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Improved Performance of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test When 500 Instead of 50 Microliters of Decontaminated Sediment Is Used

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Using 500 μl of sediment each for screening of 717 respiratory tract specimens by Gen-Probe's Amplified Mycobacterium Tuberculosis Direct Test improved the sensitivity (83.3%) of the assay, compared with our previous study using 50 μl of sediment (71.4%), without adversely affecting its specificity (99.4 and 99.0%, respectively).

The Amplified Mycobacterium Tuberculosis Direct Test kit (AMTDT; Gen-Probe, Inc., San Diego, Calif.) for the rapid detection of *Mycobacterium tuberculosis* in clinical specimens is based on the isothermal amplification of target rRNA by DNA intermediates. Detection of amplification product is achieved by using an acridinium-ester-labelled DNA probe (4). Sensitivity of AMTDT varied in different studies from 65 to 97%, whereas specificity was always high (1, 3, 4, 6–8). A low bacterial load was found to affect sensitivity of AMTDT more than that of cultures (4, 6, 10), thus limiting AMTDT's usefulness for the screening of smear-negative specimens (3).

For culture (e.g., Bactec 12B, 500 μl of sediment), a larger volume of decontaminated specimen is inoculated than used for AMTDT (50 μl of sediment). To investigate whether using 500 instead of 50 μl of sediment improved AMTDT's performance, we prospectively screened all respiratory tract specimens—i.e., sputa, tracheobronchial secretions, and bronchioloalveolar washings (BAL)—submitted to the mycobacteriology laboratory within a 6-month period (1 April to 30 September 1994) by five participating hospitals. The specimens were decontaminated by the sodium dodecyl sulfate-sodium hydroxide method, examined for acid-fast bacilli by fluorescence microscopy, and cultured with Bactec 12B and Loewenstein-Jensen medium as previously described (3). Mycobacteria were identified to the species level by PCR-restriction fragment length polymorphism analysis (9) and by conventional methods (5). For AMTDT, 500 μl of sediment was centrifuged (5 min at 13,000 rpm [11,000 × *g*] in an Eppendorf Microfuge). Fifty microliters of the pellet was resuspended in 200 μl of Specimen Dilution Buffer and processed according to the manufacturer's protocol as previously described (3). Results were recorded in relative light units (RLU; cutoff, 30,000 RLU).

Sociodemographic and clinical data were abstracted from clinical microbiology records. Results obtained by AMTDT were validated against culture and clinical patient data. Sensitivity and specificity of AMTDT using 500 μl of sediment were compared with our previous study using 50 μl of sediment (3).

A total of 717 respiratory tract specimens from 263 patients was evaluated (Table 1). Of these, 667 (93%) specimens were culture and AMTDT negative (range, 1,179 to 27,562 RLU; median, 4,188 RLU). Twenty-one (3%) cultures from nine patients yielded nontuberculous mycobacteria (*M. avium*, $n = 9$; *M. kansasii*, $n = 10$; *M. peregrinum*, $n = 1$; *Mycobacterium* spp., $n = 1$), all of which were AMTDT negative (range, 2,958 to 6,023 RLU; median, 4,369 RLU). *M. tuberculosis* was recovered from 24 (3%) specimens of 10 patients (Table 2). For three of these patients (30%), only one of three cultures performed yielded *M. tuberculosis*. Twenty (83.3%) of the specimens that were positive for *M. tuberculosis* by culture were detected also by AMTDT. Two of the patients missed by AMTDT had only one positive culture of three. For patient 7, RLU values suggested the presence of enzyme-inhibiting activity in the samples, potentially leading to false-negative results in two of the four specimens submitted (Table 2). Overall, the sensitivity and specificity of the modified AMTDT using 500 μl of sediment were 83.3 and 99.3%, respectively. Given a prevalence of 3.0% of *M. tuberculosis* isolates in this study population, the positive and negative predictive values were 80.0 and 99.4%, respectively (Table 3).

M. tuberculosis did not grow in five AMTDT-positive speci-

TABLE 1. Comparison of clinical specimens evaluated in the two study periods

Study period	Total no. of specimens	No. of samples (%)[a]								
		Specimen type			Microscopy		*M. tuberculosis*			
		Sputum	TBS[b]	BAL	Positive	Negative	Present[c]	Absent[c]	Smear positive	Smear negative
1993	617	510 (83)	55 (9)	52 (8)	24 (4)	593 (96)	21 (3)	596 (97)	14 (2)	7 (1)
1994	717	632 (88)	50 (7)	35 (5)	33 (5)	684 (95)	24 (3)	693 (97)	14 (2)	10 (1)

[a] Statistical values by the chi-square test are as follows: specimen type, $P = 0.01$; microscopy, $P = 0.6$; present and absent, $P = 0.9$; smear positive and smear negative, $P = 0.8$.
[b] TBS, tracheobronchial secretion.
[c] As determined by culture as reference.

