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Comparative Evaluation of Initial and New Versions of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for Direct Detection of *Mycobacterium tuberculosis* in Respiratory and Nonrespiratory Specimens

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We evaluated the initial version of the Amplified Mycobacterium Tuberculosis Direct Test (Gen-Probe) (AMTDT 1) and the new version of AMTDT (AMTDT 2) for the detection of *Mycobacterium tuberculosis* directly from respiratory and nonrespiratory samples and compared the results with those of culture and staining methods. The assays were applied to 410 respiratory and 272 nonrespiratory samples collected from 515 patients. The combination of the culture results and clinical diagnosis was considered to be the "gold standard." Ninety-five respiratory specimens were collected from 67 patients with a diagnosis of pulmonary tuberculosis (TB) and 68 nonrespiratory specimens were collected from 61 patients with a diagnosis of extrapulmonary TB. With respiratory specimens, the sensitivity, specificity, and positive and negative predictive values were 83, 100, 100, and 96%, respectively, for AMTDT 1 and 94.7, 100, 100, and 98.4%, respectively, for AMTDT 2. With nonrespiratory specimens, the sensitivity, specificity, and positive and negative predictive values were 83, 100, 100, and 94%, respectively, for AMTDT 1 and 86.8, 100, 100, and 98.4%, respectively, for AMTDT 2. The overall results of AMTDT 1 and AMTDT 2 were concordant for 97% (661 of 682) of the samples. Statistically significant differences in sensitivities were found between AMTDT 1 and AMTDT 2 with respiratory specimens. It was concluded that although both nucleic acid amplification methods are rapid, sensitive, and specific for the detection of *M. tuberculosis* complex in all types of clinical samples, AMTDT 2 appeared to be more sensitive than AMTDT 1 when applied to smear-negative specimens. In contrast AMTDT 2 is more susceptible than AMTDT 1 to inhibitory substances in the amplification reaction. The turnaround time of AMTDT 2 is shorter (3.5 h) than that for AMTDT 1 (5 h).

For the detection of *Mycobacterium tuberculosis*, microscopic examination of acid-fast-stained smears and culture are still the methods of choice in most diagnostic microbiology laboratories. However, both of these methods have drawbacks. Direct staining for acid-fast bacilli takes less than an hour but lacks sensitivity (6). Moreover, a positive result by this test does not discriminate between the *Mycobacterium* species. Culture for *M. tuberculosis* is sensitive and specific but may require 6 to 10 weeks of incubation. The recently developed nucleic acid amplification methods may provide us with very sensitive, specific, and rapid tests for the detection of *M. tuberculosis*, thus combining the advantages of both of the classical methods.

Since the introduction of nucleic acid amplification assays into diagnostic mycobacteriology, many publications have confirmed the sensitivity and specificity of in-house and commercial assays (2, 7, 10, 23, 24, 26, 27, 30, 31). The Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (AMTDT) uses transcription-mediated amplification and hybridization procedures to qualitatively detect *M. tuberculosis* complex rRNA. This commercially available test has been reported to be a reasonably reliable tool for the diagnosis of pulmonary and extrapulmonary tuberculosis (2, 18, 25, 31). The sensitivity of AMTDT varied from 65 to 97% in different studies, whereas the specificity was always high (2, 3, 18, 21, 26). A low bacterial load was found to affect the sensitivity of AMTDT more than that of culture (18, 21, 31), thus limiting the usefulness of AMTDT for the screening of smear-negative specimens (3). In this initial version of AMTDT (AMTDT 1), 50 μl of the pretreated specimen is used for amplification and detection reaction. Recently, Gen-Probe Inc. has developed the second version of AMTDT (AMTDT 2), which has an enhanced protocol. This new version incorporates three main changes: (i) an increase in the initial amount of pretreated specimen from 45 to 450 μl, (ii) a reduction in the incubation time of the amplification reaction (60 to 30 min), and (iii) the elimination of the termination reaction.

The purpose of the present study was to compare the initial and new versions of Gen-Probe AMTDTs (AMTDT 1 and 2, respectively) with culture and staining techniques for the direct detection of *M. tuberculosis* in respiratory and nonrespiratory specimens.

