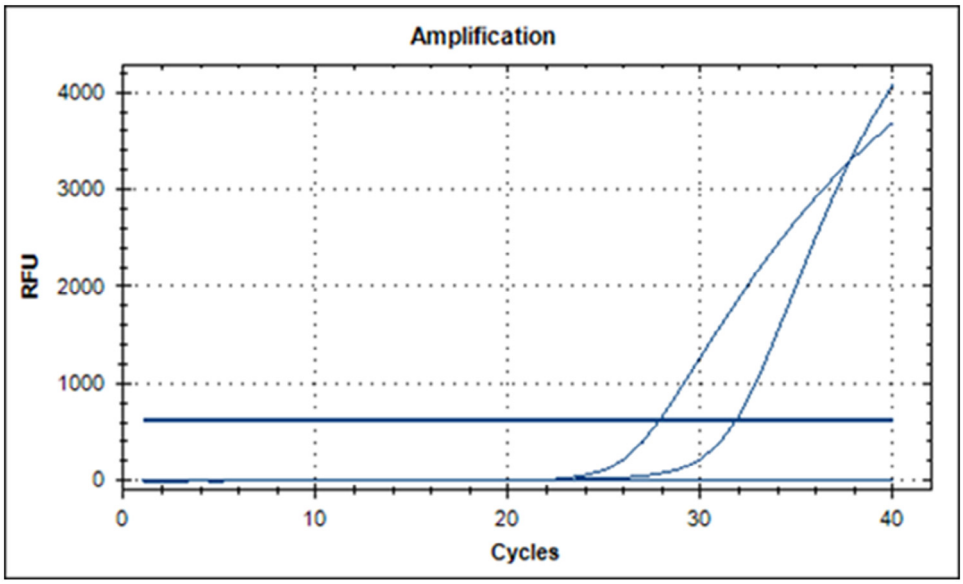

Fig 1. Ct in real-time polymerase chain reaction. RFU is plotted against the cycle number. The horizontal solid line indicates the baseline or threshold. The cycle number at which the fluorescence exceeds the background is termed Ct. Two samples are shown with a Ct of 28 and 32, respectively. *Ct*, cycle threshold; *RFU*, relative fluorescence.

Reverse-transcription PCR includes an initial step that converts the RNA target to its complimentary DNA (cDNA). Subsequent thermocycling and amplification of cDNA is performed by PCR.

Nested or hemi-nested PCR are modifications made to increase the sensitivity and specificity of the target amplification. In nested PCR, 2 primer pairs are used for amplification: the first pair amplifies an original target, and the second pair binds to sequences within the product of the first pair and amplifies a segment shorter than the original target. The rationale is to select for a specific amplification product by the use of a second primer pair within the first amplicon, therefore increasing the sensitivity and specificity of the assay. All amplification-based assays incorporate a detection method, whereby the products of amplification are recorded. Several modifications of the basic PCR technology and the detection of amplified products have been used in the laboratory, but perhaps the most useful is the real-time PCR assay (rt-PCR).

Real-time-PCR has revolutionized the direct detection of pathogens from clinical specimens.[3,4] This technology incorporates the use of fluorescent dyes in the amplification reaction and uses optics that monitor the fluorescence emission at the end of each cycle.[5] The inclusion of software that examines and interprets the reaction kinetics in real-time and generates an amplification plot for the reaction expedites the process of target detection significantly (Fig 1). By using multiple fluorescent dyes, rt-PCR can be multiplexed; therefore, detection of several targets can be achieved in the same reaction.[2,6] For example, the Xpert CT/NG assay (Cepheid, Sunnyvale, CA) uses specific primers for both *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, therefore facilitating detection of either, or both, from the same specimen.[7]

Interpretation of rt-PCR results requires an understanding of what is demonstrated in Figure 1. Each reaction has a threshold fluorescent signal which is akin to background fluorescence. The cycle at which the fluorescence of the target-specimen crosses and rises above the threshold fluorescence is known as the cycle threshold (Ct). The Ct is used as a criterion for result interpretation; cutoffs for positive and negative are set at a certain Ct based on validation results during assay design. Many rt-PCR chemistries also use an additional melt curve for specific identification of amplified product. A melt curve is generated by increasing the temperature slowly while measuring the fluorescence. As temperature increases, the DNA strand is denatured at a temperature specific to the sequence of the DNA segment (melting temperature) and leads to a decrease in fluorescence (Fig 2). The temperature at which this occurs provides an indication of the size of the amplicon, therefore enabling differentiation from nonspecific amplification. Melt curves can also be used to differentiate targets if the amplicons are designed, such that they have different melting temperature. Specific details of the various methods will be individually discussed in each disease topic.

General advantages of PCR include the ability to detect even low numbers of pathogens providing increased sensitivity. In contrast with culture, which requires 48 hours to 6 weeks for growth of fungi and *Mycobacterium tuberculosis* (MTB) PCR can provide results in a few hours. It is important to note, however, that amplification of DNA does not depend on the viability of the organism; although this could be an advantage in some situations, positive PCR results should be interpreted with caution because a nonviable pathogen may not be contributing to disease pathogenesis. Similarly, PCR assays may not be suitable for follow-up of treated infections or as tests of cure. These assays are also often unable to differentiate between asymptomatic carriage and infection. For example, a PCR assay for *Haemophilus influenzae* on a throat swab would be of limited value because *H influenzae* is often a commensal in the upper respiratory tract.[8]

Previously, molecular testing required multiple steps using complex chemistry and was most suited to be performed in a specialized molecular laboratory by trained personnel. The complex nature of previous versions of molecular assays had an additional disadvantage of possible extraneous contamination leading to false-positive results. Several advances in technology, including automation and nanotechnology, have simplified the inherent complexities of earlier methodologies. In fact, many recently developed assays require very little hands-on time and incorporate extraction amplification and detection in a single module. With rt-PCR, because the amplification and detection are performed within the same closed reaction vessel, there is less manipulation of reagents and, consequently, reduced opportunities for release of amplified DNA into the environment. Because all of the products of PCR are con-

