The role of DNA amplification technology in the diagnosis of infectious diseases

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Abstract

NUCLEIC ACID AMPLIFICATION AND DETECTION METHODS developed in the past decade are useful for the diagnosis and management of a variety of infectious diseases. The most widely used of these methods is the polymerase chain reaction (PCR). PCR assays can detect rapidly and accurately the presence of fastidious and slow-growing microorganisms, such as *Chlamydia*, mycoplasmas, mycobacteria, herpesviruses and enteroviruses, directly from clinical specimens. Commercial PCR assays for the diagnosis of tuberculosis and genital *C. trachomatis* infection are now routinely used in many diagnostic laboratories. Assays have also been developed that can detect antimicrobial resistance and are used to identify the cause of infection by organisms that cannot be cultivated. The value of viral load measurement by nucleic acid amplification in the management of patients with HIV infection or hepatitis C has also been well established. However, evaluations of this technology for rapid microbial diagnosis have generally been limited by small samples, and the cost of these assays may be as high as Can$125 per test. As nucleic acid amplification methods continue to evolve, their role in the diagnosis and management of patients with infectious diseases and their impact on clinical outcomes will become better defined.

Cases

A 58-year-old woman is being assessed for a 4-week history of low-grade fever and cough. A chest radiograph indicates the presence of disease in the left upper lobe airspace. Microscopic examination of a sputum specimen reveals a moderate number of acid-fast bacilli. Does this represent tuberculosis or the presence of nontuberculous mycobacteria?

A 19-year-old student is admitted to hospital with meningitis. Before her admission she had received 3 courses of oral cefaclor therapy. In consequence her blood and cerebrospinal fluid cultures are negative. She is responding to empiric antimicrobial therapy. Should her family or her roommates receive chemoprophylaxis for possible exposure to *Neisseria meningitidis*?

A 60-year-old man is admitted to hospital with the onset of encephalitis. Should he receive high-dose intravenous acyclovir therapy for presumed infection with herpes simplex virus?

Each of these clinical scenarios presents the medical practitioner with a problem that involves establishing a diagnosis of infection in a setting where routine laboratory investigations are likely to be nondiagnostic or will not provide results in a timely manner. In the past decade molecular techniques have been developed that allow the amplification and detection of minute amounts of nucleic acid sequences from tissues or body fluids. These nucleic acid amplification methods can create millions of identical copies of a DNA or RNA "target" sequence in a matter of hours. The ability to determine whether specific DNA or RNA sequences are present in clinical samples using molecular technology has dramatically changed our approach to the laboratory diagnosis of many diseases. For example, these methods have been useful in the diagnosis of genetic disorders such as sickle cell anemia, β-thalassemia and cystic fibrosis.[1] Recently the development of nucleic acid amplification technology has also had a significant impact on the diagnosis and management of many infectious diseases, including those represented by the 3 hypothetical cases described here.[2]

Several strategies for the amplification of nucleic acids have been described, including amplification of the nucleic acid target (e.g., polymerase chain reaction [PCR], strand-displacement amplification, self-sustaining sequence replication), amplification of a nucleic acid probe (e.g., ligase chain reaction, Qβ replicase) and signal amplification (e.g., branched-probe DNA assay). PCR is now the most widely used amplification method.

As these molecular methods are further refined and become more widely available in the next few years, physicians will need to understand their clinical applications and be aware of their potential advantages, limitations and clinical utility. In this paper we describe the principles behind PCR-based diagnosis and its applications for the diagnosis of infectious diseases. We review PCR tests that are currently available commercially and discuss assays that are under development. However, it is beyond the scope of this article to describe other nucleic acid amplification methods or to include a complete list of all PCR assays that have been developed; other recent reviews offer additional details.[2-4]

Polymerase chain reaction

PCR can amplify minute amounts of target DNA within a few hours.[1–3] Applications in microbiology and infectious diseases have included the diagnosis of infection due to slow-growing or fastidious microorganisms, detection of infectious agents that cannot be cultured and rapid identification of antimicrobial resistance.

The essential materials, reagents and equipment required for nucleic acid amplification and detection by PCR are summarized in Table 1. Nucleic acid amplification is

Table 1: Reagents and equipment required for the polymerase chain reaction (PCR)

PCR target or "template"	The segment of nucleic acid (DNA or RNA) that is to be amplified
Nucleotides	Building blocks from which nucleic acids are constructed: adenine, guanine, cytosine, thymine and uracil
Primer	A short sequence of nucleotides complementary to, and binding (annealing) to, known sequences of the target nucleic acid; essential for "priming" the amplification reaction
Taq DNA polymerase	A heat-stable enzyme that makes a new complementary copy of the target nucleic acid by adding nucleotides to the annealed primer
Reverse transcriptase	An enzyme that converts RNA into a complementary DNA sequence (used in reverse transcription PCR)
Thermocycler	The equipment in which PCR reactions occur; it is able to change rapidly to the different temperatures required for repeated PCR cycles

performed in a thermocycler, which is an instrument that can hold the assay's reagents and allows the reactions to occur at the various temperatures required. In the initial step of the procedure, nucleic acid (e.g., DNA) is extracted from the microorganism or clinical specimen of interest. Heat (90°C–95°C) is used to separate the extracted double-stranded DNA into single strands (denaturation). Cooling to 55°C then allows primers specifically designed to flank the target nucleic acid sequence to adhere to the target DNA (annealing). Following this, the enzyme *Taq* polymerase and nucleotides are added to create new DNA fragments complementary to the target DNA (extension). This completes one cycle of PCR. This process of denaturation, annealing and extension is repeated numerous times in the thermocycler. At the end of each cycle each newly synthesized DNA sequence acts as a new target for the next cycle, so that after 30 cycles millions of copies of the original target DNA are created (Fig. 1). The result is the accumulation of a specific PCR product with sequences located between the 2 flanking primers.

