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Clinical Evaluation of the Enhanced Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for Rapid Diagnosis of Tuberculosis in Prison Inmates†

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The reliability of the enhanced Amplified Mycobacterium Tuberculosis Direct Test (E-MTD; Gen-Probe, Inc., San Diego, Calif.) for rapid diagnosis of pulmonary tuberculosis (TB) was evaluated by testing 1,004 respiratory specimens from 489 Texas prison inmates. Results were compared to those of mycobacterial culture (BACTEC TB 460 and Middlebrook 7H11 biplates), smear for acid-fast bacilli (AFB; auramine O), and clinical course. After chart review, three patients (nine specimens) who were on antituberculosis therapy before the study began were excluded from final analysis. Of the remaining 995 specimens, 21 were AFB smear positive: 13 grew *Mycobacterium tuberculosis* complex (MTBC), 6 grew nontuberculous mycobacteria, and 2 (from two patients diagnosed with TB and started on therapy after the study began) were culture negative. Twenty-eight specimens (20 patients) were positive for MTBC by culture and E-MTD. Seven specimens (seven patients) were positive by culture alone; three were from patients who had other E-MTD-positive specimens, two were false-positive cultures, and two were false-negative E-MTD results. Eight specimens were positive by E-MTD only; four specimens (four patients) were false-positive E-MTD results, and four specimens were from two patients with earlier E-MTD-positive specimens that grew MTBC. Thus, there were 22 patients with TB (10 smear positive and 12 smear negative). The sensitivity and specificity of the AFB smear for diagnosis of TB, by patient, were 45.5 and 98.9%, respectively. After resolving discrepancies, these same values for E-MTD were 90.9 and 99.1% overall, 100 and 100% for the smear-positive patients, and 83.3 and 99.1% for the smear-negative patients. Excluding the one smear-negative patient whose E-MTD-negative, MTBC culture-positive specimen contained inhibitory substances, the sensitivity of E-MTD was 95.2% overall and 90.9% in smear-negative patients. The specificity and positive predictive value of E-MTD can be improved, without altering other performance characteristics, by modifying the equivocal zone recommended by the manufacturer. These data suggest that E-MTD is a reliable method for rapid diagnosis of pulmonary TB, irrespective of the AFB smear result. Guidelines for the most appropriate use of E-MTD with smear-negative patients are needed.

Tuberculosis remains a public health problem in the United States, despite a constant decline in incidence in each of the past several years. A key aspect of tuberculosis control is rapid identification of infectious patients, a process which for many years has been based on staining smears for acid-fast bacilli (AFB) and culturing for mycobacteria with a liquid and a solid medium. AFB smear results should be available within 24 h, but a positive result is not specific for tuberculosis. Mycobacterial culture and identification results, which provide a specific diagnosis, are not available for 2 to 3 weeks or longer. In response to the need for a more rapid diagnostic test, a few manufacturers have developed nucleic acid amplification tests specific for *Mycobacterium tuberculosis* complex (MTBC). Currently, two such tests (Amplified Mycobacterium Tuberculosis Direct Test [MTD; Gen-Probe, Inc., San Diego, Calif.] and AMPLICOR *Mycobacterium tuberculosis* Test [Roche Molecular Systems, Branchburg, N.J.]) are commercially available in the United States for detection of MTBC in AFB smear-positive respiratory specimens (1–3, 5–7, 9, 10, 12).

Recently, Gen-Probe modified the MTD, increasing the sample volume, decreasing the amplification time, and eliminating the hybridization controls and the amplification termination step. This enhanced version of the MTD (E-MTD) was approved by the Food and Drug Administration (FDA) for testing AFB smear-positive respiratory specimens in May 1998. The only published report of which we are aware comparing the original and enhanced versions of the MTD indicated that the E-MTD was more sensitive than the original test (i.e., 94.7 versus 83% overall) and just as specific (i.e., 100% for both assays) (4). These data suggest that the E-MTD has a potential role in the diagnosis of AFB smear-negative as well as smear-positive pulmonary tuberculosis. The purpose of this study was to evaluate the performance of the E-MTD, irrespective of the AFB smear result, in a population considered to be at high risk for tuberculosis, i.e., inmates of the Texas Department of Criminal Justice (TDCJ) prisons.

