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Clinical Evaluation of the Roche AMPLICOR PCR *Mycobacterium tuberculosis* Test for Detection of *M. tuberculosis* in Respiratory Specimens

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The reliability of the Roche AMPLICOR *Mycobacterium tuberculosis* test (AMPLICOR MTB) for the diagnosis of pulmonary tuberculosis was evaluated by testing 956 respiratory specimens from 502 patients and comparing results with results by culture and medical history. Of those 135 specimens that were culture positive for mycobacteria, 61 specimens from 31 patients grew *M. tuberculosis*. Fifty-two specimens were smear positive for acid-fast bacteria (AFB); *M. tuberculosis* was isolated from 41 of these specimens. On initial testing, the sensitivity and specificity of the AMPLICOR MTB assay, compared with culture, were 78.7 and 99.3%, respectively. After resolution of discrepancies (by review of medical history), the sensitivity, specificity, and positive and negative predictive values of the AMPLICOR MTB assay were 79.4, 99.6, 92.6, and 98.6%, respectively. Two specimens from two patients with no clinical evidence of tuberculosis were AMPLICOR MTB positive and culture positive for *Mycobacterium avium* complex. For AFB smear-positive specimens, the sensitivity, specificity, and positive and negative predictive values of AMPLICOR MTB were 97.6, 100, 100, and 90.9%, respectively. For AFB smear-negative specimens, the sensitivity, specificity, and positive and negative predictive values of AMPLICOR MTB were 40.0, 99.5, 69.2, and 98.7%, respectively. Our results support the use of AMPLICOR MTB for rapid diagnosis of tuberculosis in patients whose respiratory specimens are AFB smear positive. Further studies are needed to determine the most clinically relevant and cost-effective use of this assay with AFB smear-negative specimens.

After years of decline, tuberculosis has re-emerged as a serious public health problem in the United States (3). Factors contributing to the resurgence include the human immunodeficiency virus epidemic, immigration of persons from countries with a high incidence of tuberculosis, and an increase in the medically underserved population (2). To help control the spread of tuberculosis, rapid diagnosis is desirable.

Acid-fast staining of smears is a rapid technique, but it has a low sensitivity (approximately 10^4 bacteria per ml of specimen are necessary for a positive result), and it does not differentiate between species of *Mycobacterium* (7). Currently, diagnosis of tuberculosis requires growth of the organism on solid or in a liquid medium, which can take up to 6 to 8 weeks, followed by the use of nucleic acid probes, high-performance liquid chromatography, or conventional biochemical tests for identification. Because optimal patient management requires early initiation of drug therapy and isolation of infectious individuals as soon as possible, a technique which provides rapid, reliable detection of *Mycobacterium tuberculosis* is needed. To accomplish this, Roche Molecular Systems Inc. (Branchburg, N.J.) has developed a nucleic acid amplification (PCR) test, AMPLICOR MTB, for evaluation of respiratory specimens. The purpose of this study was to compare the AMPLICOR MTB test for the detection of *M. tuberculosis* with culture and staining techniques.

MATERIALS AND METHODS

Clinical specimens. From November 1994 to March 1995, 956 respiratory specimens (808 sputum specimens, 90 bronchoalveolar lavage fluid specimens, 55 tracheal aspirate specimens, 1 throat specimen, 1 protected specimen brush, and 1 lung biopsy specimen) from 502 patients were examined by the Clinical Microbiology Laboratory of the University of Texas Medical Branch, Galveston, Tex.