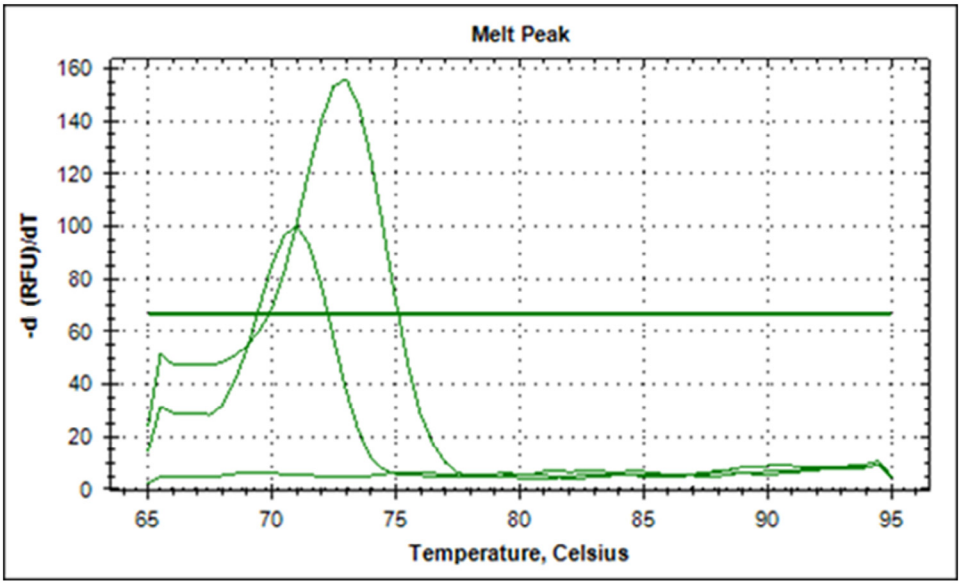

Fig 2. Melt curve use in real-time polymerase chain reaction. The fluorescence is plotted against the temperature, and the peak indicates the melting temperature or the temperature at which the double-stranded DNA dissociates, resulting in reduction in fluorescence. -d(RFU)/dt plots the change in relative fluorescence units over time.

tained, this system is less prone to contamination, decreasing the likelihood of false-positive results on subsequent runs. Moreover, amplification and detection are in real time, which significantly reduces the time required for assay completion. The simplicity of these compact, Food and Drug Administration (FDA)–approved, cartridge-based instruments has accelerated the implementation of molecular assays in the clinical laboratory, especially with features such as random access, which make instruments more user friendly. Some of these instruments are capable of high throughput testing, which is especially beneficial during high-volume periods such as influenza season. Most of these platforms are designated as closed systems, meaning that they have been approved by regulatory agencies to run a specific set of assays on a specified specimen type.[9] These assays are often designated as moderately complex. They are typically sample to result assays, with minimal sample preparation needed before testing. Instruments with software included provide a positive or negative report requiring limited interpretation from laboratory personnel. With the advent of these new types of assays, testing that may have previously been referred to a larger laboratory can be reclaimed by smaller laboratories with minimal technical expertise.

Isothermal amplification techniques

Although PCR is a highly sensitive and efficient method to amplify a target region of the DNA or RNA, the repeated thermal cycling requires both time and energy for amplification. Isothermal amplification methods are preferred as point-of-care tests, especially in resource limited areas, because they are more energy efficient. Two common isothermal amplification methods in common use are nucleic acid sequence–based amplification (NASBA) and transcription-mediated amplification (TMA). In both of these methods, RNA amplification occurs based on a retroviral replication model.[10-12] The RNA target is reverse transcribed into cDNA using a reverse transcriptase enzyme and a primer containing the T7 promoter region. Then, the RNA strand in the RNA-cDNA hybrid is degraded either by endogenous RNAase H activity (as in TMA) or by the use of added RNAase H enzyme (as in NASBA). The cDNA strand then gets amplified by DNA polymerase activity of the reverse transcriptase, and the use of a second primer produces a double-stranded DNA containing the T7 promoter sequence. This leads to synthesis of 100-1,000 copies of the RNA target sequence, which then continue the cycle of amplification. The subsequent detection of the amplicons is achieved by hybridization assays using chemiluminescent or fluorescent probes.[13] Both TMA and NASBA are primer-driven synthesis of target nucleic acid; therefore, primer design is important during assay development. However, because single-stranded RNA is the product, unlike conventional PCR, temperature-driven denaturation and primer annealing is not required, making the reaction isothermal. Theoretically, in approximately 30 minutes, 10^9 copies are generated, therefore enabling sensitive detection of RNA targets in shorter time. The use of a multienzyme complex system and the inherent difficulty of this method to amplify double-stranded DNA that require denaturation are deterrents to its widespread use. Additionally, this is end point amplification because chemiluminescent probes are used to detect the amplified product after hybridization, and subsequent protection of the hybridized product adds a level of complexity to the assay. Isothermal reactions, including these and others, such as strand displacement amplification and loop-mediated isothermal amplification (LAMP), are also used in commercial assays[14,15] and are particularly favored in resource-limited areas where thermocycling equipment may not be easily available.[15] The most extensively used technology in resource-limited settings is the LAMP. It has been widely evaluated for the diagnosis of malaria and tuberculosis (TB).[16,17] The sensitivity and specificity of malaria detection has been reported to be as high as 95%-99% using LAMP technology. In recent studies, the sensitivity and specificity of LAMP technology was found to be comparable with the GeneXpert TB/RIF assay (Cepheid, Sunnyvale, CA).[16] With the price of the assay ranging from $1.00-$5.30, depending on whether it is developed locally or purchased commercially, LAMP assays are an attractive point-of-care diagnostic in the field. The major hindrance to use of isothermal amplification techniques is the complex primer design and the need for a sensitive detection strategy.[17]

Disadvantages of nucleic acid amplification tests (NAATs) include the cost of initial instrument setup for some commercial assay

systems compared with conventional culture methods. Another potential concern is the interpretation of these assays; although the actual result is easy to record, the clinical interpretation of many of these assays can be challenging.

SIGNAL AMPLIFICATION ASSAYS

Signal amplification technology was designed to avoid the potential for contamination of reactions by the target nucleic acid. Instead of amplification of the DNA or RNA by use of enzymes, these assays amplify the signal generated by the probe hybridized to the target DNA. Because there is no amplification of target involved, the likelihood of extraneous contamination is reduced, and for the same reason, the signal is proportional to the amount of target, making it useful for quantitative assays.[18] Although target amplification assays are inherently more sensitive, signal amplification assays are often preferred, especially when measuring viral RNA in blood because reverse transcription and conversion to cDNA is not required. The branched DNA assay, hybrid capture assay, and Cleavase Invader assays are examples of signal amplification–based assays that have been used in quantitative assays for HIV and for detection of HPV, *N gonorrhea* and *C trachomatis*.[18-20]

NONAMPLIFICATION ASSAYS

Use of nucleic acid probes, short fragments of DNA or RNA or peptide nucleic acid, that bind to the target of interest can be used for detection of pathogens without the need for amplification. These probes are usually tagged with chemiluminescent, fluorescent, or enzyme-based reporter molecules to facilitate detection. Essentially, these are hybridization reactions where the complementary strands of the probe hybridize to the target nucleic acid in the specimen and are subsequently detected by the attached reporter molecule. These methods lack the sensitivity of amplification techniques, but when a large amount of target is present, as in a positive blood culture bottle, this technique can facilitate identification of pathogens in a short period of time. The most common platforms used are the microarray-based, Verigene Blood Culture Test (Nanosphere, Northbrook, IL), that not only provides identification of bacteria, but also detects resistance mechanisms directly from a positive blood culture bottle,[21] and the in situ hybridization-based peptide nucleic acid FISH assay (AdvanDx, Woburn, MA).[22]

Microarrays

As whole genomes of microorganisms become available, the information can be used to develop new technology useful for clinical applications. Microarray is an example of such efforts and involves immobilization of a multitude of DNA fragments on a glass surface or on beads. This array is then used in conjunction with hybridization and fluorescent labeling to multiplex detection of pathogens or analysis of genomes. Arrays can be in situ, solid phase, or liquid bead based. They can be PCR-based or oligonucleotide-based arrays. The potential for multiplexing of this technology is immense and has been used for simultaneous identification and/or genotyping of microbes.[23-25]

Any discussion regarding advances in clinical microbiology would be incomplete without mention of the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). Few technologic advances have made as much impact on the identification of microbes. For almost a century, pathogen identification after a positive culture was based on morphology and metabolic profiling by the use of biochemical reactions. The MALDI-TOF uses laser pulses to ionize analyte molecules, which then desorb into a gaseous phase and are allowed to pass through an electric field where they accelerate and separate based on their mass and charge (m/z). A pattern is generated, which is then matched against a database for identification.[26] MALDI-TOF has reduced turnaround time for identification of bacteria, yeasts, and mycobacteria from >48 hours to <30 minutes. It can also be used to identify pathogens directly from blood cultures, and there are some preliminary studies showing that antimicrobial susceptibility can be predicted using this technique.[27] The advent of DNA-based identification methods, especially sequencing of the ribosomal RNA gene, made rapid and precise identification of pathogens possible. Both traditional Sanger sequencing and next-generation sequencing (NGS) technologies have been used to identify pathogens directly from both fresh and formalin-fixed specimens. This technology is also used in phylogenetic analyses of outbreak strains to reconstruct transmission and implement infection control measures. However, because it is technologically complex and expensive, its routine use in the clinical laboratory is currently impractical.[28]

With these concepts in mind, the remainder of this article will be organized according to specific diseases with the different assays used for detection or diagnosis.

Mycobacterium tuberculosis

Acid-fast smears and cultures continue to be the mainstay for diagnosing TB in the laboratory.[29] By comparison with traditional culture that takes weeks, molecular assays can detect MTB directly from sputum and other specimen types in <2 hours, which provides a strong incentive for their use in the diagnosis of TB. Although most international public health agencies stress the use of NAATs as a supplement to, rather than replacement of culture, molecular testing is being advocated as a means of early diagnosis because it can facilitate (1) timely implementation of infection control strategies such as airborne isolation, (2) early institution of appropriate therapy that can dramatically influence patient outcomes, (3) early notification of public health authorities that lead to interventions in the home, workplace, school, and other social or professional settings, and (4) discontinuation of airborne isolation precautions and reduction in the number of contact investigations for patients with negative NAATs per the Centers for Disease Control and Prevention (CDC) guidelines.[30]

There are a few commercially available assays that can be used for direct detection of MTB from respiratory specimens, including sputum[31] and bronchoalveolar lavage.[32] These include the following: Xpert TB/RIF assay (Cepheid) and Hologic Amplified Mycobacterium Tuberculosis Direct test (Hologic GenProbe Inc., San Diego, CA). The Xpert TB/RIF is a hemi-nested, rt-PCR that uses molecular beacon chemistry to detect MTB and rifampin resistance through amplification of the *rpoB* gene from clinical specimens.[30] The Hologic Amplified Mycobacterium Tuberculosis Direct Test uses the isothermal transcription-mediated amplification technique.[32] This assay does not detect rifampin resistance.[32] Acceptable specimens for this assay include sputum, bronchial lavages/aspirates, or tracheal aspirates. The limit of detection is 1 colony forming unit using DNA extracted from pure cultures, and the turnaround time of this assay is 2.5-3.5 hours.

Results from these assays can be obtained in a few hours or a day, whereas with traditional culture and susceptibility methods, these results could take 8-10 weeks or longer. This allows for improved patient outcomes and appropriate institution of therapy leading to substantial cost savings based on institutional isolation procedures.[33] In many resource-limited areas, rapid diagnosis of TB is achieved by microscopy, which is rather inexpensive but lacks sensitivity. Compared with culture, microscopy detects only 45%-80% of pulmonary TB cases.[22] Microscopy also has a low positive predictive value for TB; 50%-80% in areas where non-TB mycobacteria

are isolated.[34] In a recent meta-analysis, the Xpert TB/RIF assay had a pooled sensitivity and specificity of 89% and 99%, respectively, as an initial test replacing smear microscopy.[35] In multiple studies, the Xpert TB/RIF has been shown to outperform smear microscopy by establishing a diagnosis of TB in smear-negative cases (where sensitivity is 67%) and predicting rifampicin resistance with high accuracy.[36-38] The assay's limit of detection for MTB in sputum is 600 colony forming units/mL.[31] The assay is performed on Cepheid's GeneXpert within a cartridge where the reagents and specimen are contained within the system so no extraneous contamination or biosafety concerns remain. It requires minimal hands-on time of 15-20 minutes. This assay is especially useful in high-prevalence, low-resource areas where treatment can be initiated early after rapid diagnosis, often at the site of collection. In fact, this technology has now been endorsed by the World Health Organization as a front-line test for TB in populations where there is a high incidence of suspected multidrug-resistant TB or HIV-associated TB.[39]

Performing mycobacterial cultures is labor intensive and may not be feasible for small laboratories with minimal experience in these procedures because of a patient population with a low incidence of TB. The ease and simplicity of these assays have allowed remote laboratories with less specialized technologists to perform testing for MTB. NAAT technology has significantly improved the timeliness and accuracy of test results that guide infection control decisions for management of patients with TB infection. The Xpert TB/RIF assay (Cepheid) has been shown to significantly reduce airborne infection isolation time for individuals hospitalized without active TB.[40] Although molecular testing has improved the diagnosis of MTB, cultures are still important for detecting non- TB mycobacteria and for performing phenotypic susceptibility testing of MTB.

SEXUALLY TRANSMITTED INFECTIONS: *NEISSERIA GONORRHOEAE* AND *CHLAMYDIA TRACHOMATIS*

Molecular assays are now considered the test of choice by the CDC for diagnosis of sexually transmitted infections (STIs) in adults.[41] Because chlamydial infections can be asymptomatic, and patients often do not return for follow-up of their initial visit, a rapid diagnostic test and initiation of treatment is essential for control of STIs. Additionally, NAATs were found to be 20%-50% more sensitive for diagnosis of chlamydial infections than traditional culture methods.[41] There are several FDA-approved or FDA-cleared tests available for screening specimens for *C trachomatis*/*N gonorrhoeae* (CT/NG). All currently available assays simultaneously amplify both CT/NG using rt-PCR, TMA, or strand displacement amplification assay. Depending on the assay, either the ribosomal RNA gene or the cryptic plasmid of *C trachomatis* is targeted (Table 1). These targets are specific for *C trachomatis* and do not cross-react with any other human bacteria. For *N gonorrhoeae*, segments of the ribosomal RNA or pilin or *Opa* proteins are targeted. These are mostly specific; however, in some of the assays the primers for *N gonorrhoeae* may cross react with human commensal species of *Neisseria*. This becomes especially important when using NAATs for specimens from pharyngeal or rectal swabs.[41]

Because the reference standard for diagnosis is a less sensitive (culture) method, it has been particularly difficult to demonstrate performance characteristics of molecular assays for STIs (Table 1). Therefore, there is a lack of consensus regarding the optimal method for validation of NAATs because the sensitivity of older assays was likely overestimated because of the poor performance of standard tests at that time, and sensitivity and specificity can be biased in areas with low disease prevalence.[45,46] Until better validation methods become available, public health authorities recommend NAATs for diagnosis of CT/NG in adult men and women.[41] A significant concern of molecular assays for diagnosing STIs is that there is no microbial

Table 1
Currently used Food and Drug Administration–cleared commercial assays for diagnosis of sexually transmitted infections

Assay name	Assay format	Stability of specimen at 2°C-30°C	Nucleic acid target	Comments	References
Abbott RealTime CT/NG (Abbott Laboratories, Abbott Park, IL)	Molecular beacon probe	14 d	Specific sequence within the *Chlamydia trachomatis* cryptic plasmid *opa* gene of *Neisseria gonorrhoeae*	Does not detect plasmid-free *C trachomatis* Commensal *Neisseria* spp does not cause false-positive reaction	Chernesky et al[42]
Xpert CT/NG assay (Cepheid, Sunnyvale, CA)	Multiplexed TaqMan assay	Variable depending on specimen type (8 h-45 d)	Chromosomal DNA sequence specific for *C trachomatis* *N gonorrhoeae*	Detects plasmid-free *C trachomatis* Commensal *Neisseria* spp does not cause false-positive reaction	Gaydos et al[7]
Cobas CT/NG test (Roche Diagnostics, Indianapolis, IN)	Dual probe TaqMan assay	Variable depending on specimen type (up to 1 y)	Two distinct genes one within the chromosome and other in the cryptic plasmid A highly conserved direct repeat region of *N gonorrhoeae* called DR-9	Detects plasmid-free *C trachomatis* Commensal *Neisseria* spp does not cause false-positive reaction*	Kumamoto et al[43] and Van Der Pol et al[44]
Aptima COMBO 2 assay Aptima CT assay Aptima GC assay (Hologic GenProbe Inc., San Diego, CA)	Multiplexed TaqMan assay	24 h, 30-60 d if using transport cup	23S rRNA and 16S rRNA gene of *C trachomatis* 16S rRNA gene of *N gonorrhoeae*	Detects plasmid-free and new variant *C trachomatis* Commensal *Neisseria* spp does not cause false-positive reaction	Chernesky et al[42] and Kumamoto et al[43]
BD Diagnostics ProbeTec ET CT/GC Amplified DNA assay (BD Diagnostics, Sparks, MD)	Transcription-mediated amplification, and dual kinetic assay	30 h If urine transport cup used up to 30 d	Specific sequence within the *C trachomatis* cryptic plasmid Pilin gene of *N gonorrhoeae*	Does not detect plasmid-free *C trachomatis* *Neisseria cinerea* and *Neisseria lactamica* might cause false-positive test results	Chernesky et al[42] and Kumamoto et al[43]

*The specificity of the assay is important if rectal or throat swabs are used for detection of *C trachomatis* or *N gonorrhoeae*. The presence of commensal species of *Neisseria* in these sites can lead to false-positive result depending on the assay implemented.

isolate available to test for antimicrobial susceptibility. With the emergence of multidrug-resistant *N gonorrhoeae* and extensively drug-resistant *N gonorrhoeae*, there is rising concern that this may become an untreatable superbug.[47] Some authorities maintain that strengthening culture-based surveillance of local resistance patterns is an increasingly important tool for the detection of resistant *N gonorrhoeae* strains and monitoring their spread. For this to be accomplished, microbiologic culture is required to perform susceptibility testing. Therefore, the CDC recommends culture and susceptibility on all patients with suspected treatment failures.[41] Another area of concern when using NAATs for diagnosis is suspected sexual abuse in minors. In such cases, it is advisable to check with local regulations or authorities if NAATs are acceptable.

DIAGNOSIS AND THERAPEUTIC MONITORING OF CHRONIC VIRAL INFECTIONS (HIV AND HCV)

Molecular assays are often the only methods available for detection of some viral pathogens. The use of commercially available quantitative molecular assays has dramatically changed the management of persons with HIV and HCV. Quantitation of the number of viral copies achieved by NAATs has been extremely beneficial not only in initial disease diagnosis, but also to assess prognosis, monitor therapy, and acquire information regarding resistance markers. Molecular assays have also significantly affected the care of immunosuppressed individuals with HCV infection who have benefited from personalized treatment options based on results of quantitative PCR and genotyping of the virus.[48] A list of available assays for HIV and HCV is detailed in Table 2.