Detection of the amplified products can be done by visualization with agarose gel electrophoresis, by an enzyme immunoassay format using probe-based colorimetric detection or by fluorescence emission technology. In multiplex PCR the assay is modified to include several primer pairs specific to different DNA targets to allow amplification and detection of several pathogens at the same time.

Reverse transcription PCR is a modification of this method used when the initial template is RNA rather than DNA. In this case the enzyme reverse transcriptase first converts the RNA target into a complementary DNA copy (cDNA). This cDNA can then be amplified by standard PCR methods as described earlier. Reverse transcription PCR can be used to amplify the much higher numbers of copies of messenger or ribosomal RNA than the number of DNA copies present in bacteria or fungi, and it may detect specific expression of certain genes during the course of infection. The detection of cDNA using reverse transcription PCR of messenger RNA encoded by a pathogen could be evidence of active infection,[5] in contrast to the detection of DNA from nonviable organisms using standard PCR.

Diagnosis of infectious diseases

Examples of infectious agents that have been detected by nucleic acid amplification assays are summarized in Table 2. Assays that are currently available commercially for use in diagnostic laboratories include tests for the detection of *Chlamydia trachomatis*,[9–12] *C. pneumoniae*,[8] *Mycobacterium tuberculosis*,[14,15] *Mycoplasma pneumoniae*,[17] *Neisseria gonorrhoeae*,[19] herpes simplex virus[30] and cytomegalovirus.[24] In addition there are PCR assays available for monitoring the viral load of HIV,[31–33] hepatitis C virus[29] and hepatitis B virus.[28] Unfortunately only a few of these commercially available assays have been extensively evaluated to determine their sensitivity, specificity or clinical utility. Two tests that have
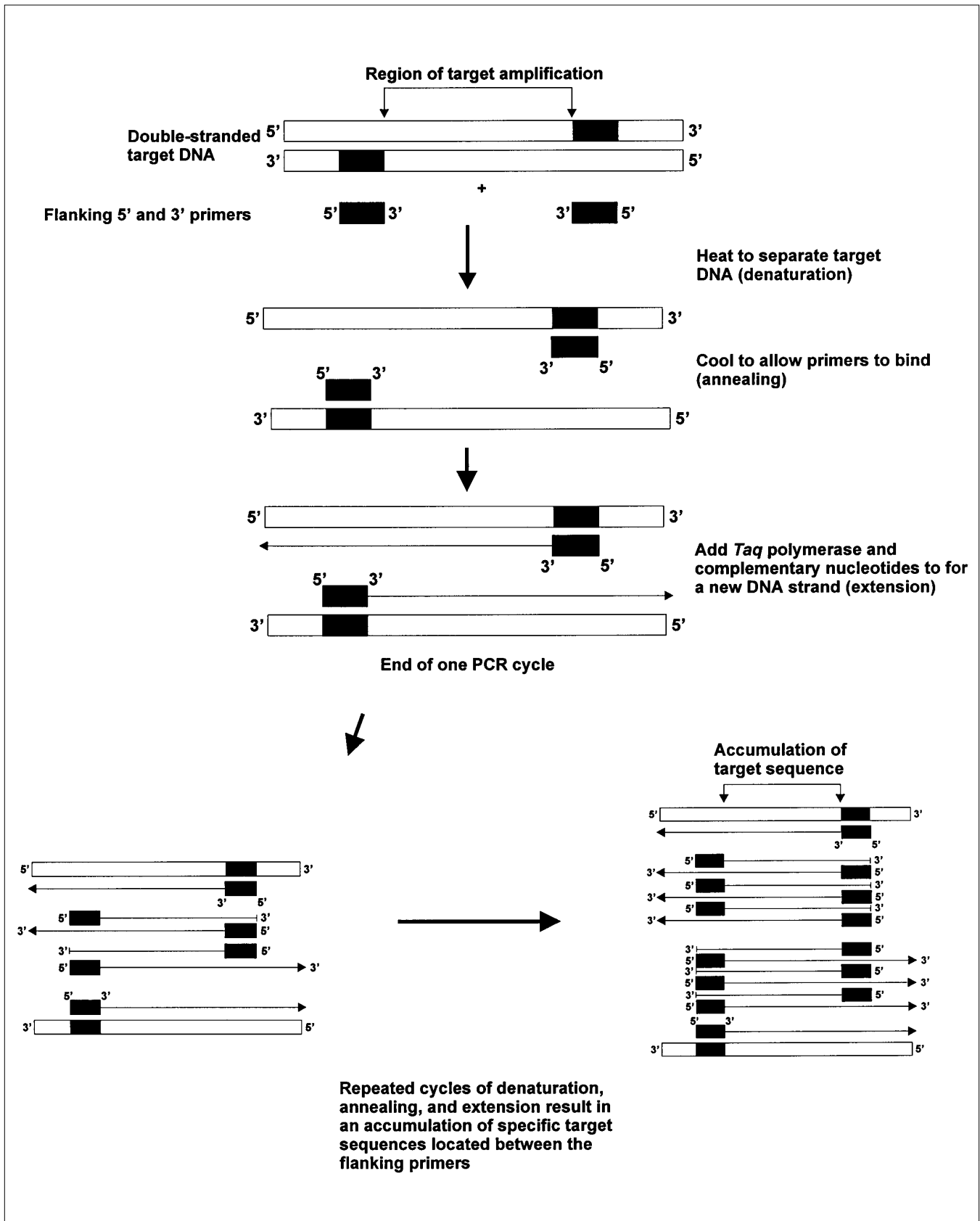
Region of target amplification

Double-stranded target DNA

Flanking 5' and 3' primers

+

Heat to separate target DNA (denaturation)

Cool to allow primers to bind (annealing)

Add *Taq* polymerase and complementary nucleotides to for a new DNA strand (extension)

End of one PCR cycle

Accumulation of target sequence

Repeated cycles of denaturation, annealing, and extension result in an accumulation of specific target sequences located between the flanking primers

Fig. 1: Schematic representation of the polymerase chain reaction (PCR).

undergone such evaluations, and are currently among the most widely used PCR assays in diagnostic microbiology laboratories, are nucleic acid amplification assays for the detection of *C. trachomatis* and *M. tuberculosis* from clinical specimens.

One of the earliest commercial tests to become available was a PCR assay for the diagnosis of *C. trachomatis* genital tract infection. *C. trachomatis* is a fastidious microorganism, requiring specialized tissue culture facilities for laboratory isolation. Direct antigen detection of the organism by enzyme immunoassay or direct immunofluorescence is technically easier than culture but may lack sensitivity and specificity.[12,39] PCR assays have been found to be significantly more accurate, with sensitivities of 90%–100% and specificities greater than 97% for the detection of *C. trachomatis* from cervical or urethral specimens.[9-11] The positive predictive values reported in these studies ranged from 89% to 100%. A major advantage of these tests is the ability to detect *Chlamydia* in urine specimens. PCR testing of freshly voided urine was found to be the most sensitive (91%) and specific (100%) method for detecting asymptomatic *C. trachomatis* infection in men.[12] In addition, these assays have been automated, allowing for the processing of large numbers of specimens. They may be used for diagnosis or STD screening. A coamplification PCR assay for the direct detection of both *N. gonorrhoeae* and *C. trachomatis* from patients with STD has also been developed.[19] The sensitivity and specificity of PCR detection of *N. gonorrhoeae* from cervical and urethral specimens were found to be greater than 90% and 96% respectively.[19]

Direct amplification tests have also had a great impact on the rapid diagnosis of tuberculosis. Conventional culture methods for the isolation of mycobacteria generally take several weeks. Commercial amplification assays have been developed to provide accurate same-day results directly from clinical specimens.[14,15,40] These methods have been found to have sensitivities of about 90%–98%, as compared with culture of specimens that are smear-positive for acid-fast bacilli.[14,15] However, the performance of these amplification assays has been suboptimal for specimens without acid-fast bacilli seen on direct microscopic examination, with reported sensitivities as low as 46%.[15,41,42] The specificity of PCR-based assays for *M. tuberculosis* is excellent (> 98%).[14,15,42] Although these assays cannot replace mycobacterial cultures, their ability to determine rapidly the presence of *M. tuberculosis* directly from respiratory tract specimens has enabled more rapid institution of effective therapy and implementation of important infection control and public health interventions.

Nucleic acid amplification assays for the detection of viruses, such as herpes simplex virus, cytomegalovirus, enteroviruses and HIV, have proved to be useful for screening and for diagnosis and management. The Canadian Blood Services has recently adopted nucleic acid amplification methods to screen donated blood for hepatitis C and HIV because of the enhanced sensitivities of these assays. PCR detection of herpes simplex virus in cerebrospinal fluid has become the method of choice for the diagnosis of herpes encephalitis, with sensitivity and specificity of 95% and 94% respectively,[30] obviating the need for a brain biopsy.[2,30,43] Enteroviruses are among the most common causes of aseptic meningitis. PCR for the diagnosis of enteroviral meningitis using cerebrospinal fluid samples has been found to be significantly more sensitive than conventional viral isolation (14% of specimens positive v. 10% positive respectively).[26,27] Moreover, the PCR assay can be completed within 1 day, whereas cultures for enteroviruses typically require up to 5

Table 2: Selected clinical applications of DNA amplification technology in infectious diseases and microbiology

Pathogens	Assay commercially available?	References
Bacteria		
Bordetella pertussis	No	Müller et al[6]
Borrelia burgdorferi	Yes	Brettschneider et al[7]
Chlamydia pneumoniae	Yes	Dalhoff et al[8]
Chlamydia trachomatis	Yes	Vincelette et al[9] Pasternack et al[10] Puolakkainen et al[11] Toye et al[12]
Escherichia coli O157:H7	No	Louie et al[13]
Mycobacterium tuberculosis	Yes	Piersimoni et al[14] D'Amato et al[15]
Mycobacterium avium complex	Yes	MacGregor et al[16]
Mycoplasma spp.	Yes	De Barbeyrac et al[17] Luki et al[18]
Neisseria gonorrhoeae	Yes	Crotchfelt et al[19]
Streptococcus pneumoniae	No	Matsumura et al[20] Chierian et al[21] Kearns et al[22]
Streptococcus pyogenes	No	Louie et al[23]
Viruses		
Cytomegalovirus	Yes	Long et al[24] Pellegrin et al[25]
Enterovirus	Yes	Van Vliet et al[26] Hadziyannis et al[27]
Hepatitis B virus	Yes	Pawlotsky et al[28]
Hepatitis C virus	Yes	Albadalejo et al[29]
Herpes simplex virus	Yes	Lakeman et al[30]
HIV	Yes	Nolte et al[31] Pachl et al[32] Segondy et al[33]
Fungi and parasites		
Cryptococcus neoformans	No	Vilgalys et al[34]
Plasmodium falciparum	No	Zhong et al[35]
Pneumocystis carinii	No	Helweg-Larsen et al[36]
Toxoplasma gondii	Yes	Burg et al[37]
Trichomonas vaginalis	No	Madico et al[38]

days for isolation of the virus. A PCR assay for cytomegalovirus is available for detection of the virus in plasma or cerebrospinal fluid specimens and has been useful in monitoring HIV and bone marrow transplant patients with cytomegalovirus infection. The performance of this test has been comparable to that of antigen assays, with reported sensitivities and specificities of 95%–98% and 98%–100% respectively.[24,25] In contrast, the sensitivity of culture detection of cytomegalovirus was only 42%.[24]

In addition to these diagnostic applications, nucleic acid amplification procedures have also been modified to allow for the quantitative measurement of viral load in order to monitor response to therapy for patients with HIV, cytomegalovirus or hepatitis C virus infection.[25,29,31–33,44,45] For example, measuring HIV viral load in serum has had a major impact on the management of HIV-infected people. Viral load measurement is of prognostic importance, predicting progression of the disease, and is used to assist in making treatment decisions.[44,45]

A number of PCR assays that are not available commercially have potentially useful applications for the diagnosis of a variety of infectious diseases (Table 2).[6,13,20–23,34–36,38] Many of these tests are likely to become available in the near future. Multiplex PCR-based assays have been developed and have the advantage of detecting multiple pathogens in a single PCR reaction. These have been used to detect common bacterial and viral causes of respiratory tract infections,[8,46–49] bacteremia[50,51] and meningitis.[20–22,52,53]

PCR technology has also been used to identify infection owing to organisms that cannot be cultured. In order to accomplish this, investigators took advantage of the observation that portions of bacterial 16S ribosomal RNA sequences are highly conserved, whereas other regions are less well conserved and are species-specific. PCR amplification of 16S rRNA sequences of bacteria that cannot be cultured from tissues of patients with diseases such as Whipple's disease and bacillary angiomatosis allowed the discovery and identification of the etiologic agents.[54,55] Furthermore, using nucleic acid amplification methods, diseases previously thought to be noninfectious have been linked to infectious agents.[56]

Detection of antimicrobial resistance

As many of the genetic mechanisms of antimicrobial resistance have become better understood, nucleic acid amplification methods have proved to be useful for the confirmation of antimicrobial resistance in laboratory isolates and for the direct detection of such resistance in clinical specimens.[57] Conventional culture and susceptibility test procedures for most pathogenic bacteria generally take 48–72 hours. The performance of these tests may be erratic because factors such as inoculum size or variability in culture conditions may affect phenotypic expression of resistance. Amplification of genetic determinants may therefore be used to confirm antimicrobial resistance based on the organism's genotype rather than relying on the variability of phenotypic expression of the resistance (Table 3). More-

Table 3: PCR-based nucleic acid amplification for detection of antimicrobial resistance

Organism	Antimicrobial resistance	Gene targets for nucleic acid amplification (references)
Methicillin-resistant *Staphylococcus aureus* and coagulase-negative staphylococci	Methicillin and all other β-lactam antibiotics	*mecA* (Vannuffel et al;[58] Murakami et al[59])
Vancomycin-resistant *Enterococcus* spp.	Vancomycin	*vanA, vanB, vanC1, vanC2, vanC3* (Satake et al;[60] Dutka-Malen et al;[61] Patel et al[62])
Streptococcus pneumoniae	Penicillin	*pbp1A* (du Plessis et al[63])
Enterobacteriaceae-producing extended-spectrum β-lactamase	Extended-spectrum penicillins and cephalosporins	SHV and TEM β-lactamase gene sequences (Arlet et al[64])
Mycobacterium tuberculosis	Isoniazid	*katG, inhA, ahpC* (Nachamkin et al[65])
	Rifampin	*rpoB* (Nachamkin et al;[65] Telenti et al[66])
Herpes simplex virus	Acyclovir	Thymidine kinase gene sequences (Sasadeusz et al[67])
Cytomegalovirus	Ganciclovir	Viral phosphotransferase gene (UL97), DNA polymerase gene (UL54) (Smith et al[68])
HIV	Reverse transcriptase inhibitors	Reverse transcriptase gene (Stuyver et al[69])
	Protease inhibitors	Protease gene (Vasudevachari et al[70])

over, these tests can be done within hours, providing clinically relevant information days before conventional susceptibility test results become available. Molecular assays to detect antimicrobial resistance directly from clinical samples have also been described.[58,60]

PCR-based methods for the detection of antimicrobial resistance have been applied to bacteria including methicillin-resistant *Staphylococcus aureus*,[58,59] vancomycin-resistant enterococci[60–62] and multidrug-resistant *M. tuberculosis*.[65,66] Detection of resistance to antiviral agents by molecular methods has also been described for acyclovir-resistant herpesviruses[67] and HIV resistant to reverse transcriptase inhibitors[69] and to protease inhibitors.[70] Currently none of these assays are available commercially, but they have been used in a number of reference and research laboratories.

The identification of methicillin resistance in *S. aureus* represents an ideal application of nucleic acid amplification methods. Methicillin-resistant *S. aureus* is an important hospital-acquired pathogen capable of causing life-threatening infections and nosocomial outbreaks. The incidence of infections from this pathogen in Canadian hospitals has increased dramatically in the past few years. Thus, the rapid and accurate identification of the pathogen is critical for patient management and for infection control programs in hospitals. However, the reliable detection of methicillin-resistant *S. aureus* using culture and susceptibility tests may be problematic because expression of resistance is usually heterogeneous and is influenced by culture conditions, especially in strains with low-level resistance.[71] All strains of methicillin-resistant *S. aureus* produce a unique penicillin-binding protein (PBP2′) that is encoded by a chromosomal gene, *mecA*. The *mecA* gene is not present in susceptible strains. PCR has been used successfully to amplify and detect *mecA* gene sequences from clinical isolates within a few hours.[59,72,73] These methods have also been used to detect methicillin-resistant *S. aureus* directly from clinical specimens such as blood cultures[74] and endotracheal aspirates.[58]

Vancomycin-resistant enterococci have also emerged as important nosocomial pathogens in North American hospitals. Identification using culture and susceptibility tests is even more problematic than that of methicillin-resistant *S. aureus*, primarily because of difficulties in detecting low levels of resistance[75] and because accurate identification using conventional laboratory procedures may take as long as 4–6 days. Vancomycin resistance in enterococci is mediated by one of several genes: *vanA*, *vanB*, *vanB2*, *vanC1*, *vanC2*, *vanC3* or *vanD*. PCR assays have been developed to recognize the *vanA*, *vanB* and *vanC* genotypes and have demonstrated value in characterizing enterococci in the laboratory when conventional laboratory test results have been inconclusive.[62,76] Another potential use of the assay is to assist in epidemiologic studies in the setting of an outbreak.[77] Finally, the ability to detect rapidly and accurately vancomycin-resistant enterococci directly from rectal swab specimens has also been reported.[60]

Incorporation of DNA amplification technology into the diagnostic microbiology laboratory

