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Detection and Identification of *Mycobacterium tuberculosis* Directly from Sputum Sediments by Amplicor PCR

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Sputum specimens received for the diagnosis of tuberculosis or other mycobacterial infections were tested by a PCR-based assay and culture techniques. Results of the PCR assay (Amplicor *Mycobacterium tuberculosis* Test) were compared with results of standard culture techniques with cultures held for 6 weeks. One thousand nine specimens were included: 301 retrospective specimens (frozen at −70°C and later tested by PCR) and 708 prospective specimens (tested within 1 day of processing). One hundred sixty-two (16%) of the specimens were culture positive for *M. tuberculosis*; 83 (51%) of these were also fluorochrome stain positive. The sensitivity and specificity of the Amplicor PCR compared with those of culture were 83% (134 of 162 specimens) and 97% (800 of 827 specimens), respectively. The sensitivity for fluorochrome stain-positive specimens was 99%, and that for fluorochrome stain-negative specimens was 66%. The great majority of the 28 PCR-negative, culture-positive specimens were low positives; 27 were smear negative and 19 contained <100 CFU of *M. tuberculosis* per ml. The 27 PCR-positive, culture-negative specimens included 24 that were positive by repeat testing by alternate primer PCR and were from patients with tuberculosis on antimicrobial therapy. With these considered culture misses, the final sensitivities of PCR and culture were 85, and 87%, respectively, while the specificities were 99.6 and 100%, respectively. After normal laboratory processing of sputum specimens, the Amplicor PCR assay can be completed in 8 h. Thus, it is possible to have results available within 10 h of specimen submission.

The laboratory diagnosis of *Mycobacterium tuberculosis* currently depends on acid-fast staining and culture of processed sputum specimens, which are technologies that have been used for decades in the United States (20). While these techniques have been continuously refined and improved, they still have severe limitations. Microscopic examination of acid-fast smears has a sensitivity and specificity low enough to be useful only as a presumptive screening test. However, because acid-fast smear results are available rapidly they are used to manage patient care and to make public health decisions. Culture, considered the most accurate test because of its high degrees of sensitivity and specificity, is labor-intensive and slow. Clinical laboratories hold cultures for 6 to 8 weeks to achieve the maximum sensitivity (20). Radiometric liquid culture (BACTEC), the most rapid culture technique that is widely used, requires an average of 13 days to become positive (2). The most sensitive and rapid culture and staining techniques available are not used by all laboratories because of funding, staffing, and training difficulties (16). The recent increase in tuberculosis cases in the United States and the emergence of multidrug-resistant strains have demonstrated the weaknesses in the currently used techniques and underscored the need for more rapid and accurate methods of laboratory diagnosis (8).

Assays that rapidly amplify and detect specific regions of bacterial DNA or RNA have provided the techniques that promise to make improvements in the laboratory detection and identification of *M. tuberculosis*. PCR has been used to detect *M. tuberculosis* in respiratory samples (1, 3, 4, 6, 9, 10, 13–15, 19, 21–23, 25, 29, 30) and other clinical samples (10–12, 18, 26, 28). The laboratories that use PCR tests have produced their own tests with a wide variety of primers, probes, and extraction, amplification, and detection techniques. This has led to wide variations in reported assay performances. The clinical sensitivity of PCR compared with that of culture has been reported to be from 74% to greater than 100%, with actual detection limits of <1 to 100 CFU (1, 3, 6, 13–15, 21, 23, 25, 29, 30). In some instances, test production and diagnostic testing in the same facility can lead to poor sensitivity because of the lack of assay optimization or low specificity because of amplicon contamination (24). These drawbacks may be eliminated by use of a commercially available PCR kit. Because of the personnel and funding available for research and development, standardization of the assay protocol, and dedicated production facilities and stringent quality control programs, commercially developed diagnostic PCR tests for *M. tuberculosis* have the potential for better overall performance characteristics in clinical laboratories. They are also available for use by laboratories which cannot produce in-house PCR assays. The potential for this has been demonstrated by the consistent performance characteristics of the Gen-Probe Mycobacterium Tuberculosis Direct Test, a commercially developed rRNA amplification assay for *M. tuberculosis*. When it was tested at several sites, this assay demonstrated a sensitivity of 78 to 98% and a specificity of 96 to 100% (1, 5, 17, 22). This report describes a combined retrospective and prospective clinical trial of the Roche Amplicor *Mycobacterium tuberculosis* Test, a commercially developed PCR assay for *M. tuberculosis*.

MATERIALS AND METHODS

Clinical specimens and culture techniques. Specimens were collected from patients being screened for tuberculosis or other pulmonary mycobacterial infections or patients being followed during antituberculosis therapy at the Orange County Health Care Agency Pulmonary Disease Clinic. A total of 1,009 specimens were included in the study. The 301 retrospective specimens included 103 sequential culture-positive and 198 sequential culture-negative specimens. The 708 prospective specimens were sequential by date and time received. Nine hundred fourteen specimens were induced sputum samples taken with an Ultra-Neb 99 nebulizer (DeVilbiss, Somerset, Pa.) with 0.45% NaCl solution and a 50-ml sterile conical tube collection kit (Sage Products, Crystal Lake, Ill.). The remaining 95 specimens were sputum samples collected in the same collection kit. Specimens were held at 4°C, received by the laboratory within 2 h, and

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TABLE 1. Number of positive specimens and sensitivity of Amplicor PCR and fluorochrome staining by concentration of *M. tuberculosis* in specimen

Quantitative culture (CFU/ml)	No. (%) culture positive specimens	Amplicor PCR		Fluorochrome staining	
		No. of true-positive specimens	Sensitivity (%)	No. of true-positive specimens	Sensitivity (%)
1–100	62 (38)	43	69	16	26
>100–500	30 (19)	26	87	14	47
>500–1,000	19 (12)	19	100	13	68
>1,000	40 (25)	39	98	39	98
Not available	11 (7)	7	64	1	9
Total	162 (100)	134	83	83	51

processed by standard laboratory procedures. The majority of the specimens were processed within 24 h by a standard *N*-acetyl-L-cysteine sodium hydroxide method (20) and were centrifuged at 3,000 × *g* for 20 min, and then 1.0 ml of 0.2% bovine serum albumin (BBL, Cockeysville, Md.), 45.5 U of penicillin G per ml, and 9% LaMotte Wide Range Indicator (LaMotte Chemical Company, Chestertown, Md.) were added to the final pellet; this was followed by titration to pH 6.8 to 7.2 with 0.5 N HCl. For each specimen, two Lowenstein-Jensen tubes (BBL) were inoculated with 0.1 ml of specimen, and a smear was made for fluorochrome staining. A BACTEC 12B vial was inoculated with 0.5 ml for all specimens collected to establish a diagnosis of tuberculosis. The remainder of the sample was frozen at −70°C for up to 4 months for the retrospective specimens or refrigerated at 4°C for 1 day or less for the prospective specimens. Fluorochrome staining was performed by standard procedures (20). Tube cultures were examined weekly for a total of 6 weeks. BACTEC vials were tested every day for 7 days and biweekly for 6 weeks. Positive cultures were quantitated, and acid-fast isolates were identified by standard biochemical techniques (20), DNA-RNA hybridization (Accu-Probe; Gen-Probe, San Diego, Calif.), or high-performance liquid chromatography (7).

PCR assay. A 100-μl aliquot of the sediments from processed clinical specimens was tested by the Amplicor *Mycobacterium Tuberculosis* Test (Roche Diagnostic Systems, Branchburg, N.J.) according to the directions in the manufacturer's draft package insert. The assay amplifies a 584-bp sequence from the 16S rRNA gene. Testing was carried out in duplicate, and the results were calculated by using the results for the first sample only. Prior to beginning the clinical trial, all microbiologists performing the assay were qualified. They were considered qualified if they correctly tested two sets of 20 unknown samples on two successive runs. The test consists of three steps. Sample preparation uses wash, centrifugation, and heating with a lysis buffer at 60°C for 45 min. Amplification is carried out by using a 37-cycle program in a Perkin-Elmer 9600 thermal cycler. Detection is accomplished by hybridizing the biotin-labeled amplicon to probe-coated microwell plates and sequential washing and addition of an avidin-horseradish peroxidase conjugate and then the addition of a tetramethylbenxidine substrate. After the addition of the stop solution, the optical density at A_{450} was measured with a Bio-Tek EL-312 microplate reader (EL-312; Bio-Tek, Winooski, Vt.). Sample preparation resulted in 50 μl of a 1:2 dilution of the initial sample being used for the amplification step. Thus, the effective sample size for amplification was 25 μl. Each run consisted of 92 specimens, 3 negative controls, and 1 positive control. Control values met the package insert specifications for all runs. During the trial a total of two different lots of the testing materials were used.

