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Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens by a Target-Amplified Test System

GABY E. PFYFFER,[1]* PASCALE KISSLING,[1] RUTH WIRTH,[1] AND RAINER WEBER[2]

Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, CH-8028 Zurich,[1] and Division of Infectious Diseases, Department of Medicine, University Hospital, CH-8091 Zurich,[2] Switzerland

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A total of 938 respiratory specimens (633 sputa, 249 bronchial and tracheal aspirates, and 56 bronchoalveolar lavages) from 589 patients were tested for direct detection of *Mycobacterium tuberculosis* complex by the Gen-Probe amplified *Mycobacterium tuberculosis* direct test (MTD), and the results were compared with those of the conventional methods of fluorescence microscopy and cultivation (solid and radiometric media). One series of specimens (n = 515) was decontaminated with *N*-acetyl-L-cysteine (NALC)–NaOH; the other one (n = 423) was decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH. Of the specimens decontaminated with NALC, 39 were MTD and culture positive, 455 were MTD and culture negative, 18 were MTD positive and culture negative, and 3 were MTD negative and culture positive, indicating a sensitivity of 92.9% and a specificity of 96.2% for the MTD. Of the specimens decontaminated with SDS, 35 were MTD and culture positive, 372 were MTD and culture negative, 15 were MTD positive and culture negative, and 1 was MTD negative and culture positive, indicating a sensitivity of 97.2% and a specificity of 96.1% for the MTD. After resolution of discrepant results by review of the patients' clinical data, the sensitivity of the MTD was 93.9%, the specificity was 97.6%, the positive predictive value was 80.7%, and the negative predictive value was 99.3% for the NALC series; the corresponding values were 97.4, 96.9, 76.0, and 99.7%, respectively, for the SDS series. In conclusion, the MTD is a highly sensitive and specific technique for detecting *M. tuberculosis* complex within hours in both smear-positive and smear-negative respiratory specimens.

The unexpected reemergence of tuberculosis (TB) in industrialized countries and recent outbreaks of multidrug-resistant (MDR) TB as well as the dramatically enhanced vulnerability of human immunodeficiency virus-positive individuals to tubercle bacilli (1, 2, 5, 6, 9, 14, 24) underscore the urgent need for rapid and accurate detection of *Mycobacterium tuberculosis*.

Principally, newer methods such as the radiometric BACTEC technique and the commercially available DNA probes have considerably reduced turnaround time. However, the current detection methods used routinely in the clinical mycobacteriology laboratory are almost entirely growth dependent. The most recent advances in TB diagnostics have, therefore, been concentrated in the fields of direct detection of acid-fast bacilli in clinical specimens by molecular biological methods (8) and, to a lesser extent, chemical analysis of the structural components of these microorganisms (13, 18).

Amplification of mycobacterial nucleic acids by PCR is coming to be used more frequently in diagnostic laboratories (4, 10). Nevertheless, some of the reports vary greatly with respect to the ability of PCR to detect *M. tuberculosis* in clinical specimens (23). Also, many of the protocols do not easily fit into a clinical laboratory's work flow, since they can be quite demanding in terms of technical equipment and operational skills.

Recently, Gen-Probe (San Diego, Calif.) has developed a new isothermal transcription-mediated amplification system which detects *M. tuberculosis* complex directly in respiratory specimens and provides a billionfold amplification of the rRNA targets. With the transcription-mediated amplification system, as few as one mycobacterium cell equivalent can be detected (15). After amplification of a specific rRNA target via DNA intermediates, the amplicon is detected by the same hybridization protection assay (HPA) employed in the conventional Gen-Probe tests used for culture confirmation.

According to the manufacturer's suggestion, the new amplified *Mycobacterium tuberculosis* direct test (MTD) should be carried out only with sediments of sputa and bronchial aspirates which have been decontaminated according to the *N*-acetyl-L-cysteine (NALC)–NaOH or NaOH procedures recommended by the Centers for Disease Control and Prevention (Atlanta, Ga.) (16, 20). Since many TB laboratories pretreat their respiratory specimens with sodium dodecyl (lauryl) sulfate (SDS)-NaOH (21), we have performed a comparative study encompassing both decontamination methods. Our evaluation includes more than 900 clinical specimens which have been tested by the MTD and by the "gold standard," i.e., cultivation of the sediment on an egg- and agar-based medium, as well as by the BACTEC technique. Our data reveal that within 4 to 5 h, the MTD generates results which are both extremely sensitive and extremely specific, irrespective of the decontamination procedure applied.