Culture and identification. Specimens were decontaminated with *N*-acetyl-cysteine-2% sodium hydroxide and concentrated by centrifugation according to standard laboratory protocol (5). A smear of the sediment was stained with Auramine O and examined for acid-fast bacilli (AFB) (5). Middlebrook 7H10/7H11 biplates (Remel, Lenexa, Kans.) and BACTEC 12B bottles (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) were inoculated with several drops and 500 μl of sediment from each specimen, respectively. Two aliquots, each consisting of 200 μl of sediment, were frozen at −20°C for batch analysis by the AMPLICOR MTB test. Middlebrook plates were incubated in an atmosphere of 7 to 10% CO_2 at 37°C for up to 8 weeks and examined for mycobacteria weekly. BACTEC 12B bottles were incubated at 37°C and monitored for growth by the BACTEC 460 (Becton Dickinson) every 3 days for the first 2 weeks and then weekly for an additional 3 weeks. When the growth index (GI) reached 100 or greater, a smear of broth was stained with Kinyoun, and if AFB were present, the BACTEC vial was reincubated and monitored daily until a GI of 999 was reached, at which time a 100-μl aliquot was removed to perform DNA-RNA probe analysis for *M. tuberculosis* complex (AccuProbe; Gene-Probe Inc., San Diego, Calif.). A Löwenstein-Jensen tube was also inoculated with several drops of broth from the BACTEC vial. If the probe was negative, tests for identification were performed on colonies recovered on solid media. On the basis of colony morphology, appropriate RNA-DNA nucleic acid probes were selected for identification of *M. tuberculosis*, *Mycobacterium avium* complex, *Mycobacterium kansasii*, or *Mycobacterium gordonae*. If the mycobacterium was a rapid grower, biochemical tests were used to identify the organism (5). Isolates not identified with this protocol were sent to the Texas Department of Health for identification.

Roche AMPLICOR MTB test. PCR samples were thawed, mixed by using a vortex mixer, and processed according to the manufacturer's instructions. Briefly, 100 μl of sample was added to 500 μl of Sputum Wash Solution, mixed by using a vortex mixer, and centrifuged at 12,500 × *g* for 10 min. The supernatant was discarded, 100 μl of Sputum Lysis Reagent was added, and the pellet was resuspended by mixing on a vortex mixer. One positive and three negative controls, supplied with each assay kit, were included with each run. The sample and controls were incubated at 60°C in a dry-heat block for 45 min and then pulse-centrifuged at 12,500 × *g* for 10 s. Both sample and control tubes received 100 μl of Sputum Neutralization Reagent and were mixed by using a vortex mixer. To PCR tubes containing 50 μl of Master Mix (biotin-labeled deoxynucleotide triphosphates with deoxyuridine replacing deoxythymidine, *Taq* DNA polymerase, and Uracil N-Glycosylase [termed AmpErase]), 50 μl of the prepared samples or controls was added. Prepared PCR tubes were placed in the Perkin-

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TABLE 1. Initial comparison of AMPLICOR MTB test with culture for detection of *M. tuberculosis* in respiratory specimens

Specimen type (no.)	No. of specimens (no. of patients)				Sensitivity (%)	Specificity (%)
	Culture positive[a]		Culture negative[a]			
	PCR+	PCR−	PCR+	PCR−		
All (956)	48 (22)	13 (9)	6 (6)	889 (465)	78.7	99.3
Smear positive (52)	40 (16)	1 (1)	1 (1)	10 (7)	97.6	90.9
Smear negative (904)	8 (6)	12 (8)	5 (5)	879 (462)	40.0	99.4

[a] Culture positive or negative for *M. tuberculosis*.

Elmer 9600 thermal cycler (Norwalk, Conn.) and amplified as follows: hold at 50°C for 2 min, 2 cycles for 20 s at 98°C; 20 s at 62°C and 45 s at 72°C, 35 cycles at 20 s at 94°C; 20 s at 62°C and 45 s at 72°C; hold for 5 min at 72°C. Following amplification, the amplicons were denatured by the addition of 100 μl of denaturation solution for 10 min at room temperature. To each well of an eight-well microtiter plate strip coated with a capture probe specific for *M. tuberculosis* complex, 25 μl of the denatured amplicons and 100 μl of hybridization buffer was added and allowed to hybridize at 37°C for 1.5 h. Strips were washed 5 times with wash buffer, 100 μl of avidin-horseradish peroxidase conjugate was added, and the strips were incubated at 37°C for 15 min. The strips were washed 5 times, and 100 μl of peroxidase-tetramethybenzidine was added for 10 min at room temperature. The reaction was stopped with 100 μl of dilute sulfuric acid. The reaction product was read by using an EL-312E Bio-Kinetics reader (Bio-Tek Instruments, Winooski, Vt.) at 450 nm. If the control values were not within the given ranges (positive control must be greater than 2.000 A_{450}, and the negative control must be less than 0.250 A_{450}) the entire run was invalidated and the assay was repeated. A specimen reading that was equal to or greater than 0.350 A_{450} was considered positive for *M. tuberculosis* complex.

Discrepant analysis. Final classification of AMPLICOR PCR results was based on review of the patients' clinical histories in addition to culture results. AMPLICOR PCR testing also was repeated on *M. tuberculosis* culture-negative, PCR-positive samples. If the sample was negative when retested by PCR, the initial results were reported as false positive.

