JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1997, p. 307–310 Vol. 35, No. 1
0095-1137/97/$04.00+0

Direct Detection of *Mycobacterium tuberculosis* Complex in Nonrespiratory Specimens by Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test

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Received 3 July 1996/Returned for modification 15 August 1996/Accepted 18 October 1996

The Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (AMTDT) was adapted for the detection of *Mycobacterium tuberculosis* complex in 224 nonrespiratory specimens from 188 patients. The sensitivity and specificity of the AMTDT for such specimens, after resolution of discrepant results, were 85.7 and 100%, respectively. Pretreatment of nonrespiratory specimens with sodium dodecyl (lauryl) sulfate is mandatory to obtain consistent and reproducible AMTDT results. The use of 500 μl of decontaminated specimen improves the sensitivity of the test. Because the AMTDT detects stable rRNA from noncultivable bacilli, it is not useful for monitoring patients receiving treatment.

The laboratory diagnosis of tuberculosis (TB) is currently based on acid-fast staining and culture on solid and/or liquid media. Staining is a rapid screening test, but its sensitivity is low, especially in nonrespiratory specimens (7). Culture on solid media requires up to 6 weeks to detect positive specimens (1, 10). The faster BACTEC radiometric broth system has been an important addition to culturing methods (2, 10, 16). The use of high-performance liquid chromatography for the assay of mycolic acids (6) and the use of DNA probes (14, 27) have further aided in the rapid identification of *Mycobacterium tuberculosis* complex in culture.

In research laboratories, PCR has proven useful for the direct diagnosis of TB by allowing use of diverse genetic elements as the target templates (1, 5, 8, 10, 11, 23). Using noncommercial PCR assays, however, results in an unexpectedly high variation in sensitivity (8, 15, 19, 23). Another practical problem of PCR is that it does not fit easily into routine laboratory practice, as costly equipment and expertise are not always available (8, 11, 19, 23). Therefore, the search for rapid, standardized, and reliable commercial detection systems for *M. tuberculosis* continues (5, 17).

The Amplified Mycobacterium Tuberculosis Direct Test (AMTDT) (Gen-Probe Inc., San Diego, Calif.) is based on the isothermal amplification of target rRNA by DNA intermediates followed by chemiluminiscent detection of amplicons with an acridinium ester-labeled DNA probe. The AMTDT is highly sensitive and specific for detecting *M. tuberculosis* in respiratory specimens (3, 12, 18, 20, 22, 26). One drawback to using this test in the daily laboratory routine is that the manufacturers do not recommend its use with nonrespiratory specimens.

The purpose of the present study was threefold: (i) to evaluate and adapt the AMTDT for the detection of *M. tuberculosis* complex in nonrespiratory samples and compare it with standard culture and staining techniques, (ii) to investigate whether using 500 instead of 50 μl of sample improves AMTDT performance, and (iii) to study the viability of the AMTDT for the follow-up of patients receiving anti-TB treatment.

From February through December 1995, we investigated 224 consecutive nonrespiratory specimens (40 urine, 38 fecal, 28 biopsy or lymph node exudate, 1 pericardic fluid, 41 pleural exudate, 17 cerebrospinal fluid [CSF], 10 articular fluid, 17 ascitic fluid, 2 gastric juice aspirate, 13 liver or skin biopsy, 1 otic exudate, and 16 bone marrow aspirate samples) collected from 188 patients at our hospital. Upon receipt, specimens were stored at 4°C prior to processing. Gastric aspirates were immediately neutralized with trisodium phosphate buffer after retrieval. Tissue specimens were sliced and homogenized in a mortar under sterile conditions before processing. Urine and other fluid samples were previously centrifuged at 3,600 × *g* for 20 min. These samples were digested and decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH as previously described (28), the sediment obtained was washed with 30 ml of distilled water and centrifuged (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 5 min, the samples were rinsed 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, we repeated the process. For all specimens, half the sediment was stored at −80°C for the target-amplified test, and the other half was inoculated onto the culture media and used for acid-fast staining.

