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Relevance of Nucleic Acid Amplification Techniques for Diagnosis of Respiratory Tract Infections in the Clinical Laboratory

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INTRODUCTION

During the last 5 to 7 years, the advantages of diagnostic molecular techniques have been so widely publicized that increasing pressure has been placed on clinical microbiology laboratories to apply them for the detection of a wide variety of infectious agents, especially since test kits for some applications are being made commercially available. In this paper, we review the efficiency and practicability of nucleic acid amplification techniques for the diagnosis of respiratory tract infections.

Before introducing molecular techniques in the diagnostic laboratory, several strategic questions must be addressed: which organisms should be targeted; which clinical specimens should be tested; and do these molecular tests fulfill the required criteria of high sensitivity and specificity, speed, simplicity, and clinical relevance? In general, molecular diagnostic techniques are indicated (i) for the detection of organisms that cannot be grown in vitro or for which current culture techniques are too insensitive, or (ii) for the detection of organisms requiring complex media or cell cultures and/or prolonged incubation times. For respiratory infections, the following organisms meet the criteria described above: rhinoviruses, coronaviruses, hantaviruses, *Bordetella pertussis*, *Legionella* species, *Coxiella burnetii*, *Chlamydia trachomatis*, *Chlamydia psittaci*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, *Mycobacterium tuberculosis*, fungi, and *Pneumocystis carinii.*

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This review concentrates on those respiratory agents for which considerable numbers of clinical specimens have been examined. Studies concerning the development of tests for the corresponding pathogens are not considered. Respiratory disease due to cytomegalovirus is not discussed because it does not result from an airborne infection but most frequently from a reactivation of a latent infection in relation to an immunosuppressive state, in which the interpretation of the virological investigations poses particular problems.

The basic principle of any molecular diagnostic test is the detection of a specific nucleic acid sequence by hybridization to a complementary sequence, a probe, followed by detection of the hybrid (21). However, the sensitivity of nucleic acid probe tests that do not involve amplification is lower than that of classical diagnostic tests (191). This lack of sensitivity applies to the detection of respiratory pathogens including rhinoviruses (3, 16), *M. pneumoniae* (71, 102, 103, 176), *C. pneumoniae* (19), and *M. tuberculosis* (150). The main use of the nonamplification probe procedure is in the identification rather than the detection of microorganisms (32, 45).

Thereupon, techniques have been developed to amplify the target nucleic acid or the probe. Any stretch of nucleic acid can be copied by using DNA polymerase, provided that some sequence data are known to allow the design of appropriate primers. DNA replication was made possible in 1958, when Kornberg discovered the DNA polymerase (106). For many years, one of the main applications of this discovery was in the DNA-sequencing procedure of Sanger et al. (166). In 1986, Mullis et al. (132) introduced a reiterative process, PCR, which leads to an exponential increase in the production of the nucleic acid. In view of the immense number of possible appli-

cations in the most diverse fields, commercial interest was immediately awakened. Alternative nucleic acid amplification techniques were developed and patented, using different enzymes and strategies, but they are all based on reiterative reactions (29, 60, 110, 115, 216).

Nucleic acid amplification techniques can be classified by several criteria. Conceptually, there are those in which the target nucleic acid is amplified and those in which the probe is multiplied (21, 215); from a practical point of view, there are the in-house-developed applications and the commercially available tests. Target nucleic acid amplification techniques include PCR, the strand displacement amplification, and the isothermal RNA self-sustaining sequence replication reaction, from which the commercialized nucleic acid sequence-based amplification (NASBA) and the transcription-mediated amplification (TMA) are derived. The ligase chain reaction (LCR), in the so-called gapped LCR format, is a combination of target and probe amplification. The Qβ replicase amplification (QβRA) involves probe amplification only.

PCR (132) consists of a number of temperature cycles, each cycle consisting of two or three temperature steps: denaturation to ensure the separation of the target DNA duplexes, annealing to allow added synthetic oligonucleotide primers to hybridize to the DNA target, and extension to allow the added DNA polymerase to synthesize complementary DNA strands. In some protocols, annealing and extension occur at the same temperature. After a series of these temperature cycles, the specific PCR product or amplicon, consisting of the two primers bridged by the intervening nucleotide sequence, accumulates. Modifications of the basic procedure are nested PCR (149), multiplex PCR (25), and reverse transcriptase (RT) PCR (149).

In a nested PCR (149), a second round of amplification is performed, using the amplicon of the first round as a target and a pair of primers complementary to sequences within this amplicon, the amplicon of the second reaction being shorter than that of the first. The advantage of nested PCR is increased sensitivity, but this is achieved at the cost of a high risk of cross-contamination, since the tubes containing amplicons have to be opened after the first stage to add new reagents for the second stage. It also increases the specificity of the reaction, since the internal primers anneal only if the amplicon has the corresponding, expected, sequence.

In a multiplex PCR (25) several independent amplifications are carried out simultaneously in one tube with a mixture of primers. However, since the annealing temperatures for the respective primer pairs are not necessarily identical, problems of specificity of the individual reactions may result.

In an RT-PCR (149), an RNA target, usually viral RNA, is first transcribed into complementary DNA, enabling the PCR to proceed.

The TMA and NASBA (29, 60) amplify RNA via the simultaneous action of three enzymes: an RT (which also has polymerase activity), an RNase, and an RNA polymerase. The synthesis of cDNA is primed by specially designed oligonucleotide primers, one end of which is a target-specific sequence, while the other end contains a promoter for the RNA polymerase. The RT synthesizes an RNA-DNA hybrid, the RNase digests the RNA component, and the RT synthesizes double-stranded DNA; finally, the RNA polymerase produces numerous RNA copies.

In the LCR (216), after heat denaturation of the double-stranded DNA, two pairs of primers anneal to each strand of the target. A DNA ligase joins the primers, and the ligation product is released by heating and serves as template for new ligations. In the gapped LCR (110), a gap of 1 to 3 bases is left between the primers and is filled in by the action of added DNA polymerase, before the primers are covalently linked by a ligase. In subsequent cycles, the ligated primers act as targets for further annealing and ligation.

In the QβRA (115), a specifically synthesized RNA probe is used. It contains a sequence specific for a target, either DNA or RNA, a sequence to enable the capture of the probe-template hybrids, and a sequence recognized by the Qβ replicase enzyme to start replication. After annealing of the probe to the target, the hybrids are captured, and the probe is removed enzymatically and amplified by the Qβ replicase. This technique is still largely in the developmental stage, the main difficulty being the separation of nonhybridized from hybridized probe before amplification.

Each of the amplification techniques is composed of three parts: sample preparation, amplification, and product detection. The sample preparation step involves primarily the liberation and concentration of the target nucleic acid and the elimination of amplification inhibitors. A great diversity of sample preparation procedures has been described, particularly for PCR. Inhibitors occur frequently and may be difficult to eliminate: heme compounds (79) and polysaccharides in sputum (109), as well as some reagents (67) and components of swabs (207).

The amplification step should aim at maximum sensitivity and specificity through judicious choice of the primers and optimal temperatures when thermocyling is involved, offer maximum protection against contamination, and include proper positive and negative controls. The purpose of the positive control is to monitor the amplification process, particularly to detect inhibitors of the reaction. Concomitant amplification of human β-globin has been used frequently for this purpose. At the same time, it determines the presence of host cell material, which is particularly useful after elaborate sample preparation procedures. However, it requires the introduction of specific primers into the reaction, resulting in a multiplex PCR. To avoid this problem, a PCR for the globin is sometimes performed in a separate tube, but the optimal cycling temperatures for this internal control may differ from those required for the principal reaction. Therefore, specific, positive internal controls are preferred. These are modified amplicons that have been made shorter or longer and are added to each reaction tube. Their ends are identical to those of the target, and therefore they are amplified by the same reagents as the real target, but they are easily differentiated from it by being shorter or longer (6, 38, 44, 64, 96, 105, 137, 148, 151, 167, 188, 197, 198). By adding specific positive internal controls to the samples at the very start of the process, the efficacy of the sample preparation procedure can be assessed. Moreover, the addition of specific internal controls avoids the use of reference organisms or their nucleic acid as positive, external controls, thus eliminating an important possible source of contamination. The addition of a limited amount of internal control should not significantly reduce the sensitivity of the procedure, and it offers greater advantages than disadvantages (198). Internal controls allow also the quantitation of the reaction (96).

Negative controls are target-free samples, usually distilled water, which are subjected to the same manipulations as the test samples. Their purpose is to detect contaminations between reaction tubes. Indeed, after numerous exponential nucleic acid amplifications, there are ample sources of cross-contamination in the laboratory. The greater the number of manipulations, the greater the risk of cross-contamination among the specimens, especially if multiple centrifugations are required. Appropriate measures should be taken to avoid con-

TABLE 1. Diagnostic methods for respiratory viruses

Etiologic agent	Rapid conventional methods available	PCR	
		Relevant	Reference(s)
Adenoviruses	+	No	
Influenza viruses	+	No	27
Parainfluenza viruses	+	No	
RSV	+	No	146
Herpes simplex virus	+	No	
Rhinoviruses	−	Yes	7, 69, 89, 91
Coronaviruses	−	Yes	133
Enteroviruses	−	Yes	91

tamination. These measures include the use of three different rooms with restricted access for each of the reaction steps, the use of appropriate pipette tips, and cleaning of the area by UV irradiation, or the use in the PCR of dUTP instead of dTTP, allowing disintegration of unwanted, possibly contaminating, amplicons by uracil-*N′*-glycosylase (177).

Because of the exquisite sensitivity of nucleic acid amplification tests, there should be a constant awareness of the possibility of false-positive results. These not only are due to cross-contaminations in the laboratory but also may result from contaminations during sampling, particularly when organisms, such as fungi or legionellas present in the environment, are studied. Samples from treated patients may remain positive for prolonged periods (39, 63, 75). For all these reasons, confirmation of the existence of some microorganisms in subclinical infections or a carrier state becomes difficult.

In the PCR and the LCR, the amplicons can be detected by gel electrophoresis, followed or not by solid- or liquid-phase hybridization with a specific probe, by fluorescence (88), or by an enzyme immunoassay (EIA) reaction. Hybridization can increase the sensitivity of the detection 10- or 100-fold. The amplicons of NASBA and TMA are detected by hybridization or by a commercial luminescence reaction (41), and those of the QβRA can be detected by an incorporated fluorescent dye.

At present, PCR is undoubtedly the most widely used amplification technique, probably because it was the first one described and was introduced rapidly in innumerable laboratories for a wide variety of applications. Commercial formats of PCR (Roche), TMA (MTDT, GenProbe), NASBA (Organon Teknika), and LCR (Abbott) have been developed, particularly for infectious agents for which large numbers of clinical specimens are tested: sexually transmitted agents (*Neisseria gonorrhoeae*, *C. trachomatis*, human immunodeficiency viruses), hepatitis C virus, and *M. tuberculosis*. In these formats, the amplicon is detected either by a semiautomated EIA reaction (Roche) or by an electrochemiluminescence procedure or a hybridization reaction (Organon Teknika), or it is coupled to an existing acridinium ester luminescent nucleic acid probe technique (GenProbe) or a previously developed, automated EIA technique (Abbott).

In-house tests are more versatile and can easily be applied to any target by switching to the appropriate primers and, if necessary, adapting the cycling temperatures accordingly.

MOLECULAR DIAGNOSTIC TECHNIQUES FOR ACUTE RESPIRATORY TRACT INFECTIONS

Viruses

Table 1 illustrates the present situation for the diagnosis of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus (RSV) infections for which rapid conventional techniques are available: influenza virus and RSV can be detected in the clinical specimens by immunofluorescence and parainfluenza virus and adenovirus can be detected by immunofluorescence after incubation for 48 h in shell vial cultures (147). In these cases, nucleic acid amplification techniques have no added value in terms of sensitivity or rapidity. In one study (27), comparing PCR with conventional techniques for the detection of influenza virus, the authors concluded that there are no arguments for the introduction of PCR for the diagnosis of influenza virus infection. In a study by Paton et al. (146), PCR for RSV had a sensitivity of 94.6% and a specificity of 97%; the molecular technique detected 1% of cases undiagnosed by culture and EIA. Clearly, PCR does not represent significant improvement over existing methods for the detection of these viruses.

Rhinoviruses and coronaviruses grow poorly in cell culture. In addition, rapid immunofluorescence and/or culture techniques are not available for the direct detection of these viruses in clinical specimens (7, 69). Typically, rhinoviruses are isolated in roller cultures, sometimes after several blind passages, followed by acid lability testing. More than 100 serotypes are known. PCR is much more sensitive than is culture (136): Ireland et al. (89) and Johnston et al. (91) detected five and three times as many rhinoviruses by PCR, respectively, compared with the best available cell culture techniques. In another study (59), significantly more multiple-virus infections by RSV, parainfluenza viruses, and rhinoviruses were detected by RT-PCR than by culture. However, some technical details must still be worked out. To detect the large number of rhinovirus serotypes, regions within the conserved noncoding 5′ untranslated region of the genome are amplified (54), leading to cross-reactions with many enteroviruses. Several methods have been used to detect rhinoviruses specifically: a nested procedure, the use of primers spanning a region between the 5′ untranslated region and the VP2/VP4 region, hybridization with specific probes (69), and differentiation on the basis of the size of the amplicons (89, 141, 196) or sequencing (131). Nevertheless, Johnston et al. (91) could identify only 8 of 30 positive samples as rhinoviruses on the basis of either acid lability or the length of the amplicon, with 73% remaining "unclassified picornaviruses." Another problem emerging from studies on human rhinoviruses by PCR is whether healthy carriers exist: 12 and 4% of samples from asymptomatic children and adults, respectively, were positive for picornavirus by PCR (91).