Detection of PCR inhibitor. Samples that were culture positive and Roche PCR negative were tested for the presence of inhibitors of the PCR by rerunning the test on an aliquot of the sample to which 20 copies of positive control *M. tuberculosis* DNA were added after the sample preparation step.

Alternative primer PCR assay. A second PCR assay which amplified a portion of the superoxide dismutase gene was used to test all PCR-positive, culture-negative specimens. The testing was carried out by a previously described assay (31).

RESULTS

The clinical performance of the Amplicor *Mycobacterium Tuberculosis* Test was determined by comparing the Amplicor PCR results with those of standard culture and fluorochrome staining for 1,009 specimens. Twenty prospective specimens were overgrown by contaminants on culture and were excluded from analysis. Of the remaining 989 specimens, 103 (34%) of the 301 retrospective and 59 (8.6%) of the 688 prospective specimens were culture positive for *M. tuberculosis*, for an overall positivity rate of 16%. Thirty-eight percent were low-positive specimens and 31% were medium-positive specimens, while 25% were high-positive specimens (Table 1). Acid-fast bacteria other than *M. tuberculosis* were isolated from 95 specimens; 4 of these specimens were also positive by Amplicor PCR, most likely because of noncultivable *M. tuberculosis* isolates that were also present in the specimen (Table 2). The overall results presented in Table 3 indicate that when compared with culture, the sensitivity of Amplicor PCR, was 83% and the specificity was 97%, while fluorochrome staining had a sensitivity of 51% and a specificity of 99%.

As indicated in Table 1, the sensitivities of both the Amplicor PCR and fluorochrome staining varied with the concentration of *M. tuberculosis* in the specimens, as determined by semiquantitative culture. Both techniques were more sensitive with specimens containing a greater number of organisms. The sensitivity of the Amplicor PCR assay ranged from 69% for low-positive specimens (≤100 CFU/ml) to 98% for high-positive specimens (>1,000 CFU/ml). In comparison, the sensitivity of fluorochrome staining ranged from 26 to 98% for the low- and high-positive specimens, respectively. For specimens which were smear positive, the sensitivity of the Amplicor PCR was 99%; for smear-negative specimens the sensitivity was 66% (Table 4).

TABLE 2. Acid-fast organisms other than *M. tuberculosis* isolated from clinical specimens

Identification	No. isolated	No. of specimens PCR positive
Actinomadura madurae	1	0
Mycobacterium avium complex	10	0
Mycobacterium chelonae	4	0
Mycobacterium flavescens	3	1[a]
Mycobacterium fortuitum	3	0
Mycobacterium gordonae	3	0
Mycobacterium intracellulare	1	0
Mycobacterium phlei	1	0
Mycobacterium scrofulaceum	1	0
Mycobacterium terrae	1	0
Rhodococcus sp.	1	0
Tsukamurella spp.	2	0
NTM[b] (unidentified[c])	3	0
NTM (orange pigment, unidentified[c])	23	1[a]
NTM (non-pigmented, unidentified[c])	38	2[a]
Total	95	4

[a] Specimens considered a culture miss in discrepancy analysis (Table 6).
[b] NTM, nontuberculosis mycobacterium.
[c] Not identified because of the low number of organisms isolated (<10 CFU).

TABLE 3. Initial comparison of Amplicor PCR and fluorochrome staining with culture

Test and result	Culture result (no. of specimens)		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Amplicor PCR				
Positive	134	27	83	97
Negative	28	800		
Fluorochrome staining				
Positive	83	8[a]	51	99
Negative	79	819		

[a] Six specimens were also positive by PCR, one specimen was culture positive for *M. avium*, and one specimens was culture positive for *M. fortuitum*.

TABLE 4. PCR sensitivity by fluorochrome smear result for *M. tuberculosis* culture-positive specimens

Fluorochrome smear result	No. (%) of specimens			Sensitivity (%)
	Total	True positive	False negative	
Positive	83 (51)	82	1	99
Negative	79 (49)	52	27	66
Total	162 (100)	134	28	83

Twenty-eight specimens were Amplicor PCR negative and culture positive. The majority of these were low-positive specimens. Twenty-seven were smear negative and 19 contained <100 CFU of *M. tuberculosis* per ml. Two of these 28 specimens (7.1%) contained inhibitors of the PCR which caused a negative result even after the addition of *M. tuberculosis* DNA (Table 5). Since a total of 162 positive specimens were tested, the percentage of specimens with inhibitors present in sufficient quantity to cause a positive specimen to display a negative result can be calculated as 2 of 162, or 1.2%. Six specimens were Amplicor PCR positive on retesting. These specimens had culture results that were low or medium positive and had an initial negative result because of sampling error (Table 5).