Newer DNA amplification methods have the potential to significantly influence the diagnosis and management of a variety of infectious diseases. Conventional laboratory diagnostic methods require a minimum of 24 hours, and in many cases significantly longer. Moreover, cultures may yield no bacterial growth if there has been a delay in transporting the specimen to the laboratory, if the number of viable infecting organisms is low, or if the patient was taking antibiotics by the time the culture specimen was obtained. Certain pathogenic organisms, such as *Mycoplasma* species, *Chlamydia* species, rickettsia and viruses, are not easily detected by routine culture methods and require specialized procedures. Rapid nonculture diagnostic tests relying on antigen detection by immunofluorescence or enzyme immunoassay, or using DNA probes, may have variable diagnostic sensitivities or specificities as compared with culture. Molecular methods with amplification and detection of target nucleic acids have generally been found to have superior sensitivity and specificity and have the potential to provide results within hours of collecting the specimen. As described here, currently available commercial tests using PCR for the diagnosis of infections include those able to detect *C. trachomatis*, *M. tuberculosis*, HIV, herpes simplex virus, cytomegalovirus, enterovirus, hepatitis C virus and other infectious agents. Many of these assays are now routinely being used in clinical microbiology laboratories. Diagnostic test kits for many other infectious agents are under development. Pilot studies have indicated the feasibility of designing broad-range multiplex PCR assays with the capability of detecting a panel of microorganisms from clinical specimens.[48,51,78,79] PCR-based methods have also been found to identify accurately antimicrobial resistance in clinical isolates and directly from patient specimens.[57,58,60,74]

Despite the obvious advantages to these newer procedures, there may be potential limitations to DNA amplification technology in the diagnostic microbiology laboratory (Table 4). The accuracy and reproducibility of PCR assays depend on the technical expertise and experience of the operator. Specificity of the test may be affected by contamination of the specimen during laboratory processing, if nonspecific primers are selected for the assay or if PCR conditions are not optimal, allowing nonspecific products to amplify. The most common sources of contamination are from other samples or from previous amplification procedures. Contamination or amplification product carry-over of even minute amounts of nucleic acid may result in the generation of billions of DNA copies that may lead to a false-positive test result. For this reason laboratories should have separate rooms for different steps of the PCR procedure and must follow stringent quality control measures to prevent contamination or carry-over. False-negative test

results may occur because of the presence of substances in the specimen that inhibit nucleic acid extraction or amplification. Certain specimen types (e.g., blood) are more likely to contain such inhibitors. The assays may also lack sensitivity if there is a low inoculum of the microorganism present in the clinical specimen. This may be exacerbated if an inadequate sample or very small specimen volume (i.e., < 20 μL) is available for testing.

Interpretation of nucleic acid amplification test results is not always clear-cut. For example, assays may detect the residual DNA of a pathogenic microorganism even after successful treatment,[80] and it is not clear whether this represents the presence of a small number of viable organisms or amplified DNA from nonviable organisms. Therefore, PCR tests should not be used to monitor the effectiveness of a course of therapy,[39] and physicians must be aware of the laboratory testing procedures. In addition, the meaning of a positive PCR test result has not been validated for all infections. For example, it is uncertain whether a positive PCR test result for cytomegalovirus from a patient's serum represents active disease or latent infection. Similarly, detection of pneumococcal DNA in blood samples has been reported in asymptomatic children colonized with *S. pneumoniae*[81] and therefore may not always indicate an invasive infection. These observations suggest that there is a need for interpretive guidelines based on a correlation of nucleic acid amplification test results with clinical outcome.

Finally, it must be acknowledged that performance of a PCR assay is generally more expensive than conventional diagnostic laboratory methods. The requirement of separate rooms for pre-PCR and post-PCR steps in order to reduce the risk of cross-contamination means that molecular laboratories use a disproportionate amount of laboratory space. There are capital costs associated with the initial equipment purchase (about Can$15 000), reagent costs for each clinical and control sample processed (Can$8–$40) and labour expenses. Therefore, the cost of these assays has been reported to be as high as Can$125 per test.[2]

Molecular technology involving nucleic acid amplifica-

tion and detection is a promising tool for the rapid and accurate diagnosis of a variety of infectious diseases, and for the confirmation or detection, or both, of antimicrobial resistance (Table 4). Some of these tests are now widely used for the diagnosis of tuberculosis and *C. trachomatis* infection, and other assays have become important in the management of HIV infection and hepatitis C. A large number of PCR assays are still under development with the potential to provide accurate and rapid results when conventional methods are either not available, insensitive or too slow. To date, evaluations of this technology have generally been limited by small samples and have not considered how these assays should fit into routine laboratory procedures, particularly in smaller, nonreference laboratories. As this technology continues to evolve, it will be important to assess the cost-effectiveness of these procedures and their real impact on patient management and outcomes.

Competing interests: None declared.