RESULTS

Of the 956 specimens examined, 135 were culture positive for mycobacteria; 61 specimens from 31 patients grew *M. tuberculosis*. Isolates from the remaining 74 cultures were *M. avium* complex (55 specimens), *M. kansasii* (8 specimens), *M. gordonae* (4 specimens), *M. fortuitum* (5 specimens), *M. mucogenicum* (1 specimen), and a rapid grower that was not *M. fortuitum-M. chelonae* (1 specimen). Fifty-two specimens were AFB smear positive; *M. tuberculosis* was eventually isolated from 41 (17 patients), *M. avium* complex was isolated from 2, *M. kansasii* was isolated from 4, and *M. fortuitum* was isolated from 3 of these.

In all, 35 independent batches of PCR assays, comprising 1,158 specimens, were performed. The results from five batches (170 specimens) were invalidated and the batches were repeated because positive controls (from four assays) or negative controls (from one assay) did not meet acceptable criteria. As shown in Table 1, the *M. tuberculosis* PCR assay was positive for 54 specimens from 28 patients. Of these 54, 48 were culture positive for *M. tuberculosis* and 40 were AFB smear positive. Of the remaining six, two were culture positive for *M. avium* complex and four were culture negative for mycobacteria. Compared with culture, the overall sensitivity, specificity, and positive and negative predictive values of PCR were 78.7, 99.3, 88.9, and 98.6%, respectively. For AFB smear-positive specimens, PCR sensitivity and specificity were 97.6 and 90.9%, respectively, whereas the PCR sensitivity and specificity for AFB smear-negative specimens were 40.0 and 99.4%, respectively.

Of the six specimens from six patients that were culture negative but PCR positive, two had enzyme-linked immunosorbent assay readings of 1.11 and 1.78 A_{450} and were located adjacent to *M. tuberculosis* culture-positive specimens during PCR sample preparation and analysis. PCR analysis of a second aliquot of these specimens was negative. Duplicate aliquots were available for three of the four remaining specimens; all three were positive when reassayed by PCR. Medical records of these four patients were reviewed. The two patients whose sputum cultures were positive for *M. avium* had no clinical evidence of tuberculosis. Both remaining patients had a previous diagnosis of tuberculosis. One of these had been diagnosed with tuberculosis 10 days earlier, when antituberculosis therapy was started on the basis of a smear-positive sputum specimen that was subsequently PCR positive and *M. tuberculosis* culture positive. The last patient, from whom only one specimen was collected, had been diagnosed with tuberculosis 6 months earlier and had been on drug therapy since diagnosis. After resolution of these six discrepancies, 50 specimens, of which 41 were PCR positive, were considered to be from persons with tuberculosis. Of the initial six PCR-positive, *M. tuberculosis* culture-negative specimens, four remained false positive, i.e., PCR positive, tuberculosis negative. As shown in Table 2, 41 of the 42 AFB smear-positive specimens from persons with tuberculosis were PCR positive, and only 1 of these specimens (from a patient previously diagnosed with tuberculosis) was PCR negative. Of 883 AFB smear-negative specimens, 4 were PCR positive, tuberculosis negative. The resolved overall sensitivity, specificity, and positive and negative predictive values of PCR analysis (compared with culture and the patient's clinical history) were 79.4, 99.6, 92.6, and

TABLE 2. Comparison after discrepant analysis of AMPLICOR MTB test and results of acid-fast smear and patients' clinical histories for tuberculosis

Specimen type (no.)	No. of specimens (no. of patients)				Sensitivity (%)	Specificity (%)	Predictive value (%)	
	Tuberculosis positive[a]		Tuberculosis negative[a]					
	PCR+	PCR−	PCR+	PCR−			Positive	Negative
All (956)	50 (24)	13 (9)	4 (4)	889 (465)	79.4	99.6	92.6	98.6
Smear positive (52)	41 (17)	1 (1)	0 (0)	10 (7)	97.6	100	100	90.9
Smear negative (904)	9 (7)	12 (8)	4 (4)	879 (458)	42.9	99.5	69.2	98.7

[a] Tuberculosis positive or negative for *M. tuberculosis* as determined by culture or clinical history.