Clearly, there is still more to learn about the epidemiology of rhinoviruses, particularly in children, infants, and the elderly. Molecular diagnostic techniques offer the necessary tools.

A PCR based on the genomic sequences of the two known human coronavirus strains, 229E and OC43, is available (133), and it is highly likely that more, as yet uncultivated, human coronaviruses remain to be detected. No extensive studies to define better the role of coronaviruses in respiratory infections have been undertaken.

Hantavirus pulmonary syndrome, a rodent-borne infection, appeared in 1993 and 1994 in the New Mexico-Arizona-Colorado area. It is characterized by fever, myalgias, headache, and cough, followed rapidly by respiratory failure. Antibodies against heterologous hantavirus antigens were initially used to identify the causative agent, and then the hantavirus genome was detected by PCR in autopsy specimens (135). Specific genetic recombinant-derived proteins were prepared from viral genomic sequences amplified from tissues obtained from patients who died of confirmed hantavirus illness (108). Since the virus has not yet been cultured, PCR with specific primers and serology are the only diagnostic possibilities.

Rapid diagnostic techniques for respiratory pathogens are not only important for clinical-epidemiological reasons but are also useful so that treatment can be appropriately initiated within the first 24 h or halted when the symptoms are found to be caused by another microbial agent.

Bacteria

***Bordetella pertussis*.** Despite the routine immunization of children, pertussis continues to be an important disease in infants and young children. During the last 2 years, there has been a resurgence of pertussis in the United States (24), Italy, the Russian Federation, and Sweden (165). In 1994, approximately 3,500 to 4,000 cases were reported to the Centers for Disease Control and Prevention in the United States (24). These figures probably underestimate the true incidence of pertussis because of the difficulty in confirming the diagnosis (70, 182). The major reservoir for pertussis now appears to be previously vaccinated adolescents and adults with atypical and often unrecognized symptoms of pertussis. Making the clinical diagnosis of pertussis in this reservoir is more challenging because many of these patients do not have the classic coughing paroxysms or "whoops."

The conventional laboratory diagnosis of pertussis has relied on culture, direct immunofluorescence, and serologic testing. Each of these methods has problems with either sensitivity or specificity (47, 70, 182). Diagnosis by culture is specific but not very sensitive since most individuals are culture negative at the time when clinical symptoms are apparent. Direct immunofluorescence is prone to a large number of false-positive results, and when used on a single specimen, serologic testing is often nonspecific. Follow-up confirmation with a second specimen would result in a 3- to 4-week delay in the diagnosis. These problems have led to an inability to confirm the diagnosis in many patients, and therefore nucleic acid amplification techniques, in practice PCR, have been used (8, 40, 47, 68, 72, 73, 169, 170, 199). The presence of a repetitive gene element in *B. pertussis* increases the sensitivity of the PCR. The reaction allows also a clear-cut distinction between the pathogenic *B. pertussis* and the usually nonpathogenic *B. parapertussis* (199).

An unexpected origin of false-positive PCR results for *B. pertussis* was described by Taranger et al. (187). Pharyngeal samples were obtained in a room that was grossly contaminated with pertussis DNA because killed, whole-cell pertussis vaccine was administered in the same room.

In a recent report (123), several aspects of PCR-based detection of *B. pertussis* were discussed. The main conclusions, which we can support, were that (i) there are no comparative studies between the different PCR procedures; (ii) although the PCR procedures used in different laboratories can detect 80 to 100% of the culture-positive samples, the percentage of PCR-positive samples that were culture negative differed by 13 to 88%; (iii) there is need for rigorous control of false-positive and false-negative results; (iv) questionable results must be confirmed by a second method; and (v) PCR-positive results are acceptable only for individuals with classical symptoms of pertussis. The clinical and epidemiological significance of a PCR-positive result in someone with mild or no symptoms should be interpreted with caution, and, if possible, other markers, such as serologic tests or epidemiologic data should be used in addition. Finally, it is too early to recommend a standard PCR technique for the detection of *B. pertussis* in clinical specimens, because no comparative studies have been done.

***Legionella* species.** Legionellae are ubiquitously distributed in natural and man-made water systems (49, 206). Respiratory infections caused by *Legionella* spp. often occur in immunodeficient persons. Cultures of bronchoalveolar lavage specimens take a minimum of 48 to 72 h to grow, and plates should be incubated for 7 days. Jaulhac et al. (90) applied PCR retrospectively to frozen bronchoalveolar lavage specimens. They confirmed all culture-positive specimens and found additional specimens positive by PCR from patients whose clinical features were in accordance with legionellosis. Kessler et al. (98), in a prospective study combining a rapid DNA extraction procedure with a commercial kit for the amplification and detection of legionellae in environmental samples, detected the organisms in all specimens later confirmed by culture. In another study (125), legionellae were detected by PCR but not by conventional culture.

In an effort to detect *Legionella* infections by the examination of specimens obtained by less invasive procedures, Maiwald et al. (120) examined urine specimens from experimentally infected guinea pigs and patients by an EIA and by PCR. PCR was more sensitive than EIA in detecting legionellae, and two urine samples were intermittently positive, indicating that DNA is not continuously excreted. The advantage of PCR over EIA is that PCR is a genus-specific reaction whereas antigen detection must be performed with a variety of serogroup reagents to cover the spectrum of possible causative species. The authors concluded that a more detailed prospective study of hospitalized patients with pneumonia is warranted. Their results also illustrate the recurring problem of contamination associated with amplification techniques, since 3 of 30 control samples from patients with urinary tract infections were positive, possibly as a result of contamination by hospital water.

The need for nucleic acid amplification techniques for *Legionella* infections can be questioned in view of their relatively easy isolation from respiratory specimens within a moderate time span and the ability to prevent nosocomial legionellosis by control of legionellae in the hospital plumbing system (114). PCR may be more suitable for the detection of legionellae in environmental specimens to avoid overgrowth by contaminating organisms (119).

***Coxiella burnetii*.** *C. burnetii* is a fastidious intracellular bacterium. Different strains show heterogeneity in their growth conditions, with some being very difficult to culture in vitro. The isolation of *C. burnetii* was greatly improved and facilitated by application of the shell vial assay technique (159), which produced results within 6 days. A PCR for *C. burnetii* (181) has been shown to be very sensitive and specific and is able to produce results within 6 h. It can be applied to inoculated shell vials or directly to clinical specimens. For the time being, this procedure will remain restricted to reference laboratories in countries or areas where the disease does occur, as illustrated recently by To et al. (194).

***Chlamydia* species.** Three *Chlamydia* species are responsible for human respiratory infections: *C. psittaci* and *C. pneumoniae* in adults and older children, and *C. trachomatis* in newborns, who are infected during delivery.

The last organism has been implicated, by serology (2), in 3 to 18% of all cases of infant pneumonitis. Although nucleic acid amplification techniques for the detection of *C. trachomatis* in genitourinary specimens have been intensively studied, there are no such studies on respiratory specimens. It could well be that the techniques used for genitourinary specimens cannot be applied unchanged to respiratory specimens, particularly the specimen preparation procedure (41).

C. psittaci may be an important human pathogen in some areas and may be underdiagnosed on the basis of serologic testing alone. Since respiratory infections by *C. trachomatis* and *C. psittaci* occur sporadically, there has been less need or

opportunity for the application of amplification techniques for these infections. Several research groups have developed a two-step procedure for the successive detection of organisms belonging to this genus and their subsequent identification to the species level, by the amplification of a common genus-specific DNA sequence followed by digestion with restriction enzymes (80, 160, 210) or by a nested PCR (195). None of these procedures has been applied on a significant scale.

The role of *C. pneumoniae* in disease was discovered relatively recently, but the insensitivity of cell culture techniques has hampered extensive clinical and epidemiological investigations. In addition, serologic tests are labor-intensive, since they rely on microimmunofluorescence tests for detection of both immunoglobulin M (IgM) and IgG. Serologic investigations seem to indicate that the culture technique fails to detect many infections. However, taking into account the shortcomings of serologic testing, in terms of specificity and sensitivity (58), it can be surmised that the techniques available fail to diagnose *C. pneumoniae* infections to an unknown extent, although the organism does not seem to be a common cause of respiratory infection in children (65). Therefore, several PCR primer sets have been developed to detect either outer membrane or 16S rRNA coding genes (10, 19, 55, 58, 66, 143, 157, 160).

One of the difficulties in evaluating nucleic acid amplification tests for the diagnosis of *C. pneumoniae* infections is the choice of the reference or "gold standard." Because culture is relatively insensitive, many studies refer to serologic results, considering the presence of IgM, a fourfold increase in antibody titers during and after the acute disease episode, or an IgG titer of at least 512 to be significant. The presence of clinical symptoms cannot be taken into account, since asymptomatic infections by *C. pneumoniae* have been documented by culture and PCR (84).

In addition to this problem of the appropriate reference method to use for the detection of *C. pneumoniae*, inhibitors of PCR are common components of the specimens. Some solutions have been proposed, including the use of samples such as gargled water, throat swabs, or nasopharyngeal swabs instead of nasopharyngeal aspirates or sputum (157, 195), alternative sample treatment methods (62, 117), and introduction of a nested PCR (11).

In all studies in which they were compared, PCR detected 10 to 20% more cases than culture, but in turn serologic determination detected 10 to 20% more cases than PCR. In one study (58), when compared with the combination of a positive culture and direct immunofluorescence test, the PCR had a sensitivity of 76.5% and a specificity of 99%; when compared with the combination of a positive PCR and direct immunofluorescence test, the sensitivity of culture was 87.5%. In the same study, only 8 acute-phase serum specimens (23%) of the 35 *C. pneumoniae* culture- or PCR-positive patients had a diagnostic antibody titer, as did 18.8% of those from 80 asymptomatic persons. Thom et al. (192) diagnosed 21 cases by serologic testing among 743 middle-aged and older patients; 15 of the patients were positive by PCR. Gaydos et al. (56) studied 132 *C. pneumoniae* culture-negative BAL specimens from 108 immunocompromised patients. A total of 20 *C. pneumoniae* infections were diagnosed: 8 by PCR, 4 by PCR and serologic testing, and 8 by serologic testing alone. In this study, PCR and serologic testing had a sensitivity and specificity of 33.3 and 91%, respectively, and both detected 60% of the cases. Thus, it seems that both conventional culture and PCR diagnose only a fraction of the total number of cases and that the diagnosis of individual infections by serology is by no means straightforward, due to the occurrence of many false-negative and false-positive results.

Many aspects of the diagnosis of *C. pneumoniae* infections by amplification techniques remain to be explored. There is need for an internal control; for comparisons of different types of samples, sample preparation methods, and primers; and for several amplification techniques to be performed on the same specimens.

Mycoplasma pneumoniae. *M. pneumoniae* grows slowly in vitro, requiring 2 to 4 weeks for colonies to appear. Therefore, research laboratories have identified several genomic sequences suitable for amplification, including the P1 gene (87), the 16S rRNA gene (201), and a species-specific protein gene (116). In clinical studies, the sensitivity and specificity of amplifications based on these sequences were 90 to 94% and 97 to 100%, respectively (34, 57, 86, 94, 112, 116, 122, 176, 178, 193, 201). PCR also detected *M. pneumoniae* in specimens from 1 to 3% of healthy subjects (116, 193) or convalescent patients, raising the possibility of a carrier state or persistence of the organism in the respiratory tree. In a recent study (86), 371 nasopharyngeal aspirates from children with acute respiratory infections were examined for viruses by rapid conventional techniques and for the presence of *M. pneumoniae* by culture and several different PCR protocols in two laboratories. Each laboratory applied one sample preparation method: freezing-boiling or isothiocyanate treatment, followed by phenol-chloroform extraction. Prepared samples were exchanged between laboratories. In both laboratories, identical primers were used in the PCR directed against the P1 gene, while one laboratory also used primers against the 16S rRNA gene. A specific internal control for the P1 amplification was included (198). Samples were defined as positive if (i) culture was positive for *M. pneumoniae*, (ii) culture and PCR for the P1 and/or the 16S rRNA genes were positive, or (iii) PCR was positive for both the P1 and 16S genes after a particular extraction procedure. Samples positive by PCR for only one of the primer pairs were considered as contaminants. Compared with PCR, culture had a sensitivity of 61%. For the PCR, depending on the preparation method used, sensitivity with the P1 primers was 76.9 to 92.3% on inspection of the electrophoresis gel and 92.3% after hybridization. The specificity was 100%. Depending on the sample preparation method, amplification of the 16S rRNA gene had a sensitivity of 53.8 to 84.6% on visual inspection of the electrophoresis gel and 69.2 to 92.3% after hybridization. The specificity was 100%. It was concluded that, provided a specific positive internal control is used, sample preparation by freezing-boiling combined with PCR for the P1 gene and amplicon detection by visual inspection of the electrophoresis gel could be recommended for clinical use, although the best results were obtained by hybridization with a labeled probe. False-positive results occurred in 0.2% of the reactions. It remains to be seen whether the finding of Resnikov et al. (163) that throat swabs contain significantly fewer PCR inhibitors than do nasopharyngeal aspirates is confirmed and that the effect does not simply result from dilution.

In the same study by Ieven et al. (86), *M. pneumoniae* was found in 3.5% of the samples but significantly more often (6.9%) in those from children older than 2 years of age. *M. pneumoniae* was the third most common etiologic agent of acute respiratory infections in children, after RSV and influenza virus. In lower respiratory infections, such as bronchopneumonia and pneumonia, *M. pneumoniae* was found as frequently as RSV. PCR is unquestionably an important step forward for the diagnosis of *M. pneumoniae* infections.