References

1. Saiki RK, Scharf SJ, Faloona F, Mullis KB, Horn GT, Erlich HA, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985;230:1350-4.
2. Fredricks DN, Relman DA. Application of polymerase chain reactions to the diagnosis of infectious diseases. *Clin Infect Dis* 1999;29:475-88.
3. Birkenmeyer LG, Mushahwar IK. DNA probe amplification methods. *J Virol Methods* 1991;35:117-26.
4. Tang YW, Procop GW, Persong DH. Molecular diagnostics of infectious diseases. *Clin Chem* 1997;43:2021-38.
5. Naber SP. Molecular pathology — diagnosis of infectious disease. *N Engl J Med* 1994;331:1212-5.
6. Müller FMC, Hoppe JE, von König CHW. Laboratory diagnosis of pertussis: state of the art in 1997. *J Clin Microbiol* 1997;35:2435-43.
7. Brettschneider S, Bruckbauer H, Klugbauer N, Hofmann H. Diagnostic value of PCR for detection of *Borrelia burgdorferi* in skin biopsy and urine samples from patients with skin borreliosis. *J Clin Microbiol* 1998;36:2658-65.
8. Dalhoff K, Maass M. *Chlamydia pneumoniae* pneumonia in hospitalized patients. Clinical characteristics and diagnostic value of polymerase chain reaction detection in BAL. *Chest* 1996;110:351-6.
9. Vincelette J, Schirm J, Bogard M, Bourgault AM, Luijt DS, Bianchi A, et al. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR Test for detection of *Chlamydia trachomatis* in urogenital specimens. *J Clin Microbiol* 1999;37:74-80.
10. Pasternack R, Vuorinen P, Pitkäjärvi T, Koskela M, Miettinen A. Comparison of manual Amplicor PCR, Cobas Amplicor PCR, and LCx assays for detection of *Chlamydia trachomatis* infection in women by using urine specimens. *J Clin Microbiol* 1997;35:402-5.
11. Puolakkainen M, Hiltunen-Back E, Reunala T, Suhonen S, Lähteenmäki P, Lehtinen M, et al. Comparison of performances of two commercially available tests, a PCR assay and a ligase chain reaction test, in detection of urogenital *Chlamydia trachomatis* infection. *J Clin Microbiol* 1998;36:1489-93.
12. Toye B, Peeling RW, Jessamine P, Claman P, Gemmill I. Diagnosis of *Chlamydia trachomatis* infections in asymptomatic men and women by PCR assay. *J Clin Microbiol* 1996;34:1396-400.
13. Louie M, Read S, Simor AE, Holland J, Louie L, Ziebell K, et al. Application of multiplex PCR for detection of non-O157 verocytotoxin-producing *Escherichia coli* in bloody stools: identification of serogroups O26 and O111. *J Clin Microbiol* 1998;36:3375-7.
14. Piersimoni C, Callegaro A, Nista D, Bornigia S, De Conti F, Santini G, et al. Comparative evaluation of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *J Clin Microbiol* 1997;35:193-6.
15. D'Amato RF, Wallman AA, Hochstein LH, Colaninno PM, Scardamaglia M, Ardila E, et al. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. *J Clin Microbiol* 1995;33:1832-4.
16. MacGregor RR, Dreyer K, Herman S, Hocknell PK, Nghiem L, Tevere VJ, et al. Use of PCR in detection of *Mycobacterium avium* complex (MAC) bacteremia: sensitivity of the assay and effect of treatment for MAC infection on

Table 4: Potential advantages and limitations of PCR in the diagnosis of infectious diseases

Advantages	Limitations
High sensitivity	Potential for false-positive test results (e.g., by amplification of "contaminating" DNA)
High specificity	
Good reproducibility	
Ability to detect the presence of infecting microorganisms that may not be identified by conventional methods	Potential for false-negative test results (e.g., because of presence of PCR inhibitors interfering with nucleic acid amplification)
Rapidity, able to provide same-day results	Interpretation of positive PCR test results not yet validated for all infectious diseases (e.g., latent v. active infection)
	Technically complex procedures
	Expensive equipment and reagents

concentrations of human immunodeficiency virus in plasma. *J Clin Microbiol* 1999;37:90-4.

17. De Barbeyrac B, Bernet-Poggi C, Fébrer F, Renaudin H, Dupon M, Bébéar C. Detection of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in clinical samples by polymerase chain reaction. *Clin Infect Dis* 1993;17(Suppl 1):S83-9.

18. Luki N, Lebel P, Boucher M, Doray B, Turgeon J, Brousseau R. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. *Eur J Clin Microbiol Infect Dis* 1998;17:255-63.

19. Crotchfelt KA, Welsh LE, DeBonville D, Rosenstraus M, Quinn TC. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. *J Clin Microbiol* 1997;35:1536-40.

20. Matsumura SO, Louie L, Louie M, Simor AE. Direct multiplex PCR for the rapid detection of bacterial pathogens associated with acute meningitis [abstract D-25]. In: *Program and Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy*; 1998 Sept 24–27; San Diego. San Diego: American Society for Microbiology; 1998. p. 134.

21. Chierian T, Lalitha MK, Manoharan A, Thomas K, Volken RH, Steinhoff MC. PCR-enzyme immunoassay for detection of *Streptococcus pneumoniae* DNA in cerebrospinal fluid samples from patients with culture-negative meningitis. *J Clin Microbiol* 1998;36:3605-8.

22. Kearns AM, Freeman R, Murphy OH, Seiders PR, Steward M, Wheeler J. Rapid PCR-based detection of *Streptococcus pneumoniae* DNA in cerebrospinal fluid. *J Clin Microbiol* 1999;37:3434.

23. Louie L, Simor AE, Louie M, McGeer A, Low DE. Diagnosis of group A streptococcal necrotizing fasciitis by using PCR to amplify the streptococcal pyrogenic exotoxin B gene. *J Clin Microbiol* 1998;36:1769-71.

24. Long CM, Drew L, Miner R, Jekic-McMullen D, Impraim C, Kao SY. Detection of cytomegalovirus in plasma and cerebrospinal fluid specimens from human immunodeficiency virus-infected patients by the AMPLICOR CMV Test. *J Clin Microbiol* 1998;36:2434-8.

25. Pellegrin I, Garrigue I, Binquet C, Chene G, Neau D, Bonot P, et al. Evaluation of new quantitative assays for diagnosis and monitoring of cytomegalovirus disease in human immunodeficiency virus-positive patients. *J Clin Microbiol* 1999;37:3124-32.

26. Van Vliet KE, Glimåker M, Lebon P, Klapper PE, Taylor CE, Ciardi M, et al. Multicenter evaluation of the Amplicor Enterovirus PCR Test with cerebrospinal fluid from patients with aseptic meningitis. *J Clin Microbiol* 1998; 36:2652-7.

27. Hadziyannis E, Cornish N, Starkey C, Procop GW, Yen-Lieberman B. Amplicor Enterovirus polymerase chain reaction in patients with aseptic meningitis. A sensitive test limited by amplification inhibitors. *Arch Pathol Lab Med* 1999;123:882-4.

