Clinical Utility of the Polymerase Chain Reaction in the Diagnosis of Infections due to *Mycobacterium tuberculosis* *


**Objective:** To evaluate the clinical utility of the polymerase chain reaction (PCR) in the diagnosis of infections due to *Mycobacterium tuberculosis*

**Design:** Clinical specimens were assayed by PCR for the presence of the insertion element IS6110, a DNA sequence unique to the *M. tuberculosis* complex of organisms. The PCR results were then correlated with acid-fast bacilli (AFB) smears, cultures, pathology, and clinical histories.

**Setting:** Bellevue Hospital, a large municipal teaching hospital

**Patients:** Inpatients on the Bellevue Chest Service

**Measurements and results:** Sixty-five patients were evaluated. The PCR for *M. tuberculosis* was positive in 37 patients and negative in 28. When correlated with smears, cultures, pathology, and clinical history, the sensitivity of PCR for a diagnosis of active tuberculosis (TB) was 100 percent. However, the specificity for a diagnosis of active TB was only 70 percent, as the PCR assay was positive in a number of patients with only prior, treated TB, or asymptomatic tuberculous infection. For a diagnosis of any TB infection (active, treated, or asymptomatic), sensitivity of PCR was 87.5 percent and specificity was 90 percent.

**Conclusions:** The PCR assay for TB is extremely sensitive, but it lacks specificity for a diagnosis of active TB. Its role in clinical practice will likely be limited to well-defined situations, such as HIV-positive patients with intrathoracic adenopathy, and it may be most useful in excluding active TB from consideration in selected patients. Given the cost of the assay and the labor intensity it requires, it should not be part of the routine initial evaluation of patients with suspected pulmonary TB.

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The current tuberculosis (TB) epidemic in New York City and elsewhere is marked by several features that make rapid diagnosis of the infection important. The high incidence of multidrug resistance, the close association between TB and HIV infection, and the preponderance of cases among homeless individuals who often live in temporary shelter housing where the potential for spread of infection is great, demand as rapid identification of cases as is possible in order to institute proper therapeutic and infection control measures. Current rapid identification of pulmonary TB depends chiefly on examination of sputum samples by light microscopy after appropriate staining techniques have been applied. However, vast experience with this technique indicates that only about 75 percent of cases of active pulmonary TB will be identified by examination of sputum smears, and the yield of such examinations is highest in patients with cavitary lesions on chest radiographs. This is of particular note considering that in patients with concurrent TB and HIV infection, there is a preponderance of atypical chest radiograph findings, such as pleural disease and mediastinal lymphadenopathy, neither of which is usually associated with positive sputum smears. There have been several reports of delayed diagnosis of TB in patients with the acquired immunodeficiency syndrome; these delays may in part be responsible for the extremely high mortality seen in patients with HIV and TB infection that has been observed recently.

The polymerase chain reaction (PCR) is an extremely specific and sensitive method of DNA amplification that is capable of identifying and amplifying as little as a single copy of a given DNA sequence to the point where the DNA is easily detectable by gel electrophoresis. Polymerase chain reaction offers a possible alternative to conventional diagnostic techniques that may be clinically useful in a variety of disorders. Biologic specimens such as sputum, blood, bronchoalveolar lavage (BAL) fluid, and cerebrospinal fluid are all amenable to PCR analysis, and numerous opportunistic pathogens have been successfully identified in these specimens to date.

The PCR assays for *Mycobacterium tuberculosis* have been possible since the identification of DNA sequences unique to the *M. tuberculosis* complex of...
organisms. Most investigators have chosen the insertion element IS6110, which is present in several copies in most *M tuberculosis* strains, as the amplification target. However, initial reports on the use of PCR assays for the diagnosis of active TB have yielded conflicting results: whereas all series report an extremely high sensitivity for the assay, the specificity of the assay varies in different studies, with some investigators finding that PCR readily detects mycobacterial DNA in cases of prior or treated infection. The current report details our experience with the PCR assay as it has been applied to a variety of clinical samples in a population with a high prevalence of TB. We describe the clinical utility of this assay based on our experience, and outline scenarios in which the PCR assay may be useful in establishing or ruling out a possible diagnosis of TB.