Mycobacterium tuberculosis. Amplification techniques for the diagnosis of tuberculosis have attracted considerable interest, particularly with the hope of shortening the time required to detect and identify *M. tuberculosis* in respiratory specimens

TABLE 2. Evaluation of PCR for *M. tuberculosis* in different studies

Study (reference)	No. of specimens	Prevalence (%)[a]	Sensitivity (%)		Specificity (%)		PPV (%)[b]	
			C[c]	R[c]	C	R	C	R
Abe et al. (1)	135	28	81.3	84.2	94.2	100	81.3	84
Beige et al. (9)	103	47	98		70		75	
Clarridge et al. (28)	>5,000	4.4	83.6	86.1	98.7	100	94.2	98.4
Forbes and Hicks (51)	734	11		85.2		97.7		83.3
Kocagöz et al. (104)	78	49		87		96		97
Miller et al. (126)	750	21	78.2	92.3				100
Miyazaki et al. (129)	323	13	97		92	100	82	100
Nolte et al. (137)	313	40	91		100		100	
Shawar et al. (175)	384	18	74	80	95	97	77	86
Yuen et al. (218)	519	8	96		85	100		

[a] Prevalence of positive specimens based on culture results.
[b] PPV, positive predictive value.
[c] C, crude results; R, revised results after discrepancy analysis.

such as sputum or BAL samples. It is in this field of clinical microbiology that most amplification procedures, developed both in-house and in commercialized formats, have been evaluated.

(i) Technical aspects. Many different DNA amplification targets have been proposed, such as genes encoding the 32-kDa (179), the 38-kDa (129, 219), and the 65-kDa (145, 152) antigens and the *dnaJ* (183, 184), *groEl*, and *mtb-4* genes (104, 220). Some of these are genus or group specific, with species identification requiring subsequent restriction enzyme treatment or hybridization. The target most frequently amplified is the IS*986* or IS*6110* repetitive element (43, 77), which is present at 10 to 16 copies in most *M. tuberculosis* complex isolates, thereby increasing the sensitivity of the amplification reaction. In comparative studies, tests with the IS*6110* primers were generally more sensitive and more specific than those with IS*986* (37, 76, 208). Recently, however, *M. tuberculosis* isolates without this insertion element have been discovered in Southeast Asia (33, 202, 219).

Numerous techniques for sample preparation have been proposed, including boiling; freezing-boiling; shaking with glass beads (100); sonication (17); chloroform (213), proteinase K or "chelex" (36) treatments and combinations of these treatments; resin treatment (4); and more complex nucleic acid extraction methods (14). The commercial kits furnish their own sample treatment reagent.

Some PCRs are performed with dUTP instead of dTTP, allowing decontamination with uracil-*N'*-glycosylase (217). Both single and nested PCR formats (129, 152, 176, 213) have been applied, sometimes with the explicit purpose of overcoming PCR inhibitors.

Internal controls have been used (6, 38, 44, 105, 137, 139). However, they were only occasionally added to the specimens before the DNA extraction procedure, as was done by Kolk et al. (105). By being present during the entire procedure, an internal control not only detects inhibitors but also monitors the efficacy of the sample preparation method. Inhibitors have been detected in 3.7 to 16% of clinical samples (28, 51, 139). Curiously, Nolte et al. (137) detected inhibitors in 17% of the samples with β-globin primers but only in 10% with a specific internal control.

(ii) Results on sputum specimens with in-house PCR tests. Table 2 presents the results of nine studies in which IS*6110* was used as the amplification target. Some of these studies were done on a series of specimens with a high prevalence of positive samples. It should be remembered that for a constant rate of false-positive tests, the positive predictive value of a test decreases drastically when the prevalence of infection is low, as is the case in industrialized countries. In a population with a prevalence of <5% (in most Western European countries [139], the prevalence of positive samples is 3 to 4%), false-positive rates of 1 to 5% can lead to overdiagnosis of 50% or more of cases.

In general, the authors of the studies present their results first as "crude results", i.e., as produced by the test and thereafter as "revised results," i.e., after considering the discrepancies between the test results and the corresponding clinical information. Some authors include culture-negative, clinically diagnosed cases of tuberculosis among the "true-positives," sometimes even based on favorable response to anti-tuberculosis treatment, and thereby increase the specificity and positive predictive value of the test. None of them formulated a standard definition of a positive case except for Noordhoek et al. (139), who used the following definition of a true-positive specimen: (i) *M. tuberculosis* was cultured; or (ii) direct microscopy and PCR were positive but culture was negative; or (iii) direct microscopy and culture were negative but PCR was positive and other material from the patient was positive on culture or had been positive in the past.

None of the published studies observed a statistically significant difference between culture and the amplification technique (99). However, sensitivity and specificity are calculated as a function of the culture technique, since this is the reference method used in the absence of a better definition of a positive case of tuberculosis. In the studies, specificities vary between 85 and 100% but sensitivities are usually lower, between 74 and 97%. In one study on over 5,000 specimens (28) with a 4.4% prevalence of positive results, sensitivity, specificity, and positive predictive values were 84, 99, and 94%, respectively. By applying two primer systems in a multiplex PCR, Beige et al. (9) attained a sensitivity of 98% but a specificity of only 70%.

However, the main criticism of the use of PCR for the diagnosis of tuberculosis is a result of the separate analyses of the sensitivities of smear-positive and smear-negative, culture-positive specimens in different studies (Table 3). The test sensitivity in smear-positive cases is 88 to 100% but drops to between 50 and 92% in smear-negative cases.

One of the reasons for the lack of sensitivity may be the sample preparation method. Except for one study (139), all the procedures were applied to homogenized and decontaminated specimens as used for culture. Although this may seem appropriate when amplification techniques are compared with culture, it is not logical and may not even be optimal. In all studies

TABLE 3. Results of PCR for *M. tuberculosis* for smear-positive and smear-negative specimens

Study (reference)	PCR sensitivity (%) in different studies		
	Overall	Smear and culture positive	Smear negative, culture positive
Abe et al. (1)	84	96	50
Clarridge et al. (28)	86	94	62
Forbes and Hicks (51)	85	88	71
Miller et al. (126)	92	98	78
Nolte et al. (137)	91	95	57
Shawar et al. (175)	74	90	53
Yuen et al. (218)	96	100	92

of diagnostic amplification techniques for microorganisms other than *M. tuberculosis*, samples are divided before being allocated to the reference and amplification techniques and are thereafter prepared separately as required for each. If this were done for tuberculosis, half of the original specimen would be lysed and the nucleic acid target would be solubilized, concentrated, and introduced into the amplification reaction, thus possibly maximizing the sensitivity. In the case of paucibacillary specimens, there is a delicate balance between amplification procedures and culture. Compared with the amplification procedures, a significantly greater volume of specimen is introduced into the culture media, thus favoring the latter. However, the decontamination procedures kill 70 to 90% of the viable bacilli in the inoculum (107, 217), favoring the alternative approach. This aspect of sample preparation has been studied by Goessens et al. for the detection of *C. trachomatis* in genital specimens (63) and merits investigation for tuberculosis.

Only Noordhoek et al. (139) divided the specimens into two portions, one for conventional detection methods and one for PCR, directed at the IS*6110* element. Unfortunately, their analysis was done with a mixture of respiratory and nonrespiratory specimens, including pleural fluid, urine, and biopsy specimens. The sensitivity and specificity were 92.1 and 99.8%, respectively. PCR was negative for nine smear- and culture-positive samples. The corresponding isolates were tested and did contain the IS*6110* fragment. The authors ascribe these failures to an unequal distribution of a small number of mycobacteria present in the samples, since in each of these cases, only one or two of the three Loewenstein-Jensen culture tubes that were inoculated in parallel were positive. In this study, amplification of DNA extracted from half of the sputum specimen was not superior to culture of the other half.

In this connection, the sequence capture procedure recently described by Magiapan et al. (118) for pleural fluid specimens could be a significant advance. In this procedure, biotinylated oligonucleotides hybridize with mycobacterial DNA in the specimen and are subsequently bound to avidin-coated beads, which are introduced into the PCR mixture. Of 17 samples 13, including 3 of 3 culture-positive samples and 10 of 14 culture-negative samples, gave positive PCR results. Results of the application of this procedure to sputum specimens are eagerly awaited. The use of more appropriate primers could also enhance the sensitivity of the reaction, since even for a particular DNA sequence, different primers may result in different test sensitivities (74, 220).

Efforts to increase the sensitivity by performing a PCR on 25 μl instead of 5 μl of specimen were hampered by an unacceptable increase in the level of inhibitors (6). In contrast, by increasing the sample volume in the commercially available TMA (GenProbe MTDT) from 50 to 500 μl, one group (13) increased the sensitivity from 71.4% (obtained in a previous study [12]) to 83.3% without a loss of specificity (13).

The effectiveness of PCR for tuberculosis is related to the experience and accuracy of the personnel conducting the assay. This was illustrated by an external quality control study of seven laboratories which were tested with sputum samples spiked or not spiked with *M. tuberculosis* BCG (138). Each laboratory used its own protocol for specimen treatment and amplicon detection, but in each case the amplification target was IS*6110*. In general, false-positive rates varied between 0 and 20%, but the rate in one laboratory reached 77%; sensitivities varied between 2 and 90%. A second external quality control study of 30 laboratories, organized more recently by the same authors (140), showed no improvement: 56% of participants produced false-positive results in 5 to >50% of the samples.

(iii) Results on sputum specimens with commercially available amplification tests. The commercially available PCR (Amplicor; Roche) and TMA (Mycobacterium Tuberculosis Direct Test [MTDT]; GenProbe) test give results comparable to those obtained with in-house PCR tests (Tables 4 and 5). Sensitivities vary between 70 and 100%. The results of the MTDT for smear-positive and smear-negative specimens, respectively (Table 6), are comparable to those obtained by PCR.

Schirm et al. (168) compared an in-house PCR and the commercial PCR (Amplicor) on 504 specimens. The sensitivity of the in-house test, 92.6%, was superior to that of the Amplicor system, 70.4%, although the specificity was identical for both. More samples were inhibitory in the commercial test

TABLE 4. Evaluation of the commercially available PCR (Amplicor) for *M. tuberculosis*

Study (reference)	No. of specimens	Prevalence (%)[a]	Sensitivity (%)		Specificity (%)		PPV (%)[b]	
			C[c]	R[c]	C	R	C	R
Carpentier et al. (20)	2,073	9	86		98		94.5	
D'Amato et al. (31)	985			66.7		99.7		91.7
Gleason et al. (61)	532		95		96			
Ichiyama et al. (85)	422	29	97.8		96	98.7		
Moore and Curry (130)	1,009	16	83	87	97	100		
Schirm et al. (168)	504	6	70.4		98			
Vuorinen et al. (205)	256		84.6	82.8	99.1	100		100
Wobeser et al. (214)	1,480	9.5		79		99		93

[a] Prevalence of positive specimens based on culture results.
[b] PPV, positive predictive value.
[c] C, crude results; R, revised results after discrepancy analysis.

TABLE 5. Evaluation of MTDT for the detection of *M. tuberculosis*

Study (reference)	No. of specimens	Prevalence (%)[a]	Sensitivity (%)		Specificity (%)		PPV (%)[b]	
			C[c]	R[c]	C	R	C	R
Abe et al. (1)	135	28	90.6	91.9	95.1	100	85.3	100
Bodmer et al. (12)	617	3	71.4		99		71.4	
Ichiyama et al. (85)	422	29	100		90.1	99.3		
Jonas et al. (92)	758	16	79.8	82.4	96.7	99.4	82	93.8
Miller et al. (126)	750	19	83.9	91	95.3	98.5	82	97
Pfyffer et al. (154)	938	8	92.9	93.9	96.2	97.6	68.4	94
Portaels et al. (156)	497[d]	4	86		96		50	80.7
	418[e]	71	97		69		89	
Vlaspolder et al. (203)	412	14	96.7	98.4	97.7	98.9	88.1	93.8
Vuorinen et al. (205)	256	13	84.6	86.2	98.7	100	100	

[a] Prevalence of positive specimens based on culture results.
[b] PPV, positive predictive value.
[c] C, crude results; R, revised results after discrepancy analysis.
[d] Belgian population.
[e] African population.

than in the in-house version. Both Ichiyama et al. (85) and Vuorinen et al. (205) compared the MTDT with the Amplicor PCR on the same specimens. In the Ichiyama study, the sensitivity and specificity of the MTDT were somewhat better than those obtained with Amplicor, but in the Vuorinen study, the results with the two test kits were similar (Tables 4 and 5).

The QβRA has been applied on a limited scale only (5, 174). The test is performed on a large volume of sputum, but the purification of the hybridized probe from the reaction mix is labor-intensive. PCR inhibitors do not interfere with the QβRA, but the procedure is very prone to amplicon contamination. In a study by Shah et al. on 261 sputum samples (174), the results were not superior to those of other amplification reactions: the sensitivity and specificity were 97.1 and 96.5%, respectively, and after revision were 97.3 and 97.8%, respectively.

Application of LCR (88) and NASBA (209) to tuberculosis has as yet been insufficiently evaluated.

(iv) Specimens other than sputum. PCR does not solve the problem of the bacteriological diagnosis of tuberculosis in children, who do not produce sputum. Pierre et al. (153) performed a PCR on 58 gastric aspirates, for which the classical procedures are known to have a low sensitivity. When DNA amplification was applied to two gastric aspirates from the same patient and amplified in duplicate, 25% of the specimens produced at least one positive result; when three different specimens from the same subject were examined twice, the positivity increased to 60% (in 9 of 15 children).