28. Pawlotsky JM, Bastie A, Lonjon I, Rémiré J, Darthuy F, Soussy CJ, et al. What technique should be used for routine detection and quantification of HBV DNA in clinical samples? *J Virol Methods* 1997;65:245-53.

29. Albadalejo J, Alonso R, Antinozzi R, Bogard M, Bourgault AM, Colucci G, et al. Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* 1998;36:862-5.

30. Lakeman FD, Whitley RJ, and the National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. *J Infect Dis* 1995;171:857-63.

31. Nolte FS, Boysza J, Thurmond C, Clark SW, Lennox JL. Clinical comparison of an enhanced-sensitivity branched-DNA assay and reverse transcription-PCR for quantitation of human immunodeficiency virus type-1 RNA in plasma. *J Clin Microbiol* 1998;36:716-20.

32. Pachl C, Todd JA, Kern DG, Sheridan PJ, Fong SJ, Stempien M, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;8:446-54.

33. Segondy M, Izopet J, Pellegrin I, Montes B, Dumon B, Pasquier C, et al. Comparison of the QUANTIPLEX HIV-1 RNA 2.0 Assay with the AMPLICOR HIV-1 MONITOR 1.0 Assay for quantitation of levels of human immunodeficiency virus type 1 RNA in plasma of patients receiving stavudine-didanosine combination therapy. *J Clin Microbiol* 1998;36:3392-5.

34. Vilgalys R, Hester M. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *J Bacteriol* 1990;172:4238-46.

35. Zhong KJY, Kain KC. Evaluation of a colorimetric PCR-based assay to diagnose *Plasmodium falciparum* malaria in travelers. *J Clin Microbiol* 1999;37:339-41.

36. Helweg-Larsen J, Jensen JS, Benfield T, Svendsen UG, Lundgren JD, Lundgren B. Diagnostic use of PCR for detection of *Pneumocystis carinii* in oral wash samples. *J Clin Microbiol* 1998;36:2068-72.

37. Burg JL, Grover CM, Pouletty P, Boothroyd JC. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by the polymerase chain reaction. *J Clin Microbiol* 1989;27:1787-92.

38. Madico G, Quinn TC, Rompalo A, McKee KT Jr, Gaydos CA. Diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swab samples. *J Clin Microbiol* 1998;36:3205-10.

39. Black CM. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol Rev* 1997;10:160-84.

40. Lumb R, Davies K, Dawson D, Gibb R, Gottlieb T, Kershaw C, et al. Multicenter evaluation of the Abbott Lcx *Mycobacterium tuberculosis* ligase chain reaction assay. *J Clin Microbiol* 1998;37:3102-7.

41. Cartuyvels R, De Ridder C, Jonckheere S, Verbist L, Van Eldere J. Prospective clinical evaluation of Amplicor *Mycobacterium tuberculosis* PCR test as a screening method in a low-prevalence population. *J Clin Microbiol* 1996;34: 2001-3.

42. Bennedsen J, Thomsen VO, Pfyffer GE, Funke G, Feldmann K, Beneke A, et al. Utility of PCR in diagnosing pulmonary tuberculosis. *J Clin Microbiol* 1996;34:1407-11.

43. Whitley RJ, Lakeman F. Herpes simplex virus infections of the central nervous system: therapeutic and diagnostic considerations. *Clin Infect Dis* 1995; 20:414-20.

44. Mellors JW, Munoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. *Ann Intern Med* 1997;126:946-54.

45. Hughes MD, Johnson VA, Hirsch MS, Bremer JW, Elbeik T, Erice A, et al. Monitoring plasma HIV-1 RNA levels in addition to CD4+ lymphocyte count improves assessment of antiretroviral therapeutic response. *Ann Intern Med* 1997;126:929-38.

46. Osiowy C. Direct detection of respiratory syncytial virus, parainfluenza virus, and adenovirus in clinical respiratory specimens by a multiplex reverse transcription-PCR assay. *J Clin Microbiol* 1998;36:3149-54.

47. Echevarría JE, Erdman DD, Swierkosz EM, Holloway BP, Anderson LJ. Simultaneous detection and identification of human parainfluenza viruses 1, 2, and 3 from clinical samples by multiplex PCR. *J Clin Microbiol* 1998;36: 1388-91.

48. Gröndahl B, Puppe W, Hoppe A, Kühne I, Weigl JAI, Schmitt H-J. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. *J Clin Microbiol* 1999;37:1-7.

49. Menéndez R, Córdoba J, de la Cuadra P, Cremades MJ, López-Hontagas JL, Salavert M, et al. Value of the polymerase chain reaction assay in noninvasive respiratory samples for diagnosis of community-acquired pneumonia. *Am J Respir Crit Care Med* 1999;159:1868-73.

50. Hassan-King M, Baldeh I, Adegbola R, Omosigho C, Usen SO, Oparaugo A, et al. Detection of *Haemophilus influenzae* and *Streptococcus pneumoniae* DNA in blood culture by a single PCR assay. *J Clin Microbiol* 1996;34:2030-2.

51. Ley BE, Linton CJ, Bennett DMC, Jalal H, Foot ABM, Millar MR. Detection of bacteraemia in patients with fever and neutropenia using 16S rRNA gene amplification by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1998;17:247-53.

52. Huang C, Chatterjee NK, Grady LJ. Diagnosis of viral infections of the central nervous system. *N Engl J Med* 1999;340:483-4.

53. Read SJ, Jeffery KJM, Bangham CRM. Aseptic meningitis and encephalitis: the role of PCR in the diagnostic laboratory. *J Clin Microbiol* 1997;35:691-6.

54. Relman DA, Schmidt TM, MacDermott RP, Falkow S. Identification of the uncultured bacillus of Whipple's Disease. *N Engl J Med* 1992;327:293-301.

55. Relman DA, Loutit JS, Schmidt TM, Falkow S, Tompkins LS. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. *N Engl J Med* 1990;323:1573-80.