MATERIALS AND METHODS

Clinical specimens. Respiratory specimens (expectorated and induced sputum, bronchial washings, bronchoalveolar lavage fluid, and tracheal aspirates) collected from TDCJ prison inmates for whom mycobacterial culture had been requested from September 1997 to July 1998 were included in the study. Grossly bloody specimens were excluded from the evaluation, in accordance with the test manufacturer's protocol. Specimens from inmates hospitalized at the University of Texas Medical Branch (UTMB)-TDCJ hospital in Galveston were transported directly to the UTMB microbiology laboratory and refrigerated until processed. Specimens from inmates in prison units throughout Texas were refrigerated at the unit and then placed in a cooler and transported by automobile to UTMB or

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† We dedicate this paper to the memory of Gbo Yuoh, whom we all will miss.

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mailed to UTMB by overnight express mail. All specimens were processed within 24 h of receipt in the laboratory.

Specimen processing and culture. The entire amount of specimen received (which ranged from approximately 0.5 to 5 ml) was decontaminated with 1% (final concentration) sodium hydroxide-*N*-acetylcysteine and concentrated by centrifugation at 3,000 × *g* for 20 min, according to standard procedures (8). The protocols used to limit the potential for cross-contamination during processing included using single-use containers for phosphate buffer and digestant, removing and replacing the cap from each specimen tube sequentially during the addition of specimens and reagents, and allowing the tubes to stand for a few minutes after agitation on a vortex mixer (14). Approximately 0.2 ml of the sediment was used to prepare a smear for staining with auramine O. To the remaining sediment, phosphate buffer was added to give a final volume of 2.0 ml. For culture, 0.5 ml of the suspension was inoculated into a BACTEC 12B bottle (Becton Dickinson, Sparks, Md.) and 0.2 ml was inoculated onto each side of a Middlebrook 7H11 biplate (Becton Dickinson). Two 500-μl aliquots were reserved for E-MTD testing: one was stored at 2 to 8°C and tested within 72 h of processing; the other was stored frozen at −70°C for future analysis, if necessary.

BACTEC 12B bottles were incubated at 37°C in 8% CO_2 and monitored for growth for 5 weeks by the BACTEC 460 TB instrument according to the manufacturer's instructions, as described in detail elsewhere (8), and following recommendations to help prevent cross-contamination (14). The plates were incubated at 37°C in 8% CO_2 and examined weekly for growth for 6 weeks. Isolates of mycobacteria were identified by DNA probes (AccuProbe [Gen-Probe, Inc.] for MTBC, *Mycobacterium avium* complex, *Mycobacterium kansasii*, and *Mycobacterium gordonae*) or by conventional biochemical tests (for rapidly growing mycobacteria), performed according to standard protocol (8). Isolates not identified by these procedures were referred to the Texas Department of Health for identification by high-performance liquid chromatography and/or conventional biochemical tests.

EMTD. E-MTD was performed according to the manufacturer's protocol as follows. Briefly, 50 μl of specimen dilution buffer was added to each lysing tube, after which 450 μl of well-mixed specimen, positive cell control, or negative cell control was added to the correspondingly labeled tube. The lysing tubes were vigorously agitated on a vortex mixer for 3 s and then sonicated for 15 min at ambient temperature in a water bath sonicator (Branson Ultrasonic Corp., Dansbury, Conn.) that had been previously degassed for 15 min at ambient temperature. At this point, samples and controls (referred to as lysates) were ready for amplification. Amplification tubes were labeled, and 50 μl of *Mycobacterium tuberculosis* Amplification Reagent was added to the bottom of each tube, after which 200 μl of *Mycobacterium* Oil Reagent was gently added by allowing it to run down the side of the tube. With an extended-length hydrophobically plugged pipette tip, 25 μl (each) of the sample and control lysates were transferred to the appropriate tube (the remaining lysate was stored at −70°C for further testing, if necessary). The tubes were incubated at 95°C for 15 min in a dry-heat block and then transferred to a 42°C water bath for 5 min. To each tube, 25 μl of *Mycobacterium* Enzyme Reagent was added; the tubes were capped, gently shaken, and incubated in a 42°C water bath for 30 min. After amplification, the tubes were carefully uncapped, and 100 μl of Hybridization Reagent was added. The tubes were capped with new caps, vigorously agitated on a multitube vortex mixer (VWR Scientific Products, West Chester, Pa.) for at least 30 s, and incubated in a 60°C water bath for 15 min. After hybridization, 300 μl of Selection Reagent was added. The tubes were capped with new caps, vigorously agitated on a multitube vortex mixer for at least 30 s, incubated in a 60°C water bath for 15 min, allowed to cool at ambient temperature for 5 min, and read (in relative light units [RLU]) in a luminometer (Leader 50; Gen-Probe, Inc.). The interpretation of the results was as follows: <30,000 RLU was considered negative; ≥500,000 RLU was considered positive; and 30,000 to 500,000 RLU was considered equivocal. For samples yielding an equivocal result, a second aliquot of processed (i.e., decontaminated and concentrated) specimen was tested; if the second result was ≥30,000 RLU, the sample was considered positive, whereas if it was <30,000 RLU, it was considered negative. The results of an E-MTD assay were considered acceptable when the negative cell control was <20,000 RLU and the positive cell control was ≥500,000 RLU. E-MTD results were not reported because at the time of the study the test was not FDA approved.