98.6%, respectively. For AFB smear-positive specimens, the sensitivity, specificity, and positive and negative predictive values of AMPLICOR MTB were 97.6, 100, 100, and 90.9%, respectively. For AFB smear-negative specimens, the sensitivity, specificity, and positive and negative predictive values of AMPLICOR MTB were 42.9, 99.5, 69.2, and 98.7%, respectively.

DISCUSSION

Our experience with the AMPLICOR MTB test is similar to those of other investigators (1, 4, 6, 8). The sensitivity of the PCR test has been 95% or greater for AFB smear-positive samples but much lower (43 to 66%) for AFB smear-negative specimens (4, 6). Our overall sensitivity of 79% (after resolution of discrepancies) is close to the 83% reported by Moore and Curry (6), higher than the 67% reported by D'Amato and colleagues (4), but lower than the 95% reported by Beavis et al. (1). The results of Beavis et al., however, reflect the fact that 93% of the specimens in their evaluation that were culture positive for *M. tuberculosis* were AFB smear positive (1). In contrast, the sensitivity of the AFB smear for diagnosis of tuberculosis was 70.5% in our evaluation and only 50 to 51% in the studies by Moore and Curry and D'Amato et al. (4, 6).

The specificity of the AMPLICOR MTB test appears to be excellent: greater than 99% (6). In our experience, after initial testing, the specificity was 99.3%; there were six possible false-positive results. Two of these six samples were adjacent to one that had a very high enzyme immunoassay reading (i.e., 3.50 or greater), and the PCR result for both was negative upon retesting, thus indicating initial cross-contamination and underscoring the importance of patient history and repeat analysis for specimens with discrepant PCR and AFB smear results. Another two potentially false-positive specimens were collected from patients who had been previously diagnosed with tuberculosis (10 days and 6 months earlier) and who were receiving antituberculosis therapy; both were reclassified as true positives. This same scenario (i.e., MTB PCR-positive, culture-negative specimens from persons known to have tuberculosis) has been reported by others (4, 8). Exactly what these results mean in regard to patient care, however, is not clear at this time. The PCR result is a true positive, but how this information should be used in the clinical setting has not yet been established. Further investigation of this issue is needed. The final two false-positive specimens were culture positive for *M. avium* complex, which has not been previously reported. The reason for this discrepancy is unclear.

To determine the potential value of AMPLICOR MTB for diagnosis of tuberculosis in patients whose sputum smears are negative for AFB, the medical records of the seven patients from whom the eight PCR-positive, AFB smear-negative, *M. tuberculosis* culture-positive specimens were collected were reviewed. Four of these patients were known to have tuberculosis, and in each case cultures were ordered to monitor efficacy of therapy. For these patients, a positive PCR result would not have altered their care. The other three patients, however, did not have a specific diagnosis at the time the positive PCR result could have been available. All three patients were infected with the human immunodeficiency virus, and all had both pulmonary (noncavitary) and extrapulmonary tuberculosis. Two of these three patients were hospitalized, and for both, antituberculosis therapy would have been started 1 week earlier on the basis of a positive PCR result. In both patients, an invasive procedure (i.e., fine-needle aspiration of an enlarged lymph node) was required for diagnosis. One patient died 3 days after appropriate treatment was begun, but it is impossible to determine what effect an earlier diagnosis would have had on the outcome. The third patient was managed as an outpatient. In this case, appropriate therapy could have been initiated 3 weeks earlier had the PCR result been available.

In summary, our findings and those of others support the use of AMPLICOR MTB for rapid diagnosis of tuberculosis in patients whose respiratory specimens are AFB smear positive. Not all patients with AFB smear-positive sputum samples have tuberculosis (19 of 28 patients [68%] in our study); therefore, a rapid yes or no answer would allow optimal patient management, including the best use of AFB isolation rooms. Data from our study and others also indicate that, at this time, PCR cannot replace mycobacterial culture. The sensitivity of the PCR assay for AFB smear-negative specimens is approximately 50%, and an isolate is necessary for susceptibility testing. Therefore, because PCR must be a supplemental test, requiring additional resources, recommendations for its appropriate use are needed. Issues that should be addressed regarding the role of PCR in diagnosis as well as management of tuberculosis include optimal use of PCR in testing AFB smear-negative specimens, because a rapid diagnosis clearly will benefit certain patients whose sputum smears are AFB negative, and its role in evaluation of response to therapy.

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