The diagnosis of tuberculosis by detection of *M. tuberculosis* in peripheral blood mononuclear cells, even by a molecular amplification technique, is still impractical (164), although there has been one promising study (171). The technique is more sensitive, although not optimal, in human immunodeficiency virus-infected patients, particularly in the presence of disseminated disease (50).

Since the lack of sensitivity is the main shortcoming of the amplification techniques and the specificity is more satisfactory, the tests can be useful for organism identification. When culture in a liquid medium is combined with automated growth detection and an amplification method, the time for the diagnosis of *M. tuberculosis* can be shortened to a mean of 14 days (52). PCR and MTDT assays on clinical specimens may also be useful when there is a need for rapid differentiation between *M. tuberculosis* and nontuberculous mycobacterial infections, such as in AIDS patients in industrialized countries (172).

(v) Critique of published studies. The published studies illustrate some shortcomings in design as well as in analysis. There should be no mixtures of respiratory and other specimens, and specimens from patients being treated should not be included. Mycobacterial DNA can be detected for a long time after the start of treatment and in the absence of positive cultures in human (75) and experimental (39) models of tuberculosis. Specimens should be divided, and each portion should be prepared independently for culture and amplification. Some patients may produce sputum with unequally distributed bacilli and/or may not excrete them continuously, and the decontamination procedures may kill variable proportions of the organisms; therefore, three specimens per patient, collected at different times or days, should be examined by each method. A definition of positivity, based on microbiological rather than clinical evidence, should be established. Culture-negative, amplification-positive specimens should be retested by an amplification reaction targeted at an alternative nucleic acid fragment to reveal false-positive results, as done by Herrera and Segovia (78). The sensitivity of the amplification method should be calculated for both the number of positive specimens and the number of positive patients.

(vi) Conclusions concerning amplification techniques for diagnostic purposes. At present, the conclusions published by the Centers for Disease Control and Prevention in 1993 (23) are still valid: a particular technique cannot be replaced by a

TABLE 6. Results of MTDT for the detection of *M. tuberculosis* in smear positive and smear negative specimens

Study (reference)	MTDT sensitivity (%) in different studies		
	Overall	Smear and culture positive	Smear negative, culture positive
Abe et al. (1)	92	100	70
Bodmer et al. (12)	71	100	14[a]
Jonas et al. (92)	82	100	54[b]
Miller et al. (126)	91	94	63
Pfyffer et al. (154)	95	100	80[b]
Portaels et al. (156)	86[c]	89	85
	97[d]	97	100

[a] 86% of these were positive only in liquid medium.
[b] ≤100 CFU/ml in culture.
[c] Belgian population.
[d] African population.

different one if the latter is not at least equivalent to the former and at most has the same cost. At present, amplification methods for *M. tuberculosis* cannot replace the conventional diagnostic techniques, especially since strains should still be cultured for susceptibility testing. The decision of the U.S. Food and Drug Administration is equally justified: use of the rapid MTDT should be restricted to smear-positive samples from untreated patients with tuberculosis and used only in conjunction with traditional sputum examination. It should not be used for smear-negative sputum samples or for other specimens such as pleural or cerebrospinal fluid (53).

(vii) Amplification techniques for *M. tuberculosis* drug susceptibility tests. Because the molecular basis of rifampin resistance is known (97, 189, 190, 212), up to 97% of the rifampin-resistant strains can now also be identified by PCR (35, 48, 83, 211). There is one important limitation to this test: it does not measure the proportion of rifampin-resistant mutants among the isolated strain. Only when the proportion is higher than 1% is the corresponding disease resistant to rifampin therapy. Only further studies will determine how frequently isolates with a low proportion of rifampin-resistant mutants are detected by this technique. Since rifampin resistance develops mostly in isolates that are already isoniazid resistant, the recognition of rifampin resistance lends a high suspicion of multidrug resistance.

Cultures remain necessary to identify rifampin-resistant strains not detected by the PCR, to test for susceptibility to other drugs, and to allow other investigations such as restriction fragment length polymorphism for epidemiologic purposes.

Fungi

Fungal respiratory infections may be due to dimorphic fungi such as *Histoplasma* spp., *Blastomyces* spp., or *Coccidioides immitis*, and they occur sporadically in defined geographic areas. We are not aware of any effort to diagnose these infections by molecular diagnostic techniques.

A second group of fungal respiratory infections are caused by ubiquitous saprophytic fungi, occur 10 times more frequently in immunocompromised individuals (204) than in nonimmunocompromised persons, and are common among patients in intensive care units. *Candida albicans* and *Aspergillus* spp. are the most frequent etiologic agents (204), and mixed infections with bacteria and cytomegalovirus occur in a significant proportion of cases. To shorten the time required for diagnosis, amplification reactions have been developed. Amplification targets have been genes coding for specific proteins (30, 95, 161, 186), 18S rDNA (15, 81, 82, 121, 124, 144), the 26S intergenic spacer region (180), or mitochondrial DNA (127). The last two represent repeated sequences, and thus their use increases the test sensitivity. In their work, Bretagne et al. (15) constructed an internal control. In some studies, primers were directed at a limited number (161) or a wide range of species; in the latter case, this was followed by treatment with restriction enzymes to obtain group identifications (82, 121, 173).

Molecular diagnostic techniques have been applied on BAL specimens and protected brush specimens to shorten the time for diagnosis, and on blood (26, 81, 128, 155) and/or urine (161) specimens in an effort to obtain a diagnosis through less invasive procedures. Only a few preliminary tests on detecting *Aspergillus* spp. in urine specimens have been performed (161). *C. albicans* was detected in seeded blood specimens (18, 81, 128), in blood samples from experimentally infected animals (95, 200), and in human blood in one study (95). The sensitivity of the PCR for *C. albicans* was disappointing: 79% (95), 73% (26), and 46% (158). Two possible reasons for this lack of sensitivity have been mentioned: the difficulty in releasing DNA from *C. albicans* cells, a critical need when they are present in small numbers (162); and the small volume of the specimen used in the amplification reaction (158). PCR has been used more frequently for classification and identification of *Candida* spp. than for their detection (93).

Spreadbury et al. (180) obtained a low sensitivity (80%) and specificity (72%) for the detection of *Aspergillus fumigatus* in clinical specimens, while Bretagne et al. (15), investigating a series of 55 specimens, obtained 25% false-positive results, i.e., detection of amplicons specific for *Aspergillus* spp. in immunocompromised patients who did not develop aspergillosis during follow-up. The authors point out numerous possibilities for contamination by environmental fungi during the preparation and storage of the reagents and the collection, transport, and manipulation of the specimens. Furthermore, the unsolved problem in the investigation of respiratory specimens for yeasts and molds is to distinguish between colonization and infection (15, 124, 134, 186). This differentiation might be possible in the future if genes related to virulence or invasiveness could be identified. At present, molecular diagnostic techniques do not improve the diagnosis of fungal infection by classical procedures.

Pneumocystis carinii

Several studies have confirmed the greater sensitivity of PCR over immunofluorescence for the detection of *Pneumocystis carinii* (22, 42, 46, 101, 111, 142, 185). Although the specificity of the assays is usually high, in one study *P. carinii* was detected in the absence of clinical symptoms (46). This could mean that colonization by *P. carinii* may occur, if contamination of samples in this study can be excluded. The conclusion of Tamburini et al. (185) that *P. carinii* should be sought in BAL specimens by the classical immunofluorescence microscopic technique and that amplification methods should be used only in exceptional cases, when the classical method remains negative, seems reasonable. In the presence of a high clinical suspicion of disease, PCR may have some utility, since claims have been made concerning the detection of *P. carinii* in sputum and two-thirds of blood specimens from patients with a generalized infection (113).

CONCLUSION

The statement that molecular diagnostic techniques, particularly PCR, are able to detect and amplify specifically a single molecule in solution in an olympic-sized swimming pool is nice but also illustrates one of the main difficulties of the procedure: how to introduce the contents of the swimming pool, or the one molecule it contains, into a 2-ml amplification vial.

The main problems facing molecular diagnostic techniques are the false-positive and false-negative results. The former may be avoided by the use of the correct controls in optimal working circumstances, i.e., good laboratory practice (177). Furthermore, any new or unusual findings should be confirmed by an independent amplification reaction. Laboratories engaging in molecular diagnostic techniques should first attain a proficiency level that excludes contamination.

Only when this technical level is reached is it possible to tackle the next problem—the test sensitivity. Much work remains to be done on this aspect. The sensitivity of use of oropharyngeal swabs and nasopharyngeal aspirates for the recovery of pathogens should be compared.

The unknown nature of most inhibitors in clinical specimens

certainly does not facilitate the development of techniques to eliminate them. Efforts to increase the sensitivity of a test by increasing the sample volume in the reaction mixture may increase the interference by inhibitors in some tests but apparently not in others. The extent to which procedures intended to concentrate the amplification target also concentrate inhibitors is unknown, as is the amount of target nucleic acid that is lost during procedures intended to eliminate inhibitors. The latter quantity could be determined by the addition of specific positive internal controls. New applications of amplification reactions should not be introduced without inclusion of specific positive internal controls. An optimal sample preparation method should be simple and rapid, and its ability to concentrate the target and eliminate inhibitors should not be nullified by its being too elaborate and time-consuming.

Compared with classical methods, nucleic acid amplification techniques are definitely more sensitive for the detection of some respiratory disease agents, particularly rhinoviruses, coronaviruses, *B. pertussis*, *M. pneumoniae*, and *C. pneumoniae*. These techniques are indispensable, not only for epidemiological studies but, for the last two organisms, also for clinical diagnostic purposes. However, in view of the results obtained in studies of other organisms, in which the sensitivity of the molecular diagnostic techniques is suboptimal, it can be surmised that the results for these agents are impressive only because the classical methods are particularly insensitive.

The great enthusiasm aroused by molecular diagnostic techniques in the field of tuberculosis detection should be tempered by the knowledge that the expectations concerning their high sensitivity and specificity have not yet been fulfilled. These problems must be addressed before amplification techniques can replace the classical diagnostic techniques. The lack of sensitivity of PCR for *M. tuberculosis* could result from the use of very small sample volumes in the reactions and an irregular dispersion of the organisms in paucibacillary samples. These shortcomings suggest the need for improved sample preparation techniques or the performance of more than one test on each sample.

The introduction of amplification techniques into the clinical diagnostic laboratory is also affected by the staff and space available and, if the decision is made to introduce them, whether they will be added to or replace existing procedures.

In conclusion, laboratories can apply molecular diagnostic techniques only if they comply with stringent external quality control requirements. As far as respiratory disease agents are concerned, amplification procedures should be limited to those listed above for which traditional culture methods are very insensitive and, depending on the geographical location, *Coxiella burnetii* and *Chlamydia psittaci*. For *M. tuberculosis*, they may be useful in some cases when an urgent identification is required if used in conjunction with culture in liquid medium and automated growth monitoring and for the rapid detection of most rifampin-resistant, and hence multiresistant, *M. tuberculosis* isolates.

We think that molecular diagnostic techniques are currently at a stage analogous to that of the clinical bacteriological techniques in the 1960s, before they were improved by many studies and gradually became standardized over the next two decades.