Protocol for detection of inhibitory substances. Specimens that grew MTBC but were E-MTD negative were analyzed for the presence of inhibitors or interfering substances as follows. The lysate from the specimen in question was thawed at ambient temperature, and 25 μl was added to an amplification tube containing 50 μl of amplification reagent and 200 μl of oil reagent. The tube was then seeded with 5 μl of the positive cell control lysate, placed in the 95°C heat block for 15 min, and tested as described in the preceding paragraphs. In addition, if a sufficient quantity of processed specimen was available, 450 μl of that specimen and 50 μl of the amplification positive cell control were added to a lysing tube containing 50 μl of specimen dilution buffer. Lysis, amplification, and detection were then performed as described in the previous section. A positive and a negative control were processed and tested as previously described, and to serve as a positive control for the seeded lysates, 5 μl of positive cell control lysate was added to a second negative control tube. The interpretation of the results was as follows: ≥30,000 RLU was considered negative for inhibitory substances that prevent amplification and <30,000 RLU was considered to show that substances that inhibit amplification were present.

Resolution of discrepancies. For all patients who had specimens with discordant MTBC culture and E-MTD results and no specimens for which both tests were positive, the following steps were taken. The medical record (including initial symptoms, the Mantoux test result [if it was performed and the result was recorded], chest radiograph findings, response to therapy, and admitting and discharge diagnoses) was reviewed. Laboratory records were examined for those patients with a single positive culture, and if there was evidence suggesting the possibility of cross-contamination, isolates were sent to the Texas Center for Infectious Disease, San Antonio, for molecular fingerprinting by IS*6110* restriction fragment length polymorphism analysis and TBN12 typing. For E-MTD-negative specimens that grew MTBC and for which the culture results was considered a true positive, additional processed sample and lysate were tested for inhibitory substances.

Data analysis. The sensitivity, specificity, and positive and negative predictive values of E-MTD were calculated after initial testing (i.e., before resolution of discrepancies between E-MTD and culture results), using the culture result as the reference method, both by specimen and by patient; after resolution of discrepancies, they were calculated by patient only. For analysis by patient, all specimens for each patient were included. If any specimen from a patient was positive by AFB smear, culture (for MTBC), or E-MTD, that patient was considered positive for that particular test. For example, if the specimen from day 1 was culture negative for MTBC but E-MTD positive, and the specimen from day 2 was positive for MTBC by both culture and E-MTD, the culture and E-MTD results for that patient were considered concordant. Additionally, if the first specimen was smear, culture, and E-MTD positive and any of the subsequent specimens were positive by smear alone or smear and E-MTD, the patient would be considered smear positive with concordant E-MTD and culture results.

RESULTS

A total of 1,005 respiratory specimens were collected from 489 TDCJ prison inmates (214 hospitalized at the UTMB-TDCJ hospital and 275 outpatients incarcerated in prison units throughout Texas). One grossly bloody specimen had to be excluded, based on the test manufacturer's recommendations. Only one specimen each was collected from 180 patients; two specimens each were collected from 146 patients; three each were collected from 144 patients; four each were collected from 15 patients; and five specimens each were collected from 4 patients. Eighty-eight of the 1,004 specimens tested (8.7%) grew mycobacteria: 36 specimens of MTBC (from 25 of the 489 patients [5.1%]), 24 specimens of *Mycobacterium fortuitum-Mycobacterium chelonae* complex (from 19 patients), 13 specimens of *M. avium* complex (from 10 patients), 8 specimens of *M. gordonae* (from 8 patients), 4 specimens of *M. kansasii* (from 3 patients), 2 specimens of *Mycobacterium mucogenicum* (from 2 patients), and 1 specimen of *Mycobacterium vaccae* (from 1 patient).

On initial testing, 40 of the 1,004 specimens (4.1%) from 26 patients were positive for MTBC by the E-MTD (Table 1). Twenty-eight of these from 20 patients were MTBC culture positive; the remaining 12 (from 8 patients) were negative for mycobacteria (Table 2). The sensitivity, specificity, and positive and negative predictive values of the E-MTD for diagnosis of tuberculosis, based on these initial results, are shown in Table 1 by specimen and by patient.