MATERIALS AND METHODS

Patients and clinical specimens. From November 1996 to May 1997, we investigated 682 respiratory and nonrespiratory specimens collected from 515 patients at the Hospital Universitario Germans Trias i Pujol, Barcelona, Spain. Only clinical samples from patients suspected of having pulmonary and/or ex-

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trapulmonary tuberculosis were included in the study. Four hundred ten respiratory specimens (335 expectorated sputum specimens, 45 bronchial and tracheal aspirate specimens, and 30 bronchoalveolar lavage specimens) and 272 nonrespiratory specimens comprising 73 urine specimens, 29 pleural fluid specimens, 26 ascitic fluid specimens, 21 cerebrospinal fluid (CSF) specimens, 14 articular fluid specimens, 4 pericardial fluid specimens, 16 bone marrow aspirate specimens, 38 biopsy or lymph node exudate specimens, 16 purulent exudate specimens, 12 tissue biopsy specimens, and 23 gastric juice aspirate specimens were included in the study. Once collected, the specimens were kept at 4°C prior to processing. Gastric juice aspirates were immediately neutralized with trisodium phosphate buffer after retrieval.

Decontamination procedures. Biopsy specimens were sliced and homogenized in a mortar with 2 ml of 0.9% NaCl under sterile conditions before processing. Urine and other fluid samples were previously centrifuged at 3,300 × *g* for 20 min. All samples with the exception of bone marrow aspirates were digested and decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH as described previously (29, 30, 32). Briefly, 2 to 3 ml of a specimen was transferred to a 50-ml plastic centrifuge tube, and an equal volume of SDS-NaOH digestant solution (1% NaOH, 3% SDS [Fluka Chemical Company, Buchs, Switzerland]) was added; after being vortexed, the samples were vigorously shaken for 30 min. To neutralize the specimen a 1.43% H_3PO_4 solution (containing 0.006% bromocresol purple as a pH indicator) was added. After a centrifugation step (3,300 × *g*, 20 min), the supernatant was removed, the pellet was suspended with 30 ml of sterile distilled water and centrifuged again (3,300 × *g*, 20 min), and the supernatant was removed. Bone marrow aspirates were received in Isolator tubes (Wampole Laboratories, Cranbury, N.J.), and 1 ml of sample was treated with 100 μl of 10% SDS. After being vortexed for 10 min at room temperature, bone marrow aspirates were washed with 30 ml of distilled water and centrifuged (3,300 × *g*, 20 min), and the supernatant was removed. If the sediment still had hemorrhagic contents, the process was repeated. The cell pellets from all pretreated specimens were finally resuspended in 2.2 ml of 0.067 M phosphate buffer (pH 6.8). For all specimens, half of the sediment was stored at −80°C for the amplification techniques, and the other half was used for acid-fast staining and culture.

Microscopy. Smears were screened by staining with auramine-rhodamine fluorochrome. Positive slides were confirmed by the Ziehl-Neelsen technique (6, 19).

Culture. Equal aliquots (approximately 250 μl) of the processed sediment were inoculated onto two solid slants, Löwenstein-Jensen and Coletsos (an egg-based medium containing pyruvate, salts solution, asparagine, glutamate, glycerin, and malachite green; with this medium the detection times for *M. bovis* and dysgonic strains of *M. tuberculosis* are faster [8]) slants, and the slants were incubated at 37°C for 8 weeks in a humidified atmosphere. In addition, 500 μl of the sediment was inoculated into BACTEC 12B medium (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) supplemented with 0.1 ml of a mixture of antimicrobial agents (polymyxin B, azlocillin, nalidixic acid, trimethoprim, and amphotericin B) and incubated at 37°C for up to 8 weeks. Solid media were read weekly, and BACTEC cultures were read twice weekly for the first 2 weeks and once weekly thereafter. A growth index of >100 was considered positive for BACTEC cultures, and smears for Ziehl-Neelsen staining and culture on Löwenstein-Jensen and Coletsos media were prepared to detect acid-fast bacilli (AFB).

Identification of mycobacteria. Routine biochemical methods (17), gas-liquid chromatography (22), and the Accuprobe culture confirmation tests (Gen-Probe Inc., San Diego, Calif.) (16) were used for the identification of isolates.

AMTDTs. The AMTDT uses an isothermal enzymatic amplification system of target rRNA via DNA intermediates. Detection of amplicons is achieved by using an acridinium ester-labeled DNA probe. The tests were performed according to the instructions on the package insert. The AMTDT protocols consisted of the following steps. For lysis, 50 and 450 μl of the pretreated specimens were used in the initial and new versions, respectively, and were added to 200 and 50 μl of specimen dilution buffer, respectively, in a lysing tube, and the mixtures were sonicated for 15 min in a water bath sonicator (Branson 1200; Branson Ultrasonics Corporation, Danbury, Conn.) at room temperature. For amplification, 25 and 50 μl of reconstituted amplification reagent were used for AMTDT 1 and AMTDT 2, respectively. After the reaction, the tubes were covered with 200 μl of mineral oil. Fifty and 25 μl of lysate for AMTDT 1 and AMTDT 2, respectively, was transferred to the amplification tubes, and the tubes were incubated at 95°C for 15 min and then cooled at 42°C for 5 min. An enzyme reagent mixture (25 μl for both tests) was added, and the mixture was incubated at 42°C for 2 h for AMTDT 1 and 45 min for AMTDT 2. To terminate amplification (AMTDT 1 only), 20 μl of the termination reagent was added to each tube, and the mixtures were kept at 42°C for another 10 min. For detection by both tests, the reconstituted acridinium-labeled probe (100 μl) was added to each tube, and the tubes were incubated at 60°C for 15 min, and then the selection reagent (300 μl) was added and the mixtures were reincubated at 60°C for 10 min. All temperature-controlled incubation steps were carried out in heating blocks. All runs included AMTDT amplification-positive and -negative controls and hybridization-positive and -negative controls. Prior to being read in a luminometer (LEADER 50; Gen-Probe), the tubes were cooled at room temperature for 5 to 10 min. The cutoff value was set at 30,000 relative light units (RLUs). Samples with values of >30,000 RLUs were considered positive; samples with values of <30,000 RLUs were considered negative. An RLU ratio of sample RLUs/cutoff RLUs of ≥1.0 was considered a positive result, as recommended by the manufacturer.

Evaluation of inhibition of AMTDTs. All available specimens that were positive for growth of *M. tuberculosis* but negative by the AMTDTs and a group of randomly selected AFB culture-positive and -negative specimens were analyzed for inhibition of the AMTDTs. This was achieved by performing a second analysis by the AMTDTs but with the addition of known amounts of RNA. The duplicate vial contained 5 μl (i.e., 1/10) of the amount of the amplification-positive control provided by the manufacturer and 45 μl of lysate specimen for AMTDT 1 and 50 μl of the amplification-positive control with 450 μl of lysate specimen for AMTDT 2.

Clinical data for the patients. For those specimens for which the results of the culture and the amplification techniques were discrepant, clinical data and other microbiological results for the patient were analyzed. Clinical assessment included the patient's history, signs, symptoms, chest X-ray results, and laboratory results; cytological and histological results for the specimens; result of the tuberculin skin test; and a history of the drugs that had been administered. Moreover, all specimens with discrepant results were retested with new aliquots of the same pretreated specimens.

Statistical analysis. The sensitivities, specificities, and positive predictive values (PPVs), and negative predictive values (NPVs) of the amplification techniques were calculated by contrasting the results with the culture results and the patient's clinical data. Statistical comparisons were calculated by using the chi-square test; a P value of <0.05 was considered significant.

RESULTS

The initial and new versions of AMTDT were evaluated for their abilities to detect *M. tuberculosis* complex in 410 respiratory specimens from 275 patients and 272 nonrespiratory specimens from 240 patients. These patients were suspected of having pulmonary and/or extrapulmonary mycobacterial infections. The clinical performance of the amplification assays was determined by comparison of the results with those of standard culture and staining techniques. The results for respiratory and nonrespiratory specimens were analyzed separately.