MATERIALS AND METHODS

Specimens. From February to July 1993, respiratory specimens (sputa, bronchial and tracheal aspirates, and bronchoalveolar lavages) were obtained from patients admitted to the University Hospital of Zurich and other hospitals in the metropolitan area. Clinical samples were also collected from patients consulting private physicians. All patients had respiratory symptoms, and some of them (ca. 20%) were also suspected of having TB. Upon receipt, specimens were kept at 4°C prior to processing (two processings per day).

Decontamination procedures. Respiratory specimens in 50-ml plastic centrifuge tubes were adjusted to 10 ml with

* Corresponding author. Mailing address: Swiss National Center for Mycobacteria, Department of Medical Microbiology, University of Zurich, Gloriastrasse 30, CH-8028 Zurich, Switzerland. Phone: 41-1-257 27 86. Fax: 41-1-261 87 90.

sterile distilled water. One series of clinical specimens (the NALC series) (n = 515) was decontaminated with NALC-sodium hydroxide according to the procedure recommended by the Centers for Disease Control and Prevention (16); the other series (the SDS series) (n = 423) was treated with SDS-sodium hydroxide (21).

(i) NALC series (phase I). To 10 ml of a specimen an equal volume of digestant (3% NaOH, 1.45% sodium citrate, 0.5% NALC [Sigma Chemical Company, St. Louis, Mo.]) was added. After being vortexed, the mixture was shaken for 15 min, neutralized by being adjusted to 50 ml with sterile 0.067 M phosphate buffer (pH 6.8), and centrifuged at 3,300 × g for 15 min. The supernatant was discarded, and the sediment was resuspended in 2 ml of phosphate buffer.

(ii) SDS series (phase II). To 10 ml of a specimen an equal volume of digestant (1% NaOH, 3.16% SDS [Fluka Chemical Company, Buchs, Switzerland]) was added; after being vortexed, the samples were vigorously shaken for 30 min and then left in the rack for another 10 min. Ten milliliters of 1.43% H_3PO_4 (containing 0.006% bromcresol purple as a pH indicator) was added to neutralize the specimen. After a centrifugation step (3,300 × g, 20 min), the pellet was suspended in 10 ml of H_2O and centrifuged again (15 min). The sediment was finally resuspended in 2 ml of 0.067 M phosphate buffer (pH 6.8).

In both protocols, one half of the sediment was kept at −80°C for the target-amplified test, and the other half was inoculated onto the culture media and used for acid-fast staining.

Culture. The processed sediment (0.25 ml) was inoculated onto a Löwenstein-Jensen slant (produced in our department) and onto a Middlebrook 7H10-sel 7H11 agar plate (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and incubated at 36 ± 1°C for 9 weeks in 6% CO_2. In addition, 0.5 ml of the sediment was cultivated by the radiometric BACTEC technique (17). Each BACTEC Middlebrook 7H12 medium vial (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) was supplemented with 0.1 ml of an antimicrobial mixture (PANTA; Becton Dickinson) containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin. The vials were incubated at 36 ± 1°C for 7 weeks in 6% CO_2.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome. Positive slides were confirmed by Ziehl-Neelsen staining (16).

Identification of mycobacteria. Routine biochemical methods (16, 20) and the Accuprobe culture confirmation kits (Gen-Probe) were employed for the identification of isolates.

MTD. The MTD protocol consisted of the following steps. (i) For lysis, 50 μl of a decontaminated sample was added to 200 μl of specimen dilution buffer in a lysing tube, and the mixture was sonicated for 15 min in a water bath sonicator at room temperature. (ii) For amplification, 25 μl of reconstituted amplification reagent was placed in a reaction tube and covered with 200 μl of oil. Fifty microliters of lysate (or MTD amplification-positive control and MTD amplification-negative control) was transferred to the amplification tube, incubated at 95°C for 15 min, and then cooled at 42°C for 5 min. An enzyme reagent mix (25 μl) was added, and the mixture was incubated at 42°C for 2 h. To terminate amplification, 20 μl of the termination reagent was added to each tube, and the mixtures were kept at 42°C for another 10 min. (iii) For detection, the reconstituted acridinium ester-labeled probe (100 μl) was added to the tubes, and they were incubated at 60°C for 15 min; 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. Appropriate HPA-positive and HPA-negative control experiments were run according to the recommendations of the manufacturer. Prior to being read in a luminometer (LEADER 50; Gen-Probe), the tubes were cooled at room temperature for 5 min.

TABLE 1. Types and numbers of clinical specimens tested

Type of clinical specimen	No. of specimens decontaminated with:	
	NALC-NaOH (n = 515)	SDS-NaOH (n = 423)
Sputum	357	276
Bronchial aspirate	121	110
Tracheal aspirate	11	7
Bronchoalveolar lavage	26	30

HPA-positive and HPA-negative controls were included in each series at the beginning of each run. Furthermore, all runs included MTD amplification-positive and -negative controls, which were measured after the HPA controls and at the end of each run.

The cutoff value was set at 30,000 relative light units (RLU). Samples with values of ≥30,000 RLU were considered positive; samples with values of <30,000 RLU were considered negative.

Patients' clinical data. In those cases in which discrepant results for the MTD and the culture (gold standard) were obtained, the responsible physicians were contacted and the clinical data of the patient were evaluated. Clinical assessment included the patient's history, signs, symptoms, chest X ray, laboratory results, and follow-up observation as well as the results obtained with additional specimens from the patient that were sent to the mycobacteriology laboratory.

Statistical analysis. The chi-square test was done by using Epi Info (version 5.0; Centers for Disease Control and Prevention).

RESULTS

A total of 589 patients consecutively enrolled were evaluated for *M. tuberculosis* disease by a newly developed transcription-mediated amplification assay for respiratory specimens. The MTD was used to test a total of 938 clinical specimens (633 sputa, 231 bronchial and 18 tracheal aspirates, and 56 bronchoalveolar lavages). Roughly one half of the patients' specimens (n = 515) were decontaminated by the NALC-NaOH procedure; the other half (n = 423) were decontaminated with SDS-NaOH (Table 1).

Positive and negative results could be very clearly discriminated by the magnitude of RLU. Almost 80% of all positive results had values of >2,000,000 RLU. Conversely, negative results were usually far below the cutoff value of 30,000 RLU (91% had values of <10,000 RLU).

Specimens decontaminated with NALC. Of the NALC series, 39 specimens were MTD positive and culture positive, and 455 were negative by both methods. There were 21 discrepant results: 18 specimens were positive by the MTD and negative by culture, and three were negative by the MTD and positive by culture. On the basis of these data, the overall sensitivity of the MTD was 92.9%, its specificity was 96.2%, its positive predictive value (PPV) was 68.4%, and its negative predictive value (NPV) was 99.3% (Table 2).

Of the 18 specimens which were MTD positive and culture negative (Table 3), 4 came from patients who had developed

TABLE 2. Detection of *M. tuberculosis* in respiratory specimens by MTD and culture (before resolution of discrepant results)

Specimen group and MTD result	No. of specimens with culture result		Sensitivity (%)[a]	Specificity (%)[a]	PPV (%)	NPV (%)
	Positive	Negative				
NALC series (n = 515)			92.9	96.2	68.4	99.3
Positive	39	18				
Negative	3	455				
SDS series (n = 423)			97.2	96.1	70.0	99.7
Positive	35	15				
Negative	1	372				

[a] No statistically significant difference (chi-square test).

active pulmonary TB 1 to 8 months before, whereas 1 came from a patient with active MDR TB diagnosed 30 months before. All of these patients were still under anti-TB medication and had previously delivered other specimens to our laboratory which were culture positive. These five specimens were considered true positive. There were six specimens from five patients with TB which was active 25 to 53 years before. Of these patients, a 76-year-old woman had two positive MTD results with two different specimens. Since radiological and clinical findings indicated a reactivation of the disease, these two specimens were also considered true positive. The specimens from four patients with a history of TB as well as those from seven patients with other respiratory problems at the time of examination were considered false positive because both follow-up observation and culture results did not confirm the presence of active TB. The three MTD-negative but culture-positive samples originated from patients with MDR TB. We had previously analyzed multiple specimens from all these patients which were both culture positive and MTD positive. After a review of discrepant results (Table 3) the adjusted sensitivity, specificity, PPV, and NPV of the MTD were 93.9, 97.6, 80.7, and 99.3%, respectively (Table 4).

Specimens decontaminated with SDS. Before evaluating the SDS-decontaminated series, we checked whether the washing step (after the neutralization of the specimen) in the standard procedure protocol was sufficient to remove all traces of SDS. Pilot runs showed that additional washing steps (one to two times) with the final pellet were not required: the RLU values

TABLE 3. Analysis of discrepant results between MTD (NALC series) and culture

Specimen no.	Patient's age (yr) and sex[a]	RLU value	MTD result	Culture result	No. of positive cultures/no. of specimens tested[b]	Type of specimen[c]	Clinical diagnosis[d]	Comments	Final interpretation of MTD[e]
1	65, F	3,309,793	+	−	2/4	BA	Active TB	Therapy of TB for 2 mo	TP
2	28, M	3,272,865	+	−	4/7	SP	Active TB	Therapy of TB for 1 mo	TP
3	67, M	3,359,980	+	−	7/9	BA	Active TB	Therapy of TB for 2 mo + isolation of *M. fortuitum*	TP
4	49, F	3,224,254	+	−	7/10	SP	Active TB	Therapy of TB for 8 mo	TP
5	44, M	3,103,834	+	−	61/62	SP	Active MDR TB[f]	Therapy of TB for 30 mo	TP
6[g]	44, M	4,539	−	+	61/62	SP	Active MDR TB[f]	Therapy of TB for 30 mo	FN
7	46, M	10,539	−	+	7/7	SP	Active MDR TB[h]	Therapy of TB for 3 mo	FN
8	35, F	10,389	−	+	15/15	SP	Active MDR TB[i]	Therapy of TB for 1 mo	FN
9	76, F	2,594,788	+	−	0/3	SP	Pneumonia of left upper lobe and presumed reactivation of TB	TB in 1940; therapy of TB started[j]	TP
10[k]	76, F	1,472,076	+	−	0/3	TA	Pneumonia of left upper lobe and presumed reactivation of TB	TB in 1940; therapy of TB started[j]	TP
11	74, M	38,027	+	−	0/1	SP	Pneumococcal pneumonia	TB in 1949	FP
12	69, M	914,448	+	−	0/5	TA	Pneumonia	TB in 1944, 1950, and 1953	FP
13	70, F	3,288,736	+	−	0/1	BA	Transient hemoptysis	TB in 1944	FP
14	68, M	2,531,908	+	−	0/1	BA	Transient cough	TB in 1968	FP
15	72, M	66,001	+	−	0/2	BAL	Lung cancer, abscess	Thickened pleura	FP
16	62, M	3,151,828	+	−	0/2	BA	Tracheobronchitis		FP
17	35, M	3,301,921	+	−	0/1	BAL	Bronchitis		FP
18	86, F	1,228,890	+	−	0/5	SP	Bronchitis	Isolation of *M. gordonae* and *M. xenopi*	FP
19	29, M	3,272,786	+	−	0/3	SP	Chronic cough	Human immunodeficiency virus infection	FP
20	79, M	103,319	+	−	0/3	SP	Bronchitis	Alcohol abuse	FP
21	3, M	655,774	+	−	0/2	BAL	Pneumonia	Septic granulomatosis	FP

[a] F, female; M, male.
[b] Specimens tested were not all necessarily included in the study period.
[c] BA, bronchial aspirate; SP, sputum; TA, tracheal aspirate; BAL, bronchoalveolar lavage.
[d] Assessment based on signs, symptoms, routine laboratory results, chest X ray, results of TB cultures, and follow-up.
[e] Active TB or reactivation of TB is not excluded in patients 11 to 21, but the presence of tuberculosis could not be proven by means of positive TB cultures during follow-up of 1 to 6 months. TP, true positive; FN, false negative; FP, false positive.
[f] Resistant to isoniazid, rifampin, ethambutol, and pyrazinamide.
[g] Obtained from same patient as specimen 5.
[h] Resistant to isoniazid, rifampin, and pyrazinamide.
[i] Resistant to isoniazid, rifampin, and ethambutol.
[j] Clinician started treatment of suspected TB on the basis of clinical assessment (without knowledge of the result of the MTD).
[k] Obtained from same patient as specimen 9.

TABLE 4. MTD compared with culture and clinical assessment of patients (after resolution of discrepant results[a])

Specimen group and MTD result	Final interpretation (no. of specimens)		Sensitivity[b] (%)	Specificity[b] (%)	PPV (%)	NPV (%)
	True positive	Negative				
NALC series, revised (n = 515)			93.9	97.6	80.7	99.3
Positive	46	11				
Negative	3	455				
SDS series, revised (n = 423)			97.4	96.9	76.0	99.7
Positive	38	12				
Negative	1	372				

[a] See Materials and Methods.
[b] No statistically significant difference (chi-square test).

obtained for each series did not differ significantly (data not shown).

Of the SDS-decontaminated samples, 35 specimens were MTD positive and culture positive and 372 were negative by both methods. In total, there were 16 discrepant results in this group: 15 specimens were MTD positive but negative by culture, and for 1 specimen the opposite held true. On the basis of these data the following values were obtained: sensitivity, 97.2%; specificity, 96.1%; PPV, 70.0%; and NPV, 99.7% (Table 2).

Of the 15 specimens which were MTD positive and culture negative (Table 5), 3 originated from patients who had been treated for active TB for 2 to 3 months. These specimens were considered true positive. Twelve specimens were considered false positive. Of these, four were obtained from three patients with a history of TB (active 44 to 50 years before). Three patients without a history of TB themselves had family members suffering from active pulmonary TB. Of the remaining five patients with respiratory problems at the time of examination, three were immunosuppressed. The only specimen with a false-negative result in this series was obtained from a patient who had active lung TB caused by a strain which is fully susceptible. From this patient two other respiratory specimens which were MTD and culture positive were obtained concurrently. After resolution of the discrepancies (Table 5), the sensitivity, specificity, PPV, and NPV of the MTD increased to 97.4, 96.9, 76.0, and 99.7%, respectively (Table 4).

When both series (NALC and SDS) were evaluated for each patient, a sensitivity of 97.8% and a specificity of 97.1% were found.

Both series comprised 58 smear-positive specimens which were, without exception, MTD positive. Among the 20 smear-negative specimens which were culture positive the MTD failed to detect 4 specimens. On the other hand, the MTD detected 33 additional smear-negative samples which were culture negative; among these cases at least eight clinical profiles were consistent with a certain diagnosis of TB.

Among the 938 specimens tested, 24 isolates of nontuber-

TABLE 5. Analysis of discrepant results between MTD (SDS series) and culture

Specimen no.	Patient's age (yr), sex[a]	RLU value	MTD result	Culture result	No. of positive cultures/no. of specimens tested[b]	Type of specimen[c]	Clinical diagnosis[d]	Comments	Final interpretation of MTD[e]
1	42, F	1,041,626	+	−	4/6	SP	Reactivation of TB	Therapy of TB for 2 mo	TP
2	64, F	3,117,786	+	−	3/5	BA	Active TB	Therapy of TB for 3 mo	TP
3	46, M	1,209,803	+	−	4/7	SP	Active TB	Therapy of TB for 2 mo	TP
4	63, M	3,500	−	+	2/5	SP	Active TB[f]	Therapy of TB for 1 mo	FN
5	77, M	182,785	+	−	0/4	SP	Pneumothorax	TB in 1944	FP
6	63, F	2,793,048	+	−	0/3	SP	Bronchitis	TB in 1945, 1952, and 1954	FP
7[g]	63, F	1,284,276	+	−	0/3	SP	Bronchitis	TB in 1945, 1952, and 1954	FP
8	77, F	36,790	+	−	0/1	BA	Ischemic heart disease	TB in 1950	FP
9	27, F	35,170	+	−	0/2	SP	Transient cough and hemoptysis	Mother had active TB at time of MTD	FP
10	64, F	46,059	+	−	0/3	SP	Transient cough	History of TB? Brother had TB	FP
11	49, M	71,482	+	−	0/4	BAL	*Pneumocystis carinii* pneumonia	Immunosuppression, heart transplantation 5 mo before	FP
12	44, F	399,401	+	−	0/1	SP	Transient cough		FP
13	38, M	86,822	+	−	0/1	BAL	Pneumonia	Human immunodeficiency virus infection	FP
14	77, M	72,969	+	−	0/1	BA	Lung cancer	Brother died of presumed TB	FP
15	35, F	57,340	+	−	0/2	SP	Pneumonia, lung abscess	Leukemia	FP
16	41, M	65,262	+	−	0/1	BA	Pneumonia	Laryngeal cancer	FP

[a] F, female; M, male.
[b] Specimens tested are not all necessarily included in the study period.
[c] SP, sputum; BA, bronchial aspirate; BAL, bronchoalveolar lavage.
[d] Assessment based on signs, symptoms, routine laboratory results, chest X ray, results of TB cultures, and follow-up.
[e] Active TB or reactivation of TB is not excluded in patients 5 to 16, but the presence of tuberculosis could not be proven by means of positive TB cultures during follow-up of 1 to 6 months. TP, true positive; FN, false negative; FP, false positive.
[f] Fully susceptible strain (Inh^s, Rmp^s, Emb^s, Pza^s).
[g] Obtained from same patient as specimen 6.

culous mycobacteria (NTM) (*M. avium* complex [8 isolates], *M. gordonae* [6 isolates], *M. xenopi* [5 isolates], and *M. flavescens*, *M. fortuitum*, *M. kansasii*, and *M. nonchromogenicum* [1 isolate each] as well as an unspecified NTM) were detected by conventional culture, but none were detected by the MTD.

DISCUSSION

Rapid diagnosis of pulmonary TB by molecular biological methods is predominantly based on amplification of mycobacterial DNA by PCR (3, 4, 7, 10–12, 19). Although the revolutionary potential of this technique in the diagnosis of infectious diseases cannot be denied, at present a test in kit form which would allow the use of this method in the clinical mycobacteriology laboratory is still being sought.

The Gen-Probe amplified nucleic acid probe test is a novel molecular biological alternative. This test is based on the same principles as the culture confirmation method (Accuprobe), i.e., in-solution rRNA targeting and HPA detection, but a preceding enzymatic rRNA amplification step which satisfies the urgent need for greater sensitivity has been added. Targeting rRNA rather than DNA gives the process a distinct head start in terms of bacterial numbers: the transcription-mediated amplification technique starts with ca. 2,000 rRNA targets per cell and creates billions of amplicons in just 2 h (15). As a consequence, it minimizes failures that can occur when only low numbers of microorganisms are present.

The high sensitivity of the MTD and the high signal-to-noise ratio of the HPA are nicely evidenced by the large window that exists between most MTD-positive and MTD-negative samples (cutoff, 30,000 RLU). Of the MTD-positive specimens, only 11 had low, though positive, RLU values of approximately 40,000 to 80,000, whereas the great majority of specimens had values above 2,000,000 RLU (see Results). Contrariwise, of the 827 MTD-negative specimens, more than 90% had RLU values of less than 10,000. Finally, RLU values for NTM were similarly low: for all 24 specimens from which NTM were subsequently obtained in culture the RLU values ranged between 3,000 and 14,000.

Our comprehensive study encompassing more than 900 clinical specimens demonstrates that the MTD is indeed highly sensitive and specific (Tables 2 and 4). Among the 33 MTD false-positive results (from 31 patients), i.e., specimens which were MTD positive but negative in culture, 8 discrepant results could be unambiguously resolved (Tables 3 and 5). These patients had active TB and underwent treatment. A history of TB was found for eight patients (10 specimens). Although clinical assessment and the short-term follow-up (1 to 6 months) could not with certainty exclude current TB in these patients, the specimens from seven patients were considered false positive. Epidemiological studies have yet to determine whether or when this kind of patient will develop a clinically manifest reactivation of pulmonary TB or whether a positive MTD result in these cases, together with the respiratory symptoms, might indicate the need to start preventive chemotherapy with isoniazid. For the young woman who presented with transient hemoptysis and a cough after having visited her tuberculous mother (Table 5, specimen 9), the MTD may indicate an acute infection with *M. tuberculosis*. We have, however, also considered this MTD result false positive, because follow-up observation of the patient did not suggest the presence of active TB. The remaining 14 specimens were similarly considered false positive although the follow-up period was too short to eventually exclude an active TB. For these latter cases it has to be assessed (i) whether the positive MTD result might indicate a high probability of the development of active TB in the near future, (ii) whether the result reflects a latent infection due to *M. tuberculosis* in this patient group, or (iii) whether the MTD result was indeed false positive.

Thus, the final number of false-positive MTD results was 23 of 938, which indicates increases in sensitivity, specificity, PPV, and NPV (Table 4). The test detected all 58 smear-positive specimens and 75% of the smear-negative specimens which were culture positive. Furthermore, when the MTD-positive, culture-negative specimens which turned out to be true positives are taken into account (Tables 3 and 5), it is seen that 85% of all smear-negative samples could be detected by the MTD.

On the basis of our data, the test's sensitivity is superior to that of the culture approach: culture sensitivities were 85.7% (NALC series) and 92.3% (SDS series) versus 93.9 and 97.4%, respectively, for the MTD. The sensitivities for cultures obtained from SDS-treated specimens and for those from specimens decontaminated with NALC are, however, not statistically significant (chi-square test; $P > 0.05$). Similarly, the MTD is possibly also superior to most of the PCR-based studies reported hitherto. The PCR-based studies indeed show mixed results; although some excellent procedures for the detection of *M. tuberculosis* in respiratory specimens by PCR which yield both high specificity and high sensitivity have been developed (83.5 and 99.0% [7], 93.9 and 94.3% [11], and 87.2 and 97.7% [12], respectively), there are still other investigators reporting assays which are disappointingly insensitive (55 and 74%, with specificity levels of 98 and 95%, respectively, depending on the detection procedure [22]). Apparently, most difficulties arise with microscopically negative specimens (57% sensitivity [19]).

The four false-negative results (three in the NALC series and one in the SDS series) may be explained by (i) the presence of possible amplification inhibitors in the sample, analogous to those reported for PCR protocols in which inhibition of the enzyme reaction is a well-known drawback (18, 21, 24); (ii) nonuniform distribution of microorganisms in the test suspension, since *M. tuberculosis* is known to form cords and clumps (14, 15); and (iii) the failure of RNA synthesis due to poor viability of the cells as demonstrated by the extremely long period until detection of these particular isolates in culture (6 to 8 weeks) and their overall poor growth (one to three colonies formed on solid media). On the other hand, it is less likely that any medication led to nondetectability of the mycobacteria, as the majority of the positive specimens in this study was obtained from patients receiving standard anti-TB therapy. Also, a false-negative MTD result should not necessarily be exclusively attributed to multidrug resistance, since such a result was also observed with a fully susceptible strain of *M. tuberculosis* (Table 5).

Respiratory specimens do not necessarily need to be processed by the NALC procedure. Our data for the SDS series show that the values obtained compare well with those obtained with the NALC series, i.e., the sensitivity and the NPV were even higher (97.4 versus 93.3% and 99.7 versus 99.3%, respectively [Table 4]). However, no statistically significant difference in culture sensitivity between the two decontamination series ($P > 0.05$) was found. Extensive washing after SDS decontamination seems to be crucial. Obviously, the washing step in our standard protocol (after neutralization of the sediment) is sufficient to remove all remaining traces of the detergent which might interfere with the enzymes of the assay.

Several main points emerge from the data presented in this study. (i) Compared with traditional methods, the MTD has a short detection time for *M. tuberculosis* (several weeks versus less than 1 day). (ii) The MTD is characterized by excellent

sensitivity and specificity, making it an ideal tool for TB infections in which only few bacteria are present. Furthermore, with a NPV close to 100%, the test could help to eliminate uncertain diagnosis and time-consuming confirmation for the laboratory and avoid both unnecessary treatment and costly hospitalization of the patient. (iii) All steps from amplification to HPA detection can be carried out in a single-test-tube format. This not only significantly minimizes concerns about cross-contamination but also makes this test easy to perform and easy to implement in a clinical mycobacteriology laboratory. (iv) Respiratory specimens can be processed by either the NALC or the SDS decontamination protocol. (v) The specificity of the MTD has to be interpreted with some caution, as the culture method, which was adopted as the gold standard, obviously has its limitations: in our study, the culture method failed to detect *M. tuberculosis* in at least eight samples from patients with active TB, while MTD results were positive. (vi) Performing the MTD does not free a laboratory from establishing cultures, since NTM cannot be detected by the MTD. Also, growth is still required for routine susceptibility testing. (vii) Results of the MTD should always be interpreted in conjunction with culture and acid-fast smear test results and other clinical data available to the physician.

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ADDENDUM

From the 77-year-old woman whose bronchial aspirate was considered false positive (Table 5, specimen 8), we received follow-up specimens 5 months later (i.e., in October 1993). In the meantime, cultures from cerebrospinal fluid and a bronchoalveolar lavage yielded *M. tuberculosis.*

REFERENCES

1. **Beck-Sagué, C., S. