Chapter 47
Interpretation and Relevance of Advanced Technique Results

Charles W. Stratton and Yi-Wei Tang

Introduction

Advanced techniques in the field of diagnostic microbiology have made amazing progress over the past two decades due largely to a technological revolution in the molecular aspects of microbiology [1, 2]. In particular, rapid molecular methods for nucleic acid amplification and characterization combined with automation and user-friendly software have significantly broadened the diagnostic capabilities of modern clinical microbiology laboratories. Molecular methods such as nucleic acid amplification tests (NAATs) rapidly are being developed and introduced in the clinical laboratory setting. Indeed, every section of the clinical microbiology laboratory, including bacteriology, mycology, mycobacteriology, parasitology, and virology, have benefited from these advanced techniques. Because of the rapid development and adaptation of these molecular techniques, the interpretation and relevance of the results produced by such molecular methods has lagged somewhat behind. The purpose of this chapter is to review and discuss the interpretation and relevance of results produced by these advanced molecular techniques. Moreover, this chapter will address the “myths” of NAATs, as these myths can markedly influence the interpretation and relevance of these results.
Myths of Nucleic Acid Amplification Tests

Myth 1: NAATs Are Extremely Sensitive

There are two aspects on sensitivity: analytical, which is determined by the limit of detection in a given specimen, and diagnostic/clinical, which is determined by the percentage of target in a patient (true positive) population. Most of NAATs possess excellent analytical sensitivity reaching ten copies of target genomes per reaction. This has made the NAATs essential for the detection of microbial pathogens from specimens such as cerebrospinal fluid (CSF) where there may be extremely low microbial loads [3–6]. However, in certain situations, the sensitivities of NAATs are inferior to conventional culture techniques in which a large volume of a specimen easily can be evaluated. Examples include blood cultures and sputum cultures for Mycobacterium tuberculosis [7–10]. Current NAATs are usually performed using very small volumes; until the processing of such specimens is greatly improved, the sensitivity of these NAATs will remain lower than conventional culture techniques.

Currently, most NAATs are designed in a monoplex format, i.e., one primer set for one specific microbial pathogen. Although the use of multiplex PCR amplification techniques is increasing in the clinical diagnostic field, these multiplex methods account for the minority of molecular testing. In conventional microbial culture techniques, a chocolate agar plate or a mixed cell line would allow the recovery of many different pathogens and/or multiple pathogens if they were present in a tested specimen. From this perspective, the diagnostic sensitivity of a monoplex NAAT may not be sufficiently high since it only detects the one pathogen that is being tested for rather than many different pathogens and/or multiple pathogens.

Myth 2: Real-Time PCR Is Extremely Sensitive as well as Objective

The term “real-time PCR” indicates a PCR procedure in which nucleic acid amplification and amplification product detection happen simultaneously. Real-time PCR methods often incorporate a FRET procedure, which allows the amplicon detection and identification to occur in real time in relation to the nucleic acid amplification. This avoids carryover amplicon contamination since the reaction is occurring in closed system. Moreover, real-time PCR allows accurate quantification of the nucleic acid. However, NAATs based on real-time PCR methods are not necessarily more sensitive than other NAATs [11]. In contrast, due to the nonspecific spontaneous FRET procedure, real-time PCR assays may be less sensitive due to the raised cutoff values. The arithmetic, spontaneous increase in fluorescent background emissions interferes with the exponential, specific energy emissions during the simultaneous detection procedure, producing potentially false-positive results. To overcome this nonspecific issue, the system has to either increase the cycle threshold (Ct) cutoff value or decrease the cycle numbers during the amplification, which results in decreased sensitivity.
Real-time PCR does provide a nice qualification procedure with a wide range covered; however, it is not ideal for qualitative assay measurements as there are no objective criteria for determining the cutoff point. This can be problematic when the microbial load in the tested specimen is extremely low. For example, HSV and Chlamydia pneumoniae detection in CSF specimens by real-time PCR is not as objective and sensitive in comparison to end detection PCR procedures [4, 12]. In the later procedures, a separate detection and identification is used after amplification in which additional signal amplification (e.g., antigen–antibody linking) can be incorporated to further enhance the test sensitivity [12, 13].

**Myth 3: NAATs Are Useful Tests for Assessing Therapeutic Efficacy**

NAATs are often considered to be useful tests for assessing therapeutic efficacy. However, this is not true. NAATs detect microbial organism-specific nucleic acids; therefore, a positive NAAT result can occur with both alive and dead microorganisms, which is particularly true for those pathogens that have protective cell wall. The best example of this is the detection of M. tuberculosis DNA in sputum where the dead microbial pathogen DNA can remain un-degraded due to the fatty acid-rich cell walls [14, 15]. Unlike the results of a function-based testing method, such as mycobacterial cultures, in the clinical setting, a positive PCR result after anti-tuberculosis therapy does not necessarily mean treatment failure. Therefore, DNA-targeted NAATs are usually not considered to be tests of cure. This is also true for sexually transmitted pathogens such as Chlamydia trachomatis and Neisseria gonorrhoea [16].

Although DNA-targeted NAATs are not useful for therapeutic monitoring, mRNA-targeted NAATs may be used for assessment of antimicrobial treatment therapy. For example, the application of mRNA-targeted NAATs has been demonstrated for monitoring of tuberculosis therapy. Anti-TB therapy regimen selection is largely empiric. Treatment may not be modified until weeks or months later as results of antimicrobial susceptibility tests become available. Because the half-life of bacterial mRNA is extremely short compared to rRNA or genomic DNA, molecular assays that target mycobacterial mRNA better reflect mycobacterial viability. The ability of mRNA-based assays to distinguish viable from nonviable organisms have demonstrated that such assays are useful in monitoring the efficacy of anti-TB therapy [14, 15].

**Myth 4: NAAT Results Allow Direct and Objective Interpretation**

Conventional microbiology methods are generally direct and objective in terms of interpretation of the results. A blood culture that is positive for Staphylococcus aureus strongly suggests that the patient has a staphylococcal bacteremia. A positive hepatitis A IgM antibody detected in serum implies a recent infection caused by this virus. However, positive NAAT results can be somewhat indirect and subjective.
since the exact meaning of a positive NAAT result is only that the microbe-specific nucleic acid exists in the tested specimen. This can be caused by contamination, colonization and/or infection. Even when an infection is established, it can be either an acute or chronic infection.

Higher sensitivity may not always be clinically relevant. One good example is seen with the varicella-zoster virus (VZV). Previously, a positive VZV result, by either DFA or culture, would be interpreted as VZV being the causative pathogen and would be considered clinically relevant. PCR-based NAATs have increased VZV detection several hundred-fold such that a positive NAAT result for VZV from a skin or mucosal lesion specimen may be unclear as to the exact causative relationship of this virus to infection [17].

Clinical Relevance and Interpretation of Molecular Tests

The Use of Molecular Assays for Diagnosing Bloodstream Infections

Bloodstream infections have long been recognized as among the most severe manifestations of bacterial disease and were first described in 1940 by Keefer in his sentinel paper “The Clinical Significance of Bacteremia” [18]. The importance of the rapid diagnosis of bloodstream infections is not argued and serves to illustrate many of the issues involved in the interpretation and relevance of advanced techniques in diagnostic microbiology.

By 1940 when Keefer pointed out the clinical relevance of bacteremia, blood cultures were well established for the evaluation of febrile patients [19]. Since then, the techniques and pitfalls for blood cultures as well as the clinical implications of positive blood cultures have been well documented [20–23]. Not surprisingly, molecular and other non-culture-based methods for the rapid diagnosis of bloodstream infections have been widely evaluated [8, 10]. These studies along with earlier studies of blood cultures have illustrated some important points regarding the limitations of molecular assays for diagnosing bloodstream infections, which are described below:

Limitations of Molecular Assays for Diagnosing Bloodstream Infections

Interpretation of DNAemia

The detection of circulating microbial DNA (i.e., DNAemia) is, per se, a new diagnostic parameter that may or may not represent the presence of viable microorganisms in blood [10, 24]. For example, interpretation of DNAemia with coagulase-negative
Interpretation and Relevance of Advanced Technique Results

staphylococci is problematic due to a false-positive rate that ranges from 60 to 80\% [22, 25]. In contrast, interpretation of DNAemia with *Ehrlichia* species is not a problem due to a true-positive rate of 100\% [26]. Interpretation of DNAemia has also been a problem in some studies where DNAemia is detected by PCR but not by blood cultures [24]. A number of these “false-positive” PCR results have been considered clinically significant, based on either retrospective chart review or subsequent isolation of the pathogen from other relevant clinical specimens [10, 27–32]. Clearly, the continued clinical investigation of microbial DNAemia during sepsis and other critical illnesses is needed and will provide a better understanding of the biology of the microbial circulating DNA that underpins such molecular diagnostic techniques [10, 24, 33].

### Molecular Detection of Resistance Determinants

Another important issue for molecular diagnostic techniques is the need for molecular detection of resistance determinants [10, 24]. Antimicrobial susceptibility testing is recognized as important for confirming susceptibility to chosen empirical antimicrobial agents as well as for detecting resistance in individual microbial isolates [34]. Current methods for antimicrobial susceptibility testing continue to be based for the most part on the detection of microbial growth or lack of growth in the presence of the antimicrobial agent being tested [34, 35]. The direct detection of resistance genes by molecular methods such as PCR to date has limitations due to the fact that relatively few resistance genes are firmly associated with phenotypic resistance [34–36]. For example, resistance genes associated with phenotypic resistance that can be found in Gram-positive cocci include *meca*, *vanA*, and *vanB*. In contrast, the lack of consensus sequences among acetyltransferases and adenyltransferase genes from Gram-negative bacilli makes the molecular detection of aminoglycoside resistance difficult. Although molecular methods for antimicrobial susceptibility testing are ascending, phenotypic methods for determining the level of susceptibility of bacterial isolates to antimicrobial agents are likely to remain clinically relevant for many years.

### Volume of Blood Tested

The volume of blood cultured is known to be an important variable in blood cultures because the number of microorganisms in blood may small [37–41]. Typically in adults, there are fewer than 10 CFU/ml, and there may be less than 1 CFU/ml. In septic neonates, there is a sizeable subset with less than 4 CFU/ml [40]. Clearly the volume of blood tested by molecular methods will also be important. Moreover, the Poisson distribution of these microorganisms is such that they are not evenly distributed [37, 42]. This increases the likelihood that sampling a small volume of blood will miss a microorganism that is causing sepsis. Volume-related issues may explain the lower sensitivity seen with a molecular method (66.7\%) than seen with conventional
blood cultures in a study of neonatal sepsis [31]. The Poisson distribution may explain the moderate concordance between blood cultures and a molecular method reported in a study of post-surgical sepsis in adults [27].

**Contamination of Blood Samples**

The sample of blood collected to assess bacteremia and/or fungemia, whether this analysis is done by blood culture or by a molecular method, can be contaminated with microorganisms from the skin during venipuncture and/or from indwelling vascular devices if the blood is obtained from such a device [23]. False-positive blood cultures have been recognized as a troublesome issue for decades, and such contamination will be no less important for molecular methods.

**The Use of Molecular Assays for Diagnosing Tuberculosis**

Tuberculosis remains one of the most important public health issues in the world. Tuberculosis results in approximately 1.7 million deaths each year, and the number of new cases worldwide is estimated at more than nine million; this is higher than at any other time in history [43]. Yet control of this treatable infection has been handicapped until recently by the lack of new diagnostic tests for the detection of *M. tuberculosis* and drug resistance [44]. The development of molecular assays for the detection of *M. tuberculosis* as well as simultaneous detection of resistance to isoniazid and/or rifampin promises to greatly assist TB control efforts although there are important limitations of these molecular methods that must be understood when interpreting the results and considering the relevance of such molecular techniques [44–46]. Indeed, none of these molecular methods eliminates the need for mycobacterial cultures, and all require a laboratory infrastructure that can accommodate molecular testing. Specific limitations of these molecular methods in both interpretation and relevance will be described below.

**The Limitations of Molecular Assays for Diagnosing Tuberculosis**

**Limited Sensitivities**

There currently are a number of different molecular assays for detecting the presence of *M. tuberculosis* in sputum. These include PCR, transcription-mediated amplification, loop-mediated isothermal amplification [47], and Xpert MTB/RIF [47]. In comparison to mycobacterial culture, these molecular assays possess sensitivities approaching 90%. In general, these molecular methods work better with
Interpretation and Relevance of Advanced Technique Results

smear-positive than with smear-negative sputum specimens; none are more sensitive than mycobacterial cultures. The sensitivity for patients with smear-negative sputum can be increased by the use of bronchial aspirates [48] or bronchial lavage fluid [49], but is still not as sensitive as mycobacterial cultures.

Molecular Detection of Resistance Determinants

There currently are a number of different molecular assays for detecting gene mutations associated with resistance to a particular antituberculosis drug [50–54]. There are always gaps between basic research and clinical application as some of the drug-resistance mechanisms remain unknown while new resistance-related mutations are emerging. In addition, all molecular assays basically include a DNA amplification step and are categorized by the manner in which the amplified DNA is detected except for sequencing, which has some distinct advantages over the other methods. None of these methods, including sequencing, are able to detect all resistant strains although sequencing comes the closest to doing so. The major limitation of these molecular methods, except sequencing, is that they detect only known mutations in a defined site or region, as their design is dependent upon known mutations. The advantage of sequencing for molecular detection of mutations of drug resistance can be seen by a recent report from the Centers for Disease Control and Prevention [45]. This study used DNA sequencing to detect resistance to the first-line antituberculosis drugs isoniazid, rifampin, pyrazinamide, and ethambutol and to the second-line drugs amikacin, capreomycin, kanamycin, ciprofloxacin, and ofloxacin. The molecular data were compared to phenotypic data. Sensitivity and specificity values for the first-line and second-line drug loci were, in general, excellent and supported the use of DNA sequencing to detect drug resistance in the *M. tuberculosis* complex.

Misidentification

Although uncommon, misidentification has been reported with molecular assays for tuberculosis [55, 56]. In one of these reported cases [55], a patient presented with inguinal lymphadenopathy as well as erythema nodosum-like lesions on his legs and forearms. A biopsy of an enlarged inguinal lymph node demonstrated caseating granulomata and numerous acid-fast bacilli on Ziehl-Neelsen staining; a portion of this node was sent for mycobacterial culture and molecular analysis. In addition, a skin biopsy of a forearm nodule was done; this revealed acid-fast bacilli that were morphologically typical of *Mycobacterium leprae*. A diagnosis of leprosy was made based on the clinical presentation and the skin biopsy results. However, the lymph node sent for mycobacterial culture and molecular analysis was positive by the Gen-Probe Amplified *M. tuberculosis* Direct (MTD) test (BBL). Although leprosy was still considered to be a correct diagnosis due to the clinical presentation and the skin biopsy findings, the possibility of this patient also having tuberculosis
could not be ruled out until the culture results were known. Therefore, the patient was treated for both leprosy and tuberculosis until cultures at 7 weeks as well as additional PCR testing of lymph node material for \textit{M. tuberculosis} were reported to be negative. A root cause analysis was done in order to investigate this misidentification. \textit{M. leprae} culture material was obtained from the National Hansen’s Disease Programs at Louisiana State University; these mycobacterial organisms were tested with the Gen-Probe MTD test and were positive at a concentration of $5 \times 10^5$ organisms per ml, but were indeterminate at a concentration of $5 \times 10^4$ organisms per ml. The investigators concluded that a high concentration of \textit{M. leprae} in a clinical specimen could lead to a false-positive result with the Gen-Probe MTD test [55].

\textbf{The Use of Molecular Assays for Diagnosing Respiratory Tract Infections}

There is no doubt that respiratory tract infection other than those caused by \textit{M. tuberculosis} also are of considerable clinical importance. Lower respiratory tract infections continue to be a leading cause of death due to infectious diseases in the United States as well as worldwide [57]. Hospital-acquired pneumonia is considered to be one of the most difficult treatment challenges in infectious diseases in part because results of culture and antimicrobial susceptibility testing can take 48 h or longer [58]. Viral respiratory tract infections caused by pathogens such as the severe acute respiratory syndrome coronavirus (SARS-CoV) [59] and novel A/H1N1 influenza virus [60] can cause epidemic viral pneumonia in which some patients have respiratory failure with a significant risk of mortality [61]. Respiratory tract infections are also important in the ambulatory setting because of the documented overuse of antimicrobial agents in this patient population [62].

Despite the obvious clinical importance of respiratory tract infections, the diagnosis of lower respiratory tract infections has always been problematic due, in large part, to issues related to the optimal collection and evaluation of sputum. The diagnostic value of detecting \textit{M. tuberculosis} in the sputum was recognized as early as 1884 [63], and evaluation of sputum became the cornerstone for the diagnosis of tuberculosis [43, 44]. Post-mortem studies in the late 1890s and early 1900s then established the role of other microorganisms such as \textit{Streptococcus pneumoniae}, \textit{Haemophilus influenzae}, \textit{S. aureus}, and \textit{Klebsiella pneumoniae} in non-tuberculous infections of the respiratory tract [64–66]. In 1902, the use of the Gram’s stain was described [67]. The microscopic examination of sputum was followed by the introduction of sputum cultures for the diagnosis of bacterial pneumonia [68–70]. Of note in these early reports describing sputum cultures was the recognition that collection of the sputum was important. For example, Hastings and Niles in a 1911 publication [69] point out that, “Exudates formed in portions of the respiratory tract that are normally sterile may be collected and treated in a way that will prevent contamination.” These investigators further define a “\textit{clean sputum}, i.e., one containing
only two or three types of bacteria and free from buccal squamous cells, and a dirty sputum, i.e., one containing a varied bacterial and fungoid flora and buccal squamous cells, are readily recognized on microscopic examination.” They also state that, “A dirty sputum is not suitable for bacterial examination and should be discarded for a second or third clean specimen from the same patient.” Luetscher opines in his paper [70] that, “The patient should be instructed to expectorate into the bottle or dish only what he is certain comes from his ‘boots,’ and also be made to understand that very little is wanted, but that that little must be choice.” These astute observations remain relevant a century later.

Clearly, the pitfalls of collecting expectorated sputum specimens suitable for microscopic examination and cultures were recognized early in the twentieth century. In the 1960s, these pitfalls were again being articulated and addressed [71–76]. In particular, contamination by microorganisms present in the upper respiratory tract (i.e., nasal-oral-pharyngeal regions) was considered to be a major issue with expectorated sputum [77, 78]. Because of these pitfalls, a number of alternative methods have been used to obtain better sputum specimens. Bronchoscopy, although introduced early in the twentieth century and used on occasion for aspirating pus from larger airways [79], was not widely used for obtaining sputum for microscopy and culture until the 1970s when fiberoptic bronchoscopy became available [80]. Fiberoptic bronchoscopy also resulted in the use of bronchoalveolar lavage for diagnosing acute bacterial pneumonias [81]. Other methods adopted for obtaining uncontaminated sputum included transtracheal aspiration [72], percutaneous needle biopsy [76], and open-lung biopsy [71].

Despite these continued attempts to obtain appropriate sputum specimens that are more clinically relevant, the usefulness of sputum cultures has continued to be questioned in numerous reports [82–88]. Indeed, the Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults recommend that pretreatment Gram stain and culture should be performed only if a good quality sputum sample can be obtained and quality performance measures for collection, transport, and processing of this sputum sample can be assured [89]. It must be remembered that sputum collection is the “weakest link” in the “chain” of evidence that provides the etiologic diagnosis of pneumonia.

Assuming that sputum collection is done correctly, the next issue is making sure that any microbial pathogen present in the sputum can be identified. It is not surprising that molecular assays for the detection and characterization of microorganisms rapidly emerged in the clinical microbiology laboratory as an important adjunct to traditional culture methods [90, 91]. It was quickly realized that molecular assays such as NAATs offered significant advantages over conventional methods for the detection of Mycoplasma pneumoniae [92, 93], Legionella species [94], and Chlamydia species [95]; moreover, these three respiratory pathogens did not require concomitant susceptibility testing results from clinical isolates. Similarly, the advantage of NAATs for the laboratory diagnosis of pertussis was recognized very early [96, 97]; PCR testing is now considered by the CDC to be an important tool for diagnosis of pertussis especially in the setting of the current resurgence of pertussis
disease as it can provide timely results with improved sensitivity over culture [98]. The inherent problems associated with the detection and identification of respiratory viruses by culture and/or serologic methods also resulted in the early application of molecular assays for rapid detection and characterization of respiratory viruses [99]. Both user-developed and commercial molecular methods have quickly evolved and now allow rapid identification of multiple common viral pathogens causing respiratory tract infections [100–102]. In addition to identification of viral respiratory pathogens, it was appreciated that rapid molecular assays would also offer significant advantages for diagnosing recognized bacterial pulmonary pathogens causing community-acquired pneumonia [57, 93, 103, 104]. Indeed, initial studies in which rapid molecular assays were combined with conventional diagnostic methods have demonstrated that this approach increased the etiological diagnosis of lower respiratory tract infections considerably [105, 106]. This was especially true for patients with adequate collection of sputum [105]. Of interest was the observation that NAATs increased both the diagnostic and treatment costs [106]. Finally, the diagnosis of hospital-acquired pneumonia is another potential area where the use of rapid molecular assays for respiratory pathogens may prove useful [58]. Currently clinical trials are needed to provide evidence for which molecular assays are best as well as how this molecular information should be applied in the clinical setting.

The Limitations of Molecular Assays for Diagnosing Respiratory Tract Infections

Sputum/Specimen Collection

Clearly the same limitations of conventional sputum culture methods for diagnosing respiratory tract infections are also limitations for molecular methods. In particular, the collection of sputum continues to be the most important aspect for the diagnosis of lower respiratory tract infections even when molecular assays are used [58]. These new molecular methods will not guarantee that the microbiology laboratory will receive the optimal sputum sample to analyze.

Complexity of Pulmonary Microbiome

Another important aspect of molecular assays for the diagnosis of respiratory infections is that these methods are clearly going to reveal the complexities of the pulmonary microbiome. Indeed, recent applications of molecular assays have revealed a more diverse microbiota than previously recognized in the airways of patients with chronic pulmonary disease [107, 108]. For example, comprehensive profiling of the airway bacterial communities was accomplished using a culture-independent microarray, the 16S rRNA PhyloChip, of a cohort of COPD patients...
requiring ventilatory support and antimicrobial therapy for exacerbation-related respiratory failure [109]. PhylolChip analysis demonstrated the presence of over 1,200 bacterial taxa representing 140 distinct families, including many that were not previously detected in airway diseases. A core community of 75 bacterial taxa was noted in all patients; many of these microorganisms were known pathogens in airway diseases.

**Colonization Versus Infection**

Given the fact that the pulmonary microbiome is more complex than previously appreciated, the obvious question then becomes which microorganisms are colonizing and which are causing infection. One might also ask if there is any real difference between colonization versus infection in the airways. Molecular identification of bacteria in the lower airways of preterm infants has revealed that early bacterial colonization of the airways with diverse species occurs within the first 3 days of life of intubated preterm infants [110]. Such neonatal airway colonization with Gram-negative bacilli is associated with a cytokine response as well as with severe bronchopulmonary dysplasia [111, 112]. The etiologic role of neonatal colonization in children with non-cystic fibrosis bronchiectasis is unclear at this time [113–115], but molecular methods may provide further insight into the pathogenesis of this disorder. Similarly, the etiologic role of bacterial colonization in the pathogenesis of chronic obstructive pulmonary disease [107–109, 116, 117] is currently being elucidated with the assistance of molecular methods.

**Simultaneous Detection of Multiple Pathogens**

The extreme sensitivity of molecular methods such as NAATs may result in simultaneous detection of multiple pathogens from sputum specimens. Detection of multiple pathogens in sputum by molecular methods has already been reported in community-acquired pneumonia [105] where mixed infections were frequently seen: these most commonly were *S. pneumoniae* together with a respiratory virus. These findings are not unexpected; a number of studies have reported an association between viral respiratory tract infections and invasive pneumococcal disease [118–120]. Molecular diagnostic methods employed in other studies of respiratory tract infections have confirmed the etiologic role viral respiratory tract infections and bacterial pneumonia [121–123].

**Accuracy of Assay Development**

An important issue for NAATs is whether the amplification products truly represent the target microorganism [104]. Molecular methods that employ DNA sequencing are often considered completely accurate with 100% sensitivity and specificity.
This, unfortunately, is not the case. There are a variety of technical factors such as the influence of contaminating DNA from other sources on the sequencing template, the selection of the primers used for the amplification, the quality of the base-calling software, and the method used for compiling the “consensus sequence” from multiple forward and reverse reactions [104, 124–127]. Inappropriately chosen gene targets and regions will result in false-positives and negatives. The insertion sequence element IS481, found in several hundred copies in the *Bordetella pertussis* genome, is frequently used as a target for *B. pertussis* detection and has a much greater analytical sensitivity than assays with single-copy target sequences, such as that of the pertussis toxin promoter [128]. However, false-positive results have been reported due to the smaller copy numbers of IS481 existing in non-pertussis *Bordetella* species [129, 130]. The accuracy of assay development is often not appreciated by the non-molecular microbiologist or the clinician.

**The Use of Molecular Assays for Diagnosing Enteric Infections**

Most acute diarrheal illnesses are self-limited or viral [131]. For afebrile patients who present with watery non-bloody diarrhea of less than 24 h duration, microbiologic investigation is usually unnecessary [131, 132]. In contrast, patients with a diarrheal illness lasting for more than one day, especially when the illness is accompanied by fever, bloody stools, recent antimicrobial use, hospitalization, or systemic illness, should have a microbiologic evaluation of their diarrheal stool [131–134]. The microbiologic stool evaluation for such enteric infections has for many decades relied upon the analysis of bacterial cultures and/or microscopy to detect ova and parasites [134]. For nosocomial diarrhea or patients with a history of recent use of antimicrobial agents prior to the onset of diarrhea, the microbiologic stool evaluation should focus on the diagnosis of toxigenic *Clostridium difficile* [135]. For persistent diarrhea in patients with a history of international travel, the microbiologic stool evaluation may require special selective and differential agar such as thiosulfate citrate bile salts sucrose (TSCB) agar for *Vibrio* species [136]. Finally, the noroviruses are the most common cause of non-bacterial enteritis worldwide: the laboratory diagnosis of noroviruses depends on the detection of virus particles by EM, detection of viral antigens by EIA, or detection of viral RNA by real-time PCR [137].

Given the complexity of conventional methods for the microbiologic evaluation of a stool specimen from a patient with a diarrheal illness, it is not surprising that determining the microbiologic etiology of an enteric infection remains an elusive goal [138]. It is no wonder that molecular methods have been applied to the diagnosis of acute infectious diarrhea [138, 139]. Enteric infections due to the broad range of potential pathogens such as viruses, bacteria, protozoa, and helminths are well suited for multiplex molecular assays. Indeed, monoplex and multiplex molecular assays for many of these enteric pathogens have already been described [140–146]. Therefore, it will be important for both clinicians and microbiologists to appreciate the limitations of these molecular assays.
The Limitations of Molecular Assays for Diagnosing Enteric Infections

Lack of a Gold Standard for the Microbiologic Cause of Enteric Infections

The absence of a gold standard for the microbiologic cause of enteric infections means that the clinical significance of a detected pathogen may not always be clear [138]. Although conventional wisdom suggests that there should be one main pathogen causing an enteric infection in a patient, the detection of multiple pathogens in some patients will challenge this thinking [138]. This is apt to be particularly true for parasitic enteric infections. Moreover, the detection of RNA or DNA in a stool specimen does not necessarily mean a viable or an infectious pathogen.

Complexity of the Human Gut Microbiome

Molecular assays including high-throughput sequencing techniques have begun to identify the vast communities of bacteria that inhabit the skin and gut in humans [147]. Despite these methods, the human gut remains relatively unexplored [147, 148]. This complexity is likely to be a factor in the use of NAATs for diagnosing enteric infections if for no other reason than the influence of contaminating DNA from these gut microbes on the sequencing template.

Issues with Nucleic Acid Extraction

The molecular diagnosis of an enteric infection will usually begin with extraction of nucleic acid from the specimen. Because this specimen is generally a diarrheal stool sample, the extraction step becomes a critical step in this molecular diagnostic process. This is because stool is a complex mixture with multiple and diverse nucleic acids and amplification inhibitors. Investigators have noted that detection of a given target will be reduced several logs when the target is placed in a stool mixture [138]. This may result in enteric pathogens present in low numbers being missed. This is the reason that some investigators have used molecular methods following isolation of potential enteric bacterial pathogens from stool [149]. In addition, extraction of DNA from ova and parasites may be more difficult than extracting DNA from bacteria [140]. Concentration of ova and parasites that may be present in low numbers may be required, as it is for microscopic evaluation.

Requirement for Multiplex PCR

Over 50 pathogens currently are recognized a potential causes of enteric infections [138]. This means that a multiplex PCR such as the Luminex bead method must be used. Even a multiplex approach will likely require the use of a diagnostic algorithm
or the use of several multiplex assays. The use of multiplex assays will create several technical problems that include difficulty with discrimination of multiple targets in a single reaction and reduced sensitivity. Multiplex assays also will cause some problems with interpretation due to detection of multiple pathogens. For example, one study that reexamined stool samples using PCR found that the detection rate increased for both viral and bacterial pathogens, but the detection rate for multiple pathogens also increased [150].

**Requirement for Quantitative PCR**

Molecular assays due to their high sensitivity may detect low levels of enteric pathogens with unclear clinical significance. For example, *Giardia* species are known to occur in stool at high rates in persons without diarrhea [151]. Therefore, the use of quantitative PCR methods may be needed in order to provide information that will be useful for interpreting the clinical significance; the assumption being that a higher burden is more likely to be associated with disease [138]. Ultimately, this relationship of higher burden and symptoms of disease will need to be verified for many enteric pathogens for which this relationship has not yet been determined.

**Molecular Detection of Resistant Determinants**

Antimicrobial resistance is increasing for many bacterial pathogens and is likely to happen with enteric pathogens such as *Shigella*, *Salmonella*, and *Campylobacter*. Detection of resistance determinants may be necessary in the future and is likely to be difficult from stool samples due to the diversity of microorganisms present in stool [138].

**The Use of Molecular Assays for Diagnosing Tissue Infections**

The use of molecular assays for diagnosing tissue infections is another area that is rapidly evolving. For example, molecular assays have proven quite successful in the diagnosis of infectious endocarditis [151–160]. Indeed, a number of fastidious microorganisms causing endocarditis have been identified using molecular assays; these include *Tropheryma whippelii* [151], *Bartonella quintana* [153, 158, 160], *Bartonella henselae* [158], and *Coxiella burnetii* [158]. This success has resulted in molecular assays being included in the best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis [161]. Molecular assays of tissue have been useful for diagnosing necrotizing fasciitis caused by group A streptococci when cultures were negative or not available [162, 163].

Finally, molecular assays for fungal pathogens also have been widely studied and have the potential to be useful in the diagnosis of fungal tissue infections [164–174]. Fungal pathogens identified from tissue by molecular assays include *Paracoccidioides*.
brasiliensis [165], Histoplasma capsulatum [164], Coccidioides immitis [174], Blastomyces dermatitidis [174], Aspergillus fumigatus [166], Absidia corymbifera [166], Rhisopus arrhizus [166], and Candida species [171]. NNATs have been used to detect a variety of DNA and RNA viral pathogens in formalin-fixed, paraffin-embedded (FFPE) tissue specimens [175–180]. The use of molecular assays for diagnosing tissue infections will only increase over time; therefore, the limitations of these molecular assays should be appreciated.

**Limitations in the Use of Molecular Assays for Diagnosing Tissue Infections**

**Fresh/Frozen Tissue Versus Formalin-Fixed, Paraffin-Embedded Tissue**

Fresh/frozen tissue is best for molecular testing and should be available if molecular testing is considered at the time of biopsy [181, 182]. In contrast, FFPE tissue often is the only tissue available when molecular testing is considered as an afterthought. Accordingly, one of the most important limitations in the use of molecular assays for diagnosing tissue infections is considering these assays at the time of biopsy so that fresh tissue can be used or frozen for use later. The difference in sensitivity for PCR testing can be seen by a study in which fresh nonembedded tissues were found to have sensitivities for PCR detection of fungi of 97 % versus only 68 % for FFPE tissue [181]. The reason for this decreased sensitivity is that nucleic acids obtained from FFPE tissue are frequently damaged (i.e., cross-linked) and may contain PCR inhibitors [183, 184]. If FFPE tissue must be used, a housekeeping human gene must be amplified as a control [179, 184].

**Wide Diversity of Potential Microbial Pathogens**

The wide diversity of potential microbial pathogens that could potentially be detected in tissue is readily apparent. These pathogens could be viral, bacterial, fungal, or parasitic. This diversity will greatly influence the DNA targets and the PCR primers used as well as whether monoplex or multiplex PCR methods will be used. For example, species-specific identification of a wide range of clinically relevant fungal pathogens using Luminex technology required up to three different probes for each fungal pathogen using the internal transcribed spacer (ITS2) region, which is highly variable among genomes of individual fungal species [185].

**Choice of DNA Target, PCR Primers, and Amplification Method**

The choice of the DNA target is important [167]. In general, molecular assays that target multicopy genes provide the greatest sensitivity. Amplification methods
should provide objective endpoint assessments for the PDR test used. PCR primers are important. For example, there is insufficient variation in the internal transcribed spacer (ITS1) region to differentiate certain species of fungal pathogens [186]; therefore, analysis of other regions such as ITS2 should be considered. False-positive results have been described with certain primer for *H. capsulatum* [164]. False-negative results have been found for *C. immitis* from FFPE tissue (73 % sensitivity) versus fresh tissue (93 % sensitivity) suggesting a primer problem, degradation, or inhibitors [164]. Finally, it is estimated that approximately 10–20 % of the sequences in GenBank are misidentified [187]. Currently there are relatively few commercial kits available for molecular testing using tissue specimens. If laboratory-developed PCR assays for tissues are used, they must be evaluated, verified, and validated by the laboratory before the results can be used for clinical diagnosis and patient care [186, 188].

**Issues with Nucleic Acid Extraction**

DNA extraction from FFPE tissues is difficult and requires special protocols [187]. The amount of DNA extracted is usually quite small; reported methods show an amplification success rate between 60 % and 80 %. Commercial DNA extraction kits have been evaluated [187]; one method (TaKaRa) was noted to extract DNA for 69 of the 74 FFPE tissue samples from which a housekeeping gene could be amplified. Moreover, this method was cost-effective and had a non-laborious protocol. Successful extraction of RNA from FFPE specimens depends on the prompt original tissue processing and a well-developed extraction protocol [175, 179, 180, 184].

**Low Number of Pathogens and/or Random Distribution in Tissue**

When the number of pathogens is scant in tissues, the amount of DNA obtained may be insufficient to perform a PCR assay. Moreover, these pathogens are often randomly distributed in the tissue [37, 42]. When FFPE tissue is used, a punch biopsy can be used to take a sample from an area noted to have inflammation and/or microorganisms by a stained slide from the same tissue block. The stained slide can be marked and then used to direct the location for the punch biopsy sample from the tissue block. Use of fresh or frozen tissue is more problematic as the selection of tissue will be random and may not contain microorganisms.

**Simultaneous Detection of Multiple Pathogens**

As would be expected, molecular assays already have been noted to detect mixed infections. This may present difficulty in interpretation of the results. In particular, microbial diversity in endocarditis has been noted with cultivation-independent molecular techniques [188]. Multiple pathogens detected by molecular assays have also been reported in fungal infections [166].
Concluding Remarks

Outcomes from infectious diseases are directly related to the length of time required for identification of the microbial pathogen. Until recently, clinical microbiology laboratories have been handicapped by conventional, slow multistep culture-based techniques that require prolonged incubation times for many pathogens and are not able to isolate others. Clinicians unable by clinical judgment or diagnostic results to quickly and accurately identify a pathogen causing infection must adopt a conservative approach involving empiric therapy with broad-spectrum antimicrobial agents. Fortunately, this cumbersome approach is rapidly changing because of the introduction of molecular diagnostic techniques. Indeed, molecular assays such as NAATs have initiated a revolution in the field of diagnostic microbiology due to their high sensitivity, specificity, rapid test turnaround time as well as potential high throughput and automation. Molecular assays have been heralded as the “diagnostic tool for the millennium” [189, 190]. However, molecular assays also bring some uncertainty such as that caused by false-positive results due to background contamination from exogenous sources of DNA [190, 191]. For example, one study using a universal 16S rRNA PCR assays detected eubacterial DNA in blood samples from healthy subjects [192]. NAATs also may give false-negative results due to two principle reasons: (1) the relatively small sample required for PCR reactions and (2) technical problems associated with PCR processing [193]. Moreover, the results of molecular assays may be difficult to interpret and apply in the clinical setting. As NAATs are increasingly used in routine clinical microbiology laboratories, interpretation is expected to be more difficult as new tests are developed and more complicated multiplex assays emerge. For example, clinical relevance of positive NAATs in paraffin block specimens and multiple microbial organisms found in any specimen will need careful interpretation. As the usefulness of these molecular assays is determined by usage over time, communication between the clinician and the microbiology laboratory is always suggested whenever an interpretation is needed. Finally, both the clinical microbiologist and the clinician must acquire a working knowledge of the principles, diagnostic value, and limitations of these molecular assays [194, 195].

References

1. Tang YW, Stratton CW (2006) Advanced techniques in diagnostic microbiology. Springer Science, New York, NY
2. Tang YW, Persing DH (2009) Diagnostic microbiology. In: Schaechter M (ed) Encyclopedia of microbiology, 3rd edn. Elsevier, Oxford, pp 308–320
3. Tang YW (2007) Laboratory diagnosis of central nervous system infections by molecular amplification techniques. Exp Opin Med Diagn 1:489–509
4. Smalling TW, Sefers SE, Li HJ, Tang YW (2002) Molecular approaches to detecting herpes simplex virus and enteroviruses in the central nervous system. J Clin Microbiol 40:2317–2322
5. Tang YW, Mitchell PS, Espy MJ, Smith TF, Persing DH (1999) Molecular diagnosis of herpes simplex virus infections in the central nervous system. J Clin Microbiol 37:2127–2136
6. Yamamoto Y (2002) PCR in diagnosis of infection: detection of bacteria in cerebrospinal fluids. Clin Diagn Lab Immunol 9:508–514
7. Balasingham SV, Davidsen T, Szpinda I, Frye SA, Tonjum T (2009) Molecular diagnostics in tuberculosis: basis and implications for therapy. Mol Diagn Ther 13:137–151. doi:10.2165/01250444-200913030-00001
8. Ecker DJ, Sampath R, Li H et al (2010) New technology for rapid molecular diagnosis of bloodstream infections. Expert Rev Mol Diagn 10:399–415
9. Crump JA, Tuohy MJ, Morrissey AB et al (2011) Performance of nucleic acid amplification following extraction of 5 mL of whole blood for the diagnosis of Mycobacterium tuberculosis bacteremia. J Clin Microbiol 26:26
10. Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clementi M (2010) The era of molecular and other non-culture-based methods in diagnosis of sepsis. Clin Microbiol Rev 23:235–251
11. Bastien P, Procop GW, Reischl U (2008) Quantitative real-time PCR is not more sensitive than "conventional" PCR. J Clin Microbiol 46:1897–1900, Epub 9 Apr 2008
12. Tang YW, Sirram S, Li H et al (2009) Qualitative and quantitative detection of Chlamyphilia pneumoniae DNA in cerebrospinal fluid from multiple sclerosis patients and controls. PLoS One 4:e5200, Epub 9 Apr 2009
13. Tang YW, Rys PN, Rutledge BJ, Mitchell PS, Smith TF, Persing DH (1998) Comparative evaluation of colorimetric microtiter plate systems for detection of herpes simplex virus in cerebrospinal fluid. J Clin Microbiol 36:2714–2717
14. Li L, Mahan CS, Palaci M et al (2010) Sputum Mycobacterium tuberculosis mRNA as a marker of bacteriologic clearance in response to antituberculosis therapy. Monitoring therapeutic efficacy by real-time detection of Mycobacterium tuberculosis mRNA in sputum. J Clin Microbiol 48:46–51, Epub 18 Nov 2009
15. Mdivani N, Li H, Akhalaia M et al (2009) Monitoring therapeutic efficacy by real-time detection of Mycobacterium tuberculosis mRNA in sputum. Clin Chem 55:1694–1700, Epub 2 Jul 2009
16. Gaydos CA, Crotchfelt KA, Howell MR, Kralian S, Hauptman P, Quinn TC (1998) Molecular amplification assays to detect chlamydial infections in urine specimens from high school female students and to monitor the persistence of chlamydial DNA after therapy. J Infect Dis 177:417–424
17. Schmid DS, Jumaan AO (2010) Impact of varicella vaccine on varicella-zoster virus dynamics. Clin Microbiol Rev 23:202–217
18. Keefer CS (1941) The clinical significance of bacteremia. NY State Med J 41:976–981
19. Fox H, Forrester JS (1940) Clinical blood culture. An analysis of over 5,000 cases. Am J Clin Pathol 10:493–504
20. Bryan CS (1989) Clinical implications of positive blood cultures. Clin Microbiol Rev 2:329–353
21. Weinstein MP, Roller LB, Murphy JR, Lichtenstein KA (1983) The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev Infect Dis 5:35–53
22. Weinstein MP, Towns ML, Quartey SM et al (1997) The clinical significance of positive blood cultures in the 1990s: a prospective comprehensive evaluation of the microbiology, epidemiology, and outcome of bacteremia and fungemia in adults. Clin Infect Dis 24:584–602
23. Hall KK, Lyman JA (2006) Updated review of blood culture contamination. Clin Microbiol Rev 19:788–802
24. Struelens MJ (2010) Detection of microbial DNAemia: does it matter for sepsis management? Intensive Care Med 36:193–195
25. Schifman RB, Bachner P, Howanitz PJ (1996) Blood culture quality improvement: a College of American Pathologists Q-Probes study involving 909 institutions and 289 572 blood culture sets. Arch Pathol Lab Med 120:999–1002
26. Eshoo MW, Crowder CD, Li H et al (2010) Detection and identification of Ehrlichia species in blood by use of PCR and electrospray ionization mass spectrometry. J Clin Microbiol 48:472–478
27. Bloos F, Hinder F, Becker K et al (2010) A multicenter trial to compare blood culture with polymerase chain reaction in severe human sepsis. Intensive Care Med 36:241–247
28. Dierkes C, Ehrenstein B, Siebig S, Linde HJ, Reischl U, Salzberger B (2009) Clinical impact of a commercially available multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis. BMC Infect Dis 9:126
29. Louie RF, Tang Z, Albertson TE, Cohen S, Tran NK, Kost GJ (2008) Multiplex polymerase chain reaction detection enhancement of bacteremia and fungemia. Crit Care Med 36:1487–1492
30. Mancini N, Clerici D, Diotti R et al (2008) Molecular diagnosis of sepsis in neutropenic patients with haematological malignancies. J Med Microbiol 57:601–604
31. Reier-Nilsen T, Farstad T, Nakstad B, Lauvra V, Steinbakk M (2009) Comparison of broad range 16 S rDNA PCR and conventional blood culture for diagnosis of sepsis in the newborn: a case control study. BMC Pediatr 9:5
32. Westh H, Lisby G, Breyssse F et al (2009) Multiplex real-time PCR and blood culture for identification of bloodstream pathogens in patients with suspected sepsis. Clin Microbiol Infect 15:544–551
33. Dark PM, Dean P, Warhurst G (2009) Bench-to-bedside review: the promise of rapid infection diagnosis during sepsis using polymerase chain reaction-based pathogen detection. Crit Care 13:217
34. Jorgensen JH, Ferraro MJ (2009) Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 49:1749–1755
35. Holland TL, Woods CW, Joyce M (2009) Antibacterial susceptibility testing in the clinical laboratory. Infect Dis Clin North Am 23:757–790, vii
36. Tenover FC (1989) DNA probes for antimicrobial susceptibility testing. Clin Lab Med 9:341–347
37. Forster LI (2003) Measurement uncertainty in microbiology. J AOAC Int 86:1089–1094
38. Hall MM, Ilstrup DM, Washington JA 2nd (1976) Effect of volume of blood cultured on detection of bacteremia. J Clin Microbiol 3:643–645
39. Mermel LA, Maki DG (1993) Detection of bacteremia in adults: consequences of culturing an inadequate volume of blood. Ann Intern Med 119:270–272
40. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP (1996) Volume of blood required to detect common neonatal pathogens. J Pediatr 129:275–278
41. Wilson ML, Weinstein MP (1994) General principles in the laboratory detection of bacteremia and fungemia. Clin Lab Med 14:69–82
42. Sun X, Kurosu S, Shintani H (2006) The expanded application of most probable number to the quantitative evaluation of extremely low microbial count. PDA J Pharm Sci Technol 60:124–134
43. Lawn SD, Zumla AI (2011) Tuberculosis. Lancet 378:57–72
44. Wilson ML (2011) Recent advances in the laboratory detection of Mycobacterium tuberculosis complex and drug resistance. Clin Infect Dis 52:1350–1355
45. Campbell PJ, Morlock GP, Sikes RD et al (2011) Molecular detection of mutations associated with first- and second-line drug resistance compared with conventional drug susceptibility testing of Mycobacterium tuberculosis. Antimicrob Agents Chemother 55:2032–2041
46. Woods GL (1999) Molecular methods in the detection and identification of mycobacterial infections. Arch Pathol Lab Med 123:1002–1006
47. Boehme CC, Nabeta P, Henostroza G et al (2007) Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. J Clin Microbiol 45:1936–1940
48. Min JW, Yoon HI, Park KU, Song JH, Lee CT, Lee JH (2010) Real-time polymerase chain reaction in bronchial aspirate for rapid detection of sputum smear-negative tuberculosis. Int J Tuberc Lung Dis 14:852–858
49. Kibiki GS, Mulder B, van der Ven AJ et al (2007) Laboratory diagnosis of pulmonary tuberculosis in TB and HIV endemic settings and the contribution of real time PCR for M. tuberculosis in bronchoalveolar lavage fluid. Trop Med Int Health 12:1210–1217
50. Massire C, Ivy CA, Lovari R et al (2011) Simultaneous identification of mycobacterial isolates to the species level and determination of tuberculosis drug resistance by PCR followed by electrospray ionization mass spectrometry. J Clin Microbiol 49:908–917, Epub 29 Dec 2010
51. Gegia M, Mdivani N, Mendes RE et al (2008) Prevalence of and molecular basis for tuberculosis drug resistance in the Republic of Georgia: validation of a QIAplex system for detection of drug resistance-related mutations. Antimicrob Agents Chemother 52:725–729, Epub 10 Dec 2007
52. Cooksey RC, Morlock GP, Glickman S, Crawford JT (1997) Evaluation of a line probe assay kit for characterization of rpoB mutations in rifampin-resistant Mycobacterium tuberculosis isolates from New York City. J Clin Microbiol 35:1281–1283
53. Abebe G, Paasch F, Apers L, Rigouts L, Colebunders R (2011) Tuberculosis drug resistance testing by molecular methods: opportunities and challenges in resource limited settings. J Microbiol Methods 84:155–160
54. Boehme CC, Nabet P, Hillemann D et al (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005–1015
55. Chedore P, Broukhanski G, Shainhouse Z, Jamieson F (2006) False-positive amplified Mycobacterium tuberculosis direct test results for samples containing Mycobacterium leprae. J Clin Microbiol 44:612–613
56. Lefmann M, Moter A, Schweickert B, Gobel UB (2005) Misidentification of Mycobacterium leprae as Mycobacterium intracellulare by the COBAS AMPLICOR M. intracellulare test. J Clin Microbiol 43:1928–1929
57. Bartlett JG (2011) Diagnostic tests for agents of community-acquired pneumonia. Clin Infect Dis 52(Suppl 4):S296–S304
58. Endimiani A, Hujer KM, Hujer AM et al (2011) Are we ready for novel detection methods to treat respiratory pathogens in hospital-acquired pneumonia? Clin Infect Dis 52(Suppl 4):S373–S383
59. Gabbett HS (1884) The diagnostic value of the discovery of Koch’s Bacilli in sputum. Br Med J 1:805–808
60. Smith WH (1898) A Case of Pneumonia due to the Bacillus Mucosus Capsulatus. (B. of Friedlander.). J Boston Soc Med Sci 2:174–179
61. Wollstein M (1905) The bacteriology of broncho- and lobular pneumonia in infancy. J Exp Med 6:391–400
62. Gaensler EA, Moister VB, Hamm J (1964) Open-lung biopsy in diffuse pulmonary disease. N Engl J Med 270:1319–1331
63. Kalinske RW, Parker RH, Brandt D, Hoeprich PD (1965) Transtracheal aspiration in diagnosis of lower respiratory tract diseases. Antimicrob Agents Chemother (Bethesda) 5:30–36
73. Morrow GW Jr, Andersen HA, Geraci JE (1964) The diagnosis and management of acute infectious pneumonia. Med Clin North Am 48:829–838
74. Mulder J (1964) Clinical significance of bacteriologic examination of sputum in cases of acute and chronic bacterial disease of respiratory tract. Adv Intern Med 12:233–255
75. Shulman JA, Phillips LA, Petersdorf RG (1965) Errors and hazards in the diagnosis and treatment of bacterial pneumonias. Ann Intern Med 62:41–58
76. Manfredi F, Rosenbaum D, Behnke RH (1963) Percutaneous needle biopsy of the lung in diffuse pulmonary diseases. Ann Intern Med 58:773–778
77. Hewitt WL (1970) Bacteria in sputum–contaminants or culprits? Calif Med 112:60–62
78. Hoeprich PD (1970) Etiologic diagnosis of lower respiratory tract infections. Calif Med 112:1–8
79. Jackson C (1925) Bronchoscopy for disease. Br Med J 2:699
80. Wimberley N, Faling LJ, Bartlett JG (1979) A fiberoptic bronchoscopy technique to obtain uncontaminated lower airway secretions for bacterial culture. Am Rev Respir Dis 119:337–343
81. Thorpe JE, Baughman RP, Frame PT, Wesseler TA, Staneck JL (1987) Bronchoalveolar lavage for diagnosing acute bacterial pneumonia. J Infect Dis 155:855–861
82. Coster JF, Barrett-Connor E (1972) The nonvalue of sputum culture in the diagnosis of pneumonia. Am Rev Respir Dis 105:139–140
83. Ewig S, Schlochtermeier M, Goke N, Niederman MS (2002) Applying sputum as a diagnostic tool in pneumonia: limited yield, minimal impact on treatment decisions. Chest 121:1486–1492
84. Heineman HS, Chawla JK, Lopton WM (1977) Misinformation from sputum cultures without microscopic examination. J Clin Microbiol 6:518–527
85. Lenton JA, Lucks DA (1987) Nonvalue of sputum culture in the management of lower respiratory tract infections. J Clin Microbiol 25:758–762
86. Lidman C, Burman LG, Lagergren A, Ortvist A (2002) Limited value of routine microbiological diagnostics in patients hospitalized for community-acquired pneumonia. Scand J Infect Dis 34:873–879
87. Miyashita N, Shimizu H, Ouchi K et al (2008) Assessment of the usefulness of sputum Gram stain and culture for diagnosis of community-acquired pneumonia requiring hospitalization. Med Sci Monit 14:CR171–CR176
88. Theerthakarai R, El-Halees W, Ismail M, Solis RA, Khan MA (2001) Nonvalue of the initial microbiological studies in the management of nonsevere community-acquired pneumonia. Chest 119:181–184
89. Mandell LA, Wunderink RG, Anzueto A et al (2007) Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44(Suppl 2):S27–S72
90. Murdoch DR (2003) Nucleic acid amplification tests for the diagnosis of pneumonia. Clin Infect Dis 36:1162–1170, Epub 22 Apr 2003
91. Murdoch DR (2004) Molecular genetic methods in the diagnosis of lower respiratory tract infections. APMIS 112:713–727
92. Blackmore TK, Reznikov M, Gordon DL (1995) Clinical utility of the polymerase chain reaction to diagnose Mycoplasma pneumoniae infection. Pathology 27:177–181
93. Deng J, Zheng Y, Zhao R, Wright PF, Stratton CW, Tang YW (2009) Culture versus polymerase chain reaction for the etiologic diagnosis of community-acquired pneumonia in antibiotic-pretreated pediatric patients. Pediatr Infect Dis J 28:53–55
94. Jaulhac B, Nowicki M, Bornstein N et al (1992) Detection of Legionella spp. in bronchoalveolar lavage fluids by DNA amplification. J Clin Microbiol 30:920–924
95. Tong CY, Sillis M (1993) Detection of Chlamydia pneumoniae and Chlamydia psittaci in sputum samples by PCR. J Clin Pathol 46:313–317
96. Muller FM, Hoppe JE, Wirsing von Konig CH (1997) Laboratory diagnosis of pertussis: state of the art in 1997. J Clin Microbiol 35:2435–2443
97. van der Zee A, Agterberg C, Peeters M, Mooi F, Schellekens J (1996) A clinical validation of Bordetella pertussis and Bordetella parapertussis polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. J Infect Dis 174:89–96
98. Hauk L (2011) CDC releases best practices for the use of PCR testing for diagnosing pertussis. Am Fam Physician 84:1176
99. Yan Y, Zhang S, Tang YW (2011) Molecular assays for the detection and characterization of respiratory viruses. Semin Respir Crit Care Med 32:512–526, Epub 19 Aug 2011
100. Caliendo AM (2011) Multiplex PCR and emerging technologies for the detection of respiratory pathogens. Clin Infect Dis 52:S326–S330
101. Mahony JB (2008) Detection of respiratory viruses by molecular methods. Clin Microbiol Rev 21:716–747
102. Wu W, Tang YW (2009) Emerging molecular assays for detection and characterization of respiratory viruses. Clin Lab Med 29:673–693
103. IDSA (2011) An unmet medical need: rapid molecular diagnostics tests for respiratory tract infections. Clin Infect Dis 52:S384–S395
104. Tenover FC (2011) Developing molecular amplification methods for rapid diagnosis of respiratory tract infections caused by bacterial pathogens. Clin Infect Dis 52:S338–S345
105. Johansson N, Kalin M, Tiveljung-Lindell A, Giske CG, Hedlund J (2010) Etiology of community-acquired pneumonia: increased microbiological yield with new diagnostic methods. Clin Infect Dis 50:202–209
106. Oosterheert JJ, van Loon AM, Schuurman R et al (2005) Impact of rapid detection of viral and atypical bacterial pathogens by real-time polymerase chain reaction for patients with lower respiratory tract infection. Clin Infect Dis 41:1438–1444, Epub 13 Oct 2005
107. Cabello H, Torres A, Celis R et al (1997) Bacterial colonization of distal airways in healthy subjects and chronic lung disease: a bronchoscopic study. Eur Respir J 10:1137–1144
108. Huang YJ, Lynch SV (2011) The emerging relationship between the airway microbiota and chronic respiratory disease: clinical implications. Expert Rev Respir Med 5:809–821
109. Huang YJ, Kim E, Cox MJ et al (2010) A persistent and diverse airway microbiota present during chronic obstructive pulmonary disease exacerbations. Omics 14:9–59
110. Mourani PM, Harris JK, Sontag MK, Robertson CE, Abman SH (2011) Molecular identification of bacteria in tracheal aspirate fluid from mechanically ventilated preterm infants. PLoS One 6:e25959, Epub 10 Oct 2011
111. Cordero L, Ayers LW, Davis K (1997) Neonatal airway colonization with gram-negative bacilli: association with severity of bronchopulmonary dysplasia. Pediatr Infect Dis J 16:18–23
112. De Dooy J, Ieven M, Stevens W, Schuerwegh A, Mahieu L (2004) Endotracheal colonization at birth is associated with a pathogen-dependent pro- and antiinflammatory cytokine response in ventilated preterm infants: a prospective cohort study. Pediatr Res 56:547–552, Epub 4 Aug 2004
113. King P (2011) Pathogenesis of bronchiectasis. Paediatr Respir Rev 12:104–110, Epub 24 Nov 2010
114. Redding GJ (2009) Bronchiectasis in children. Pediatr Clin North Am 56:157–171, xi
115. Staffler P, Carr SB (2010) Non-cystic fibrosis bronchiectasis: its diagnosis and management. Arch Dis Child Educ Pract Ed 95:73–82
116. Sethi S, Maloney J, Grove L, Wrona C, Berenson CS (2006) Airway inflammation and bronchial bacterial colonization in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 173:991–998, Epub 10 Feb 2006
117. van Alphen L, Jansen HM, Dankert J (1995) Virulence factors in the colonization and persistence of bacteria in the airways. Am J Respir Crit Care Med 151:2094–2099, discussion 9–100
118. Murdoch DR, Jennings LC (2009) Association of respiratory virus activity and environmental factors with the incidence of invasive pneumococcal disease. J Infect 58:37–46, Epub 29 Nov 2008
119. Stensballe LG, Hjuler T, Andersen A et al (2008) Hospitalization for respiratory syncytial virus infection and invasive pneumococcal disease in Danish children aged <2 years: a population-based cohort study. Clin Infect Dis 46:1165–1171
120. Watson M, Gilmore R, Menzies R, Ferson M, McIntyre P (2006) The association of respiratory viruses, temperature, and other climatic parameters with the incidence of invasive pneumococcal disease in Sydney, Australia. Clin Infect Dis 42:211–215, Epub 12 Dec 2005
Interpretation and Relevance of Advanced Technique Results

121. Creer DD, Dilworth JP, Gillespie SH et al (2006) Aetiological role of viral and bacterial infections in acute adult lower respiratory tract infection (LRTI) in primary care. Thorax 61:75–79, Epub 14 Oct 2005

122. Johnstone J, Majumdar SR, Fox JD, Marrie TJ (2008) Viral infection in adults hospitalized with community-acquired pneumonia: prevalence, pathogens, and presentation. Chest 134:1141–1148, Epub 8 Aug 2008

123. Vu HT, Yoshida LM, Suzuki M et al (2011) Association between nasopharyngeal load of Streptococcus pneumoniae, viral coinfection, and radiologically confirmed pneumonia in Vietnamese children. Pediatr Infect Dis J 30:11–18

124. Bonfield JK, Staden R (1995) The application of numerical estimates of base calling accuracy to DNA sequencing projects. Nucleic Acids Res 23:1406–1410

125. Churchill GA, Waterman MS (1992) The accuracy of DNA sequences: estimating sequence quality. Genomics 14:89–98

126. Ewing B, Green P (1998) Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8:186–194

127. Richterich P (1998) Estimation of errors in “raw” DNA sequences: a validation study. Genome Res 8:251–259

128. Andre P, Caro V, Njamkepo E, Wendelboe AM, Van Rie A, Guiso N (2008) Comparison of serological and real-time PCR assays to diagnose Bordetella pertussis infection in 2007. J Clin Microbiol 46:1672–1677, Epub 26 Mar 2008

129. Loeffelholz MJ, Thompson CJ, Long KS, Gilchrist MJ (2000) Detection of Bordetella holmesii using Bordetella pertussis IS481 PCR assay. J Clin Microbiol 38:467

130. Register KB, Sanden GN (2006) Prevalence and sequence variants of IS481 in Bordetella bronchiseptica: implications for IS481-based detection of Bordetella pertussis. J Clin Microbiol 44:4577–4583, Epub 25 Oct 2006

131. Hoshiko M (1994) Laboratory diagnosis of infectious diarrhea. Pediatr Ann 23:570–574

132. Thielman NM, Guerrant RL (2004) Clinical practice. Acute infectious diarrhea. N Engl J Med 350:38–47

133. Guerrant RL, Van Gilder T, Steiner TS et al (2001) Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 32:331–351, Epub 30 Jan 2001

134. Pawlowski SW, Warren CA, Guerrant R (2009) Diagnosis and treatment of acute or persistent diarrhea. Gastroenterology 136:1874–1886, Epub 7 May 2009

135. Kufelnicka AM, Kirn TJ (2011) Effective utilization of evolving methods for the laboratory diagnosis of Clostridium difficile infection. Clin Infect Dis 52:1451–1457

136. Ryan ET, Madoff LC, Ferraro MJ (2011) Case records of the Massachusetts General Hospital. Case 20–2011. A 30-year-old man with diarrhea after a trip to the Dominican Republic. N Engl J Med 364:2536–2541

137. Patel MM, Hall AJ, Vinje J, Parashar UD (2009) Noroviruses: a comprehensive review. J Clin Virol 44:1–8, Epub 11 Dec 2008

138. Platts-Mills JA, Operario DJ, Houpt ER (2011) Molecular diagnosis of diarrhea: current status and future potential. Curr Infect Dis Rep 26:26

139. Echeverria P, Sethabutr O, Serichantaregs O (1993) Modern diagnosis (with molecular tests) of acute infectious diarrhea. Gastroenterol Clin North Am 22:661–682

140. Calderaro A, Gorrini C, Montecchini S et al (2010) Evaluation of a real-time polymerase chain reaction assay for the laboratory diagnosis of giardiasis. Diagn Microbiol Infect Dis 66:261–267, Epub 10 Nov 2009

141. Liu J, Gratz J, Maro A et al (2011) Simultaneous detection of six diarrhea-causing bacterial pathogens with an in house PCR-Luminex Assay. J Clin Microbiol 9:9

142. Liu J, Kibiki G, Maro V et al (2011) Multiplex reverse transcription PCR Luminex assay for detection and quantitation of viral agents of gastroenteritis. J Clin Virol 50:308–313, Epub 21 Jan 2011

143. O’Leary J, Corcoran D, Lucey B (2009) Comparison of the EntericBio multiplex PCR system with routine culture for detection of bacterial enteric pathogens. J Clin Microbiol 47:3449–3453, Epub 2 Sept 2009
144. Taniuchi M, Verweij JJ, Noor Z et al (2011) High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. Am J Trop Med Hyg 84: 332–337
145. Taniuchi M, Verweij JJ, Sethabutr O et al (2011) Multiplex polymerase chain reaction method to detect Cyclospora, Cystoisospora, and Microsporidia in stool samples. Diagn Microbiol Infect Dis 71:386–390, Epub 6 Oct 2011
146. Wolffs PF, Bruggeman CA, van Well GT, van Loo IH (2011) Replacing traditional diagnostics of fecal viral pathogens by a comprehensive panel of real-time PCRs. J Clin Microbiol 49:1926–1931, Epub 23 Mar 2011
147. Parfrey LW, Walters WA, Knight R (2011) Microbial eukaryotes in the human microbiome: ecology, evolution, and future directions. Front Microbiol 2:153, Epub 11 Jul 2011
148. Walter J, Ley R (2011) The human gut microbiome: ecology and recent evolutionary changes. Annu Rev Microbiol 65:411–429
149. Be Y, Li H, Lu X, Stratton CW, Tang YW (2010) Mass spectrometry biotyper system identifies enteric bacterial pathogens directly from colonies grown on selective stool culture media. J Clin Microbiol 48:3888–3892, Epub 15 Sept 2010
150. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J (2007) Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case–control Infectious Intestinal Disease Study (1993–1996). Eur J Clin Microbiol Infect Dis 26:311–323
151. Goldenberger D, Kunzli A, Vogt P, Zbinden R, Altwegg M (1997) Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. J Clin Microbiol 35:2733–2739
152. Khulordava I, Miller G, Haas D et al (2003) Identification of the bacterial etiology of culture-negative endocarditis by amplification and sequencing of a small ribosomal RNA gene. Diagn Microbiol Infect Dis 46:9–11
153. Kotilainen P, Heiro M, Jalava J et al (2006) Aetiological diagnosis of infective endocarditis by direct amplification of rRNA genes from surgically removed valve tissue. An 11-year experience in a Finnish teaching hospital. Ann Med 38:263–273
154. Lang S, Watkin RW, Lambert PA, Bonser RS, Littler WA, Elliott TS (2004) Evaluation of PCR in the molecular diagnosis of endocarditis. J Infect 48:269–275
155. Lisby G, Gutschik E, Durack DT (2002) Molecular methods for diagnosis of infective endocarditis. Infect Dis Clin North Am 16:393–412, x
156. Madershahian N, Strauch JT, Breuer M, Bruhin R, Straube E, Wahlers T (2005) Polymerase chain reaction amplification as a diagnostic tool in culture-negative multiple-valve endocarditis. Ann Thorac Surg 79:e21–e22
157. Mencacci A, Leli C, Cardaccia A et al (2011) Comparison of conventional culture with SeptiFast real-time PCR for microbial pathogen detection in clinical specimens other than blood. J Med Microbiol 60:1774–1778, Epub 11 Aug 2011
158. Tang YW (2009) Duplex PCR assay simultaneously detecting and differentiating Bartonella quintana, B. henselae, and Coxiella burnetii in surgical heart valve specimens. J Clin Microbiol 47:2647–2650, Epub 24 Jun 2009
159. Wilck MB, Wu Y, Howe JG, Crouch JY, Edberg SC (2001) Endocarditis caused by culture-negative organisms visible by Brown and Brenn staining: utility of PCR and DNA sequencing for diagnosis. J Clin Microbiol 39:2025–2027
160. Rahimian J, Raoult D, Tang YW, Hanna BA (2006) Bartonella quintana endocarditis with positive serology for Coxiella burnetii. J Infect 53:e151–e153, Epub 27 Dec 2005
161. Houptkian P, Raoult D (2003) Diagnostic methods. Current best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis. Cardiol Clin 21: 207–217
162. Louie L, Simor AE, Louie M, McGeer A, Low DE (1998) Diagnosis of group A streptococcal necrotizing fasciitis by using PCR to amplify the streptococcal pyrogenic exotoxin B gene. J Clin Microbiol 36:1769–1771
93547 Interpretation and Relevance of Advanced Technique Results

163. Muldrew KL, Simpson JF, Stratton CW, Tang YW (2005) Molecular diagnosis of necrotizing fasciitis by 16 S rRNA gene sequencing and superantigen gene detection. J Mol Diagn 7: 641–645

164. Bialek R, Feucht A, Aepinus C et al (2002) Evaluation of two nested PCR assays for detection of Histoplasma capsulatum DNA in human tissue. J Clin Microbiol 40: 1644–1647

165. Bialek R, Ibricevic A, Aepinus C et al (2000) Detection of Paracoccidioides brasilensis in tissue samples by a nested PCR assay. J Clin Microbiol 38: 2940–2942

166. Bialek R, Konrad F, Kern J et al (2005) PCR based identification and discrimination of agents of mucormycosis and aspergillosis in paraffin wax embedded tissue. J Clin Pathol 58: 1180–1184

167. Chen SC, Halliday CL, Meyer W (2002) A review of nucleic acid-based diagnostic tests for systemic mycoses with an emphasis on polymerase chain reaction-based assays. Med Mycol 40: 333–357

168. Hammond SP, Bialek R, Milner DA, Petschnigg EM, Baden LR, Marty FM (2011) Molecular methods to improve diagnosis and identification of mucormycosis. J Clin Microbiol 49: 2151–2153, Epub 20 Apr 2011

169. Lass-Florl C, Follett SA, Moody A, Denning DW (2011) Detection of Aspergillus in lung and other tissue samples using the MycAssay Aspergillus real-time PCR kit. Can J Microbiol 57: 765–768, Epub 23 Aug 2011

170. Rickerts V, Just-Nubling G, Konrad F et al (2006) Diagnosis of invasive aspergillosis and mucormycosis in immunocompromised patients by seminested PCR assay of tissue samples. Eur J Clin Microbiol Infect Dis 25: 8–13

171. Rickerts V, Khot PD, Myerson D, Ko DL, Lambrecht E, Fredricks DN (2011) Comparison of quantitative real time PCR with Sequencing and ribosomal RNA-FISH for the identification of fungi in formalin fixed, paraffin-embedded tissue specimens. BMC Infect Dis 11: 202

172. Rickerts V, Loeffler J, Bohme A, Einsele H, Just-Nubling G (2001) Diagnosis of disseminated zygomycosis using a polymerase chain reaction assay. Eur J Clin Microbiol Infect Dis 20: 744–745

173. Rickerts V, Mousset S, Lambrecht E et al (2007) Comparison of histopathological analysis, culture, and polymerase chain reaction assays to detect invasive mold infections from biopsy specimens. Clin Infect Dis 44: 1078–1083, Epub 5 Mar 2007

174. Bialek R, Gonzalez GM, Begerow D, Zelck UE (2005) Coccidioidomycosis and blastomycosis: advances in molecular diagnosis. FEMS Immunol Med Microbiol 45: 355–360

175. Bhatnagar J, Guarnier J, Paddock CD et al (2007) Detection of West Nile virus in formalin-fixed, paraffin-embedded human tissues by RT-PCR: a useful adjunct to conventional tissue-based diagnostic methods. J Clin Virol 38: 106–111, Epub 8 Dec 2006

176. Boyd AS, Stasko TS, Tang YW (2011) Basaloid squamous cell carcinoma of the skin. J Am Acad Dermatol 64: 144–151

177. Sato M, Li H, Ikizler MR et al (2009) Detection of viruses in human adenoid tissues by use of multiplex PCR. J Clin Microbiol 47: 771–773, Epub 30 Dec 2008

178. Smeets SJ, Hesseling AT, Speel EJ et al (2007) A novel algorithm for reliable detection of human papillomavirus in paraffin embedded head and neck cancer specimen. Int J Cancer 121: 2465–2472

179. Ullrich T, Tang YW, Correa H, Hill M, Matta P, Weitkamp JH (2012) Absence of gastrointestinal pathogens in ileum tissue resected for necrotizing enterocolitis. Pediatr Infect Dis J 31(4): 413–414

180. Vogt S, Schneider-Stock R, Klauck S, Roessner A, Rocken C (2003) Detection of hepatitis C virus RNA in formalin-fixed, paraffin-embedded thin-needle liver biopsy specimens. Am J Clin Pathol 120: 536–543

181. Lau A, Chen S, Sorrell T et al (2007) Development and clinical application of a panfungal PCR assay to detect and identify fungal DNA in tissue specimens. J Clin Microbiol 45: 380–385, Epub 22 Nov 2006

182. Mrazek C, Lass-Florl C (2011) Biopsy procedures for molecular tissue diagnosis of invasive fungal infections. Curr Infect Dis Rep 13: 504–509
183. Cabaret O, Toussain G, Abermil N et al (2011) Degradation of fungal DNA in formalin-fixed paraffin-embedded sinus fungal balls hampers reliable sequence-based identification of fungi. Med Mycol 49:329–332, Epub 18 Oct 2010
184. Foss RD, Guha-Thakurta N, Conran RM, Gutman P (1994) Effects of fixative and fixation time on the extraction and polymerase chain reaction amplification of RNA from paraffin-embedded tissue. Comparison of two housekeeping gene mRNA controls. Diagn Mol Pathol 3:148–155
185. Landlinger C, Preuner S, Willinger B et al (2009) Species-specific identification of a wide range of clinically relevant fungal pathogens by use of Luminex xMAP technology. J Clin Microbiol 47:1063–1073, Epub 25 Feb 2009
186. CLSI (2008) Interpretive criteria for identification of bacteria and fungi by DNA target sequencing: approved guideline. Clinical and Laboratory Standards Institute, Wayne, PA
187. Munoz-Cadavid C, Rudd S, Zaki SR et al (2010) Improving molecular detection of fungal DNA in formalin-fixed paraffin-embedded tissues: comparison of five tissue DNA extraction methods using panfungal PCR. J Clin Microbiol 48:2147–2153, Epub 14 Apr 2010
188. Wolff TY, Moser C, Bundgaard H, Holby N, Nielsen PH, Thomsen TR (2011) Detection of microbial diversity in endocarditis using cultivation-independent molecular techniques. Scand J Infect Dis 43:857–869, Epub 26 Aug 2011
189. Burd EM (2010) Validation of laboratory-developed molecular assays for infectious diseases. Clin Microbiol Rev 23:550–576
190. Pitt TL, Saunders NA (2000) Molecular bacteriology: a diagnostic tool for the millennium. J Clin Pathol 53:71–75
191. Borst A, Box AT, Fluit AC (2004) False-positive results and contamination in nucleic acid amplification assays: suggestions for a prevent and destroy strategy. Eur J Clin Microbiol Infect Dis 23:289–299, Epub 10 Mar 2004
192. Aslanzadeh J (2004) Preventing PCR amplification carryover contamination in a clinical laboratory. Ann Clin Lab Sci 34:389–396
193. Nikkari S, McLaughlin IJ, Bi W, Dodge DE, Relman DA (2001) Does blood of healthy subjects contain bacterial ribosomal DNA? J Clin Microbiol 39:1956–1959
194. Wolk D, Mitchell S, Patel R (2001) Principles of molecular microbiology testing methods. Infect Dis Clin North Am 15:1157–1204
195. Yang S, Rothman RE (2004) PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. Lancet Infect Dis 4:337–348