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Diagnosis of Tuberculosis: Available Technologies, Limitations, and Possibilities

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Rapid diagnosis and treatment are important for preventing transmission of *Mycobacterium tuberculosis*. However, the diagnosis of tuberculosis continues to pose serious problems, mainly because of difficulties in differentiating between patients with active tuberculosis and those with healed lesions, normal mycobacterium boris BCG (Bacillus Calmette Guerin) vaccinated individuals, and unvaccinated Manteux positives. Physicians still rely on conventional methods such as Ziehl-Neelsen (ZN) staining, fluorochrome staining, sputum culture, gastric lavage, and other non-traditional methods. Although the tuberculin test has aided in the diagnosis of tuberculosis for more than 85 years, its interpretation is difficult because sensitization with nontuberculous mycobacteria leads to false-positive tests. There have been numerous unsuccessful attempts to develop clinically useful serodiagnostic kits for tuberculosis. A number of proteinaceous and nonprotein antigens (such as acyltrehaloses and phenolglycolipids) have been explored from time to time for the development of such assays but they have not proved to be clinically useful. It has been difficult to develop an ELISA utilizing a suitable antigen because *M. tuberculosis* shares a large number of antigenic proteins with other microorganisms that may or may not be pathogenic. With the advent of molecular biology techniques, there have been significant advances in nucleic acid-based amplification and hybridization, which are helping to rectify existing flaws in the diagnosis of tuberculosis. The detection of mycobacterial DNA in clinical samples by polymerase chain reaction (PCR) is a promising approach for the rapid diagnosis of tuberculous infection. However, the PCR results must be corrected for the presence of inhibitors as well as for DNA contamination. In the modern era of genetics, marked by proteomics and genomics, the day is not far off when DNA chip-based hybridization assays will instantly reveal mycobacterial infections. J. Clin. Lab. Anal. 17: 155–163, 2003. © 2003 Wiley-Liss, Inc.

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INTRODUCTION

Myobacteria were the first bacterial pathogens to be described in humans, and continue to produce devastating illness even today. The discovery of the causative organism of tuberculosis dates back to 1882, when Robert Koch [58] described the isolation of tubercle

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bacillus. Since then, a large number of mycobacterial species responsible for causing pulmonary and extrapulmonary infections have been identified in humans as well as in animals (1). These include two major pathogenic organisms, *Mycobacterium tuberculosis* and *M. leprae*, which are known to cause tuberculosis and leprosy, respectively, in humans. Tuberculosis displays all of the principal characteristic features of a global epidemic disease and is rampant throughout the world. One out of three people on this planet is believed to be infected with *M. tuberculosis*, leading to eight million cases of active tuberculosis per year and approximately three million deaths annually. The emergence of epidemic multi-drug-resistant strains of *M. tuberculosis* in conjunction with HIV infection has made this problem all the more serious. Globally there are about 4.6 million cases of dual HIV and tuberculosis infection. Significant mortality and morbidity rates have been reported in various parts of the world, including developed as well as developing nations (2,3). Tuberculosis has become a major concern worldwide, and without commitment and action at national and international levels, tuberculosis will claim about 30 million more lives in the next decade and there will be about 90 million new cases of active tuberculosis. Such a vast epidemic creates challenges as it raises the demand for public health solutions. The currently available remedies for fighting tuberculosis are inadequate. The ultimate goal of biomedical tuberculosis research around the world should be to lessen the public health burden of this disease by developing improved diagnostic and therapeutic intervention strategies (4).

PROBLEMS ASSOCIATED WITH DIAGNOSIS

With the threat of such an epidemic looming, and despite an enormous amount of research since the time of Koch, we still have no simple, rapid, sensitive, and specific test to differentiate most or all patients with active tuberculosis from those with quiescent lesions, previous vaccination, or other diseases, or even from those who are completely healthy (5). Timely and accurate identification (screening) of persons infected with *M. tuberculosis* and rapid laboratory confirmation of tuberculosis are two effective public health measures that can be taken to combat the tuberculosis epidemic. Errors in diagnosis based predominantly on X-ray images and/or symptoms are common. While infectious cases are frequently missed, some people are mistakenly diagnosed with tuberculosis and are inappropriately treated. The pool of infection is growing and the resulting risk of becoming infected is tremendous. To ward off such risks there is a dire need to develop strategies for identifying infection with the best possible precision.