The smears were stained with auramine-rhodamine fluorochrome as a screening method (16). Positive slides were confirmed to be positive by the Ziehl-Neelsen stain. Clinical specimens were inoculated onto a Löwenstein-Jensen slant, onto a Coletsos slant (9), and into BACTEC 12B medium. Slants and vials were incubated at 35 to 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. Routine biochemical methods (16), gas-liquid chromatography

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(21), and Accuprobe culture confirmation tests (Gen-Probe Inc.) were employed for the identification of isolates.

The AMTDT was performed with 50 μl of pretreated (SDS-NaOH) specimen according to the manufacturer's package insert. Each run included positive and negative amplification controls and positive and negative hybridization controls. A positive cutoff of 30,000 relative light units was used. All specimens with discrepant results were retested with 50 μl of the same decontaminated sample. Additionally, false-negative samples were reevaluated by AMTDT using 500 μl of the pellet as previously described (4). Briefly, 500 μl of decontaminated sample was centrifuged (5 min at 11,000 × *g* in an Eppendorf Microfuge), and 50 μl of the pellet was resuspended in 200 μl of specimen dilution buffer and processed according to the manufacturer's instructions. Cultures were the standards against which AMTDT results were compared.

In the cases where results from the culture and the AMTDT were discrepant, clinical data and histological and other microbiological results of the patient were evaluated. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the AMTDT were calculated in comparison with culture results and, separately, in comparison with culture results plus the patients' clinical data. Statistical comparisons were performed by using a chi-square test.

Two hundred twenty-four specimens from 188 patients with clinical signs or symptoms of extrapulmonary TB were tested. The sources of these specimens and detailed results of smears for acid-fast bacilli, cultures, and AMTDTs are given in Table 1. Seventy-one specimens from 60 patients (13 smear positive) were AMTDT positive and culture positive for *M. tuberculosis*, and 121 specimens (all smear negative and culture negative) from 100 patients were AMTDT negative. Twelve specimens (eight smear positive) were AMTDT negative and culture positive for nontuberculous mycobacteria (NTM); the species of NTM identified from these specimens were *M. avium-M. intracellulare* complex (four specimens), *M. genavense* (three specimens), *M. xenopi* (one specimen), *M. kansasii* (one specimen), and *M. gordonae* (three specimens). All NTM isolates from smear-positive specimens corresponded to patients with clinical pictures compatible with extrapulmonary or disseminated mycobacteriosis.

Results of 20 nonrespiratory specimens from 16 patients were discrepant (Table 2). Thirteen specimens (12 smear negative) were AMTDT negative and culture positive for *M. tuberculosis*. Twelve of these specimens gave cultures with ≤10 colonies of *M. tuberculosis*, and one gave ≤100 colonies; these 13 specimens were considered false negatives. Six AMTDT-positive and culture-negative samples corresponded to patients with active TB who were receiving anti-TB treatment at the moment they were enrolled in the study. Five samples corresponded to patients with other previous or intercurrent positive cultures for *M. tuberculosis*. These six specimens were considered true positives. One AMTDT-positive but culture-negative lymph node specimen remained inconclusive (Table 2). Of the 76 patients with extrapulmonary TB, 44 also had pulmonary disease.

The sensitivity, specificity, PPV, and NPV of the AMTDT for nonrespiratory specimens were 85.7, 100, 100, and 91.1%, respectively. For smear-positive and smear-negative specimens culture positive for *M. tuberculosis* the sensitivities of AMTDT were 95 and 83%, respectively. Comparisons of culture and AMTDT results for different types of extrapulmonary specimens are given in Table 3.

Discrepant results were retested with a new aliquot (50 μl) of the same processed specimen. After repeat testing, results for seven AMTDT-positive and culture-negative specimens were confirmed; six were considered true positives, while one remained inconclusive. Thirteen AMTDT-negative and culture-positive specimens for *M. tuberculosis* were also confirmed as true negatives after repeating the AMTDT. However, 9 of these 13 specimens were AMTDT positive when 500 μl of sediment was used (Table 2). Overall, the sensitivity of the modified AMTDT using 500 μl of sediment was 95% ($P < 0.001$).

Twelve specimens from patients treated for active TB were selected for inclusion in the study. Six were AMTDT positive and culture positive for *M. tuberculosis*, and all were obtained from five human immunodeficiency virus-positive patients with multidrug-resistant TB. The other six specimens, from six patients treated for active TB for 1 to 7 months, were AMTDT positive, smear positive, and culture negative.

The AMTDT has recently been evaluated in comparison to culture methods and acid-fast bacillus smear for respiratory specimens (1, 3, 18, 20, 25). For more than 5,000 respiratory

TABLE 1. Comparison of results obtained in 224 nonrespiratory specimens by microscopy, culture, and Gen-Probe AMTDT

Type of specimen	*n*	Microscopy result	Culture		AMTDT result
			Result	Species identified	
Urine	3	Pos.[b]	Pos.	*M. tuberculosis*	Pos.
Urine	6	Neg.[c]	Pos.	*M. tuberculosis*	Pos.
Urine	6	Neg.	Pos.	*M. tuberculosis*	Neg.
Urine	3	Neg.	Pos.	*M. gordonae*	Neg.
Urine	22	Neg.	Neg.		Neg.
Fecal	4	Pos.	Pos.	*M. tuberculosis*	Pos.
Fecal	3	Pos.	Pos.	MAIC[a]	Neg.
Fecal	1	Pos.	Pos.	*M. tuberculosis*	Neg.
Fecal	3	Pos.	Neg.	*M. genavense*	Neg.
Fecal	1	Pos.	Pos.	*M. xenopi*	Neg.
Fecal	1	Pos.	Neg.		Pos.
Fecal	2	Neg.	Pos.	*M. tuberculosis*	Pos.
Fecal	1	Neg.	Pos.	MAIC	Neg.
Fecal	22	Neg.	Neg.		Neg.
Lymph node	5	Pos.	Pos.	*M. tuberculosis*	Pos.
Lymph node	3	Pos.	Neg.		Pos.
Lymph node	9	Neg.	Pos.	*M. tuberculosis*	Pos.
Lymph node	3	Neg.	Pos.	*M. tuberculosis*	Neg.
Lymph node	1	Neg.	Neg.		Pos.
Lymph node	7	Neg.	Neg.		Neg.
Pericardic fluid	1	Neg.	Pos.	*M. tuberculosis*	Pos.
Pleural exudate	1	Pos.	Pos.	*M. tuberculosis*	Pos.
Pleural exudate	1	Pos.	Pos.	*M. kansasii*	Neg.
Pleural exudate	12	Neg.	Pos.	*M. tuberculosis*	Pos.
Pleural exudate	27	Neg.	Neg.		Neg.
CSF	5	Neg.	Pos.	*M. tuberculosis*	Pos.
CSF	3	Neg.	Pos.	*M. tuberculosis*	Neg.
CSF	9	Neg.	Neg.		Neg.
Articular fluid	5	Neg.	Pos.	*M. tuberculosis*	Pos.
Articular fluid	5	Neg.	Neg.		Neg.
Ascitic fluid	11	Neg.	Pos.	*M. tuberculosis*	Pos.
Ascitic fluid	6	Neg.	Neg.		Neg.
Gastric aspirate	1	Neg.	Pos.	*M. tuberculosis*	Pos.
Gastric aspirate	1	Neg.	Neg.		Neg.
Tissue biopsy	2	Pos.	Neg.		Pos.
Tissue biopsy	1	Neg.	Pos.	*M. tuberculosis*	Pos.
Tissue biopsy	10	Neg.	Neg.		Neg.
Bone marrow	4	Neg.	Pos.	*M. tuberculosis*	Pos.
Bone marrow	12	Neg.	Neg.		Neg.
Otic exudate	1	Neg.	Pos.	*M. tuberculosis*	Pos.

[a] MAIC, *M. avium-M. intracellulare* complex.
[b] Pos., positive.
[c] Neg., negative.

TABLE 2. Analysis of discrepant results in nonrespiratory specimens[a]

Type of specimen	*n*	Result of:				Antituberculosis treatment (duration)	*M. tuberculosis* isolate in other specimens	Final result
		Microscopy	Culture[b]	AMTDT with:				
				50 μl	500 μl[c]			
Urine	4	Neg.[d]	Pos.[e]	Neg.	Pos.	No	Yes	True Pos.
Urine	2	Neg.	Pos.	Neg.	Neg.	No	No	False Neg.
Fecal	1	Pos.	Pos.	Neg.	Neg.	Yes (5 days)	Yes	False Neg.
Fecal	1	Pos.	Neg.	Pos.	—	Yes (1 mo)	Yes	True Pos.
Lymph node	2	Neg.	Pos.	Neg.	Pos.	No	No	True Pos.
Lymph node	1	Neg.	Pos.	Neg.	Neg.	No	No	False Neg.
Lymph node[f]	1	Pos.	Neg.	Pos.	—	Yes (7 mo)	Yes	True Pos.
Lymph node[f]	1	Pos.	Neg.	Pos.	—	Yes (1 mo)	No	True Pos.
Lymph node[f]	1	Pos.	Neg.	Pos.	—	Yes (3 mo)	Yes	True Pos.
Lymph node[g]	1	Neg.	Neg.	Pos.	—	No	No	Inconclusive
CSF	3	Neg.	Pos.	Neg.	Pos.	No	Yes	True Pos.
Tissue biopsy	2	Pos.	Neg.	Pos.	—	Yes (4 mo)	Yes	True Pos.

[a] All patients had a clinical diagnosis of TB.
[b] For *M. tuberculosis.*
[c] Retested only the 13 specimens that were AMTDT negative and culture positive for *M. tuberculosis*. —, not retested.
[d] Neg., negative.
[e] Pos., positive.
[f] Specimens from three patients with active cervical tuberculous lymphadenitis, with compatible histology (granulomatous inflammation) and good response to TB treatment.
[g] From one patient whose histology showed granulomatous inflammation; response to TB treatment was good, and the patient probably had active cervical tuberculous lymphadenitis.

specimens the AMTDT has yielded overall sensitivities between 82 and 97% and specificities between 97 and 100%. In contrast, there is considerably less experience with direct detection of *M. tuberculosis* in nonrespiratory specimens with the AMTDT (13, 25, 30). Paradoxically, however, it is precisely extrapulmonary TB (tuberculous pleuritis, peritonitis, meningitis, and lymph node TB, etc.) for which a rapid and accurate laboratory diagnosis is of prime importance. Recent evaluations of AMTDT performance have reported complete inhibition of the test when applied to a CSF specimen and a pleural aspirate specimen spiked with *M. bovis* BCG cells (12) and an unacceptable sensitivity of 20% for pleural exudates compared with culture on Löwenstein-Jensen medium (30). Very recently, however, the AMTDT was successfully adapted by Pfyffer et al. (25) for use with CSF and other nonrespiratory specimens. The need for proper sample preparation for amplification procedures to eliminate inhibitors that interfere with detection has been demonstrated in several studies (1, 3, 13, 24–26).

TABLE 3. Results of AMTDT and culture in nonrespiratory specimens

Type of specimen and AMTDT result	No. of cultures		Total
	Positive	Negative	
Urine			
Positive	9	0	9
Negative	9[a]	22	31
Total	18	22	40
Fecal			
Positive	6	1[b]	7
Negative	9[c]	22	31
Total	15	23	38
Lymph node			
Positive	14	4[d]	18
Negative	3	7	10
Total	17	11	28
Sterile fluid			
Positive	35	0	35
Negative	4[e]	47	51
Total	39	47	86
Other			
Positive	7	2[b]	9
Negative	0	23	23
Total	7	25	32

[a] *M. tuberculosis* ($n = 6$) and *M. gordonae* ($n = 3$).
[b] TB treatment initiated for extrapulmonary disease.
[c] *M. avium-M. intracellulare* ($n = 4$), *M. genavense* ($n = 3$), *M. xenopi* ($n = 1$), and *M. tuberculosis* ($n = 1$).
[d] Three specimens from three patients with TB treatment initiated for active cervical tuberculous lymphadenitis; the other specimen corresponded to a patient who probably had active cervical tuberculous lymphadenitis (Table 2).
[e] *M. tuberculosis* ($n = 3$) and *M. kansasii* ($n = 1$).

The difficulties of using the AMTDT in detecting *M. tuberculosis* in nonrespiratory specimens, then, is well-known (12, 25, 30). In the present study, two different SDS pretreatment procedures of such specimens, including some containing a high proportion of interfering compounds such as from urine, feces, fluids, and bone marrow aspirates, are described. Pretreatment with SDS-NaOH in 86 sterile body fluid samples (41 pleural exudates, 17 CSFs, 10 articular fluids, 17 ascitic fluids, and 1 pericardic fluid) yielded values for sensitivity, specificity, PPV, and NPV of 92.1, 100, 100, and 94.1%, respectively, for the AMTDT. Pretreatment with SDS, involving extensive washing which is sufficient to remove any traces of detergent that might react with the assay, likewise proved successful in detecting specimens small loads of TB bacilli in bone marrow aspirates, although too few specimens were tested to permit statistical analysis (Table 1). Other authors (25, 26) have similarly reported success in pretreating samples with SDS, a detergent which denatures proteins and enzymes and eliminates most of the inhibitory compounds present in body fluids. As for nonrespiratory specimens other than body fluids and bone marrow aspirates, we found that pretreatment with SDS is also mandatory to obtain consistent and reproducible AMTDT results. The sensitivity and specificity of AMTDT of 85.7 and 100%, respectively, obtained after SDS treatment, were acceptable for detection of *M. tuberculosis* in all our pretreated

samples; these results were similar to those of Pfyffer et al. (25), who achieved a sensitivity and specificity of 93.1 and 97.7%, respectively, in specimens pretreated with SDS-NaOH, in contrast with those of Vlaspolder et al. (30), who reported a sensitivity of only 20% in pleural exudates pretreated with *N*-acetyl-L-cysteine–NaOH.

The 13 false-negative AMTDT results that we report (six urine samples, one fecal sample, three lymph nodes, and three CSF samples) (Table 2) illustrated another sampling problem commonly encountered with small loads of TB bacilli in nonrespiratory specimens as a result of the tendency of the bacilli to clump together. As previously described (4, 25), increasing the initial amount of specimen from 50 to 500 μl and subjecting the specimen to subsequent centrifugation provides the AMTDT with a larger target. Our data show clearly that screening 500 instead 50 μl of sediment improves the sensitivity of the test (85.7 and 95.6% in 50- and 500-μl nonrespiratory specimens, respectively) without adversely affecting specificity.

We conclude that because the AMTDT is able to detect stable rRNA from noncultivable bacilli in pretreated nonrespiratory specimens, the test is not useful for monitoring patients receiving treatment. However, before this can be considered, additional studies need to be realized to corroborate the existing data in patients undergoing antimicrobial therapy. Published reports (18, 25, 26, 30) clearly show that a patient receiving such treatment can remain AMTDT positive after cultures become negative, possibly because 16S rRNA molecules are protected after cell death by ribosomal proteins (29). Despite this handicap, we believe that the AMTDT can be used in diagnosis of partially treated patients arriving with inconclusive medical data that are often culture negative and may be acid-fast staining positive or negative. Our findings underscore the need to design a faster, more sensitive direct test to replace acid-fast staining and culture so that patients in treatment can be monitored adequately.

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