The 27 specimens that were Amplicor PCR positive and culture negative were further investigated by repeat testing, alternate primer PCR, and review of laboratory and patient records (Table 6). The specimens were from 22 patients, all of whom were diagnosed with pulmonary tuberculosis and were on antimicrobial therapy. One specimen was negative on repeat testing and negative by an alternate primer PCR. The result for this specimen was considered false positive. By using the criteria of a positive result on repeat testing, a positive alternate primer PCR result, a patient history of concurrent therapy for tuberculosis, and the history of a previous or subsequent isolate, 24 of the 27 specimens were considered culture misses. All four specimens from which an acid-fast organism other than *M. tuberculosis* was isolated (Table 2) met the criteria for being considered a culture miss (Table 6).

As a result of the analysis of specimens with discrepant results, the results for 24 specimens that were missed by culture were considered true positive, while those for 3 specimens were considered false positive by Amplicor PCR. By using these final results, the initial results from Table 3 were recalculated and are presented in Table 7. The resolved sensitivity and specificity of culture were 87 and 100%, respectively, and the resolved sensitivity and specificity of Amplicor PCR were 85 and 99.6%, respectively.

DISCUSSION

The Amplicor *Mycobacterium Tuberculosis* Test is the first PCR test that has been developed in kit form for use in the clinical laboratory for the detection of *M. tuberculosis*. Pending U.S. Food and Drug Administration clearance, the test will soon become widely available in the United States. The assay procedure itself has been simplified from research-based procedures and is not technically demanding for clinical laboratory personnel. However, several modifications of normal clinical laboratory procedures need to be made to perform the test successfully. Three separate work areas for the different segments of the test, dedicated pipettes, and extreme care in pipetting in the first transfers are necessary. In our laboratory a full plate run (96 specimens and controls) can be completed in 8 h. Since decontamination and processing of a batch of respiratory specimens require 2 h, it is possible to incorporate this assay into the normal work flow, with the ability to report results out in a maximum time of one 10-h shift or two 8-h shifts.

The initial sensitivity of the Amplicor PCR test compared with that of culture was 83%. Sixty-eight percent of the samples missed were low-positive specimens containing less than 100 CFU of *M. tuberculosis* per ml, and 14% were medium positives containing 100 to 500 CFU/ml. The sensitivity for specimens containing ≤100 CFU of *M. tuberculosis* per ml was 69%, and the sensitivity for smear-negative, culture-positive specimens was 66%. This is in contrast to the high degree of sensitivity for medium- and high-positive specimens (87% to 100%) and for smear-positive specimens (99%). This lower degree of sensitivity for low-positive specimens is most likely the result of the low effective inoculum for the PCR test compared with that for culture. The effective inoculum for PCR, 25 μl, is only 12.5% of the 200 μl inoculated into two tubes containing solid medium or 5% of the 500 μl inoculated into a BACTEC 12B vial. Since 38% of our positive samples contained ≤100 CFU of *M. tuberculosis* per ml, a larger sample size would increase the chance of *M. tuberculosis* being present in the sample analyzed and would increase the overall sensitivity of the Amplicor PCR test. A similar result was seen in earlier studies in our laboratory with the Gen-Probe Mycobacterium Tuberculosis Direct Test, an rRNA amplification assay, which used similar effective inoculum sizes (17). It is expected that the manufacturers of these tests will make improvements in the future, including a larger sample size, since it would be a simple way to increase the sensitivity of the test for low-positive specimens. Samples containing inhibitors of the PCR made up only 7% of false-negative samples or 1.2% of all positive specimens. Thus, in the patient population used for the present study, inhibitors were a minor factor in the overall sensitivity of the test. This rate of specimens containing inhibitors is similar to the 3% reported by Andersen et al. (3) but much lower than the 15% reported by Forbes and Hicks (14). The low level of inhibition may be due to a combination of the low sample volume and the manufacturer's proprietary sample treatment buffer.