Further analysis of the 11 patients (all AFB smear negative) with discrepant culture and E-MTD results (defined in Materials and Methods) indicated that 3, each with three specimens, had been on antituberculous therapy intermittently for 2 to 6 months before the study began. These three patients were excluded from the final analysis, because currently it is not known how to interpret the E-MTD results and the manufacturer recommends that specimens from patients known to have been on therapy for more than 7 days in the prior 6 months not be tested. All specimens from one patient were E-MTD and smear negative, and one was MTBC culture positive. For the second patient, all specimens were culture and smear negative and two were E-MTD positive. For the third, all specimens were culture negative, two were E-MTD positive, and one was smear positive.

TABLE 1. Performance of E-MTD, by specimen and by patient, after initial testing[a]

Parameter[b]	E-MTD result	No. with MTBC culture result of:		Sensitivity (%)	Specificity (%)	PPV (%)[c]	NPV (%)[d]
		Positive	Negative				
By specimen							
Overall	Positive	28	12	77.8	98.8	70.0	99.2
	Negative	8	956				
AFB^+	Positive	13	2	100	77.8	86.7	100
	Negative	0	7				
AFB^-	Positive	15	10	65.2	99.0	60.0	99.2
	Negative	8	949				
By patient							
Overall	Positive	20	6	80.0	98.7	76.9	98.9
	Negative	5	458				
AFB^+	Positive	10	1	100	83.3	90.9	100
	Negative	0	5				
AFB^-	Positive	10	5	66.7	98.9	66.7	98.9
	Negative	5	453				

[a] Before discrepancies were resolved.
[b] AFB^+, positive smear for AFB; AFB^-, negative smear for AFB.
[c] PPV, positive predictive value.
[d] NPV, negative predictive value.

Two of the 11 patients, both of whom had AIDS, were considered to have false-positive MTBC culture results (one specimen each). One of these patients was a 41-year-old female who had pulmonary tuberculosis diagnosed and treated in 1991. During the month prior to her most recent admission, she experienced anterior chest pain, shortness of breath, dry cough, and a 15-lb weight loss. On the day of admission, non-Hodgkin's lymphoma was diagnosed by bone marrow biopsy. An induced sputum specimen collected on hospital day 2 grew MTBC approximately 1 month later (BACTEC 12B bottle only); no mycobacteria were isolated from an expectorated sputum specimen collected on hospital day 3. The patient was treated with chemotherapeutic agents, developed tumor-lysis syndrome and *Staphylococcus aureus* sepsis, and died 9 days after admission (no antituberculous medications were given). Autopsy revealed widespread lymphoma with extensive necrosis. The lungs were congested and hemorrhagic, and in the left upper lobe there were a few small, completely organized granulomas that were negative for AFB by staining sections with the Ziehl-Neelsen stain. Mycobacterial cultures of the granulomas were negative. Unfortunately, when the discrepancy between E-MTD and culture results was recognized, the isolate from the induced sputum specimen was not available for restriction fragment length polymorphism fingerprint analysis. However, because no active tuberculosis was present at autopsy, performed only 7 days after the specimen that grew MTBC was collected, and no antituberculous therapy had been administered, the culture result was categorized as a false positive.

The other patient with a false-positive MTBC culture was a 31-year-old male with generalized weakness, malaise, and hematuria for 2 weeks. Chest radiographs on hospital days 1 and 3, respectively, showed a 1-cm-diameter ill-defined nodule in the left upper lobe and patchy bilateral opacities, suggestive of septic pulmonary emboli. Cultures of urine and blood grew *S. aureus*. The patient developed renal failure that was treated with hemodialysis. On hospital day 24, tracheal suction material and bronchoalveolar lavage fluid were submitted for mycobacterial cultures. The BACTEC 12B culture of the tracheal suction specimen and an adjacent bottle became positive approximately 3 weeks later (the companion solid medium and the bronchoalveolar lavage fluid culture of the patient in question were negative for mycobacteria). Cross-contamination in the laboratory was suspected. Molecular fingerprinting of isolates from the two adjacent bottles and a third isolate recovered earlier from a specimen processed on the same day as the tracheal suction specimen confirmed that the IS*6110* pattern of the isolate from the patient in question was identical to that of the third isolate. The pattern of these two identical isolates was different from that of the adjacent isolate and from that of the MTBC control strain used for susceptibility testing, which had been fingerprinted on two occasions in the past.

For the remaining six patients who underwent chart review, the E-MTD result was considered a false positive in four cases and a false negative in two cases. All specimens from patients with false-positive E-MTD results were smear negative. Three of the four patients had two specimens submitted, only one of which was E-MTD positive; only one specimen was collected from the fourth patient. Separate aliquots of all four specimens were retested. Three of the four (all with results between 500,000 and 1 million RLU) were negative (<30,000 RLU) on retesting; the fourth (1,839,328 RLU) remained positive (1,252,321 RLU). Of the two patients with false-negative E-MTD results, one had five specimens and the other had three specimens, and for both patients only one specimen grew MTBC.

Six MTBC culture-positive, E-MTD-negative specimens were tested for inhibitors: three from patients with one or more earlier E-MTD-positive specimens that grew MTBC, one from a patient who had been diagnosed with tuberculosis before the study began (and therefore was excluded from the final analysis), and two from patients each of whom had one specimen with a false-negative E-MTD result. Only one of these six specimens contained substances that inhibited amplification (by both methods), based on the criteria defined by Gen-Probe. The patient from whom this specimen was collected was a 27-year-old male at a prison unit who had no pulmonary symptoms. Five sputum specimens were evaluated; all were AFB smear negative, and only the one tested for inhibitors grew MTBC. The patient's initial chest radiograph showed a small nodule (approximately 4-cm diameter) in the right apex; there were no follow-up radiographs during therapy. No source of possible laboratory contamination could be identified. Al-

TABLE 2. Summary of test results for all specimens from patients with one or more E-MTD-positive, MTBC culture-negative specimens after initial testing[a]

Patient no.	AFB smear	MTBC culture	E-MTD	Comments
946462	−	−	+	FP MTD (798,722 RLU)
	−	−	−	
604283	−	−	+	FP MTD (928,435 RLU)
	−	−	−	
230220	−	−	+	FP MTD (1,839,328 RLU)
223791	−	−	+	FP MTD (533,779 RLU)
	−	−	−	
827951	−	−	+	Known TB; on therapy
	−	−	+	
	−	−	−	
145814	+	−	+	Known TB; on therapy
	−	−	+	
	−	−	−	
803975	+	+	+	Smear-positive patient with concordant culture and MTD results
	−	−	+	
	+	+	+	
	+	−	+	
	−	−	+	
675136	−	+	+	Smear-positive patient with concordant culture and MTD results
	+	−	−	
	−	−	+	

[a] FP, false positive; TB, tuberculosis; +, positive; −, negative.

TABLE 3. Performance of E-MTD, by patient, after resolution of discrepancies and modification of interpretive results

Parameter[a]	E-MTD result	No. of patients with MTBC culture result of:		Sensitivity (%)	Specificity (%)	PPV (%)[b]	NPV (%)[c]
		Positive	Negative				
Resolved							
Overall	Positive	20	4	90.9	99.1	83.3	99.6
	Negative	2	460				
AFB^+	Positive	10	0	100	100	100	100
	Negative	0	5				
AFB^-	Positive	10	4	83.3	99.1	71.4	99.6
	Negative	2	455				
With expanded equivocal zone							
Overall	Positive	20	1	90.9	99.8	95.2	99.6
	Negative	2	463				
AFB^-	Positive	10	1	83.3	99.8	90.9	99.6
	Negative	2	458				

[a] AFB^+, positive smear for AFB; AFB^-, negative smear for AFB. Data and performance characteristics for smear-positive patients are the same for both categories. For the second category, the equivocal zone was changed from 30,000 to 500,000 RLU to 30,000 to 1 million RLU.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.

though the E-MTD result for this patient was categorized as a false negative, it actually cannot be interpreted based on the inhibition assay. For the other five specimens evaluated for inhibitors, the inhibitory-assay results were less than 1 million RLU, and the results for all except one were less than 500,000 RLU, compared to results of 3.5 million RLU for the seeded positive controls, perhaps suggesting the presence of some type of interfering substance(s) in the clinical samples.

After chart review, there were 22 patients with tuberculosis. Twenty-one (2.1%) specimens (15 patients) were AFB smear positive; 13 (61.9%) of the corresponding cultures (10 patients) grew MTBC, 6 cultures (28.6%; 5 patients) grew nontuberculous mycobacteria, and 2 cultures (both were from patients recently diagnosed with tuberculosis and placed on therapy after the study began) were culture negative. Including the last two specimens among those from patients with tuberculosis, the sensitivity and specificity, respectively, of the AFB smear for diagnosis of tuberculosis were 45.5 and 99.4% by specimen and 45.5 and 98.9% by patient.