Respiratory specimens. Of the 410 respiratory specimens examined, 95 were culture positive for *M. tuberculosis*. Twenty-six specimens (19 specimens were smear positive) were culture positive for nontuberculous mycobacteria (NTM). The species of NTM identified from these specimens were *M. avium* complex (n = 13), *M. kansasii* (n = 9), *M. genavense* (n = 3), and *M. gordonae* (n = 1). Two hundred eighty-nine specimens (all smear negative) were culture negative.

A comparison of the amplification results for smears and cultures is summarized in Table 1. Eighty-two specimens (48 smear-positive and 34 smear-negative specimens) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis*, and 289 specimens (all specimens were smear negative and culture negative) were AMTDT 1 and AMTDT 2 negative. The 26 specimens yielding isolates of NTM were negative by both amplification assays. In total, 15 specimens had discrepant results. Three specimens (all smear negative) were AMTDT 1 and AMTDT 2 negative and culture positive for *M. tuberculosis*. Ten specimens (all smear negative) were AMTDT 1 negative, AMTDT 2 positive, and culture positive for *M. tuberculosis*. Two specimens (smear negative) were AMTDT 1 positive, AMTDT 2 negative, and culture positive for *M. tuberculosis*. These 15 specimens exhibited positive radiometric culture results within a period of 22 to 45 days (average time, 34 days) and showed 2 to 10 colonies in solid media (average time, 46 days). These 15 specimens with discrepant results were retested, with new aliquots of the same processed specimens being used. The results were confirmed and were considered false negative: 13 specimens for AMTDT 1 and 5 specimens for AMTDT 2.

After obtaining a combination of culture results and clinical data for the patients, a total of 95 specimens were collected from 67 patients with a diagnosis of pulmonary tuberculosis. In summary, given that the overall positivity rate was 23.2% (95 of

TABLE 1. Detection of *M. tuberculosis* complex in respiratory specimens by initial and new versions of AMTDT

Microscopy result	Test and result	No. of specimens with the following culture result:		
		Positive[a]	Negative	Total
Positive	AMTDT 1			
	Positive	48	0	48
	Negative	0	19[b]	19
	Total	48	19	67
	AMTDT 2			
	Positive	48	0	48
	Negative	0	19[b]	19
	Total	48	19	67
Negative	AMTDT 1			
	Positive	34	0	34
	Negative	13	296[c]	309
	Total	47	296	343
	AMTDT 2			
	Positive	42	0	42
	Negative	5	296[c]	301
	Total	47	296	343
Total	AMTDT 1			
	Positive	82	0	82
	Negative	13	315[d]	328
	Total	95	315	410
	AMTDT 2			
	Positive	90	0	90
	Negative	5	315[d]	320
	Total	95	315	410

[a] Only data for specimens with culture-positive results for *M. tuberculosis* are included.
[b] Nineteen specimens were AMTDT negative and culture positive for NTM.
[c] Seven specimens were AMTDT negative and culture positive for NTM.
[d] Twenty-six specimens were AMTDT negative and culture positive for NTM.

TABLE 3. Detection of *M. tuberculosis* complex in nonrespiratory specimens by initial and new versions of AMTDT

Microscopy result	Test and result	No. of specimens with the following culture result:		
		Positive[a]	Negative	Total
Positive	AMTDT 1			
	Positive	21	0	21
	Negative	0	3[b]	3
	Total	21	3	24
	AMTDT 2			
	Positive	21	0	21
	Negative	0	3[b]	3
	Total	21	3	24
Negative	AMTDT 1			
	Positive	36	0	36
	Negative	11	201[c]	212
	Total	47	201	248
	AMTDT 2			
	Positive	38	0	38
	Negative	9	201[c]	210
	Total	47	201	248
Total	AMTDT 1			
	Positive	57	0	57
	Negative	11	204[d]	215
	Total	68	204	272
	AMTDT 2			
	Positive	59	0	59
	Negative	9	204[d]	213
	Total	68	204	272

[a] Only data for specimens with culture-positive results for *M. tuberculosis* are included.
[b] Three specimens were AMTDT negative and culture positive for NTM.
[c] Six specimens were AMTDT negative and culture positive for NTM.
[d] Nine specimens were AMTDT negative and culture positive for NTM.