Traditional Diagnostic Methods

Ziehl-Neelsen (ZN) and other tests

The simplest rapid diagnostic method is the detection of acid fast bacilli by microscopy. However, this test is characterized by poor sensitivity. About 40–60% of patients with pulmonary disease and 75% of patients with extrapulmonary disease go undiagnosed by this traditional method. A minimum number of 10^4/mL bacilli are required for microscopy, and this method cannot discriminate *M. tuberculosis* from other *mycobacteria*.

Culture method

The current methods used in clinical laboratories are growth-dependent and may take 6–8 weeks to produce a negative/positive result.

The aspiration of gastric contents for examination by smear culture is another diagnostic method, but it cannot be employed on a large scale. Gastric lavage cannot be used because it is difficult to collect proper swab specimens. The introduction of the fiber-optic bronchoscope has made lung biopsy, bronchial lavage, and brushing simple and safe procedures, but unfortunately the equipment is not universally available and the procedure to collect specimens is painful.

Radiometric culture

The BACTEC 460 radiometric system (Becton Dickinson Instrument Systems, Sparks, MD) is an automated method for detecting $^{14}CO_2$ liberated by bacteria during metabolism and decarboxylation of ^{14}C-labeled substrates. For detection of mycobacteria, the system uses ^{14}C-labeled palmitic acid as the substrate in modified Middlebrook 7H12 broth. With the BACTEC system, the number of positive cultures may or may not be higher than that obtained by conventional culture on solid media. The BACTEC system is expensive, and the disposal of radioactive waste precludes its use in peripheral health centers. The use of BACTEC 12B bottles (Becton Dickinson Diagnostic Instrument System) in conjunction with Accuprobe for TB (Geneprobe Inc., San Diego, CA) can shorten the time required to identify this organism, but these procedures still require 1–3 weeks to produce results and are not cost-effective.

Examination of sputum culture is the most reliable method for detecting pulmonary tuberculosis in a clinical setting, but the process is lengthy and cumbersome, and requires the use of mycobacterial culture facilities.

Supportive tests

The most commonly used method in the history of tuberculosis diagnosis is the Mantoux test. However, positive results do not provide conclusive evidence of active tuberculosis, and negative tests do not exclude it. Sensitization with nontuberculous bacteria leads to false-positive results and the test is difficult to interpret. A positive tuberculin test may be due to active tuberculosis, past infection, BCG vaccination, or sensitization by environmental mycobacteria. Therefore, this test is more helpful in places where BCG vaccination is no longer used routinely, and in heavily afflicted populations it is ineffective. Recently, a recombinant antigen (DPPD) encoded by a gene unique to the *M. tuberculosis* complex organisms proved to be better than PPD in the Mantoux test (6). It can facilitate a more specific diagnosis of tuberculosis since the DPPD gene is not present in nontuberculous bacilli. Other tests based on hematological features, such as a high erythrocyte sedimentation rate (ESR), mild anemia, elevated lymphocyte count, and assay of acute phase reactants, are no more than suggestive. All of these drawbacks led to increased interest in serological diagnostic techniques for tuberculosis.

Nontraditional Methods

There have been numerous unsuccessful attempts to develop clinically useful serodiagnostic methods for the detection of tuberculosis. Although a large number of published reports clearly show that antibody levels are significantly higher in tuberculosis patients than in the general population, little consideration has been given to the value of antibody tests in various operational situations. The crucial factor from the diagnostic point of view is the degree of overlap between those with active disease and those without. In the case of *M. tuberculosis*, there is apparently no dominant specific antigen; indeed, most of the antibody response in the infected host is directed toward shared mycobacterial antigens (5). Certainly there is a need to explore more markers and tools for tuberculosis detection. A few such new markers and tools are described below.

Tuberculostearic acid

One easily detectable marker/component of *M. tuberculosis* is tuberculostearic acid, which can be detected in femtomole quantities by gas-liquid chromatography (7). The presence of tuberculostearic acid in cerebrospinal fluid is thought to be a diagnostic marker for tuberculous meningitis (8–10) and has been suggested to be useful in diagnosing pulmonary tuberculosis as well (11,12). However, and important concern with pulmonary specimens is that organisms other than *M. tuberculosis* may produce components that will generate a false-positive signal. Also, the availability of gas-liquid chromatography is a remote possibility in peripheral clinical laboratories.

Immunodiagnostic approaches

ELISA. Enzyme immunoassays are useful for the early diagnosis of all forms of tuberculosis. The specificity of these assays has improved significantly with the availability of purified and recombinant antigens, and monoclonal antibody-based enzyme immunoassays. However, antibody-based assays often fail to discriminate between infected and exposed subjects, and caution must be exercised when interpreting the results.

Interest in developing a reliably specific and sensitive serodiagnostic test encouraged the search for tuberculosis-specific proteins and relevant monoclonal antibodies. Preliminary approaches identified the 38kDa, 19kDa, and 16kDa proteins as prominent immunogens (13). Antibody to 38kDa antigen is elevated in multibacillary pulmonary tuberculosis patients, while antibody to HSP 71 is equally increased in sputum smear-negative pulmonary disease patients (14). Antibodies to the 16kDa antigen are selectively increased in chronically exposed household contacts of patients and hospital workers (15,16). Antibodies to lipoarabinomannan (LAM) and the 16kDa antigen are elevated in the cerebrospinal fluid of patients with tuberculosis meningitis (17). Recurrent and extensive radiographic pulmonary tuberculosis with poor prognosis is associated with high anti-38kDa and low anti-16kDa antibody levels. Patients with less pulmonary cavitations harbor a high anti-19kDa tier, while bacteriological relapse during treatment indicates a rise in antibodies to the 16kDa antigen. The most important antigens studied to date include the 65, 30/31, 23, 19, 16, 14, and 12 kDa proteins and 38 kDa lipoprotein as a serological marker for active tuberculosis. Recently, isocitrate dehydrogenase, encoded by the icd2 gene of *M. bovis* BCG was reported to be a sensitive diagnostic reagent in antibody-based assays (18).

Although the diagnostic antigen of choice currently is undoubtedly the 38kDa protein, patients with lepromatous leprosy also show a considerable amount of anti-38kDa antibodies (19). The 19kDa protein is compromised by its cross-reactivity with *M. avium* (20), while the antibody to the 16kDa protein can also be identified in a significant fraction of infected healthy subjects. Antibodies to 30-31kDa firbonectin binding proteins, which are cross-reactive with other species of mycobacteria, have been demonstrated in two-thirds of tuberculosis patients (21,22). The pronounced immunogenicity

of HSP 71 during paucibacillary infection could be attributed to enhanced secretion during intracellular replication, to surface expression enabling recognition by B cells, and/or to the adjuvant independence of this antigen. Antibodies to HSP 71 are directed to both linear and conformational epitopes localized at the polymorphic and largely species-specific carboxyl terminal part of molecules (14). The proportion of antibodies to the respective epitopes is yet to be determined, but the bulk of the antibodies to most antigens in the sera of tuberculosis patients is thought to be directed toward conformational epitopes, which are generally shared among mycobacterial species. Along with protein antigens, a number of nonprotein antigens have been explored from time to time. Prominent among these are glycolipids, sulpholipids, and lipopolysachharides.

Recently, a comparison of seven tests for the serodiagnosis of tuberculosis revealed that a combination of two tests—ICT tuberculosis and PATHOZYME-MYCO IgG-yielded the best results, with a sensitivity of 66% and a specificity of 86% (23).

Nonproteinaceous antigens as diagnostic agents in ELISA

Lipid-based molecules. Because mycobacteria are rich in lipids ($\sim$250 enzymes code for fatty acid metabolism, as compared to 50 in *E. coli*), a number of lipid-based molecules have been found to be antigenic. Trehalose-based glycolipids are found in a variety of structural forms in the lipids of mycobacteria and related bacteria. Antibodies to cord factor (trehalose-6, 6′ dimycolate) are considered to be active serological markers.

Other phospholipids. Antituberculophospholipid antibodies as serological markers have been reported to have a sensitivity and specificity of 86.9% and 100%, respectively (59). Acyltrehaloses are among the strongest antigenic glycolipids of *M. tuberculosis*. A phenolglycolipid antigen (PLG Tb1) has also been mentioned as a possible valuable diagnostic antigen (24). Although there is a vast array of available antigens, no single antigen reagent has 100% sensitivity; hence, future research should identify the best combination of antigens for the serodiagnosis of tuberculosis. Such combinations could be utilized to aid in the diagnosis of *M. tuberculosis*.

γ-Interferon

Recently, the γ-interferon assay was assessed as a potential candidate to replace the Mantoux skin test (25). The assay was evaluated in groups of immigrants, health-care workers, and *M. tuberculosis* and *M. avium* complex MAC) patients. The efficacy of the assay was not significantly different from that of the Mantoux test in cases of active tuberculosis, and it detected three of the seven cases of MAC colonization. In patients with active tuberculosis, the assay had a sensitivity of 77%, and was not significantly higher in extrapulmonary compared to pulmonary cases (83% vs. 74%). Quantiferon sensitivity was not significantly different for smear-negative or –positive cases (80% vs. 71%). However, the assay requires the use of laboratory facilities to stimulate viable lymphocytes, and an enzyme iummunoassay to quantify IFN-γ.

Approaches based on molecular biology

With the advent of molecular biology techniques, nucleic acid-based amplification and hybridization assays are helping to rectify existing flaws in tuberculosis diagnosis. These assays are based on the detection of biomolecules in patient specimens. The sensitivity of such assays can be increased by using a signal amplification system, as described below.

Branched DNA signal amplification. In this procedure, a bifunctional oligonucleotide probe is constructed that contains a sequence specific for the target species, and a sequence to which a second oligonucleotide can bind (26). The key feature of the second oligonucleotide is that it has many binding sites for a third oligonucleotide that carries an enzyme (e.g., alkaline phosphatase) that modulates the detectable signal. Theoretically, such a procedure could amplify a hybridization signal 10- to 100-fold, which might improve the detection limit of the hybridization assays to as few as 100 to 1,000 organisms per specimen. These tests have yet to be evaluated in clinical specimens or in a clinical trial.

Gene amplification. This molecular biology tool has been found to be a valuable alternative for organism detection. These nucleic acid (DNA or RNA) based amplification techniques can detect and identify mycobacteria directly in clinical samples. A target molecule is amplified to a detectable level and is hybridized with a probe. Several procedures have been described for use with *M. tuberculosis*, including strand displacement amplification (SDA) (27), polymerase chain reaction (PCR) amplification (28), transcription-mediated amplification (TMA) (29), oligonucleotide ligation amplification, and Q-beta replicase amplification (30). The first four of these amplification systems are the best developed systems for mycobacteria and are described below.

In general, each amplification system can (1) produce a clear positive signal from specimens containing as few as one to 10 bacilli; (2) clearly distinguish

M. tuberculosis from other mycobacterial species and common respiratory specimen contaminants; (3) detect *M. tuberculosis* in specimens containing a large excess of nucleic acids from human cells or other mycobacterium species; and (4) be completed in less than 1 day. These assays have been used with a variety of clinical specimens. Because of the need to culture organisms for drug susceptibility tests or to identify mycobacterium species other than *M. tuberculosis*, the assays usually are designed to be used with specimens that have been processed for culture, such as by the N-acetyl-L-cysteine/NaOH procedure for sputum specimens (31). After the specimen is collected, the second step for most assays is lysis of the mycobacteria, which can be accomplished by a variety of methods, including sonication, boiling, treatment with sodium dodecyl sulfate (SDS) plus lysozyme and heat, proteinase K, chaotropic salts, etc. Because inhibitors of enzymatic amplification reactions are found in a small percentage (1–5%) of processed sputum specimens, the lysis step is often followed by a nucleic acid purification step. Also, most assays include internal controls to assess amplification efficiency and the presence of inhibitors.

Strand displacement amplification (SDA). SDA is an isothermal amplification process developed by Becton Dickinson (BD Research Center, Research Triangle Park, NC). It takes advantage of the ability of the Klenow fragment of E. coli DNA polymerase to start at the site of a single-stranded nick in double-stranded DNA, extend one strand from the 3′ end, and displace the downstream strand of DNA (1,32). The replicated DNA and the displaced strands are then substrates for additional rounds of oligonucleotide annealing, nicking, and strand displacement such that the amplification proceeds in a geometric manner and can produce 10^7- to 10^8-fold amplification in about 2 h. The specificity of the SDA reaction is based on the choice of primers to direct the DNA synthesis. When coupled with a chemiluminescence-based hybridization detection system, the entire assay can be completed within 4 hr after a processed specimen is obtained.

Species-specific SDA assays have been developed for *M. tuberculosis*, *M. avium*, and *M. Kansasii*. An assay that detects many members of the *mycobacterium* genus (a genus-specific assay) has also been developed. This assay can be multiplexed (i.e., the amplifications can be done in a single tube, and the products can be distinguished by the detection system) without significant loss of sensitivity (1). Thus, one can have a single two-step assay to detect and differentiate between the two most commonly encountered acidfast bacteria in smear-positive specimens: *M. tuberculosis* and *M. avium*. One potential concern with genus-specific assays is that the signal produced by one species (e.g., *M. gordonae*, a common contaminant of sputum specimens) may mask the signal from a second species (e.g., *M. tuberculosis*). Thus, the ability to detect mixed infections is an important but untested feature of the assay.