Role of molecular assays for diagnosis and management of HCV infections

With a global prevalence of 1.6% and 115 million people infected, HCV accounts for a major proportion of chronic viral hepatitis cases worldwide.[49] Public health efforts to control HCV primarily involve case finding by diagnosis of acute and chronic infections.[50] Because of the availability of new treatment modalities, sustained virologic response is now achievable. Molecular assays play a key role in both the diagnosis and management of HCV. In fact, the virus was originally identified using molecular techniques because this flavivirus is difficult to culture.[51] The current diagnostic algorithm for HCV includes the use of a serologic assay for detection of antibodies; if positive, this is followed by an NAAT to confirm the stage of infection. A positive NAAT indicates chronic, active infection with HCV. A negative NAAT, in the presence of anti-HCV antibodies, indicates spontaneous resolution of infection. However, a repeat NAAT is advisable in 6-12 months because low-level viremia is not uncommon in chronic HCV. Additionally, viral RNA can be detected in serum as early as 1-3 weeks after exposure and is useful in evaluating occupational exposure to HCV. Routine use of quantitative NAAT is also recommended before initiation of therapy for chronic HCV and for monitoring the virologic response to therapy. Because qualitative NAATs should detect 50 HCV RNA IU/mL with equal sensitivity for all genotypes and because most quantitative PCR assays for HCV have a lower limit of detection of 30 RNA copies/mL, most clinical laboratories have switched to using quantitative assays.[52]

Determination of HCV genotype becomes important when determining the type of therapy to be instituted. However, newer

Table 2
Comparison of commercially available molecular assays for HCV and HIV

Assay name	Assay format	Primary use	Nature of assay	Sensitivity/specificity
Cobas Ampliprep/Cobas Taqman (Roche)	TaqMan dual-probe real-time reverse transcription PCR	Diagnosis of HCV infection and blood donor screening	Qualitative and Quantitative for presence of HCV RNA and for viral RNA quantitation	15 IU/mL/100%, sensitive and specific for all 6 genotypes
Cobas MPX Test (Roche)	TaqMan multidye multiplex real-time PCR assay	HIV, HCV, and HBV, primarily for donor screening	Qualitative for diagnosis and screening of 3 viruses	2.3, 6.8, and 46.2 IU/mL for HBV, HCV, and HIV-1, respectively
Abbott Real-time HCV (Abbott Laboratories, Abbott Park, IL)	Real-time PCR assay for quantitative detection of HCV	Quantitative determination of HCV viral load for diagnosis and monitoring of therapy in serum and plasma	A real-time PCR assay that allows for quantitative detection of viral RNA of all genotypes 1-6 of HCV	12 IU/mL for all genotypes for HCV
Abbott Real-time HCV Genotype II (Abbott Laboratories)	Real-time PCR assay with primers to detect genotypes of HCV	Detect genotype of HCV virus for therapeutic planning in serum and plasma	Real-time PCR using 3 primer sets and multiple dyes and an armored RNA internal control to detect and differentiate genotypes 1a and 1b, 2-5 of HCV	500 IU/mL for all detected genotypes, >99% sensitivity
VERSANT HIV-1 RNA 3.0 Assay (Siemens Healthcare Inc., West Sacramento, CA)	Branched DNA assay	Viral quantification from serum and plasma	Real-time PCR assay for viral RNA quantification from serum and plasma	75 copies/mL
AmpliPrep/COBAS TaqMan HIV-1 (Roche)	Real-time reverse transcription PCR amplification	Viral quantification from serum and plasma	Real-time PCR assay for viral RNA quantification from serum and plasma	50 copies/mL
NucliSens HIV-1 RNA QT assay (BioMérieux, Salt Lake City, UT)	Isothermal NASBA technology	Viral quantification from serum and plasma	Real-time PCR assay for viral RNA quantification from serum and plasma	176 copies/mL
Abbott RealTime HIV-1 m2000rt (Abbott Laboratories)	Real-time PCR assay	Viral quantification from serum and plasma	Real-time PCR assay for viral RNA quantification from serum and plasma	50 copies/mL
ViroSeq HIV-1 Genotyping System (Abbott Laboratories)	Sequence determination of protease and reverse transcriptase gene	Determination of viral genotype from human serum and plasma	Multiple reactions and 4-dye termination system to determine sequence from each patient	NA
TruGene HIV-1 Genotyping Kit and OpenGene DNA Sequencing System (Siemens Healthcare Diagnostics)	Sequence determination of protease and reverse transcriptase gene	Determination of viral genotype from human serum and plasma	Multiple reactions and a dye primer system to determine sequence	NA

HBV, hepatitis B virus; *HCV*, hepatitis C virus; *NA*, not applicable; *NASBA*, nucleic acid sequence–based amplification; *PCR*, polymerase chain reaction.

antiviral agents may obviate the need for genotyping of HCV in the future.[50] Finally, similar to many RNA viruses, HCV is prone to replication error and leads to development of viral quasispecies during treatment. Sequencing is currently the method of choice for identification of complex viral genotypes circulating in the serum for tailoring therapy and for identifying viral genotypes that may be resistant because of mutations.[53]

Role of molecular assays for diagnosis and management of HIV infections

The initial diagnosis of HIV infection is achieved primarily by serologic assays. The current fifth-generation HIV assay tests for antibodies to HIV-1, HIV-2, and p24 antigenemia simultaneously. It has significantly reduced the window period to diagnosis of HIV infection to as low as 15 days after exposure. Molecular assays for diagnosis of HIV are primarily useful in very early infection, especially after occupational exposure, and in testing infants born to HIV-positive mothers where maternal antibodies preclude the use of serologic assays. NAATs are primarily used for monitoring progression of disease and response to therapy during HIV infection. As with HCV, genotyping is important for recognizing drug-resistant mutants that arise during therapy.[54]

HOSPITAL-ACQUIRED INFECTIONS

Methicillin resistant Staphylococcus aureus

Perhaps the most significant impact of a molecular test for the prevention of hospital-acquired infection has been the success of the nasal methicillin-resistant *S aureus* (MRSA) screen. Active surveillance screening for MRSA with a molecular assay is a common practice in most hospital laboratories and especially in states with a mandatory requirement for MRSA screening and reporting. Because MRSA-colonized patients are reservoirs for self-infection and disease transmission, molecular assays contribute to early identification and isolation of carriers, elimination of carriage, and reduced potential for transmission.

The success of active surveillance testing was demonstrated by researchers in Illinois who used a rt-PCR test for nasal MRSA surveillance on >65,000 patients during 3 study periods: 12-month baseline, 12-month intensive care unit–only testing, and 18-month universal admission testing. Clinical cultures were performed to detect MRSA infection. The primary outcome was change in clinical MRSA disease. They found no significant reduction in MRSA clinical disease during the intensive care unit testing period and a 69.6% disease reduction with universal admission testing. They concluded that active surveillance testing, combined with contact isolation or with isolation plus decolonization, achieved a low MRSA health care–associated infection rate of 0.23 infections per 1,000 patient days.[55]

Although detection of MRSA by rt-PCR involves amplification of the *mec*A gene, using a singleplex rt-PCR, the application of such methods in direct specimens from nonsterile sites, such as a nasal swab, is not as straightforward. This is because of 2 reasons: first, the presence of commensal coagulase-negative staphylococci in the nose that may also possess the *mecA* gene causes primers that target the *mecA* gene to produce false-positive results. Commercial assays, such as the Xpert MRSA (Cepheid) and the GeneOhm MRSA (BD Diagnostics, Sparks, MD), use primers directed to the SCC *mec-OrfX* junction, the region of the *S aureus* chromosome where the *mec*A gene is incorporated. This ensures detection only of MRSA because coagulase-negative staphylococci do not have that target.