410 specimens), the sensitivity, specificity, PPV, and NPV were 83, 100, 100, and 96%, respectively, for AMTDT 1 and 94.7, 100, 100, and 98.4%, respectively, for AMTDT 2 (Table 2). For 48 (50.5%) specimens with smear-positive results and 47 (49.5%) specimens with smear-negative results, the sensitivities were 100 and 72.3%, respectively, for AMTDT 1 and 100 and 83%, respectively, for AMTDT 2.

TABLE 2. Comparison of confirmed results for respiratory specimens by AMTDT 1 and AMTDT 2

Test and result	No. of specimens with the following confirmed result:			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative	Total				
AMTDT 1							
Positive	82	0	82	83.0	100	100	96.0
Negative	13	315[a]	328				
Total	95	315	410				
AMTDT 2							
Positive	90	0	90	94.7	100	100	98.4
Negative	5	315[a]	320				
Total	95	315	410				

[a] Twenty-six specimens were culture positive for NTM.

The overall results of AMTDT 1 and AMTDT 2 were concordant for 97.3% (399 of 410) of the samples. Statistically significant differences in sensitivities were found between AMTDT 1 and AMTDT 2 ($P = 0.047$).

Nonrespiratory specimens. Of the 272 specimens examined, 68 were culture positive for *M. tuberculosis*. Of these, 21 (30.9%) specimens were smear positive and 47 (69.1%) specimens were smear negative. Nine specimens (three specimens were smear positive) were culture positive for NTM; the species of NTM identified from these specimens were *M. avium* complex ($n = 3$), *M. kansasii* ($n = 5$), and *M. gordonae* ($n = 1$). One hundred ninety-five specimens (all smear negative) were culture negative (Table 3).

The amplification results showed that 57 specimens (21 specimens were smear positive) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis* and that 195 specimens (all smear negative and culture negative) were AMTDT 1 and AMTDT 2 negative. The nine specimens infected with isolates of NTM were AMTDT 1 and AMTDT 2 negative. Fifteen specimens had discrepant results (Table 4). Six of these specimens with smear-negative results were AMTDT 1 negative, AMTDT 2 positive, and culture positive for *M. tuberculosis*. Four specimens smear negative and cul-

TABLE 4. Discrepant results obtained for nonrespiratory specimens[a]

Type of specimen	No. of specimens	AMTDT 1 result	AMTDT 2 result	No. of specimens inhibiting amplification (test)
Urine	1	Pos.	Neg.	1 (AMTDT 2)
Urine	3	Neg.	Pos.	
Urine	2	Neg.	Neg.	
Lymph node	3	Pos.	Neg.	3 (AMTDT 2)
Lymph node	1	Neg.	Pos.	
Lymph node	1	Neg.	Neg.	
CSF	1	Neg.	Neg.	
CSF	1	Neg.	Pos.	
Ascitic fluid	1	Neg.	Pos.	
Gastric aspirate	1	Neg.	Neg.	

[a] All specimens were negative for *M. tuberculosis* by microscopy but positive for *M. tuberculosis* by culture. Neg., negative; Pos., positive.

ture positive for *M. tuberculosis* were AMTDT 1 positive and AMTDT 2 negative. Five specimens were AMTDT 1 and AMTDT 2 negative, smear negative, and culture positive for *M. tuberculosis*. These 15 specimens exhibited positive radiometric culture results within a period of 20 to 45 days (average time, 33 days) and showed 1 to 10 colonies on solid media (average time, 45 days). These 15 specimens with discrepant results were retested, with new aliquots of the same processed specimens being used. The results were confirmed and were considered false negative: 11 for AMTDT 1 and 9 for AMTDT 2 (Table 3).

Of the 73 urine specimens tested, 8 (4 specimens were smear positive) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis*. Fifty-eight specimens were AMTDT 1 and AMTDT 2 negative and culture negative. One specimen (smear negative) was AMTDT 1 and AMTDT 2 negative and culture positive for *M. gordonae*. Two specimens (smear negative) were AMTDT 1 and AMTDT 2 negative and culture positive for *M. tuberculosis*. One smear-negative specimen was AMTDT 1 positive, AMTDT 2 negative, and culture positive for *M. tuberculosis*; and three specimens (smear negative) were AMTDT 1 negative, AMTDT 2 positive, and culture positive for *M. tuberculosis*. After review of the clinical data for the patients, the sensitivity and specificity of AMTDT 1 for urine specimens were 64.3 and 100%, respectively; the sensitivity and specificity of AMTDT 2 were 78.6 and 100%, respectively.