REFERENCES

1. **Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and S. Goto.** 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and GenProbe amplified Mycobacterium tuberculosis direct test. J. Clin. Microbiol. **31:**3270–3274.
2. **Alexander, E. R., and H. R. Harrison.** 1983. Role of *Chlamydia trachomatis* in perinatal infections. Rev. Infect. Dis. **5:**713–719.
3. **Al-Nakib, W., P. G. Higgins, G. I. Barrow, D. A. J. Tyrell, K. Andries, G. Vanden Bussche, N. Taylor, and P. A. J. Janssen.** 1989. The suppression of colds in human volunteers challenged with rhinovirus by a new synthetic drug (R61837). Antimicrob. Agents Chemother. **33:**522–525.
4. **Amicosante, M., L. Richeldi, G. Trenti, G. Paone, M. Campa, A. Bisetti, and C. Saltini.** 1995. Inactivation of polymerase inhibitors for *Mycobacterium tuberculosis* DNA amplification in sputum by using capture resin. J. Clin. Microbiol. **33:**629–630.
5. **An, Q., D. Buxton, A. Hendrickx, L. Robinson, J. Shah, L. Lu, M. Vera-Garcia, W. King, and D. M. Olive.** 1995. Comparison of amplified Qβ replicase and PCR assays for detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **33:**860–867.
6. **Andersen, A. B., S. Thybo, P. Godfrey-Faussett, and N. G. Stoker.** 1993. Polymerase chain reaction for detection of *Mycobacterium tuberculosis* in sputum. Eur. J. Clin. Microbiol. Infect. Dis. **12:**922–927.
7. **Arruda, E., and F. G. Hayden.** 1993. Detection of human rhinovirus RNA in nasal washings by PCR. Mol. Cell. Probes **7:**373–379.
8. **Backman, A., B. Johansson, and P. Olcen.** 1994. Nested PCR optimized for detection of *Bordetella pertussis* in clinical nasopharyngeal samples. J. Clin. Microbiol. **32:**2544–2548.
9. **Beige, J., J. Lokies, T. Schaberg, U. Finckh, M. Fischer, H. Mauch, H. Lode, B. Kohler, and A. Rolfs.** 1995. Clinical evaluation of a *Mycobacterium tuberculosis* PCR assay. J. Clin. Microbiol. **33:**90–95.
10. **Black, C. M., J. A. Tharpe, and H. Russell.** 1992. Distinguishing Chlamydia species by restriction analysis of the major outer membrane protein gene. Mol. Cell. Probes **6:**395–400.
11. **Black, C. M., P. I. Fields, T. O. Messmer, and B. P. Berdal.** 1994. Detection of *Chlamydia pneumoniae* in clinical specimens by polymerase chain reaction using nested primers. Eur. J. Clin. Microbiol. Infect. Dis. **13:**752–756.
12. **Bodmer, T., A. Gurtner, K. Schopfer, and L. Matter.** 1994. Screening of respiratory tract specimens for the presence of *Mycobacterium tuberculosis* by using the Gen-Probe amplified *Mycobacterium tuberculosis* direct test. J. Clin. Microbiol. **32:**1483–1487.
13. **Bodmer, T., E. Möckl, K. Mühlemann, and L. Matter.** 1996. Improved performance of GenProbe amplified *Mycobacterium tuberculosis* direct test when 500 instead of 50 microliters of decontaminated sediment is used. J. Clin. Microbiol. **34:**222–223.
14. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple methods for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
15. **Bretagne, S., J.-M. Costa, A. Marmorat-Khuong, F. Poron, C. Cordonnier, M. Vidaud, and J. Fleury-Feith.** 1995. Detection of *Aspergillus* species DNA in bronchoalveolar lavage samples by competitive PCR. J. Clin. Microbiol. **33:**1164–1168.
16. **Bruce, C. B., W. Al-Nakib, J. W. Almond, and D. A. J. Tyrrell.** 1989. Use of nucleotide probes to detect rhinovirus RNA. Arch. Virol. **105:**179–187.
17. **Buck, G. E., L. O'Hara, and J. T. Summersgill.** 1992. Rapid, simple method for treating clinical specimens containing *Mycobacterium tuberculosis* to remove DNA for polymerase chain reaction. J. Clin. Microbiol. **30:**1331–1334.
18. **Burgener-Kairuz, P., J. P. Zuber, P. Jaunin, T. G. Buchman, J. Bille, and M. Rossier.** 1994. Rapid detection and identification of *Candida albicans* and *Torulopsis* (*Candida*) *glabrata* in clinical specimens by species specific nested PCR amplification of a cytochrome P-450 lanosterol-alpha-demethylase (L1A1) gene fragment. J. Clin. Microbiol. **32:**1902–1907.
19. **Campbell, L. A., M. Perez-Melgosa, D. J. Hamilton, C. C. Kuo, and J. T. Grayston.** 1992. Detection of *Chlamydia pneumoniae* by polymerase chain reaction. J. Clin. Microbiol. **30:**434–439.
20. **Carpentier, E., B. Drouillard, M. Dailloux, D. Moinard, E. Vallee, B. Dutilh, J. Mangein, E. Bergogne-Berezin, and B. Carbonelle.** 1995. Diagnosis of tuberculosis by Amplicor *Mycobacterium tuberculosis* test: a multicenter study. J. Clin. Microbiol. **33:**3106–3110.
21. **Cartwright, C. P.** 1994. Techniques and diagnostic applications of in vivo nucleic acid amplification. Clin. Microbiol. Newsl. **16:**33–40.
22. **Cartwright, C. P., N. A. Nelson, and V. J. Gill.** 1994. Development and evaluation of a rapid and simple procedure for the detection of *Pneumocystis carinii* by PCR. J. Clin. Microbiol. **32:**1634–1638.
23. **Centers for Disease Control and Prevention.** 1993. Diagnosis of tuberculosis by nucleic acid amplification methods applied to clinical specimens. Morbid. Mortal. Weekly Rep. **42:**686.
24. **Centers for Disease Control and Prevention.** 1995. Pertussis United States, January 1992–June 1995. Morbid. Mortal. Weekly Rep. **44:**525–529.
25. **Chamberlain, J. S., R. A. Gibbs, J. E. Rainier, and C. T. Caskey.** 1990. Multiplex PCR for the diagnosis of Duchenne muscular dystrophy, p. 272–281. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., San Diego, Calif.
26. **Chryssanthou, E., B. Andersson, B. Petrini, S. Lofdahl, and J. Tollemar.** 1994. Detection of *Candida albicans* DNA in serum by polymerase chain reaction. Scand. J. Infect. Dis. **26:**479–485.
27. **Claas, E. C., A. J. Van Milaan, M. J. Sprenger, M. Ruiten-Stuiver, G. I.**

Arron, P. H. Rothbarth, and N. Masurel. 1993. Prospective application of reverse transcriptase polymerase chain reaction for diagnosing influenza infections in respiratory samples from a children's hospital. J. Clin. Microbiol. **31:**2218–2221.
28. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31:**2049–2056.
29. **Compton, J.** 1991. Nucleic acid sequence-based amplification. Nature (London) **350:**91–92.
30. **Crampin, A. C., and R. C. Matthews.** 1993. Application of the polymerase chain reaction to the diagnosis of candidosis by amplification of an HSP90 gene fragment. J. Med. Microbiol. **39:**233–238.
31. **D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Calaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kyungmee, R. C. Patel, and A. Miller.** 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche Amplicor *Mycobacterium tuberculosis* PCR test. J. Med. Microbiol. **33:** 1832–1834.
32. **Daniel, T. M.** 1990. The rapid diagnosis of tuberculosis: a selected review. J. Lab. Clin. Med. **116:**277–282.
33. **Das, S., S. L. Chan, B. W. Allen, D. A. Mitchison, and D. B. Lowrie.** 1993. Application of DNA fingerprinting with IS986 to sequential mycobacterial isolates obtained from pulmonary tuberculosis patients in Hong Kong before, during and after short-course chemotherapy. Tubercle Lung Dis. **74:**47–51.
34. **De Barbeyrac, B., C. Bernet-Poggi, F. Febrer, H. Renaudin, M. Dupon, and C. Bébéar.** 1993. Detection of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in clinical samples by polymerase chain reaction. Clin. Infect. Dis. **17:**983–989.
35. **De Beenhouwer, H., Z. Lhiang, G. Jannes, W. Mijs, L. Machtelinckx, R. Rossau, H. Traore, and F. Portaels.** 1995. Rapid detection of rifampicin resistance in sputum and biopsy specimens from tuberculosis patients by PCR and line probe assay. Tubercle Lung Dis. **76:**425–430.
36. **de Lamballiere, X., C. Zandotti, C. Vignoli, C. Bollet, and P. A. de Mico.** 1992. A one step microbial DNA extraction method using "Chelex 199" suitable for gene amplification. Res. Microbiol. **143:**785–790.
37. **de Lassence, A., D. Lecossier, C. Pierre, J. Cadranel, M. Stern, and A. J. Hance.** 1992. Detection of mycobacterial DNA in pleural fluid from patients with tuberculous pleurisy by means of the polymerase chain reaction: comparison of two protocols. Thorax **47:**265–269.
38. **De Wit, D., M. Wootton, B. Allan, and L. Steyn.** 1993. Simple method for production of internal control DNA for *Mycobacterium tuberculosis* polymerase chain reaction assays. J. Clin. Microbiol. **31:**2204–2207.
39. **De Wit, D., M. Wootton, J. Dhillon, and D. A. Mitchison.** 1995. The bacterial DNA content of mouse organs in the Cornell model of dormant tuberculosis. Tubercle Lung. Dis. **76:**555–562.
40. **Douglas, E., J. G. Coote, R. Parton, and W. McPheat.** 1993. Identification of *Bordetella pertussis* in nasopharyngeal swabs by PCR amplification of a region of the adenylate cyclase gene. J. Med. Microbiol. **38:**140–144.
41. **Dumornay, W., P. M. Roblin, M. Gelling, M. R. Hammerschlag, and M. Worku.** Comparison of chemoluminometric immunoassay with culture for diagnosis of chlamydial infections in infants. J. Clin. Microbiol. **30:**1867–1869.
42. **Eisen, D., B. C. Ross, J. Fairbairn, R. J. Warren, R. W. Baird, and B. Dwyer.** 1994. Comparison of *Pneumocystis carinii* detection by toluidine blue O staining, direct immunofluorescence and DNA amplification in sputum specimens from HIV positive patients. Pathology **26:**198–200.
43. **Eisenach, K. D., M. D. Care, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
44. **Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
45. **Ellner, P. D., T. E. Kiehn, R. Cammarata, and M. Hosmer.** 1988. Rapid detection and identification of pathogenic mycobacteria by combining radiometric and nucleic acid probe methods. J. Clin. Microbiol. **26:**1349–1352.
46. **Elvin, K.** 1994. Laboratory diagnosis and occurrence of *Pneumocystis carinii*. Scand. J. Infect. Dis. **94:**S1–S34.
47. **Ewanovich, C. A., L. W. Chin, M. G. Paranchy, M. S. Peppler, R. G. Marusyk, and W. L. Albritton.** 1993. Major outbreak of pertussis in northern Alberta, Canada: analysis of discrepant direct fluorescent-antibody and culture results by using polymerase chain reaction methodology. J. Clin. Microbiol. **31:**1715–1725.
48. **Felmlee, T. A., Q. Liu, A. C. Whelen, D. Williams, S. S. Sommer, and D. H. Persin.** 1995. Genotypic detection of *Mycobacterium tuberculosis* rifampin resistance: comparison of single-stranded conformation polymorphism and dideoxy fingerprinting. J. Clin. Microbiol. **33:**1617–1623.
49. **Fliermans, C. B., W. B. Cherry, L. H. Orrison, S. J. Smith, D. L. Tison, and D. H. Pope.** 1981. Ecological distribution of *Legionella pneumophila*. Appl. Environ. Microbiol. **41:**9–16.
50. **Folgueira, L., R. Delgado, E. Palenque, J. M. Aguado, and A. R. Noriega.** 1996. Rapid diagnosis of *Mycobacterium tuberculosis* bacteremia by PCR. J. Clin. Microbiol. **34:**512–515.
51. **Forbes, B. A., and K. E. Hicks.** 1993. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol. **31:**1688–1694.
52. **Forbes, B. A., and K. E. Hicks.** 1994. Ability of PCR assay to identify *Mycobacterium tuberculosis* in Bactec 12B vials. J. Clin. Microbiol. **32:**1725–1728.
53. **Frankel, D. H.** 1996. FDA approves rapid test for smear positive tuberculosis. Lancet **347:**48.
54. **Gama, R. E., P. R. Horsnell, P. J. Hughes, C. North, C. B. Bruce, W. Al-Kakib, and G. Stanway.** 1989. Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. J. Med. Virol. **28:**73–77.
55. **Gaydos, C. A., T. C. Quinn, and J. J. Eiden.** 1992. Identification of *Chlamydia pneumoniae* by DNA amplification of the 16S rRNA gene. J. Clin. Microbiol. **30:**796–800.
56. **Gaydos, C. A., C. L. Fowler, V. J. Gill, J. J. Eiden, and T. C. Quinn.** 1993. Detection of *Chlamydia pneumoniae* by polymerase chain reaction-enzyme immunoassay in an immunocompromised population. Clin. Infect. Dis. **17:**718–723.
57. **Gaydos, C. A., J. J. Eiden, D. Oldach, L. M. Munidy, P. Auwaerter, M. L. Warner, E. Vance, A. A. Burton, and T. C. Quinn.** 1994. Diagnosis of *Mycoplasma pneumoniae* infection in patients with community-acquired pneumonia by polymerase chain reaction enzyme immunoassay. Clin. Infect. Dis. **19:**157–160.
58. **Gaydos, C. A., P. M. Roblin, M. R. Hammerschlag, C. L. Hyman, J. J. Eiden, J. Schachter, and T. C. Quinn.** 1994. Diagnostic utility of PCR, enzyme immunoassay, culture, and serology for detection of *Chlamydia pneumoniae* in symptomatic and asymptomatic patients. J. Clin. Microbiol. **32:**903–905.
59. **Gilbert, L. A., L. Dakhama, B. M. Bone, E. A. Thoma, and R. G. Hegele.** 1996. Diagnosis of viral respiratory tract infections in children using a reverse transcription-PCR panel. J. Clin. Microbiol. **34:**140–143.
60. **Gingeras, T. R., P. Prodanovich, T. Latimer, J. C. Guatelli, and D. D. Richman.** 1991. Use of self-sustained sequence replication amplification reaction to analyze and detect mutations in zidovudine-resistant human immunodeficiency virus. J. Infect. Dis. **164:**1066–1074.
61. **Gleason, K. G., M. B. Lichty, D. L. Jungkind, and O. Giger.** 1995. Evaluation of Amplicor PCR for direct detection of *Mycobacterium tuberculosis* from sputum specimens. J. Clin. Microbiol. **33:**2582–2586.
62. **Gnarpe, J., and K. Eriksson.** 1995. Sample preparation for *Chlamydia pneumoniae* PCR. APMIS **103:**307–308.
63. **Goessens, W. H. F., J. A. J. W. Kluytmans, N. den Toom, T. H. van Rysoort-Vos, B. G. M. Niesters, E. Stolz, H. A. Verbrugh, and W. G. V. Quint.** 1995. Influence of volume of sample processed on detection of *Chlamydia trachomatis* in urogenital samples by PCR. J. Clin. Microbiol. **33:**251–253.
64. **Gomeli, H., S. Tyagi, C. G. Pritchard, P. M. Lizardi, and F. R. Kramer.** 1989. Quantitative assays based on the use of replicatable hybridization probes. Clin. Chem. **35:**1826–1831.
65. **Grayston, J. T., S. P. Wang, C. C. Kuo, and L. A. Campbell.** 1989. Current knowledge on *Chlamydia pneumoniae*, strain TWAR, an important cause of pneumonia and other acute respiratory diseases. Eur. J. Clin. Microbiol. Infect. Dis. **8:**191–202.
66. **Grayston, J. T., M. B. Aldous, A. Easton, S. P. Wang, C. C. Kuo, L. A. Campbell, and J. Altman.** 1993. Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. J. Infect. Dis. **168:**1231–1235.
67. **Greenfield, L., and J. T. White.** 1993. Sample preparation methods, p. 122–137. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. Principles and applications. American Society for Microbiology, Washington, D.C.
68. **Grimpel, E., P. Begue, I. Anjak, F. Betson, and H. Guiso.** 1993. Comparison of polymerase chain reaction, culture, and Western immunoblot serology for diagnosis of *Bordetella pertussis* infection. J. Clin. Microbiol. **31:**2745–2750.
69. **Halonen, P., A. Rocha, J. Hierholzer, B. Holloway, T. Hyypiä, P. Hurskainen, and M. Pallansch.** 1995. Detection of enteroviruses and rhinoviruses in clinical specimens by PCR and liquid-phase hybridization. J. Clin. Microbiol. **33:**648–653.
70. **Halpern, S. A., R. Bortolussi, and A. J. Wort.** 1989. Evaluation of culture, immunofluorescence, and serology for the diagnosis of pertussis. J. Clin. Microbiol. **27:**752–757.
71. **Hata, D., F. Kuze, Y. Mochizuki, H. Ohkubo, S. Kanazachi, S. Maeda, N. Miwa, and M. Haruki.** 1990. Evaluation of DNA probe test for rapid diagnosis of *Mycoplasma pneumoniae* infections. J. Pediatr. **116:**273–276.
72. **He, Q., J. Mertsola, H. Soini, M. Skurnik, O. Ruuskanen, and M. K. Viljanen.** 1993. Comparison of polymerase chain reaction with culture and enzyme immunoassay for diagnosis of pertussis. J. Clin. Microbiol. **31:**642–645.
73. **He, Q., J. Mertsola, H. Soini, and M. K. Viljanen.** 1994. Sensitive and specific polymerase chain reaction assays for detection of *Bordetella pertus-*