**METHODS**

**Procurement of Clinical Samples**

Samples of sputum, BAL fluid, and cerebrospinal fluid were taken from patients on the Bellevue Chest Service who were undergoing diagnostic evaluation for a variety of pulmonary diseases. All samples were obtained in the course of routine diagnostic evaluation, and samples for PCR consisted of material that remained after all diagnostic studies for which the sample was obtained were done. All samples were provided to the person actually performing the PCR assay (N.W.S.) in a blinded fashion regarding both the suspected diagnosis and the results of any other laboratory investigations for the patients in question.

**Processing of Samples for PCR**

To each sample was added two volumes of 2 percent NaOH. Samples were centrifuged at 2,000 rpm for 15 min and the pellets were resuspended in 1 ml of TE (10 mM Tris-Cl, 1 mM EDTA) buffer, at a pH of 8.0. To each sample was then added 60 μl of 10 percent SDS and 10 μl of proteinase K (20 μg/μl), and the samples were incubated at 56°C for 1 h. DNA was then extracted from the samples using the phenol:chloroform method followed by ethanol precipitation. (Phenol: chloroform extraction was used after a comparison in a representative subgroup showed a marked decrease in sensitivity when this step was not performed.) Finally, DNA was resuspended in 40 μl of ddH2O, and an aliquot of 2.5 μl was used in each PCR reaction.

**PCR Reaction**

Nested primer pairs were used to yield a final amplification product of a 253bp segment of the insertion element IS6110, a DNA sequence specific for the *M tuberculosis* complex of organisms. The outer primer pair consisted of a sense primer 5’-ATCGGGCTCATGTCAGTGTTCAATCGAAGAGS-3’ and an antisense primer 5’-AGTTGCACCGGCCCGCATGCCCCG-CACAGGC-3’ and the inner primer pair consisted of the sense primer 5’-CGTAGGAGGATCGAGTGGCC-3’ and the antisense primer 5’-CCCTAGGCGCTCGAGTACAAA-3’. The reaction conditions included primers in a final concentration of 1 μM, MgCl₂ in a final concentration of 25 mM, and Taq polymerase (Perkin-Elmer, Emeryville, Calif) 5U/reaction. All reactions were carried out in a volume of 50 μl.

For the second (nested) PCR, an aliquot of 2.5 μl was removed from the initial reaction and directly added to the second reaction as the template DNA. In both the initial and nested reactions, negative controls were included in every experiment. The negative control reactions consisted of buffer, nucleotides, primers, magnesium, Taq polymerase, but no template. Positive controls using genomic DNA isolated from a clinical isolate of *M tuberculosis* as template were also included in each experiment. The reaction tube containing the positive control was prepared under a hood in a separate room in the laboratory to minimize contamination and product carryover. The PCR was carried out for both the outer and inner primer pairs under the following conditions for 30 cycles of amplification: denaturation at 94°C for 1 min, annealing at 50°C for 1 min, and primer extension at 72°C for 1 min. The 30 cycles were preceded by a 2-min initial denaturation at 94°C, and were followed by a 10-min terminal extension at 72°C.

The PCR products were then analyzed by electrophoresis using a 2 percent agarose gel stained with ethidium bromide and examined under ultraviolet irradiation. Using this method, as little as one femtogram of template DNA from *M tuberculosis* could be reliably detected.

**Correlation of PCR Results With Clinical Findings**

After PCR results were obtained, each patient’s clinical and laboratory record was reviewed to determine the specific clinical TB history. The results included all relevant culture reports.

**RESULTS**

There were 65 samples (representing 65 different patients) analyzed by PCR for the presence of the insertion element IS6110 of *M tuberculosis* (Table 1). These samples consisted of 33 BAL specimens and 32 sputum samples. Of the total samples analyzed, there were 28 that gave a negative result for the IS6110 sequence, and there were 37 that gave a positive PCR signal. Of the 28 samples that gave a negative result for *M tuberculosis* by PCR, no patient had clinical evidence of active TB; that is, no patient with a negative PCR result had either a positive sputum smear for acid-fast organisms, a positive mycobacterial culture, or a biopsy specimen of any disease site that showed typical pathologic features of TB.

Among the 37 patients with a positive PCR result for *M tuberculosis*, 23 patients had definite evidence of active TB; that is, either a positive sputum examination for acid-fast bacilli (AFB), a positive

| Table 1—Correlation of PCR Assay for Mycobacterium tuberculosis With Clinical Findings |
|-----------------|------------------|
| **PCR Result** | **No. of Patients** |
| Negative        | 28               |
| Evidence of active TB                | 0/28             |
| Prior TB or+PPD                  | 5/28             |
| **M. avium**       | 3/28             |
| Positive        | 37               |
| Definite active TB                | 23/37            |
| Prior TB or+PPD                  | 12/37            |
| No evidence of TB                | 3/37             |

| Infection of any type | 3/37           |

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culture result, or demonstration of typical pathologic findings (necrotizing granuloma) in a patient with a clinical presentation typical of TB. Significantly, of the 23 patients identified as having TB by PCR, only 5 had positive sputum smears, with the remainder having their conditions diagnosed by culture results or pathologic examination. Of the remaining 15 patients, 12 had a history either of prior, treated TB (8 patients) or a positive tuberculin skin (4 patients) test with no clinical history of TB or radiographic abnormalities. The remaining three patients had no history of either active TB or a positive tuberculin test.

There were 12 patients in whom AFB smears were negative, mycobacterial cultures were positive, and the PCR assay revealed evidence of M. tuberculosis (Table 2). Eight of these 12 were HIV positive, and only 1 of the patients had cavitary lesions on chest radiograph. Seven of the eight HIV-infected patients had either hilar or mediastinal lymphadenopathy as the predominant abnormality on chest radiograph. All HIV-positive patients with adenopathy had undergone fiberoptic bronchoscopy and transbronchial lung or lymph node biopsy and none of the HIV-positive patients in this group had a diagnosis of lymphoma.

Based on the above results, sensitivity and specificity values were calculated (Table 3). For a diagnosis of active TB, the overall sensitivity of the PCR assay was 100 percent, and the specificity was 70 percent. For a diagnosis of any history of tuberculous infection (positive tuberculin test, treated or distant disease, or active TB), the sensitivity was 87.5 percent and the specificity was 90 percent.

Sensitivity and specificity data were also calculated for sputum and BAL specimens separately. The sensitivity for both types of specimens for a diagnosis of active TB was 100 percent. The specificity for sputum for TB was 45 percent, (62 percent for a diagnosis of any TB history) and for BAL was 80 percent (90 percent for a diagnosis of any TB history).

**DISCUSSION**

Most previous studies on the role of the PCR in diagnosing TB have focused on technical issues regarding the assay and the ability of PCR to reliably differentiate M. tuberculosis from other mycobacterial species. In our study, we have investigated the role the assay might play in actual clinical practice. Our results indicate that the PCR is not always able to reliably differentiate active disease from either prior, treated disease or simple tuberculous infection, as manifest by a positive tuberculin skin test. Still, these data raise the possibility that PCR assays for TB can indeed have a role in specific clinical situations. Based on our study, the PCR assay for TB can be useful in suggesting a diagnosis of TB in HIV-positive patients with chest radiographs showing hilar or mediastinal adenopathy; it is also useful in patients with no history of TB who present with parenchymal abnormalities other than cavitary lesions on chest radiograph. Additionally, a negative PCR result seems quite useful in excluding TB as a diagnostic consideration.

Our data confirm the experience of several prior investigators as to the sensitivity of the PCR assay for M. tuberculosis in a variety of clinical situations and from a variety of biologic specimens. Strikingly, in our series, no patient with active TB went undiagnosed using the PCR. In addition, the specificity of the assay in regard to the ability to differentiate M. tuberculosis from mycobacteria other than TB was also excellent.

The finding that mycobacterial DNA can be amplified from clinical specimens of patients with prior, or ongoing, treated TB, or even positive tuberculin skin tests alone, is not surprising, and may be explained by several possible scenarios. The first is that in cases of active TB, the mycobacterial burden may remain substantial despite institution of therapy, though organisms may no longer be capable of producing infection. Sultan and colleagues and Riley and coworkers demonstrated that the relative infectiousness of untreated to treated patients was roughly 50 to 1, despite positive sputum.
tum acid-fast smears in both groups. Given the fact that a positive smear represents a minimum of 5,000 to 10,000 organisms per milliliter of sputum, it is to be expected that PCR, which is theoretically capable of detecting a single organism in a sample, will yield a positive result far into a course of therapy. This would be particularly likely in patients with cavitary disease, as a tuberculous cavity is estimated to contain at least \(10^9\) to \(10^{10}\) organisms.\(^{14}\) It has been estimated by several investigators that after approximately 2 to 3 weeks of antituberculosis chemotherapy, there is a reduction of 2 to 3 logs in the mycobacterial burden, which in a heavily infected individual would still leave in excess of 10,000 organisms in a cavity.\(^{15}\) In this light, the results of Eisenach and colleagues\(^9\) are perhaps somewhat unexpected, as they reported that 0 of 29 patients receiving therapy had a positive PCR result, though the chest radiographs were not described in their report.\(^9\)

Interestingly, analysis of the subgroup of patients with negative AFB smears of sputum or lavage fluid but with positive culture and PCR results yielded notable trends. This group was more likely to be HIV positive and to have chest radiographic findings of hilar or mediastinal adenopathy than other patients with TB. Thus, our data suggest that in HIV-positive patients with lymphadenopathy but negative sputum smears for AFB, PCR detection of mycobacteria in sputum or lavage fluid may provide reliable early evidence of active TB.

Our finding that several of the positive PCR results we obtained were in patients with only a history of TB or only a positive tuberculin skin test are in general agreement with data published by Walker et al.,\(^10\) who found that most patients with a history of prior TB or close contact with an active case have a positive PCR for TB when a variety of biologic specimens was examined. There are data to support these findings from early investigations in TB that establish the presence of residual mycobacterial organisms in patients with prior TB histories but no signs of active disease. For example, the landmark report by Stead\(^16\) established that the development of chronic pulmonary TB in patients with no history but only tuberculin hypersensitivity evidenced by skin testing was due not to reinfection with a new organism but rather was caused by reactivation of dormant organisms in the lungs. This requires the persistence of viable mycobacteria in the lungs for long periods of time, and it is likely that a sensitive assay such as the PCR could occasionally detect organisms. In case of recently treated but clinically inapparent disease, there is also evidence of residual mycobacteria in the lungs. Thus, the findings of positive PCR results are not unexpected in view of our understanding of the pathophysiology of clinical TB.

The finding of occasional positive PCR results in patients with a history of treated TB is also supported by pathologic studies done after the development of effective antituberculosis therapy. In one early study, Auerbach,\(^17\) found that in a number of pathologic specimens from lung resections for bronchiectasis due to TB, mycobacteria could be recovered by culture or smear in a number of cases, despite the fact that all patients had received antituberculosis chemotherapy and had months of negative findings from sputum examinations. Rozenzweig and Stead\(^18\) reported similar findings in their study of the pathogenesis of bronchiectasis. Willauer et al.\(^19\) found that 23 percent of lung resections done in patients with a history of TB but negative results of sputum examinations (smear and culture) contained viable tuberculosis organisms. Similar findings were reported in four separate studies.\(^20-23\) Thus, there is pathologic evidence for the persistence of mycobacteria (though the viability of these organisms is not easily determined) in patients with residual pulmonary abnormalities who have received prolonged antituberculosis chemotherapy resulting in repeatedly negative sputum culture. This is in accord with our findings of positive PCR results in patients with negative sputum cultures after therapy but who present with hemoptysis and bronchiectasis. Even in patients with only a tiny, healed Ghon complex and a positive tuberculin test, careful pathologic examination of the lung will reveal residual foci of tubercle bacilli despite no evidence of clinically active disease.\(^24\)