Of the 94 organic fluid specimens tested, 20 (19 were smear negative) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis*. Sixty-nine specimens (smear negative) were AMTDT 1 and AMTDT 2 negative and culture negative. Two specimens (one was smear positive) were AMTDT 1 and AMTDT 2 negative and culture positive for NTM. One specimen was AMTDT 1 and AMTDT 2 negative, smear negative, and culture positive for *M. tuberculosis*. Two smear-negative specimens were AMTDT 1 and AMTDT 2 negative and culture positive for *M. tuberculosis*. The sensitivity and specificity of AMTDT 1 for organic fluid specimens were 87 and 100%, respectively; those of AMTDT 2 were 95.6 and 100%, respectively.

Of 38 lymph node specimens tested, 13 (6 were smear negative) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis*. Twenty specimens were AMTDT 1 and AMTDT 2 negative and culture negative. Three specimens (smear negative) were AMTDT 1 positive, AMTDT 2 negative, and culture positive for *M. tuberculosis*. One specimen (smear negative) was AMTDT 1 negative, AMTDT 2 positive, and culture positive for *M. tuberculosis*. One specimen (smear negative) was AMTDT 1 and AMTDT 2 negative and culture positive for *M. tuberculosis*. The sensitivity and specificity of AMTDT 1 for lymph nodes were 88.4 and 100%, respectively; those of AMTDT were 77.8 and 100%, respectively.

The majority of all other 66 nonrespiratory specimens (23 gastric juice aspirate specimens, 15 bone marrow aspirate specimens, 16 purulent exudate specimens, and 12 tissue biopsy specimens) had AMTDT 1 and AMTDT 2 results which mainly agreed with the culture results. Twelve specimens (nine were smear positive) were AMTDT 1 and AMTDT 2 positive and culture positive for *M. tuberculosis*. Four specimens were AMTDT 1 and AMTDT 2 negative and culture positive for NTM. Forty-eight specimens were AMTDT 1 and AMTDT 2 negative, smear negative, and culture negative. Two specimens were AMTDT 1 and AMTDT 2 negative and culture positive for *M. avium* complex. One specimen (a gastric aspirate specimen) was AMTDT 1 and AMTDT 2 negative and culture positive for *M. tuberculosis*. Thus, the sensitivity and specificity of both AMTDTs were 92.3 and 100%, respectively.

In summary, after obtaining a combination of culture results and clinical data for the patients, a total of 68 specimens were collected from 61 patients with a diagnosis of extrapulmonary tuberculosis. Given that the overall rate of positivity was 25% (68 of 272 specimens), the sensitivity, specificity, PPV, and NPV with nonrespiratory specimens were 83, 100, 100, and 94%, respectively, for AMTDT 1 and 86.8, 100, 100, and 98.4%, respectively, for AMTDT 2 (Table 5). For 21 (30.9%) specimens with smear-positive results and 47 (69.1%) specimens with smear-negative results, the sensitivities of AMTDT 1 were 100 and 76.6%, respectively, and those of AMTDT 2 were 100 and 88.9%, respectively.

The overall results of AMTDT 1 and AMTDT 2 with nonrespiratory specimens were concordant for 96.3% (262 of 272) of the samples. No statistically significant differences in sensitivity and specificity were found between AMTDT 1 and AMTDT 2 ($P > 0.05$).