sis in nasopharyngeal specimens. J. Pediatr. **124:**421–426.
74. **He, Q., M. Marjamaki, H. Soini, J. Mertsola, and M. K. Viljanen.** 1994. Primers are decisive for sensitivity of PCR. BioTechniques **17:**82–87.
75. **Hellyer, T. J., T. W. Fletcher, J. H. Bates, W. W. Stead, G. L. Templeton, M. D. Cave, and K. D. Eisenach.** 1996. Strand displacement amplification and the polymerase chain reaction for monitoring response to treatment in patients with pulmonary tuberculosis. J. Infect. Dis. **173:**934–941.
76. **Hermans, P. W. M., A. R. J. Schuitema, D. Van Solingen, C. P. Verstynen, E. M. Bik, A. H. Kolk, and J. van Embden.** 1990. Specific detection of *Mycobacterium tuberculosis* complex strains by polymerase chain reaction. J. Clin. Microbiol. **28:**1204–1213.
77. **Hermans, P. W. M., D. van Soolingen, J. W. Dale, A. R. J. Schutema, R. A. McAdam, D. Catty, and J. D. A. van Embden.** 1990. Insertion element IS*986* from *Mycobacterium tuberculosis*: a useful tool for diagnosis and epidemiology of tuberculosis. J. Clin. Microbiol. **28:**2051–2058.
78. **Herrera, E. A., and M. Segovia.** 1996. Evaluation of mtp40 genomic fragment amplification for specific detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **34:**1108–1113.
79. **Higuchi, R.** 1989. Simple and rapid preparation of samples for PCR, p. 31–38. *In* H. A. Erlich (ed.), PCR technology. Principles and applications for DNA amplification. Stockton Press, New York, N.Y.
80. **Holland, S. M., C. A. Gaydos, and T. C. Quinn.** 1990. Detection and differentiation of *Chlamydia trachomatis*, *Chlamydia psittaci*, and *Chlamydia pneumoniae* by DNA amplification. J. Infect. Dis. **162:**984–987.
81. **Holmes, A. R., R. D. Cannon, M. G. Shepherd, and H. F. Jenkinson.** 1993. Detection of *Candida albicans* and other yeasts in blood by PCR. J. Clin. Microbiol. **32:**228–231.
82. **Hopfer, R. L., P. Walden, S. Setterquist, and W. E. Highsmith.** 1993. Detection and differentiation of fungi in clinical specimens using polymerase chain reaction (PCR) amplification and restriction enzyme analysis. J. Med. Vet. Mycol. **31:**65–75.
83. **Hunt, J. M., G. D. Robert, L. Stockman, T. A. Felmlee, and H. Persing.** 1994. Detection of a genetic locus encoding resistance to rifampin in mycobacterial cultures and clinical specimens. Diagn. Microbiol. Infect. Dis. **18:**219–227.
84. **Hyman, C. L., P. M. Roblin, C. A. Gaydos, T. C. Quinn, J. Schachter, and M. R. Hammerschlag.** 1995. Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: assessment by polymerase chain reaction-enzyme immunoassay and culture. Clin. Infect. Dis. **20:**1174–1178.
85. **Ichiyama, S., Y. Iinuma, Y. Tawada, S. Yamori, Y. Hasegawa, H. Shimokata, and N. Nakashima.** 1996. Evaluation of GenProbe amplified *Mycobacterium tuberculosis* direct test and Roche-PCR microwell plate hybridization method (Amplicor Mycobacterium) for direct detection of mycobacteria. J. Clin. Microbiol. **34:**130–133.
86. **Ieven, M., D. Ursi, H. Van Bever, W. Quint, H. G. M. Niesters, and H. Goossens.** 1996. The detection of *Mycoplasma pneumoniae* by two polymerase chain reactions and its role in acute respiratory tract infections in pediatric patients. J. Infect. Dis. **173:**1445–1452.
87. **Inamine, J. M., T. P. Denny, S. Loechel, U. Schaper, C. H. Huang, K. F. Botte, and P. C. Hu.** 1988. Nucleotide sequence of the P1 attachment-protein gene of *Mycoplasma pneumoniae*. Gene **64:**217–219.
88. **Iovannisci, D. M., and E. S. Winn-Deen.** 1993. Ligation amplification and fluorescence detection of *Mycobacterium tuberculosis* DNA. Mol. Cell. Probes **7:**35–43.
89. **Ireland, D. C., J. Kent, and K. G. Nicholson.** 1993. Improved detection of rhinoviruses in nasal and throat swabs by seminested RT-PCR. J. Med. Virol. **40:**96–101.
90. **Jaulhac, B., M. Nowicki, N. Bornstein, O. Meunier, G. Prevost, Y. Piemont, J. Fleurette, and H. Monteil.** 1992. Detection of *Legionella* spp. in bronchoalveolar lavage fluids by DNA amplification. J. Clin. Microbiol. **30:**920–924.
91. **Johnston, S. L., G. Sanderon, P. K. Pattemore, S. Smith, B. G. Bardin, C. B. Bruce, P. R. Lambden, D. A. J. Tyrrell, and S. T. Holgate.** 1993. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. J. Clin. Microbiol. **31:**111–117.
92. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
93. **Jordan, J. A., and M. B. Durso.** 1996. Rapid speciation of the five most medically relevant *Candida* species using PCR amplification and a microtiter plate-based detection system. Mol. Diagn. **1:**51–58.
94. **Kai, M., S. Kamiya, H. Yabe, I. Takakura, U. Shiozawa, and A. Ozawa.** 1993. Rapid detection of *Mycoplasma pneumoniae* in clinical samples by the polymerase chain reaction. J. Med. Microbiol. **38:**166–170.
95. **Kan, V. L.** 1993. Polymerase chain reaction for the diagnosis of candidemia. J. Infect. Dis. **168:**779–783.
96. **Kaneko, S., S. Murakami, M. Unoura, and K. Kobayashi.** 1992. Quantitation of hepatitis C virus RNA by competitive polymerase chain reaction. J. Med. Virol. **37:**278–282.
97. **Kapur, V., L. Li, S. Iordanescu, M. R. Hamerick, A. Wagner, B. N. Kreiwirth, and J. M. Musser.** 1994. Characterization by automated DNA sequencing of mutations in the gene (*rpoB*) encoding the RNA polymerase beta subunit in rifampin-resistant *Mycobacterium tuberculosis* strains from New York City and Texas. J. Clin. Microbiol. **32:**1095–1098.
98. **Kessler, H. H., F. F. Reinthaler, A. Pschaid, K. Pierer, B. Kleinhappl, E. Eber, and E. Marth.** 1993. Rapid detection of *Legionella* species in bronchoalveolar lavage fluids with the EnviroAmp Legionella PCR amplification and detection kit. J. Clin. Microbiol. **31:**3325–3328.
99. **Kirkwood, B. R.** 1988. Essentials of medical statistics. Blackwell Scientific Publications, Oxford, England.
100. **Kirschner, P., B. Springer, U. Vogel, A. Meier, A. Wrede, M. Kiekenbeck, F.-C. Bange, and E. Böttger.** 1993. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a 2-year experience in a clinical laboratory. J. Clin. Microbiol. **31:**2882–2889.
101. **Kitada, K., S. Oka, T. Kohjin, S. Kimura, Y. Nakamura, and K. Shimada.** 1993. *Pneumocystis carinii* pneumonia monitored by *P. carinii* shedding in sputum by the polymerase reaction. Intern. Med. **32:**370–373.
102. **Kleemola, M., T. Heiskanen-Kosma, H. Nohynek, S. Jokinen, M. Korppi, and J. Eskola.** 1993. Diagnostic efficacy of a *Mycoplasma pneumoniae* hybridization test in nasopharyngeal aspirates of children. Pediatr. Infect. Dis. J. **12:**344–345.
103. **Kleemola, S. R., J. E. Karjalainen, and R. K. Raty.** 1990. Rapid diagnosis of *Mycoplasma pneumoniae* infection: clinical evaluation of a commercial probe test. J. Infect. Dis. **162:**70–75.
104. **Kocagöz, T., E. Yilmaz, S. Özkara, S. Kocagöz, M. Hayran, M. Sachedeva, and H. F. Chambers.** 1993. Detection of *Mycobacterium tuberculosis* in sputum samples by polymerase chain reaction using a simplified procedure. J. Clin. Microbiol. **31:**1435–1438.
105. **Kolk, A. H. J., G. T. Noordhoek, O. De Leeuw, S. Kuyper, and J. A. D. van Embden.** 1994. *Mycobacterium smegmatis* strain for detection of *Mycobacterium tuberculosis* by PCR used as internal control for inhibition of amplification and for quantification of bacteria. J. Clin. Microbiol. **32:**1354–1356.
106. **Kornberg, A.** 1959. Enzymatic synthesis of deoxyribonucleic acid. Harvey Lect. **53:**83–112.
107. **Krasnow, I., and L. G. Wayne.** 1966. Sputum digestion. I. The mortality rate of tubercle bacilli in various digestion systems. Am. J. Clin. Pathol. **45:**352–355.
108. **Ksiazek, T. G., C. J. Peters, P. E. Rollin, S. Zaki, S. Nichol, C. Spiropoulou, S. Morzunov, H. Feldmann, A. Sanchez, A. S. Khan, B. W. J. Mahy, K. Wachsmuth, and J. C. Butler.** 1995. Identification of a new North American Hantavirus that causes acute pulmonary insufficiency. Am. J. Trop. Med. Hyg. **52:**117–123.
109. **Lamblin, G., H. Rahmoune, J. M. Wieruszeski, M. Lhermitte, G. Strecker, and P. Rocussel.** 1991. Structure of two sulphated oligosaccharides from respiratory mucins of a patient suffering from cystic fibrosis: a fast-atom-bombardment m.s. and ^{1}H-n.m.r. spectroscopic study. Biochem. J. **275:**199–206.
110. **Landegren, U., R. Kaiser, J. Sanders, and L. Hood.** 1988. A ligase mediated gene detection technique. Science **241:**1077–1080.
111. **Leigh, T. R., B. G. Gazzard, A. Rowbottom, and J. V. Collins.** 1993. Quantitative and qualitative comparison of DNA amplification by PCR with immunofluorescence staining for diagnosis of *Pneumocystis carinii* pneumonia. J. Clin. Microbiol. **46:**140–144.
112. **Leng, Z., G. E. Kenny, and M. C. Roberts.** 1994. Evaluation of the detection limits of PCR for identification of *Mycoplasma pneumoniae* in clinical samples. Mol. Cell. Probes **8:**125–130.
113. **Lipschik, G. Y., V. J. Gill, J. D. Lundgren, V. A. Andrawis, N. A. Nelson, J. O. Nielsen, F. P. Ognibine, and J. A. Kovacs.** 1992. Improved diagnosis of *Pneumocystis carinii* infection by polymerase chain reaction on induced sputum and blood. Lancet **340:**203–206.
114. **Liu, Z., J. E. Stout, L. Tedesco, M. Boldin, C. Hwang, W. F. Diven, and V. L. Yu.** 1994. Controlled evaluation of copper-silver ionization in eradicating *Legionella pneumophila* from a hospital water distribution system. J. Infect. Dis. **169:**919–922.
115. **Lomeli, H., S. Tyagi, C. G. Pritchard, P. M. Lizardi, and F. R. Kramer.** 1989. Quantitative assays based on the use of replicatable hybridization probes. Clin. Chem. **35:**1826–1831.
116. **Luneberg, E., J. S. Jensen, and M. Frosch.** 1993. Detection of *Mycoplasma pneumoniae* by polymerase chain reaction and nonradioactive hybridization in microtiter plates. J. Clin. Microbiol. **31:**1088–1094.
117. **Maass, M., and K. Dalhoff.** 1994. Comparison of sample preparation methods for detection of *Chlamydia pneumoniae* in broncho-alveolar lavage fluid by PCR. J. Clin. Microbiol. **32:**2616–2619.
118. **Magiapan, G., M. Vokurka, L. Schouls, J. Cadranel, D. Lecossier, J. van Embden, and A. J. Hance.** 1996. Sequence capture-PCR improves detection of mycobacterial DNA in clinical specimens. J. Clin. Microbiol. **34:**1209–1215.
119. **Maiwald, M., K. Kissel, S. Srimnang, M. von Knebel-Doebenitz, and H. G. Sonntag.** 1994. Comparison of polymerase chain reaction and conventional culture for the detection of legionellas in hospital water samples. J. Appl. Bacteriol. **76:**216–225.
120. **Maiwald, M., M. Schill, C. Stockinger, J. H. Helbig, P. C. Luck, W. Witzleb,**