Additional performance characteristics of the SDA assay are: (1) autoclaving can be used to sterilize a sample and lyse the bacteria, (2) an internal control can be included to assess amplification efficiency and the presence of inhibitors, (3) the assay is semiquantitative, and (4) the detection system can be conveniently batched in 96-well microtiter plates. The assay has not yet been evaluated in a clinical setting.

PCR. Recently, with the development of PCR, a number of investigators have reported the detection of a specific sequence for *M. tuberculosis* directly in clinical specimens (33). Amplicor PCR has been reported to be 100% specific and 83.6% sensitive for pulmonary tuberculosis. This test was also evaluated to have a sensitivity and specificity of 66.7% and 99.6%, respectively, in tuberculosis meningitis patients. It was found that a significant proportion of patients with respiratory disease or pulmonary tuberculosis are initially smear-negative but are subsequently culture-positive (60). Pulmonary tuberculosis in two-thirds of such patients can be diagnosed by PCR. Although this test has high sensitivity, it is highly expensive (at the cost of $15 per test) for poor countries such as India. A number of other target sites on *M. tuberculosis* genome have been identified for tuberculosis detection. Prominent among these are IS 6110, IS 986, the mtp40 gene, and the 65kDa antigen. However, the application of PCR requires expertise and is also very costly. A great deal of effort will be required to make the PCR technique feasible for routine application in clinical laboratories. Moreover, the amplification of dead bacterial DNA and the absence of amplifiable *M. tuberculosis* DNA in blood is a problem, except for HIV cases with bacterimia. The efficacy of PCR strictly depends on several amplification parameters, including DNA concentration, target DNA size, and the repetitiveness of the amplified sequence.

The specificity of the PCR amplification process lies in the choice of primers used. Numerous PCR-based assays for the detection and identification of individual *mycobacterium species*, such as *M. tuberculosis*, *M. leprae*, and *M. avium*, have been described (2,3,34–47). Many target sequences have been used, but the most thoroughly evaluated assays target the *M. tuberculosis*-specific repeated DNA element IS 6110 (40). In addition, a variety of two-step PCR-based assays have been described in which the first step amplifies a target sequence common to all mycobacterium species, and the

second step determines which species gave rise to the amplified product. The second step can involve species-specific hybridization probes or nucleic acid sequencing (49). In general, the amplification process can be completed in 2–4 hr after a processed specimen is obtained, and the detection assay can be completed in an additional 2–24 hr. Additional performance characteristics are: (1) the assay requires a thermocycler and thermostable DNA polymerase, (2) internal controls can be included to assess amplification efficiency and the presence of inhibitors, and (3) the assay is semi quantitative.

Ligase chain reaction LCR). The LCR DNA amplification method was recently developed as a commercial test for the detection of *M. tuberculosis*. The method has a high sensitivity for both smear-positive (100%) and smear-negative samples, and is suitable for screening of *M. tuberculosis* in high-risk patients (50). The assay has been demonstrated to provide rapid and valuable information for the diagnosis of extrapulmonary tuberculosis as well. Because this method requires costly kits and expertise, it will take quite some time for it to find widespread acceptance in routine clinical laboratories (51).

Transcription mediated amplification (TMA). TMA, an isothermal target-based amplification system developed by Gen-Probe Inc. (San Diego, CA), has been combined with a homogeneous detection method to detect. *M. tuberculosis* in clinical specimens (52). This test (the Gen-Probe amplified *M. tuberculosis* direct test (MTD) test) uses the sediments prepared by the standard NALC/NaOH method (31) and lyses the mycobacteria by sonication. Ribosomal RNA (rRNA) is amplified via TMA such that the rRNA target sequences are copied into a transcription complex by using reverse transcriptase, and then RNA polymerase is used to make numerous RNA transcripts of the target sequence from the transcription complex. The process is then repeated autocatalytically. The amplified sequences are detected by using an acridinium ester-labeled DNA probe specific for *M. tuberculosis* in a homogeneous solution hybridization assay format similar to that used in the Gen-probe Accuprobe species identification system. An important feature of the MTD assay is that it can be done entirely in one test tube, which minimizes sample manipulations and the possibility of laboratory-introduced contamination. Also, the entire assay can be completed within 3–4 hr after a processed specimen is obtained. In respiratory samples the test has a sensitivity of 75–100% and specificity of 95–100%. The test allows for rapid identification of *M. tuberculosis* in smear-positive patients and may greatly improve sensitivity over the acid fast bacilli smear alone (53).