The second issue regarding detection of MRSA from nonsterile sites involves primers targeting the SCC *mec-OrfX* junction, which is where the *mecA* gene is incorporated in the *S aureus* chromosome. The disadvantage of this methodology is that false-positive results for MRSA can occur because some *S aureus* isolates do not have a functional *mec*A gene (empty cassette variants) but are detected by these primers. Regardless of these limitations, MRSA nasal screening by PCR is widely used and has significantly reduced turnaround time from 48-72 hours to just a few hours. MRSA screening by PCR in the neonatal intensive care unit has low positive predictive value and variable reproducibility when compared with culture, especially in culture-negative patients.[56] For this reason institutions opt to use culture screening in the neonatal intensive care unit.[56]

Clostridium difficile *associated diarrhea*

C difficile is now a major cause of morbidity, prolonged hospital stay, and a significant economic burden on the health care systems. Although most NAAT assays are highly sensitive and specific, the diagnosis of *C difficile* disease by PCR is a challenge. Because asymptomatic carriage of *C difficile* is not uncommon in hospitalized patients, careful interpretation of results is required to differentiate infection from colonization. In fact, most laboratories should, and do, have specimen acceptance policies that include rejection of formed stools or repeat specimens collected within 7 days. For similar reasons, it is recommended that the test be interpreted with caution in infants and all other causes of diarrhea be ruled out in children <3 years of age.[57]

There are several FDA-approved commercial assays available that are rt-PCRs targeting the gene that encodes for toxin B (*tcdB*) either by using TaqMan probes (ProGastro Cd test; Prodesse, Waukesha, WI) or molecular beacons (GeneOhm Cdiff assay; BD Diagnostics, San Diego, CA). The Xpert *C. difficile* test (Cepheid) is different in that it is a multiplexed assay that amplifies 2 different genes specific to toxigenic strains of *C difficile*: one that encodes for toxin B (*tcdB*), and one that regulates toxin production (*tcdC*). Analytical sensitivity of the Xpert *C. difficile* assay (Cepheid) ranges from 23-460 colony forming units/swab, depending on the strain of *C difficile* (http://www.accessdata.fda.gov/cdrh_docs/reviews/K110203.pdf). Regardless of the assay format used, a positive PCR assay only indicates that the patient is positive for toxigenic strains of *C difficile*. In asymptomatic patients, PCR assays for *C difficile* should not to be used as even tests of cure.[57] The problem is compounded by the fact that there are multiplexed gastrointestinal (GI) panels, such as the BioFire FilmArray (BioMérieux, Salt Lake City, UT), that now include *C difficile* as one of the targets causing a reporting dilemma for asymptomatic carriage of *C difficile* regardless of clinical suspicion. For similar reasons, testing for *C difficile* is not recommended in children <1 year of age. Clinical illness is rarely reported before 12-24 months of age, and it is possible that neonates/infants may lack the cellular machinery to bind and process the toxins of *C difficile*.[58] Infection preventionists should be aware of the possibility of false-positive *C difficile* results and work with the laboratory and clinicians to correctly interpret these results.

Vancomycin-resistant enterococci and carbapenem-resistant Enterobacteriaceae

The most concerning mechanisms of vancomycin resistance in enterococci are due to the presence of *van*A and *van*B genes located on mobile elements, as opposed to chromosomally mediated vancomycin resistance genes (eg, *van*C).[59] Risk factors for acquiring nosocomial vancomycin-resistant enterococci (VRE) include proximity to another patient with VRE, prior antibiotic therapy, and extended hospital stay.[59] Detecting and isolating patients colonized with VRE can reduce transmission and has been shown to decrease VRE bacteremia rates.[60] Infection prevention strategies often

include screening patients for VRE carriage and isolation of positive patients to limit the spread of resistant organisms.

Available technologies for screening for VRE are as follows. Xpert vanA (Cepheid) is an rt-PCR using TaqMan probes for detection of the *van*A gene. The acceptable specimen type is a rectal swab. The limit of detection is 37 colony forming units on a (simulated) rectal swab (Xpert vanA Assay). Turnaround time is >1 hour. (http://www.accessdata.fda.gov/cdrh_docs/reviews/K092953.pdf).

GeneOhm VanR (BD Diagnostics) is a PCR assay using molecular beacons for the detection of *van*A and *van*B. The assay is run on the SmartCycler (Cepheid) which amplifies, detects, and interprets the signals. Acceptable specimen types are perianal and rectal swabs. The analytical sensitivity is approximately 1,600 colony forming units/swab.[61] Turnaround time is >2 hours. Determining VRE colonization status with rt-tPCR methods can prompt early infection control measures, such as isolation, thereby preventing nosocomial transmission. Sensitivity and specificity for the GeneOhm VanR was reported to be 93.2% and 81.9% respectively, when compared with culture.[60]

Screening for carbapenem-resistant *Enterobacteriaceae* (CRE) carriage among inpatients is an issue of heightened concern. Recent outbreaks of hospital infections associated with the spread of CRE on contaminated devices have garnered attention from the media and prompted the FDA to issue guidance and strategies to limit the occurrence of such events (http://www.cdc.gov/hai/outbreaks/cdcstatement-LA-CRE.html). CarbaR assay (Cepheid) is an rt-PCR that is capable of targeting known carbapenem resistance plasmids and has recently received FDA approval for analyzing both bacterial colonies and rectal swabs (http://www.accessdata.fda.gov/cdrh_docs/reviews/K152614.pdf). One of the caveats of current molecular assays for CRE detection is that only known sequences are detected. Therefore, with respect to CRE, where a large number of plasmids have been described, it is prudent to know the institutional prevalence of such plasmids and to conduct periodic reviews of local antibiograms.

SYNDROME-BASED DIAGNOSTIC APPROACHES

Traditionally, the differential diagnosis of meningitis, diarrhea, sepsis, and respiratory infections, including pneumonia, has depended on clinical suspicion of a probable etiologic agent followed by a series of laboratory tests to detect the pathogen. In a major paradigm shift, this stepwise approach is beginning to be replaced by complex multiplexed panels that are capable of detecting numerous targets in a single specimen. Therefore, GI, meningitis, and respiratory pathogen panels have been developed and deployed in the health care setting. These rapid, sensitive, and highly comprehensive panels target a selection of relevant pathogens in a particular specimen type. For example, the FilmArray meningitis-encephalitis panel (BioMérieux) evaluates cerebrospinal fluid specimens for 14 different pathogens (including bacteria such as *Neisseria meningitidis*, viruses such as Herpes Simplex Virus (HSV)-1 and -2, and fungi such as *Cryptococcus neoformans*/*Cryptococcus gattii*) in little over an hour. Although most of these assays are expensive, they have the advantage of simplifying specimen collection and reducing delays in diagnosis associated with recollection and retesting of specimens for multiple different pathogens. Furthermore, the use of panel-based testing often leads to a specific antimicrobial therapy rather than the broad-spectrum therapeutic approach.[62] Additionally, the economic impact of a shorter hospital stay and the institution of appropriate infection prevention measures may render such approaches even more appealing. Health care facilities now have the option to implement a strategy of limited or expanded testing based on the patient population, turnaround time, and infection prevention protocols.

Sepsis panel

Sepsis is one of the leading causes of death despite advances in critical care. Early diagnosis and therapy are critical but need to be balanced with antimicrobial stewardship efforts.[63] The nonspecific signs and symptoms make prompt laboratory diagnosis important. Although blood cultures are the mainstay of diagnosis, rapid techniques are often sought and depended on. With the advent of molecular panels that use positive blood culture as a specimen, identification of a pathogen is possible in 2 hours. Some of these panels actually detect the presence of drug resistance genes, such as the *mecA* or *vanA* gene, if the identified pathogen is MRSA or *Enterococcus* spp. Two panels that are frequently used are the Vergiene Blood Culture Test (Nanosphere) and the FilmArray Blood Culture Identification Panel (BioMérieux). The Verigene uses microarray technology to identify bacteria and some resistance mechanisms in >3 hours. The organism's DNA is hybridized to the microarray and then is detected with signal amplification. The FilmArray panel uses a nested multiplexed PCR with melt curve analysis to identify bacteria, yeast, and a few resistance mechanisms in approximately 1 hour. In conjunction with antimicrobial stewardship, these assays help direct appropriate treatment.[64] Sensitivity and specificity of detection are still limited by the targets included in the panel.[64] Additionally, in a polymicrobial culture, sensitivity may not be equal for all organisms, affecting overall performance.[64]

Although these assays have shortened the time to identification of a pathogen, they are still dependent on an initial positive blood culture. The SeptiFast system (Roche Diagnostics, Indianapolis, IN) is a probe-based rt-PCR platform that targets ribosomal sequences and detects the 25 most commonly isolated organisms directly from blood. It is not FDA cleared, but it is used clinically in Europe with some success.[65] The recently FDA-cleared T2Candida panel (T2 Biosystems, Lexington, MA) is an automated system that completes extraction, amplification, and detection of *Candida* spp directly from blood. The detection system is unique in that amplified products are detected by amplicon-induced agglomeration of supermagnetic particles and magnetic resonance (T2MR) measurement using the T2Dx Instrument (T2Biosystems). In a recent clinical trial, the system was shown to have an overall sensitivity and specificity of 91% and 99.4%, respectively, in blood culture specimens spiked with known concentrations of *Candida* spp.[66]

Respiratory pathogen panels

The multitude of existing viral pathogens that can cause respiratory disease and the steady emergence of new viruses have greatly challenged the diagnostic laboratory. Multiplex PCR assays for respiratory viruses have made a tremendous impact in the diagnostic laboratory and on infection control practice. The increased sensitivity and faster turnaround time of these assays have significantly increased the laboratory's capacity to detect respiratory viruses. This also ensures early initiation of appropriate therapy, reduction in use of antibiotics, and timely implementation of isolation precautions by infection preventionists. Multiplex PCR assays also complement syndromic surveillance by public health authorities, through identification of respiratory viruses in circulation in the community. There are several commercially available assays with variable technology, complexity, and hands-on time, but they generally have comparable performance characteristics (Table 3). Some assays such as the Verigene RV flex panel (Nanosphere) provide the option of phased release of results; therefore, results for common viruses such

Table 3
Currently available commercial panels for diagnosis of respiratory viral infections

Assay name	Assay format	Specimen	Extraction of nucleic acids required	Sensitivity/specificity	Pathogens detected	Reference
ProFAST+ Assay (Prodesse, Hologic GenProbe Inc.)	Multiplex real-time reverse transcription PCR assay	NP swabs	Yes	94%/99%	Depends on the panel used	Loeffelholz et al[67]
FilmArray (BioMérieux, Salt Lake City, UT)	Real-time PCR with melt curve analysis	NP swabs, respiratory secretions	No	84.5%/100%*	AdV; CoV HKU1, NL63; influenza virus A (H1/2009, H1, H3); influenza virus B; hMPV; PIV1, PIV2, PIV3, PIV4; RSV; RhV/EV, CoV HKU1 and NL63, *Bordetella pertussis*, *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*	Hindiyeh et al[68]
Xpert flu (Cepheid, Sunnyvale, CA)	Multiplex real-time reverse transcription PCR assay	NP swabs/nasal aspirate	No	98%/100%	Influenza A (H1), influenza A (2009 H1), influenza B	Novak-Weekley et al[69]
Simplexa Flu A/B & RSV kit (Focus Diagnostics)	Multiplex real-time reverse transcription PCR assay	NP swabs	Yes	95%/99%	Influenza A-B and RSV	Hindiyeh et al[68]
xTAG/xTAG FAST† (Luminex)	PCR, hybridization to fluorescent bead–based liquid array	NP swabs, respiratory secretions	Yes	97%/100%	AdV; influenza virus A (H1, H3); influenza virus B; hMPV; PIV1, PIV2, PIV3; RSV (A/B); RhV/EV	Hwang et al[70]
eSensor Respiratory Viral Panel (GenMark, Diagnostics, Carlsbad, CA)	PCR followed by hybridization and electrochemical detection	NP swabs, respiratory secretions	Yes	98%/99%	Influenza A (H1, 2009 H1, H3), influenza B, parainfluenza, RSV, human metapneumovirus, rhinovirus and adenovirus	Popowitch et al[71]
Verigene RV Plus (Nanosphere, Northbrook, IL)	Multiplex reverse transcription PCR followed by gold nanoparticle hybridization assay	NP swabs	No	96%/100%	Influenza A (H1, 2009 H1, H3), influenza B, RSV	Alby et al[72]
Verigene RV flex (Nanosphere)	Multiplex reverse transcription PCR followed by gold nanoparticle hybridization assay	NP swabs	No	NA	Influenza virus A (H1/2009, H1, H3); influenza virus B; hMPV; PIV1, PIV2, PIV3, PIV4; RSV; RhV, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella holmesii*	Pending