and H. G. Sonntag. 1995. Detection of Legionella DNA in human and guinea pig urine samples by the polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **14:**25–33.
121. **Makimura, K., S. Y. Murayama, and H. Yamaguchi.** 1994. Detection of a wide range of medically important fungi by the polymerase chain reaction. J. Med. Microbiol. **40:**358–364.
122. **Marmion, B. P., J. Williamson, D. A. Worswick, T. W. Kok, and R. J. Harris.** 1993. Experience with newer techniques for the laboratory detection of *Mycoplasma pneumoniae* infection. Clin. Infect. Dis. **17:**S90–S99.
123. **Meade, B. D., and A. Bollen.** 1994. Recommendations for use of the polymerase chain reaction in the diagnosis of *Bordetella pertussis* infections. J. Med. Microbiol. **41:**51–55.
124. **Melchers, W. J., P. E. Verweij, P. van den Hurk, A. van Belkum, B. E. De Pauw, J. A. Hoogkamp-Korstanje, and J. F. Meis.** 1994. General primer-mediated PCR for detection of *Aspergillus* species. J. Clin. Microbiol. **32:** 1710–1717.
125. **Miller, L. A., J. L. Beebe, J. C. Butler, W. Martin, R. Benson, R. E. Hoffman, and B. S. Fields.** 1993. Use of polymerase chain reaction in an epidemiologic investigation of Pontiac fever. J. Infect. Dis. **168:**769–772.
126. **Miller, N., S. G. Hernandez, and T. J. Cleary.** 1994. Evaluation of Gen-Probe amplified Mycobacterium tuberculosis direct test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. J. Clin. Microbiol. **32:**393–397.
127. **Miyakawa, Y., T. Mabuchi, K. Kagaya, and Y. Fukazawa.** 1992. Isolation and characterization of a species specific DNA fragment for detection of *Candida albicans* by polymerase chain reaction. J. Clin. Microbiol. **30:**894–900.
128. **Miyakawa, Y., T. Mabuchi, and Y. Fukazawa.** 1993. New method for detection of *Candida albicans* in human blood by polymerase chain reaction. J. Clin. Microbiol. **31:**3344–3347.
129. **Miyazaki, Y., H. Koga, S. Kohno, and M. Kaku.** 1993. Nested polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **31:**2228–2232.
130. **Moore, D. F., and J. I. Curry.** 1995. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by Amplicor PCR. J. Clin. Microbiol. **33:**2686–2691.
131. **Mori, J., and J. P. Clewley.** 1994. Polymerase chain reaction and sequencing for typing rhinovirus RNA. J. Med. Virol. **44:**323–329.
132. **Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich.** 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. **51:**263–273.
133. **Myint, S., S. Johnston, G. Sanderson, and H. Simpson.** 1994. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. Mol. Cell. Probes **8:**357–364.
134. **Nakamura, H., Y. Shibata, Y. Kudo, S. Saito, H. Kimura, and H. Tomoike.** 1994. Detection of *Aspergillus fumigatus* DNA by polymerase chain reaction in the clinical samples from individuals with pulmonary aspergillosis. Rinsho-Byori **42:**676–681.
135. **Nichol, S. T., C. Spiropoulou, S. Morzunov, P. E. Rollin, T. G. Ksiazek, H. Feldmann, A. Sanchez, J. Childs, S. Zaki, and C. J. Peters.** 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. Science **226:**914–917.
136. **Nicholson, K. G., J. Kent, and D. C. Ireland.** 1993. Respiratory viruses and exacerbations of asthma in adults. Br. Med. J. **307:**982–996.
137. **Nolte, F. S., B. Metchock, J. E. McGowan, Jr., A. Edwards, O. Okwumabua, C. Thurmond, P. S. Mitchell, B. Plikaytis, and T. Shinnick.** 1993. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31:**1777–1782.
138. **Noordhoek, G. T., A. H. J. Kolk, G. Bjune, D. Catty, J. W. Dale, P. E. M. Fine, P. Godfrey-Faussett, S. N. Cho, T. Shinnick, S. B. Svenson, S. Wilson, and J. D. A. van Embden.** 1994. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. J. Clin. Microbiol. **32:**277–284.
139. **Noordhoek, G. T., J. Kaan, S. Mulder, H. Wilke, and A. H. J. Kolk.** 1995. Routine application of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Pathol. **48:**810–814.
140. **Noordhoek, G. T., J. van Embden, and A. H. J. Kolk.** 1996. Reliability of nucleic acid amplification for detection of *Mycobacterium tuberculosis*: an international collaborative quality control study among 30 laboratories. J. Clin. Microbiol. **34:**2522–2525.
141. **Olive, D. M., S. Al-Mufti, W. Al-Mulla, M. A. Khan, A. Pasca, G. Stanway, and W. Al-Nakib.** 1990. Detection and differentiation of picornaviruses in clinical samples following genomic amplification. J. Gen. Virol. **71:**2141–2147.
142. **Olsson, M., K. Elvin, S. Lofdahl, and E. Linder.** 1993. Detection of *Pneumocystis carinii* DNA in sputum and bronchoalveolar lavage samples by polymerase chain reaction. J. Clin. Microbiol. **31:**221–226.
143. **Ouchi, K., T. Nakazawa, M. Karita, and Y. Kanehara.** 1994. Prevalence of *Chlamydia pneumoniae* in acute lower respiratory infection in the pediatric population in Japan. Acta Paediatr. Jpn. **36:**256–260.
144. **Palanco, A. M., J. L. Rodriguez-Tuleda, and J. V. Martinez-Suarez.** 1995. Detection of pathogenic fungi in human blood by the polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **15:**618–620.
145. **Pao, C. C., T. S. B. Yen, J. B. You, J. S. Maa, E. H. Fiss, and C. H. Chang.** 1990. Detection and amplification of *Mycobacterium tuberculosis* by DNA amplification. J. Clin. Microbiol. **28:**1877–1880.
146. **Paton, A. W., J. C. Paton, A. J. Lawrence, P. N. Goldwater, and R. J. Harris.** 1992. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by reverse transcription and polymerase chain reaction amplification. J. Clin. Microbiol. **30:**901–904.
147. **Pattyn, S. R., D. Provinciael, R. Lambrechts, and P. Ceuppens.** 1991. Rapid diagnosis of viral respiratory infections. Comparison between immunofluorescence on clinical samples and immuno-fluorescence on centrifuged cultures. Acta Clin. Belg. **46:**7–12.
148. **Pattyn, S. R., D. Ursi, M. Ieven, V. Raes, and P. Jamet.** 1992. Polymerase chain reaction amplifying DNA coding for species specific rRNA of *Mycobacterium leprae*. Int. J. Lepr. **60:**234–243.
149. **Persing, D. H.** 1993. In vitro nucleic acid amplification techniques, p. 51–87. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. Principles and applications. American Society for Microbiology, Washington, D.C.
150. **Pfaller, M. A.** 1994. Application of new technology to the detection, identification and antimicrobial susceptibility testing of mycobacteria. Am. J. Clin. Pathol. **101:**329–337.
151. **Piatak, M. J., K. C. Luk, B. Williams, and J. D. Lifson.** 1993. Quantitative competitive polymerase chain reaction for accurate quantitation of HIV DNA and RNA species. BioTechniques **14:**70–81.
152. **Pierre, C., D. Lecossier, Y. Boussougant, D. Bocart, V. Joly, P. Yeni, and A. J. Hance.** 1991. Use of a reamplification protocol improves sensitivity of detection of *Mycobacterium tuberculosis* in clinical samples by amplification of DNA. J. Clin. Microbiol. **29:**712–717.
153. **Pierre, C., C. Olivier, D. Lecossier, Y. Boussougant, P. Yeni, and A. J. Hance.** 1993. Diagnosis of primary tuberculosis in children by amplification and detection of mycobacterial DNA. Am. Rev. Respir. Dis. **147:**420–424.
154. **Pfyffer, G. A., P. Kissling, R. Wirth, and R. Weber.** 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J. Clin. Microbiol. **32:**918–923.
155. **Polanco, A. M., J. L. Rodriguez-Tudela, and J. V. Martinez-Suarez.** 1995. Detection of pathogenic fungi in human blood by the polymerase chain reaction. Eur. J. Clin. Microbiol. Infect. Dis. **14:**618–621.
156. **Portaels, F., E. Serruys, H. De Beenhouwer, J. Degraux, K. De Ridder, K. Fissette, J. Gomez-Marin, H. Goossens, F. Mühlberger, F. Nturanye, S. R. Pattyn, F. Pouthier, and A. Van Deun.** 1996. Evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for the routine diagnosis of pulmonary tuberculosis. Acta Clin. Belg. **51:**144–149.
157. **Prückl, P. R., C. Aspöck, A. Makristathis, M. L. Rotter, H. Wank, B. Willinger, and A. M. Hirschl.** 1995. Polymerase chain reaction for detection of *Chlamydia pneumoniae* in gargled-water specimens of children. Eur. J. Clin. Microbiol. Infect. Dis. **14:**141–144.
158. **Rand, K. H., H. Houck, and M. Wolff.** 1994. Detection of candidemia by polymerase chain reaction. Mol. Cell. Probes **8:**215–221.
159. **Raoult, D., G. Vestris, and M. Enea.** 1990. Isolation of 16 strains of *Coxiella burnetii* from patients by using a sensitive centrifugation cell culture system and establishment of the strains in HEL cells. J. Clin. Microbiol. **28:**2482–2484.
160. **Rasmussen, S. J., F. P. Douglas, and P. Timms.** 1992. PCR detection and differentiation of *Chlamydia pneumoniae*, *Chlamydia psittaci*, and *Chlamydia trachomatis*. Mol. Cell. Probes **6:**389–394.
161. **Reddy, L. V., A. Kumar, and V. P. Kurup.** 1993. Specific amplification of *Aspergillus fumigatus* DNA by polymerase chain reaction. Mol. Cell. Probes **7:**121–126.
162. **Reiss, E., and C. J. Morrison.** 1993. Nonculture methods for diagnosis of disseminated candidiasis. Clin. Microbiol. Rev. **6:**311–312.
163. **Resnikov, M., T. K. Blackmore, J. J. Finlay-Jones, and D. L. Gordon.** 1995. Comparison of nasopharyngeal aspirates and throat swab specimens in a polymerase chain reaction based test for *Mycoplasma pneumoniae*. Eur. J. Clin. Microbiol. Infect. Dis. **14:**58–61.
164. **Rolfs, A., J. Beige, U. Finck, B. Köhler, T. Schaberg, J. Lokies, and H. Lode.** 1995. Amplification of *Mycobacterium tuberculosis* from peripheral blood. J. Clin. Microbiol. **33:**3312–3314.
165. **Roure, C.** 1994. Le programme régional de vaccination en Europe (1991–1993). Santé **4:**145–150.
166. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
167. **Scadden, D. T., Z. Wang, and J. E. Groopman.** 1992. Quantitation of plasma human immunodeficiency virus type 1 RNA by competitive polymerase chain reaction. J. Infect. Dis. **165:**1119–1123.
168. **Schirm, J., L. A. B. Oostendorp, and J. G. Mulder.** 1995. Comparison of Amplicor, in-house PCR and conventional culture for detection of *Mycobacterium tuberculosis* in clinical samples. J. Clin. Microbiol. **33:**3221–3224.
169. **Schläpfer, G., H. P. Senn, R. Berger, and M. Just.** 1993. Use of polymerase chain reaction to detect *Bordetella pertussis* in patients with mild or atypical symptoms of infection. Eur. J. Clin. Microbiol. Infect. Dis. **12:**459–463.
170. **Schläpfer, G., J. D. Cherry, U. Heiniger, M. Uberall, S. Schmitt-Grohé, S. Laussucq, M. Just, and K. Stehr.** 1995. Polymerase chain reaction identi-