56. Ramirez JA, the *Chlamydia pneumoniae*/Atherosclerosis Study Group. Isolation of *Chlamydia pneumoniae* from the coronary artery of a patient with coronary atherosclerosis. *Ann Intern Med* 1996;125:979-82.

57. Cockerill FR III. Genetic methods for assessing antimicrobial resistance. *Antimicrob Agents Chemother* 1999;43:199-212.

58. Vannuffel P, Laterre PF, Bouyer M, Gigi J, Vandercam B, Reynaert M, et al. Rapid and specific molecular identification of methicillin-resistant *Staphylococcus aureus* in endotracheal aspirates from mechanically ventilated patients. *J Clin Microbiol* 1998;36:2366-8.

59. Murakami K, Minamide W, Wada K, Nakamura E, Teraoka H, Watanabe S. Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 1991;29:2240-4.

60. Satake S, Clark N, Rimland D, Nolte FS, Tenover FC. Detection of vancomycin-resistant enterococci in fecal samples by PCR. *J Clin Microbiol* 1997; 35:2325-30.

61. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995;33:24-7.

62. Patel R, Uhl JR, Kohner P, Hopkins MK, Cockerill FR III. Multiplex PCR detection of *vanA*, *vanB*, *vanC-1*, and *vanC-2/3* genes in enterococci. *J Clin Microbiol* 1997;35:703-7.

63. du Plessis M, Smith AM, Klugman KP. Application of *pbp1A* PCR in identification of penicillin-resistant *Streptococcus pneumoniae*. *J Clin Microbiol* 1999; 37:628-32.

64. Arlet G, Brami G, Décrè D, Flippo A, Gaillot O, Lagrange PH, et al. Molecular characterisation by PCR-restriction fragment length polymorphism of TEM β-lactamases. *FEMS Microbiol Lett* 1995;134:203-8.

65. Nachamkin I, Kang C, Weinstein MP. Detection of resistance to isoniazid,

rifampin, and streptomycin in clinical isolates of *Mycobacterium tuberculosis* by molecular methods. *Clin Infect Dis* 1997;24:894-900.

66. Telenti A, Imboden P, Marchesi F, Schmidheini T, Bodmer T. Direct, automated detection of rifampin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single-stranded conformation polymorphism analysis. *Antimicrob Agents Chemother* 1993;37:2054-8.

67. Sasadeusz J, Tufaro JF, Safrin S, Schubert K, Hubinette MM, Cheung PK, et al. Homopolymer mutational hot spots mediate herpes simplex virus resistance to acyclovir. *J Virol* 1997;71:3872-8.

68. Smith JL, Cherrington JM, Jiles RE, Futter MD, Freeman WR, Spector SA. High-level resistance of cytomegalovirus to ganciclovir is associated with alterations in both the UL97 and DNA polymerase genes. *J Infect Dis* 1997;176:69-77.

69. Stuyver L, Wyseur A, Rombout A, Louwagie J, Scarcez T, Verhofstede C, et al. Line probe assay for rapid detection of drug-selected mutations in the human immunodeficiency virus type 1 reverse transcriptase gene. *Antimicrob Agents Chemother* 1997;41:284-91.

70. Vasudevachari MB, Zhang YM, Imamichi H, Imamichi T, Falloon J, Salzman NP. Emergence of protease inhibitor resistance mutations in human immunodeficiency virus type 1 isolates from patients and rapid screening procedure for their detection. *Antimicrob Agents Chemother* 1996;40:2535-41.

71. Coudron PE, Jones DL, Dalton HP, Archer GL. Evaluation of laboratory tests for detection of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J Clin Microbiol* 1986;24:764-9.

72. Bignardi GE, Woodford N, Chapman A, Johnson AP, Speller DCE. Detection of the *mec-A* gene and phenotypic detection of resistance in *Staphylococcus aureus* isolates with borderline or low-level methicillin resistance. *J Antimicrob Chemother* 1996;37:53-63.

73. Jayaratne P, Rutherford C. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from growth on mannitol salt oxacillin agar using PCR for nosocomial surveillance. *Diagn Microbiol Infect Dis* 1999;35:13-8.

74. Kitagawa Y, Ueda M, Ando N, Endo M, Ishibiki K, Kobayashi Y, et al. Rapid diagnosis of methicillin-resistant *Staphylococcus aureus* bacteremia by nested polymerase chain reaction. *Ann Surg* 1996;224:665-71.

75. Rosenberg J, Tenover FC, Wong J, Jarvis W, Vugia DJ. Are clinical laboratories in California accurately reporting vancomycin-resistant enterococci? *J Clin Microbiol* 1997;35:2526-30.

76. Coombs GW, Kay ID, Steven RA, Pearman JW, Bertolatti D, Grubb WB. Should genotypic testing be done on all phenotypically vancomycin-resistant enterococci detected in hospitals? *J Clin Microbiol* 1999;37:1229-30.

77. Roger M, Faucher MC, Forest P, St-Antoine P, Coutlée F. Evaluation of a *vanA*-specific assay for detection of vancomycin-resistant *Enterococcus faecium* during a hospital outbreak. *J Clin Microbiol* 1999;37:3348-9.

78. Goldenberger D, Künzli A, Vogt P, Zbinden R, Altwegg M. Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. *J Clin Microbiol* 1997;35:2733-9.

79. Klausegger A, Hell M, Berger A, Zinober K, Baier S, Jones N, et al. Gram type-specific broad-range PCR amplification for rapid detection of 62 pathogenic bacteria. *J Clin Microbiol* 1999;37:464-6.

80. Kennedy N, Gillespie SH, Saruni AOS, Kisyombe G, McNerney R, Ngowi FI, et al. Polymerase chain reaction for assessing treatment response in patients with pulmonary tuberculosis. *J Infect Dis* 1994;170:713-6.

81. Dagan R, Shriker O, Hazan I, Leibovitz E, Greenberg D, Schlaeffer F, et al. Prospective study to determine clinical relevance of detection of pneumococcal DNA in sera of children by PCR. *J Clin Microbiol* 1998;36:669-73.

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