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TABLE 2. Culture-positive specimens yielding *M. tuberculosis*

Patient	Specimen	RLU	Microscopy result[a]	Culture score[b]	Culture turnaround time[c]
1	TBS[d]	2,659,524	3+	3+	7
	BAL	2,280,495	Negative	2+	13
	Sputum	1,710,867	1+	1+	14
2	TBS	3,044,343	4+	4+	5
	BAL	3,023,218	4+	4+	5
3	Sputum	971,512	1+	1+	14
	Sputum	637,639	Negative	1+	19
	Sputum	2,392,272	1+	2+	10
	Sputum	734,785	1+	1+	17
4	Sputum	3,558[e]	Negative	1+	23
5	Sputum	2,803,513	3+	3+	7
	Sputum	2,809,487	3+	3+	7
	BAL	2,897,254	3+	3+	7
6	Sputum	3,519[e]	Negative	B	29
7	Sputum	28,262[e]	Negative	1+	22
	Sputum	15,940[e]	Negative	1+	22
	Sputum	71,121	1+	2+	21
	Sputum	53,630	Negative	2+	18
8	BAL	2,537,260	1+	1+	17
9	TBS	3,141,173	1+	3+	18
	Sputum	2,754,992	Negative	1+	14
	Sputum	2,797,445	1+	3+	19
10	Sputum	224,836	Negative	1+	19
	Sputum	1,996,470	Negative	2+	18

[a] Semiquantitative reporting according to reference 5: 1+, 1 to 9 acid-fast bacilli (AFB) per 10 fields; 2+, 1 to 9 AFB per field; 3+, 10 to 90 AFB per field; 4+, >90 AFB per field. Fluorochrome staining was at ×250 magnification.

[b] Modified according to reference 5: B, growth on Bactec only; 1+, 50 to 100 colonies on slant; 2+, 100 to 200 colonies on slant; 3+, almost confluent growth on slant; 4+, confluent growth on slant.

[c] Time delay (days) until first positive (Bactec; growth index, ≥100) reading.

[d] TBS, tracheobronchial secretion.

[e] Negative; cutoff, 30,000 RLU.

mens received from two patients. In one patient, who presented with clinical tuberculosis, five specimens were submitted. All five were smear negative and remained culture negative despite prolonged incubation for 12 weeks. However, two of these specimens were AMTDT positive (2,800,235 and 200,097 RLU). The patient improved rapidly when receiving triple therapy with isoniazid, rifampin, and pyrazinamide (as recommended by current guidelines for short-term therapy) and has remained without symptoms since (follow-up time, 12 months). These AMTDT results may therefore have been true positives. For the other patient, three smear- and culture-negative specimens were AMTDT positive (64,238, 84,202, and 103,444 RLU). He was receiving antituberculous medication for culture-confirmed tuberculosis. This could explain the discrepancy between AMTDT and culture results.

In our previous study (3) using 50 μl of sediment, sensitivity, specificity, and positive and negative predictive values were 71.4, 99, 71.4, and 99%, respectively (Table 3). The two study populations did not differ significantly for median age (59 versus 64 years; Mann-Whitney test, $P = 0.07$), gender (75 versus 68% males; chi-square test, $P = 0.1$), and the prevalence of *M. tuberculosis* isolates (chi-square test, $P = 0.9$; Table 1). During the second study period, more sputum specimens and fewer BAL were received; the rate of smear-positive specimens yielding *M. tuberculosis* by culture, however, remained unchanged (Table 1).

TABLE 3. Sensitivity, specificity, and predictive values for AMTDT using 500 and 50 μl of sediment, respectively, versus culture

Sediment	% Sensitivity	CI[a]	% Specificity	CI[a]	Predictive value (%)	
					Positive	Negative
500 μl	83.3	68.4–98.2	99.3	98.7–99.9	80.0	99.4
50 μl	71.4	52.1–90.8	99	98.2–99.8	71.4	99

[a] CI, 95% confidence interval.

Our results suggest that screening 500 instead of 50 μl of sediment may improve the sensitivity of AMTDT without adversely affecting its specificity. The described modification is easily implemented. The sensitivity of AMTDT could be further improved by an internal amplification control to detect enzyme-inhibiting activity in clinical specimens. In our study, for example, such a system may have detected the two false-negative specimens of patient 7. This would have increased the sensitivity of AMTDT to 92% and resulted in a negative predictive value of 100%. An excellent negative predictive value, however, is an essential feature of a screening test. In addition, we confirmed that the availability of at least three specimens per patient can be crucial for the microbiological diagnosis of tuberculosis (2, 6).

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