fication of *Bordetella pertussis* infections in vaccinees and family members in a pertussis vaccine efficacy trial in Germany. Pediatr. Infect. Dis. J. **14:**209–214.
171. **Schluger, N. W., R. Condos, S. Lewis, and W. Rom.** 1994. Amplification of DNA of *Mycobacterium tuberculosis* from peripheral blood of patients with pulmonary tuberculosis. Lancet **344:**232–233.
172. **Schluger, N. W., D. Kinney, T. J. Harkin, and W. N. Rom.** 1994. Clinical utility of the polymerase chain reaction in the diagnosis of infections due to *Mycobacterium tuberculosis*. Chest **105:**1116–1121.
173. **Setterquist, S., and W. E. Highsmith.** 1993. Detection and differentiation of fungi in clinical specimens using polymerase chain reaction (PCR) amplification and restriction enzyme analysis. J. Med. Vet. Mycol. **31:**65–74.
174. **Shah, J. S., J. Liu, D. Buxton, A. Hendricks, L. Robinson, G. Radcliffe, W. King, D. Lane, D. M. Olive, and J. D. Klinger.** 1995. Q-beta replicase-amplified assay for detection of *Mycobacterium tuberculosis* directly from clinical specimens. J. Clin. Microbiol. **33:**1435–1441.
175. **Shawar, R. M., F. A. K. El-Zaatari, A. Nataraj, and J. E. Clarridge.** 1993. Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and non-isotopic hybridization methods. J. Clin. Microbiol. **31:**61–65.
176. **Sillis, M.** 1993. Modern methods for the diagnosis of *Mycoplasma pneumoniae* pneumonia. Rev. Med. Microbiol. **4:**24–30.
177. **Sirko, D. A., and G. D. Ehrlich.** 1994. Laboratory facilities, protocols, and operations, p. 19–43. *In* G. D. Ehrlich and S. J. Greenberg (ed.), PCR-based diagnostics. Blackwell Scientific Publications, Boston, Mass.
178. **Skakni, L., A. Sardet, J. Just, I. Landman-Parker, J. Costil, N. Maniot-Ville, F. Bricout, and A. Garberg-Chenon.** 1992. Detection of *Mycoplasma pneumoniae* in clinical samples from pediatric patients by polymerase chain reaction. J. Clin. Microbiol. **30:**2638–2643.
179. **Soini, H., M. Shurnik, K. Liippo, E. Tala, and M. K. Viljanen.** 1992. Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32 kilodalton protein. J. Clin. Microbiol. **30:** 2025–2028.
180. **Spreadbury, C., D. Holden, A. Aufauvre-Brown, B. Bainbridge, and J. Cohen.** 1993. Detection of *Aspergillus fumigatus* by polymerase chain reaction. J. Clin. Microbiol. **31:**615–621.
181. **Stein, A., and D. Raoult.** 1992. Detection of *Coxiella burnetii* by DNA amplification using polymerase chain reaction. J. Clin. Microbiol. **30:**2462–2466.
182. **Strebel, P. M., S. L. Cochi, K. M. Farizo, B. J. Plane, S. D. Hanauer, and A. L. Banghman.** 1993. Pertussis in Missouri: evaluation of nasopharyngeal culture, direct fluorescent antibody testing and clinical case definition in the diagnosis of pertussis. Clin. Infect. Dis. **16:**276–285.
183. **Takewaki, S., K. Okuzumi, H. Ishiko, K. Nakahara, A. Ohkubo, and R. Nagai.** 1993. Genus-specific polymerase chain reaction for the mycobacterial *dnaJ* gene and species specific oligonucleotide probes. J. Clin. Microbiol. **43:**446–450.
184. **Takewaki, S., K. Okuzumi, I. Manabe, M. Tanimura, K. Miyamura, K. Nakahara, Y. Yazaki, A. Ohkubo, and R. Nagai.** 1994. Nucleotide sequence comparison of the mycobacterial dnaJ gene and PCR-restriction fragment length polymorphism analysis for identification of mycobacterial species. Int. J. Syst. Bacteriol. **44:**159–166.
185. **Tamburini, E., P. Mencarini, A. De Luca, G. Maiuro, G. Ventura, A. Antinori, A. Ammassari, E. Visconti, L. Ortona, and A. Siracusano.** 1993. Diagnosis of *Pneumocystis carinii* pneumonia: specificity and sensitivity of polymerase chain reaction in comparison with immunofluorescence in bronchoalveolar lavage specimens. J. Med. Microbiol. **38:**449–453.
186. **Tang, C. M., D. W. Holden, A. Aufauvre-Brown, and J. Cohen.** 1993. The detection of *Aspergillus* spp. by the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. Am. Rev. Respir. Dis. **148:**1313–1317.
187. **Taranger, J., B. Trollfors, L. Lind, G. Zackrisson, and K. Beling-Holmquist.** 1994. Environmental contamination leading to false-positive polymerase chain reaction for pertussis. Pediatr. Infect. Dis. J. **13:**936–937.
188. **Telenti, A., P. Imboden, and D. Germann.** 1992. Comparative polymerase chain reaction using an internal standard: application to the quantitation of viral DNA. J. Virol. Methods **39:**259–268.
189. **Telenti, A., P. Imboden, F. Marchesi, D. Lowrie, S. Cole, M. J. Colston, L. Matter, K. Schopfer, and T. Bodmer.** 1993. Detection of rifampicin resistance mutations in *Mycobacterium tuberculosis*. Lancet **341:**647–650.
190. **Telenti, A., P. Imboden, F. Marchesi, T. Schmidheini, and T. Bodmer.** 1993. Direct automated detection of rifampicin-resistant *Mycobacterium tuberculosis* by polymerase chain reaction and single strand conformation polymorphism analysis. Antimicrob. Agents Chemother. **37:**2054–2058.
191. **Tenover, F. C., and E. R. Unger.** 1993. Nucleic acid probes for detection and identification of infectious agents, p. 3–25. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology. Principles and applications. American Society for Microbiology, Washington, D.C.
192. **Thom, D. H., J. T. Grayston, L. A. Campbell, V. K. Diwan, and S. P. Wang.** 1994. Respiratory infection with *Chlamydia pneumoniae* in middle-aged and older adult outpatients. Eur. J. Clin. Microbiol. Infect. Dis. **13:**785–792.
193. **Tjhie, J. H., F. J. Van Kuppeveld, R. Roosendaal, W. J. Mechers, R. Gordijn, D. M. MacLaren, J. M. Walboomers, C. J. Meijer, and A. J. van den Brule.** 1994. Direct PCR enables detection of *Mycoplasma pneumoniae* in patients with respiratory tract infections. J. Clin. Microbiol. **32:**11–16.
194. **To, H., N. Kako, G. Q. Zhang, H. Otsuka, M. Ogawa, O. Ohial, S. V. Nguyen, T. Yamaguchi, H. Fukushi, N. Nagaoka, M. Akiyama, K. Amano, and K. Hirai.** 1996. Q-fever pneumonia in children in Japan. J. Clin. Microbiol. **34:**647–651.
195. **Tong, C. Y., and M. Sillis.** 1993. Detection of *Chlamydia pneumoniae* and *Chlamydia psittaci* in sputum samples by PCR. J. Clin. Pathol. **46:**313–317.
196. **Tongersen, H., T. Skern, and D. Blaas.** 1989. Typing of human rhinoviruses based on sequence variations in the 5′ non-coding region. J. Gen. Virol. **70:**3111–3116.
197. **Ulrich, P. P., J. M. Romeo, L. J. Daniel, and G. N. Vyas.** 1993. An improved method for the detection of hepatitis C virus in plasma utilizing heminested primers and internal control RNA. PCR Methods Applic. **2:**241–249.
198. **Ursi, J. P., D. Ursi, M. Ieven, and S. R. Pattyn.** 1992. Utility of an internal control for the polymerase chain reaction: application to detection of *Mycoplasma pneumoniae* in clinical specimens. APMIS **100:**635–639.
199. **Van der Zee, A., C. Agterberg, M. Peeters, J. Schellekens, and F. R. Mooi.** 1993. Polymerase chain reaction assay for pertussis: simultaneous detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. J. Clin. Microbiol. **31:**2134–2140.
200. **van Deventer, A. J., W. H. Goessens, A. van Belkum, H. J. van Vliet, E. W. van Etten, and H. A. Verbrugh.** 1995. Improved detection of *Candida albicans* by PCR in blood of neutropenic mice with systemic candidiasis. J. Clin. Microbiol. **33:**625–628.
201. **Van Kuppeveld, F. J., K. E. Johansson, J. M. Galama, J. Kissing, G. Bolske, E. Hjelm, J. T. van der Logt, and N. J. Melchers.** 1994. 16S rRNA based polymerase chain reaction compared with culture and serological methods for diagnosis of *Mycoplasma pneumoniae* infection. Eur. J. Clin. Microbiol. Infect. Dis. **13:**401–405.
202. **van Soolingen, D., P. E. W. de Haas, P. W. M. Hermans, P. M. A. Groenen, and J. D. A. van Embden.** 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **31:**1987–1995.
203. **Vlaspolder, F., P. Singer, and C. Roggeveen.** 1995. Diagnostic value of an amplification method (Gen-probe) compared with that of culture for diagnosis of tuberculosis. J. Clin. Microbiol. **33:**2699–2703.
204. **von Eiff, M., N. Roos, W. Fegeler, C. von Eiff, M. Zühlsdorf, J. Glaser, and J. van de Loo.** 1994. Pulmonary fungal infections in immunocompromised patients: incidence and risk factors. Mycoses **37:**329–335.
205. **Vuorinen, P., A. Miettinen, R. Vuento, and O. Hallstrom.** 1995. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by GenProbe amplified *Mycobacterium tuberculosis* Direct Test and Roche Amplicor *Mycobacterium tuberculosis* test. J. Clin. Microbiol. **33:**1856–1859.
206. **Wadowsky, R. M., R. B. Yee, L. Mezmar, E. J. Wing, and J. N. Dowling.** 1982. Hot water systems as sources of *Legionella pneumophila* in hospital and nonhospital plumbing fixtures. Appl. Environ. Microbiol. **43:**1104–1110.
207. **Wadowsky, R. M., S. Laus, T. Libert, S. J. States, and G. D. Ehrlich.** 1994. Inhibition of PCR-based assay for *Bordetella pertussis* by using calcium alginate fiber and aluminum shaft components of a nasopharyngeal swab. J. Clin. Microbiol. **32:**1054–1057.
208. **Walker, D. A., I. K. Taylor, D. M. Mitchell, and R. J. Shaw.** 1992. Comparison of polymerase chain reaction amplification of two mycobacterial DNA sequences, IS *6110* and the 65 kDa antigen gene, in the diagnosis of tuberculosis. Thorax **47:**690–694.
209. **Walker, G. T., J. G. Nadeau, P. A. Spears, J. L. Schram, C. M. Nycz, and D. D. Shank.** 1994. Multiplex strand displacement amplification (SDA) and detection of DNA sequences from *Mycobacterium tuberculosis* and other mycobacteria. Nucleic Acids Res. **22:**2670–2676.
210. **Watson, M. W., P. R. Lambden, and I. N. Clarke.** 1991. Genetic diversity and identification by amplification of the chlamydial 60-kilodalton cysteine-rich outer membrane protein gene. J. Clin. Microbiol. **29:**1188–1193.
211. **Whelen, A. C., T. A. Felmlee, J. M. Hunt, D. L. Williams, G. D. Roberts, L. Stockman, and D. H. Persing.** 1995. Direct genotypic detection of *Mycobacterium tuberculosis* rifampin resistance in clinical specimens by using single-tube heminested PCR. J. Clin. Microbiol. **33:**556–561.
212. **Williams, D. L., C. Waguespack, K. Eisenach, J. T. Crawford, and F. Portaels.** 1994. Characterization of rifampin resistance in pathogenic mycobacteria. Antimicrob. Agents Chemother. **38:**2380–2386.
213. **Wilson, S. M., R. McNerney, P. M. Nye, P. D. Godfrey-Faussett, N. G. Stoker, and A. Voller.** 1993. Progress toward a simplified polymerase chain reaction and its application to diagnosis of tuberculosis. J. Clin. Microbiol. **31:**776–782.
214. **Wobeser, W., M. Krajden, J. Conly, H. Simpson, B. Yim, M. D'Costa, M. Fuksa, C. Hian-Cheong, M. Patterson, A. Philips, R. Bannatyne, A. Haddad, J. L. Brunson, and S. Krayden.** 1996. Evaluation of Roche amplicor PCR assay for *Mycobacterium tuberculosis*. J. Clin. Microbiol. **34:**134–139.
215. **Wollcott, M. J.** 1992. Advances in nucleic acid based detection methods. Clin. Microbiol. Rev. **5:**370–386.
216. **Wu, D. Y., and R. B. Wallace.** 1989. The ligase amplification reaction

(LAR)-amplification of specific DNA sequences using sequential rounds of template-dependent ligation. Genomics **4:**560–569.
217. **Yajko, D. M., C. Wagner, V. J. Tavere, T. Kacogöz, W. K. Hadley, and H. F. Chambers.** 1995. Quantitative culture of *Mycobacterium tuberculosis* from clinical sputum specimens and dilution endpoint of its detection by the amplicor PCR assay. J. Clin. Microbiol. **33:**1944–1947.
218. **Yuen, K. Y., K. S. Chan, C. M. Chan, B. S. W. Ho, L. K. Dai, P. Y. Chan, and M. H. Ng.** 1993. Use of PCR in routine diagnosis of treated and untreated pulmonary tuberculosis. J. Clin. Pathol. **46:**318–322.
219. **Yuen, L. K. W., B. C. Ross, K. M. Jackson, and B. Dwyer.** 1993. Characterization of *Mycobacterium tuberculosis* from Vietnamese patients by Southern blot hybridization. J. Clin. Microbiol. **31:**1615–1618.
220. **Zambardi, G., C. Roure, N. Boujaafar, B. Fouque, J. Freney, and J. Fleurette.** 1993. Comparison of three primer sets for the detection of *Mycobacterium tuberculosis* in clinical samples by polymerase chain reaction. Ann. Biol. Clin. Paris **51:**893–897.