Amplification inhibition. We also examined 150 specimens for the presence of inhibitors. None of the 24 specimens (13 respiratory and 11 nonrespiratory specimens) with AMTDT 1-negative results and culture-positive results for *M. tuberculosis* showed substances inhibitory to the amplification reaction. Of the 14 specimens (5 respiratory and 9 nonrespiratory specimens) with AMTDT 2-negative results and culture-positive results for *M. tuberculosis*, 5 specimens (1 respiratory and 4 nonrespiratory specimens) contained substances inhibitory

TABLE 5. Comparison of confirmed results for nonrespiratory specimens by AMTDT 1 and AMTDT 2

Test and result	No. of specimens with the following confirmed result:			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative	Total				
AMTDT 1							
Positive	57	0	57	83.0	100	100	94.0
Negative	11	204[a]	215				
Total	68	204	272				
AMTDT 2							
Positive	59	0	59	86.8	100	100	98.4
Negative	9	204[a]	213				
Total	68	204	272				

[a] Nine specimens were culture positive for NTM.

to the amplification reaction. Among the randomly selected group of specimens, none of the 125 clinical specimens of respiratory and nonrespiratory origin contained inhibitors.

DISCUSSION

One feature of the mycobacteria belonging to the *M. tuberculosis* complex which delays detection in cultures is the slow division time, about 20 h under the best culture conditions (32). When a clinical specimen is smear negative, it takes several weeks for the culture to become positive, even when the most appropriate culture techniques are used (1, 9, 17). To provide physicians with accurate and rapid bacteriological results, it is necessary to shorten the delay in reporting the presence of *M. tuberculosis* in clinical specimens. The most promising diagnostic modality for addressing this problem is PCR. Several research groups have described different PCR systems and/or have performed clinical studies based on PCR (4, 5, 7, 11, 12, 14, 20). The investigators reported widely different results with respect to specificity and sensitivity. One reason for this may be methodological differences concerning sample preparation; others may be the volume of sample used for DNA extraction and the design of the amplification and detection procedures. The main problem with DNA amplification methods is the low sensitivity found with smear-negative samples.

In the past few years, Gen-Probe has provided the ready-to-use AMTDT, a kit for the direct detection of *M. tuberculosis* in clinical specimens. This system uses the transcription-mediated amplification method to amplify target rRNA via DNA intermediates, followed by chemiluminescence detection of the amplicon with an acridinium ester-labeled DNA probe. The analytical sensitivity of the test is one cell, and the assay only detects members of the *M. tuberculosis* complex (18). AMTDT detects rRNA, which is present at a level of approximately 2,000 copies per cell (18). This advantage enhances the sensitivity of the test compared with those of tests which detect the target sequences present in only a single or a very low copy number (28).

This initial version of AMTDT (AMTDT 1) has been sufficiently evaluated against culture methods and AFB smears with respiratory specimens (2, 18, 21, 26, 31). For more than 5,000 respiratory specimens, AMTDT 1 yielded overall sensitivities of between 82 and 97% and overall specificities of between 97 and 100%. Also, the sensitivity and specificity results for AMTDT 1 with nonrespiratory specimens were shown to be as high as those reported by other investigators with respiratory specimens (13, 15, 27).

Recently, Gen-Probe has developed the second version of AMTDT (AMTDT 2), which has an enhanced protocol. The enhancements include (i) the use of a larger quantity of pretreated specimen (450 μl), (ii) a reduction in the incubation time of the amplification reaction (60 to 30 min), and (iii) elimination of the termination reaction.

The purpose of the present study was to compare AMTDT 1 with AMTDT 2 and culture and staining techniques for the direct detection of *M. tuberculosis* in respiratory and nonrespiratory specimens.

The overall results of AMTDT 1 and AMTDT 2 were concordant for 97.3 and 96.3% of the respiratory and nonrespiratory specimens, respectively.

We obtained with respiratory specimens 13 false-negative results by AMTDT 1 and 5 false-negative results by AMTDT 2. With nonrespiratory specimens there were 11 false-negative results by AMTDT 1 and 9 false-negative results by AMTDT 2. These specimens exhibited positive radiometric culture results within a period of 20 to 45 days and showed 1 to 10 colonies on solid media. With respiratory and nonrespiratory specimens, none of the specimens with false-negative results by AMTDT 1 were shown to contain substances inhibitory to the amplification reaction. With regard to the five respiratory specimens with false-negative results by AMTDT 2, only one specimen contained inhibitors. Four of the nine nonrespiratory specimens with false-negative results by AMTDT 2 were found to contain substances inhibitory to the amplification reaction. In conclusion, the 35.7% (five samples) of the 14 samples with false-negative results showed evidence of containing substances inhibitory to the amplification for AMTDT 2. Therefore, we believe that in the present study the false-negative results obtained by AMTDT 1 were not due to sample inhibition but were due to either a sampling error because of a low number of microorganisms or a nonuniform distribution of these microorganisms in the clinical samples. In contrast, the false-negative results obtained by AMTDT 2 were due both to sample inhibition and to the presence of a low number of microorganisms in the clinical sample. For that reason, the AMTDT 2 kit should include internal controls in order to assess the efficacy of each amplification reaction and to ensure that the sample is free of interfering substances. The use of internal controls will identify those samples that are inappropriate for amplification or that require further manipulation to remove inhibitory substances, and the use of internal controls will ultimately increase confidence in the reliability of negative results.

In conclusion, for respiratory specimens, the sensitivity and specificity of AMTDT 1 were 83 and 100%, respectively, and those of AMTDT 2 were 94.7 and 100%, respectively. For nonrespiratory specimens, the sensitivity and specificity of AMTDT 1 were 83 and 100%, respectively, and those of AMTDT 2 were 86.8 and 100%, respectively. For respiratory specimens only were statistically significant differences in the sensitivities found between AMTDT 1 and AMTDT 2 ($P = 0.047$).

In tests with respiratory and nonrespiratory specimens, our study population had rates of positivity for *M. tuberculosis* of 23.2 and 25%, respectively. For 48 (50.5%) respiratory specimens with smear-positive results and 47 (49.5%) specimens with smear-negative results, the sensitivities were 100 and 72.3%, respectively, for AMTDT 1 and 100 and 83%, respectively, for AMTDT 2. For 21 (30.9%) nonrespiratory specimens with smear-positive results and 47 (69.1%) specimens with smear-negative results, the sensitivities were 100 and 76.6%, respectively, for AMTDT 1 and 100 and 88.9%, respectively, for AMTDT 2.

Similar results were obtained for AMTDT 1 in a comparative evaluation with the Roche Amplicor MTB Test, carried out with 327 respiratory specimens from 236 patients (28). With a prevalence of 15% and a staining sensitivity of 70%, the sensitivities for smear-positive and smear-negative specimens were 100 and 85.7%, respectively, for AMTDT 1 and 96.7 and 50%, respectively, for the Amplicor MTB Test.

Vuorinen et al. (31) evaluated AMTDT 1 and the Amplicor MTB Test with 256 respiratory specimens from 243 patients and found a rate of positivity of 12.7% and a staining sensitivity of 76%. Lower sensitivities were obtained for smear-negative specimens by AMTDT 1 (42.9%) and the Amplicor MTB Test (28.6%).

In a further study (27) AMTDT 1 was evaluated with 1,117 respiratory specimens and 322 nonrespiratory specimens, with a rate of positivity of 12% and a staining sensitivity of 40%. For respiratory specimens, the sensitivities obtained with smear-positive and smear-negative specimens were 98.5 and 81%,

respectively, and those obtained with nonrespiratory specimens were 99.3 and 90.5%, respectively. These results are in good accordance with the results obtained in our comparative study of AMTDT 1 and AMTDT 2.

With respect to specimens smear positive and culture positive for *M. tuberculosis*, the sensitivity of both AMTDTs was 100%. It is very important to indicate that AMTDT 2 does not appear to be susceptible to inhibition by substances present in this class of specimens. Therefore, smear-positive and AMTDT-negative results can suggest the detection of a nontuberculous mycobacterium.

In conclusion, (i) AMTDT 1 and AMTDT 2 are highly sensitive and specific techniques for the rapid detection of *M. tuberculosis* in all types of clinical samples. (ii) Although AMTDT 2 is more susceptible to inhibitors than AMTDT 1, the sensitivity of AMTDT 2 is higher than that of AMTDT 1 with smear-negative specimens. (iii) In addition, the turnaround time of AMTDT 2 is shorter (3.5 h) than that of AMTDT 1 (5 h). (iv) Finally, AMTDT 2 should incorporate an internal control for the evaluation of amplification inhibitors in the clinical samples.

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