Adv, Adenovirus; *CoV*, Coronavirus; *hMPV*, human metapneumovirus; *NP*, nasopharyngeal; *PIV*, Parainfluenza virus; *RSV*, Respiratory Syncytial Virus; *RhV/EV*, Rhinovirus/Enterovirus.
*Sensitivity was not equal for all viruses included. Overall sensitivity is indicated.
†A smaller panel consisting of AdV, influenza virus A (H1, H3), influenza virus B, hMPV, RSV, and RhV/EV.

as influenza and RSV are released first and other less common agents can be suppressed until physicians request the results. There are several studies that have shown that the use of rapid respiratory pathogen panel (RPP) testing allows patients with viral or bacterial respiratory illnesses to be quickly diagnosed equating to shorter lengths of hospital stay and earlier initiation of antiviral therapy when appropriate (eg, influenza). These studies also show that the use of RPP significantly affects isolation for infection prevention and antimicrobial stewardship efforts.[73]

Point-of-care respiratory virus testing

The landscape of molecular testing is changing with the development of small, rapid analyzers that are easy to use and require little hands-on time. These features facilitate placement of the instruments outside of the laboratory, such as in clinics or emergency departments. A point-of-care test is especially useful in 2 situations: (1) where the results lead to rapid initiation or cessation of therapy, and (2) where there is a possibility that a patient might be lost to follow-up, such as in the diagnosis of STI in patients with risk factors and associated high-risk behaviors. In the case of respiratory virus testing, point-of-care test results can aid significantly in clinical decision-making. The Cobas Liat (Roche) is a small new point-of-care rt-PCR instrument. It has been clinical laboratory improvement amendments waived for influenza A-B and *Streptococcus* A testing; testing takes 20 minutes and is sample to result (https://www.cobasliat.com). One study found that compared with the Simplexa Flu A/B & RSV Direct (Focus Diagnostics, Cypress, CA), the Cobas Liat (Roche) had a 99.2% sensitivity for influenza A, 100% sensitivity for influenza B, and 100% specificity for both targets.[74]

The Alere i (Alere Inc., Waltham, MA) is a small, rapid, isothermal nucleic acid amplification system that can perform testing for influenza A-B and *Streptococcus* A. Both tests are sample to answer and are CLIA waived. The sensitivities of the Alere i for influenza A-B were 73.2% and 97.4%, respectively, when compared with a combination of the FilmArray Respiratory Panel (BioMérieux) and Prodesse ProFLU + RT-PCR (Hologic GenProbe Inc.).[75] Specificity of the Alere i for influenza A-B was 100% for each target.[75]

GI and meningitis panels

At least 2 GI panels have received FDA clearance, and there are a few others in the pipeline. The use of a multiplex panel for diagnosis is justified by the nonspecificity of symptoms and the relative insensitivity of conventional techniques, such as microscopy for detection of ova and parasites.[76] These assays use rt-PCR (FilmArray GI panel; BioMérieux) or PCR followed by bead-based hybridization (xTAG; Luminex Corp., Austin, TX) and achieve relatively high sensitivity and specificity. However, they have the drawback of picking up low-level carriage of enteropathogens, such as *Giardia*, *Escherichia coli*, *Campylobacter*, and many viruses that do not have any clinical significance especially in developing countries.[77] The cost-effectiveness of the GI panel has also been debated, as has the fact that a molecular assay leaves the laboratory without an option to perform susceptibility testing for both epidemiologic and treatment purposes. Meningitis panels are in the process of being adopted by laboratories and although data are limited, the diagnostic performance seems to be promising and the platform is considered beneficial for rapid diagnosis of meningitis.[78]

Future of infection control in the molecular era

The most promising technology with potential to change the practice of infection control in the foreseeable future is NGS. This technology, when applied to whole-genome sequencing (WGS),

has already produced significant advances in epidemiology, phylogenetics, virulence determinants, and evolution of antimicrobial resistance of microbial pathogens.[79]

There are several technologies currently in use, mostly involving DNA extraction and preparation of libraries followed by sequencing by synthesis that generates short reads (except in a few technologies, such as PacBio RSII [Pacific Biosciences, Menlo Park, CA] or Oxford Nanopore [Oxford Nanopore Technologies, Oxford, UK], that can generate long reads). The short DNA fragment reads are then mapped on to a reference genome using software. The technology and its use have been well described in several recent reviews.[80,81]

As NGS technology becomes more amenable to use in the clinical laboratory and the associated cost decreases with the advancement of the bioinformatics pipeline, there is likely to be a concomitant increase in use of WGS in prediction of antimicrobial resistance and in tracking epidemiologic spread of microbes.

An example of the use of sequence data to generate identification and susceptibility and how it can be achieved in a very limited time is elucidated in a recent publication by Bradley et al.[82] Using the Oxford Nanopore technology, the authors were able to predict susceptibility of *S aureus* and *M tuberculosis* in <3 minutes on a laptop computer. The software used (mykrobepredictor.com) provided a sensitivity and specificity of >99% for *S aureus* and 82% and 98.5%, respectively, for *M tuberculosis*.[82]

There is nothing more satisfying in the field of epidemiology than preventing the spread of a microbe during an outbreak or studying the evolution of a pathogen over time. The science of phylogeny, when applied to tracking an outbreak, has come a long way since Snow established the association between the Broad Street pump and the spread of cholera during the 1854 outbreak in London.[83] The technology of WGS has applied to studying the spread of drug-resistant organisms in a single facility[84] and also to large-scale epidemiologic studies monitoring the evolution and spread of a pathogens over time.[85,86]

Barriers to the widespread use of NGS in infection control include concerns regarding data quality and the efficient use of bioinformatics in clinical laboratories. Beyond assurance of quality control and standardization, there needs to be infrastructure and evidence of successful use of WGS in infection control. Needless to say this technology has great potential and may be of immense utility in the future.

CONCLUSIONS

The world of microbiology has changed since van Leeuwenhoek first observed the realm of microbes. We now understand more about the intricate nature of microbiomes and the complex pathogenesis of infections. We have significantly built on Koch's postulates that first allowed us to understand infectious diseases. We can now identify and precisely characterize emerging pathogens, or trace the source of outbreaks in a matter of days, as exemplified in the German outbreak of Enterohemorrhagic *E coli* O104:H4.[87] For some of the diseases previously discussed, there are numerous assays from which to choose. These choices would depend on the relative performance of the assay, cost, complexity, infrastructure of the laboratory, and patient population served. As we embark on this new path of personalizing medicine and enhanced utilization of the laboratory, the responsibilities of laboratorians, physicians, and infection preventionists increase. With the advent of newer technologies, the onus falls on us to determine the clinical utility and cost-effectiveness of these techniques, and to educate stakeholders on the efficient use and interpretation of the results for better patient care. Furthermore, we need to remain at the forefront of technology and understand the benefits and shortcomings to continue to improve the diagnosis and control of infectious diseases.

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