Reporter mycobacteriophage. A reporter mycobacteriophage that can infect only *M. tuberculosis* specifically has been designed to detect viable/living mycobacteria in a patient specimen. The specificity of the system lies in the synthesis of a large amount of the reporter product during phage growth (i.e., amplification of the product (29). Jacobs et al. (29) constructed this reporter phase, which carries the gene for the firefly enzyme luciferase. In the presence of ATP, this enzyme oxidizes luciferin to generate light, which is the reaction that makes fireflies glow in the dark (54). Mycobacteria infected with this reporter phage produce light when luciferin is added, and samples containing as few as 500–5,000 mycobacteria generate a clear positive signal (29). Although much work needs to be done develop to and evaluate this assay (e.g., construction of an *M. tuberculosis*-specific reporter phage), it promises to be an inexpensive, easy, and specific assay for detecting *M. tuberculosis* directly in specimens. This assay may also be useful for distinguishing between live and dead bacilli. The technology has also been modified to perform antimycobacterial drug screening and produce lead compounds.

CONCLUSIONS

Tuberculosis has been declared a global emergency. The main requirement for its control is the rapid and accurate identification of infected individuals. In 1994, the Centers for Disease Control and Prevention (CDC) published recommendations for rapid diagnosis in laboratories, and proposed that smear results should be reported within 24 hr, detection and identification within 10–14 days, and susceptibility within 15–30 days. Detection of *M. tuberculosis* by microscopy is difficult in specimens containing fewer than 10^4 bacteria/mL. The culture method is deemed the ''gold standard'' for diagnosing tuberculosis; however, it can take up to 6 weeks. Moreover, serological results that use various mycobacterial antigens must be carefully interpreted. However, antibodies-based tests may be valuable for diagnosing patients in areas with high prevalence of tuberculosis.

The difficulty in developing an ELISA utilizing a suitable antigen stems from the fact that *M. tuberculosis* produces a large number of antigenic proteins, some of which appear to be common to other microorganisms. The selectability to highly specific antigens is therefore required. The use of a ''cocktail'' of mycobacterial antigens and simultaneous analysis of antigen specific IgG-IgM-IgA response may offer better specificity and sensitivity. A dot-immunobinding assay was found to be superior to PCR for detecting tuberculous meningitis (55). This assay rapid, relatively easy to perform, and

suitable for routine application in peripheral health centers.

The amplification of specific gene segments is an alternative means of identifying slow-growing *M. tuberculosis*. Amplified fragments from as little as 1 fg of DNA (equivalent to one-fifth of an organism) could be resolved on ethidium bromide-stained gels. Thus PCR offers a more sensitive and rapid method for the detection of *M. tuberculosis* compared to ZN staining and culture, and results are available 24 hr after the specimen is received in the laboratory. Heating the sample in a boiling water bath to breakdown the bacterial cell wall and release the DNA is a better method than enzymatic lysis of bacteria (56). The fact that storage of sputum samples on filter paper for 5 days at room temperature had no apparent effect on the performance of nested PCR (57) indicates that the assay can be extended to samples from peripheral health centers. The sensitivity of PCR, however, is affected by the presence of DNA polymerase inhibitors in sputum specimens, and an internal standard must be incorporated in the assay system. Commercial kits based on PCR are useful for the early and rapid detection of *M. tuberculosis*. However, the risk of contamination and false-positive results remains. For the diagnosis of smear- and culture-negative pulmonary tuberculosis, using a combination of tests of high specificity can increase the sensitivity.

The diagnostic value of a given test in clinical practice depends on its positive and negative predictive values. These values vary significantly with the prevalence of disease in a community. Even the best of the tests described here had a modest sensitivity of 60% for the detection of active tuberculosis. The majority of the tests had good specificity for the Mantoux test controls but poor specificity for the anonymous controls. While a negative result would be useful in excluding disease in a population with a low prevalence of tuberculosis, a positive result could potentially aid in clinical decision-making when sera from a group of selected symptomatic patients (with a moderate to high degree of clinical suspicion of tuberculosis) is tested.

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