The initial specificity of the Amplicor PCR test was 97%. Discrepancy analysis of the 27 PCR-positive, culture-negative specimens indicated that, with the exception of 1 specimen, all were from patients being treated with antibiotics for documented pulmonary *M. tuberculosis* infections. This has also been seen in studies comparing rRNA amplification with culture (5, 17). Recently published results (19) and experience in our laboratory (unpublished data) indicate that while undergoing treatment, some patients provide specimens in which *M.*

TABLE 5. Analysis of 28 Amplicor PCR-negative, culture-positive specimens

No. of specimens	Quantitative culture (CFU/ml)	No. of specimens		
		Smear positive	Repeat PCR positive	With PCR inhibitor[a]
19	1–100	0	5	1[b]
4	>100–500	0	1	0
0	>500–1,000	0	0	0
1	>1,000	1	0	1[c]
4	Not available	0	0	0
28		1	6	2

[a] Negative PCR result after addition of *M. tuberculosis* DNA to sample.
[b] Retrospective specimen.
[c] Prospective specimen.

TABLE 6. Analysis of 27 Amplicor PCR-positive, culture-negative specimens

No. of specimens	Repeat PCR result	Smear result	Alternate primer PCR	Antibiotic treatment	Diagnosis of tuberculosis	History of a previous isolate	Final result
1	−	−	−	+	+	−	False positive
6[a]	+	+	+	+	+	+	True positive
18[b]	+	−	+	+	+	+	True positive
2	+	−	+	+	+	−	False positive

[a] Includes one specimen from which an unidentified nontuberculous mycobacterium was isolated.
[b] Includes one specimen from which *M. fortuitum* was isolated and two specimens from which a nontuberculous mycobacterium was isolated.

tuberculosis nucleic acid can be detected for some time after the patient's specimen has become culture negative. Quantitation of the difference in the time that it takes for specimens to become smear, culture, and nucleic acid amplification negative while on therapy is currently being determined. In the present study, rigorous discrepancy analysis criteria requiring both a positive alternate primer PCR result and a history of a previous *M. tuberculosis* isolate from the patient resulted in 24 of the 27 initial false-positive specimens being considered culture misses. Two of the remaining three false-positive specimens were from patients with tuberculosis who were on therapy and from whom *M. tuberculosis* had never been isolated. It is quite possible that these two specimens were also culture misses, although they did not meet our criteria for being reclassified as true-positive specimens.

Other acid-fast bacteria were isolated from 4 of the 27 specimens considered initial false positives (one *Mycobacterium fortuitum* and three unidentified nontuberculous mycobacteria) (Table 2). Several lines of evidence indicate that this is a coincidental event and not a false-positive PCR test result. First, all four specimens came from patients being treated for tuberculosis and from whom *M. tuberculosis* isolates had been isolated previously, and the patients were positive when they were retested by a second PCR with a species-specific probe (31). Second, of 95 specimens containing acid-fast isolates other that *M. tuberculosis*, only these four specimens were positive by PCR. Third, previous experiments with stock strains indicated no positive reactions with bacteria other than *M. tuberculosis* complex (*M. tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*) (unpublished data; Amplicor PCR package insert). Amplicor PCR can also detect *M. tuberculosis* isolates that may be obscured on culture because of the presence of a more rapidly growing bacterium. Of the 20 specimens removed from the study because of the lack of culture results because of bacterial and fungal overgrowth, 2 were positive by Amplicor PCR. Review of patient data determined that both of these specimens were from patients with tuberculosis who were receiving therapy and whose specimens were previously positive (and, in one patient, subsequently positive) for *M. tuberculosis* on culture. These results indicate that the Amplicor PCR can detect *M. tuberculosis* when culture results are negative because of the treatment of patients with antimicrobial agents or when other acid-fast organisms are isolated or when culture results are not available because of overgrowth by other species of bacteria or fungi. While this can be considered a diagnostic advantage of PCR testing, comparison studies are complicated because patient data must be used to determine if a PCR result is correct.

Correction of the initial results by considering 24 specimens as culture misses provides final results with sensitivities and specificities comparable to those of Amplicor PCR and culture. In our laboratory, the actual sensitivity during routine clinical use will depend on the types of samples tested. If only diagnostic specimens from patients not yet receiving antimicrobial therapy are tested, the sensitivity will be closer to the initial sensitivity of 83%. If specimens taken during therapy for follow-up or from patients with unknown previous treatment are also tested, the sensitivity will be close to the final sensitivity reported here, which is equivalent to that of culture.

Noordhoek et al. (24) reported major PCR assay performance problems in a multilaboratory study in which each laboratory used its own laboratory-developed PCR assay to test standard unknown samples containing *Mycobacterium bovis* (BCG). In addition, several recent reports have indicated that patients free of *M. tuberculosis* disease may have sputum samples with PCR results indicating positivity for *M. tuberculosis* (4, 11, 27). Two authors of those reports concluded that a positive PCR result does not correlate with active *M. tuberculosis* infection (4, 27). The low levels of sensitivity and specificity reported by Noordhoek et al. (24) and the low level of specificity reported in the other three studies were not seen in the present study. A high level of sensitivity and excellent specificity were seen at four separate sites evaluating a Gen-Prove Mycobacterium Tuberculosis Direct Test, a commercial rRNA amplification assay for *M. tuberculosis* (1, 5, 17, 22), as well as in several recent large studies that used laboratory-based PCR assays (9, 14, 23). In addition, a recent report indicates that sputum specimens from patients with pulmonary tuberculosis who respond to therapy become PCR negative and are negative at 8- and 12-month follow-ups (19).

A major cause of the low specificity in the study of Noordhoek et al. (24) was contamination of the specimens with amplicon, causing false-positive reactions. This may also be a problem with other studies reporting low levels of specificity (4, 11, 27). Amplicon carryover contamination should not be a problem with the Amplicor assay because of the use of uracil *N*-glycosylase in the first step of the amplification combined with the substitution of dUTP for TTP in the master mixture. However, because of the extreme sensitivity of PCR it is possible to contaminate specimens and produce false-positive results in other ways. For example, several low-level false-positive results in one run of our study were due to a dedicated

TABLE 7. Comparison of culture and Amplicor PCR with resolved results

Test and result	Final result (no. of specimens)		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Amplicor PCR				
Positive	158	3	85	99.6
Negative	28	800		
Culture				
Positive	162	0	87	100
Negative	24	803		

pipetter being borrowed to pipette *M. tuberculosis*-positive specimens and then its return and use in the Amplicor PCR assay with unplugged tips. This technical error was caught and corrected, even though the negative controls used in the run had normal results. To detect contamination at this low level, the use of duplicate specimens, the use of a paired negative control for each specimen, or repeat testing of positive specimens would be necessary. However, this results in an increased cost to run the test or an increase in the time to report the results. This points out that while a test with a commercially available PCR kit may be technically easy to perform, much greater diligence than is currently exercised for other types of tests currently used in laboratories must be used to ensure that the results are accurate. Many laboratory practices that are taken for granted will have to be changed to ensure the correct performance of PCR assays.

The sensitivity and specificity of the PCR test presented here indicate that the majority of *M. tuberculosis*-positive specimens can be identified rapidly if they are screened by the test. Because the sensitivity of the PCR is not equal to that of culture in all instances, a culture technique must still be used to detect any missed positive specimens and to provide an isolate for drug susceptibility testing. The results presented here indicate that, in our laboratory, screening of every specimen by PCR would detect and confirm at least 83% of the positive specimens in the same time frame that 51% of the positives are presumptively identified by our present acid-fast smear technique. However, the time and expense required to test every specimen may lead laboratories to test only specimens for which a PCR result would have the most added benefit for patient care or tuberculosis control programs. If this is the case, two specific uses of nucleic acid amplification have proved to be very helpful to our public health tuberculosis control program. The first is the determination of whether a patient who is smear positive is infected with *M. tuberculosis*. Since the sensitivity of the PCR for smear-positive specimens as reported here is 99% and has been reported by others to be 94 to 95% (9, 23), PCR can be used to rapidly determine if an infection detected by a positive smear result is caused by *M. tuberculosis*. This is especially useful with human immunodeficiency virus-positive patient populations with a high rate of infections with *Mycobacterium avium* complex and for patients who either must be moved from shared living situations for treatment or must be placed in respiratory isolation. The second is to determine if a new smear-positive result from a patient who, because of therapy, has previously turned smear negative is caused by *M. tuberculosis* or another organism. Confirming that the positive smear is a result of continued *M. tuberculosis* infection is an early indication of possible treatment failure, and appropriate measures can then be taken. This is a more specific example of the use of PCR to follow patients to determine the success of antimicrobial therapy, as suggested by Kennedy et al. (19).

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