The E-MTD was positive for 24 patients, 20 of whom had tuberculosis (i.e., 20 patients with true-positive E-MTDs, 4 patients with false-positive E-MTDs, and 2 patients with false-negative E-MTDs [including the patient whose specimen contained inhibitory substances]). For the 10 smear-positive patients, the first smear-positive specimen was positive by the E-MTD, with one exception. In this case, the first specimen evaluated was smear negative, E-MTD positive, and eventually MTBC culture positive; the second specimen was smear positive but negative by both the E-MTD and culture. Of the 12 smear-negative patients, the E-MTD was positive in 10. For 3 of these 10 patients a single specimen was submitted for evaluation. Of the seven patients with two or more specimens, the first specimen was E-MTD positive in six cases; the second was positive in the remaining case. For five of the patients with two or more specimens, only one specimen was E-MTD positive. The sensitivity, specificity, and positive and negative predictive values of the E-MTD for diagnosis of tuberculosis, by patient, were 90.9, 99.1, 83.3, and 99.6%, respectively, overall (Table 3). These same values were 100, 100, 100, and 100%, respectively, for smear-positive patients and 83.3, 99.1, 71.4, and 99.6%, respectively, for smear-negative patients. If the smear-negative patient whose E-MTD-negative specimen contained inhibitory substances is excluded from analysis, because the E-MTD result truly cannot be interpreted, the sensitivity, specificity, and positive and negative predictive values of the E-MTD are 95.2, 99.1, 83.3, and 99.8%, respectively. These performance characteristics are unchanged for smear-positive patients; for smear-negative patients they are 90.9, 99.1, 71.4, and 99.8%, respectively.

To optimize the E-MTD in our laboratory, we assessed the impact of increasing the upper limit of the equivocal zone from 500,000 to 1 million RLU (Table 4). This modification affected only three specimens in our study; for all three the initial E-MTD result was considered a false positive, based on our protocol for resolution of discrepancies. If the E-MTD result for these samples was categorized as equivocal (according to our modified criterion), a second aliquot of each was tested. The E-MTD results on the second aliquot for all three specimens were negative. Thus, by modifying the equivocal zone as described, the specificity and positive predictive value of the E-MTD improved, overall and for smear-negative patients; the sensitivity and the negative predictive value were unchanged (Table 3).

TABLE 4. Summary of E-MTD results for specimens with initial RLU values of 30,000 to 1 million

Initial RLU	No. in range	No. with repeat RLU of[a]:	
		<30,000	≥30,000
30,000–49,999	9	9	0
50,000–74,999	6	5	1[b]
75,000–99,999	4	3	1[b]
100,000–149,999	5	4	1[b]
150,000–199,999	2	0	2[b]
200,000–299,999	3	3[c]	0
300,000–399,999	2	2	0
400,000–500,000	3	0	3
500,001–750,000	1	1	0
750,001–1 million	2	2	0

[a] <30,000, negative for MTBC; ≥30,000, positive for MTBC.
[b] Specimen also positive for MTBC by culture.
[c] One specimen (272,440 RLU on initial testing) was positive for MTBC by culture.

DISCUSSION

Rapid diagnosis and prompt initiation of therapy are critical to the control of tuberculosis. For many years the AFB smear, results of which are available in 24 h or less, has been the only rapid diagnostic test available. Unfortunately the AFB smear is not an especially sensitive test nor is it specific for tuberculosis (13). Therefore, a laboratory test with performance characteristics that are superior to those of the AFB smear but with a similar turnaround time is needed. Theoretically, nucleic acid amplification tests have the capability of fulfilling these requirements. However, the FDA has approved the currently available nucleic acid amplification tests only for AFB smear-positive respiratory specimens.

In AFB smear-positive patients, antituberculous therapy typically is begun after the smear results are reported. Therefore, the nucleic acid amplification test is most beneficial in patient populations, such as ours, where a reasonable proportion of the smear-positive specimens contain nontuberculous mycobacteria (6 of 22 [27.3%] in this study). In smear-positive patients, nucleic acid amplification testing has the greatest impact on resources associated with infection control practices but can also influence patient management. Contact investigations need to be conducted only when the source patient is AFB smear positive and the nucleic acid amplification test is positive. Hospitalized smear-positive patients can be released from airborne-infection precautions, for which room charges are higher than for regular rooms (e.g., a $375.00 difference at our institution), based on a negative nucleic acid amplification test result rather than waiting for three consecutive negative AFB smears. With regard to patient management, a negative nucleic acid amplification result allows modification of therapy, directing it toward the most frequently encountered nontuberculous mycobacteria.