Taken together with results of pathologic studies, our results using the PCR to examine a variety of biologic specimens indicate a good correlation between the PCR results and the underlying pulmonary abnormality. That is, the test is negative when there is no evidence of active or prior tuberculous infection, and it is positive in all cases of active TB. In addition, there are occasional positive test results in patients who with a history of tuberculous infection or prior active TB that is clinically resolved at the time of the PCR assay.

Previous studies examining the role of PCR in the diagnosis of TB have reported extremely high sensitivity and specificity. However, the clinical circumstances of the patients from whom samples were obtained in these series were not described in detail. In the study of Eisenach et al.\(^9\) for example, nearly all patients with positive sputum cultures also had positive acid-fast smears; this is a unusual result that implies that the patients in the study fell into two groups: cavitary disease or no history of tuberc-
cular infection at all. The same may be true in the studies of Altamirano et al.\textsuperscript{25} and Cousins et al.,\textsuperscript{26} which do not provide clinical details of the patients studied. Conversely, the present study, as well as that of Walker et al.,\textsuperscript{10} included a substantial number of patients with a history of tuberculous infection or prior active disease, and the results in both reports indicate an inability of PCR to distinguish active from prior infection in a number of cases.

The differing specificity for sputum samples as compared with BAL is interesting. The apparently greater number of false-positive results from sputum analysis may reflect mycobacterial organisms that reside only in the mouth, ears, nose, or throat, and not in the lower respiratory tract, though the overall sample size may not be large enough to draw firm conclusions. Interestingly, there were two patients in whom bronchoscopic lavage specimens were negative by culture and smear, but transbronchial biopsy specimens yielded granuloma with acid-fast organisms. Both of these patients had positive PCR of their lavage fluid.

The utility of the PCR for TB depends on the clinical situation in which the assay is performed. The present study indicates that in patients with a history of infection, PCR may not be able to distinguish active from prior infection, particularly in patients with bronchiectasis. However, in patients with no history of TB, a negative result by PCR strongly implies that tubercle bacilli are not the cause of the patient’s symptoms. When the diagnosis of TB is being considered in a patient with suggestive clinical symptomatology and an abnormal chest radiograph, the combination of negative sputum acid-fast smears and a negative PCR assay for mycobacteria (performed on sputum or BAL fluid) provides considerable evidence against active TB. Such a patient may not need to be discharged from the hospital on a regimen of antituberculosis therapy pending final culture results. Our findings suggest that PCR may be of benefit in diagnosing TB only in limited, clearly defined circumstances. We did not investigate the role of the PCR assay in the diagnosis of extrapulmonary disease such as meningeal TB or pleural TB, and there is good reason to think that the test might be extremely useful in these circumstances. However, cost, labor intensity, and relatively low specificity for a diagnosis of active disease preclude the use of PCR as an initial diagnostic test in the majority of patients suspected of having active pulmonary TB.

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REFERENCES

3 Kim TC, Blackman RS, Heatwole KM, Kim T, Rochester DF. Acid-fast bacilli in sputum smears of patients with tuberculosis: prevalence and significance of negative smears pretreatment and positive smears posttreatment. Am Rev Respir Dis 1984; 129:264-68
17 Auerbach O. Pathology of tuberculosis as affected by antibiotics. Am J Surg 1955; 89:627-36
19 Willauer GJ, Fineberg C, Johnson RG, Coghlan WP. The culture of tubercle bacilli from resected specimens of ‘nega-
20 Mydlil F, Mezensky L. Über die Häufigkeit des BK-nachwieses in kavernen resezierter Lungen bei Kranken, die schon vor der Operation negativ waren. Z Tuberk 1964; 121:276-91