An important question concerning the use of nucleic acid amplification in smear-positive patients is whether infection control and management decisions can be initiated based on a single negative result. Data from this study suggest that the answer for the E-MTD is yes. The first smear-positive specimen was E-MTD positive for all patients in our study except one, and that patient had an earlier smear-negative specimen that was E-MTD positive. Thus, the negative E-MTD result for the smear-positive specimen (which was also MTBC culture negative) would not have affected this patient's care because tuberculosis would already have been diagnosed. In any case, we believe that all smear-positive, E-MTD-negative specimens should be tested for inhibitors to allow appropriate interpretation of the result, although this was not the protocol followed in this evaluation. If inhibitory substances are not detected, we believe, based on the data of Gamboa et al. (4) and our personal experience since completion of this study (unpublished observations), that the specimen does not contain MTBC. If, on the other hand, the specimen contains substances that inhibit amplification, another sample from the patient must be evaluated. Additionally, if there are concerns about possible mislabeling of the specimen or if the results do not correlate with the patient's clinical picture, a second sample should be evaluated.

The patients who will benefit most from the nucleic acid amplification test results are those whose respiratory specimens are smear negative for AFB, yet it is this group for whom these tests currently are not approved. For smear-negative patients, the nucleic acid amplification tests can confirm or exclude a diagnosis of tuberculosis much earlier in the course of their illnesses than is possible based on culture results. For example, in our study the mean time (± standard error of the mean) from receipt of the specimen in the laboratory to a culture result was 25.4 days (±3.3 days) (range, 16 to 42 days) compared to a mean of 2.4 days (±0.3 days) (range, 1 to 3 days) for the E-MTD. More rapid diagnosis benefits both the patient and those with whom the patient is in close contact, because although smear-negative patients are less infectious than those who are smear positive, they cannot be considered unequivocally noninfectious. Transmission of tuberculosis from a smear-negative source patient can occur (11).

In addition to earlier institution of therapy, nucleic acid amplification testing of smear-negative specimens can, in some cases, shorten the hospital stay and eliminate the need for invasive procedures, which are costly and pose an added risk to the patient. In our study, the hospital stay would have been shortened for two patients, based on a positive E-MTD result, by about 7 days in one case and 14 days in the other; and for both of these patients one or more invasive procedures probably would have been avoided. One patient presented with fever, anorexia, weakness, and weight loss for 4 months; a chest radiograph showed a bilateral interstitial pattern, reported as "consistent with miliary tuberculosis." Based on this information, he was started on four-drug antituberculous therapy after two sputum specimens, collected on consecutive days, were submitted for mycobacterial smear and culture. Both specimens were AFB smear negative; the second one was E-MTD positive (3,112,395 RLU) and grew MTBC 1 month later. Because of the desire for a definitive diagnosis, video-assisted thoracotomy and lung biopsy were performed 4 days after the second sputum specimen was collected. The tissue obtained was AFB smear negative but was not tested by E-MTD; MTBC was recovered from the culture 36 days later.

The second patient had fever, malaise, and night sweats; a chest radiograph showed a right upper lobe infiltrate, a loculated pleural effusion over the right lower lobe, and calcified granulomas throughout both lung fields. Two sputum specimens for mycobacterial smear and culture were collected 3 days apart. Both specimens were AFB smear negative; the first was E-MTD positive (2,396,934 RLU) and 22 days later grew MTBC, whereas the second was negative for MTBC by both E-MTD and culture. A chest tube was placed the day after the first sputum specimen was obtained. Although pleural tuberculosis was considered the most likely diagnosis, all physicians involved in the care of this patient preferred not to begin antituberculous therapy until a diagnostic specimen was procured. Thoracotomy with pleural biopsy and decortication was performed 2 weeks after the first sputum specimen (which had been E-MTD positive) was collected. Histopathologic examination of the pleural tissue showed caseating granulomas, and four-drug therapy was begun.

Previous criticisms of the performance of nucleic acid amplification tests with smear-negative specimens were their low sensitivity (approximately 50%) and the occasional false-positive results (1), which have the potential to produce an inappropriate diagnosis of tuberculosis. In our study, the sensitivity of the E-MTD for smear-negative patients was 83.3%, the same as that recently reported by Gamboa et al. (4), when the patient whose specimen contained inhibitory substances was included and 90.9% if that patient was excluded. Although we did not perform an inhibitory assay on all smear-negative specimens, knowing whether substances that inhibit amplification are present may be important for patient management. Because adding an inhibitory assay more than doubles the cost of the E-MTD, we suggest a more cost-efficient strategy that would not adversely affect patient care. If the first smear-negative sample is negative by the E-MTD, a second sample should be tested (this would be done regardless of the inhibitory-assay

result). If the second sample is E-MTD negative, then the physician caring for the patient should be consulted to determine whether the sample should be evaluated for the presence of inhibitory substances by one or both of the procedures described earlier.

With regard to specificity, of the 1,004 specimens we tested, there were only four false-positive results (specificity, 99.6%), compared with two false-positive culture results. Three of the four patients with false-positive results had one additional specimen tested, and all were E-MTD negative. In our opinion, however, this information is not particularly useful, given that 5 of the 10 smear-negative patients with tuberculosis also had two or more specimens tested, of which only one was positive by E-MTD. All four false-positive E-MTD results occurred during the first few months of our study and when testing was performed by one individual who was not formally trained by Gen-Probe technical personnel. We have had no false-positive E-MTD results for the past 10 months, during which time over 600 specimens were tested. This emphasizes the fact that, although the E-MTD is not technically difficult to perform, it is critical that testing personnel pay extremely close attention to detail. In addition, we found that the number of false-positive E-MTD results can be limited, without causing unnecessary extra testing of true-positive samples, by expanding the equivocal zone suggested by the manufacturer (Table 4). In our study, if we increased the upper limit of the equivocal zone from 500,000 to 1 million RLU, the number of false-positive results decreased from four to one (specificity, 99.9%). Review of the medical record of this patient showed that sputum for mycobacterial smear and culture was not listed among the doctor's orders, nor was tuberculosis mentioned in the doctor's notes as a possible diagnosis. Either the clerk completing the requisition form mistakenly checked "AFB culture" or laboratory personnel mistakenly ordered it. This occurrence emphasizes the fact that the E-MTD should be performed only when there is a "high suspicion" of tuberculosis, a descriptive term that should be defined by pulmonary and/or infectious disease physicians at each institution. Moreover, in general, a diagnosis of tuberculosis (or any other disease) should not be made based on a single laboratory test result. Clinical manifestations, radiographic findings, and all laboratory test results, including the E-MTD in the case of tuberculosis, must be included in the decision process.

In summary, our data indicate that the E-MTD is a reliable method for rapid diagnosis of pulmonary tuberculosis. We do not advise using the E-MTD to test specimens from patients known to have tuberculosis, as is the current recommendation, because it is unclear how to interpret the results. To optimize the performance characteristics of the assay, proper training of all testing personnel is essential, and with regard to specificity in particular, each laboratory may wish to establish an equivocal zone based on its own data. Given the sensitivity of the E-MTD compared with that of the AFB smear (90.9 versus 45.5% by patient in our study), we suggest that the E-MTD be performed not only on AFB smear-positive specimens but also on selected smear-negative specimens from patients for whom the degree of suspicion of tuberculosis is high. Given the expense of the E-MTD, testing of all smear-negative specimens is not practical. However, the scenarios for smear-negative patients in which the E-MTD would both benefit patient management and be cost-effective have not yet been defined. Therefore, until such guidelines are available, it is our opinion that laboratory directors, in consultation with pulmonary and/or infectious disease physicians, should decide if and when to use the E-MTD, based on the needs of the patient populations they serve. This approach will allow rapid diagnosis (and subsequent treatment) of all patients with tuberculosis, optimize efficient utilization of infection control resources, and potentially decrease the length of the hospital stay and eliminate the need for expensive invasive diagnostic procedures. We believe that smear-positive, E-MTD-negative specimens should be tested for inhibitory substances, and if none are detected, the specimen can be considered to contain a nontuberculous mycobacterium. With regard to smear-negative specimens, in our opinion, if the first sample is E-MTD negative, a second specimen should be tested. If the E-MTD result for the second sample is also negative, this information should be relayed to the patient's health care provider to determine if the specimen should be tested for inhibitory substances. Our data suggest that a single positive E-MTD result is sufficient for the diagnosis of tuberculosis if the clinical history and radiographic findings are consistent and that, under these same conditions, the diagnosis can be excluded based on two negative E-MTD results with a high degree of confidence (given that one or both of the first two specimens from smear-negative patients with tuberculosis in our study were E-MTD positive). These suggestions, however, are based on data from only 10 patients and therefore must be confirmed by additional studies.

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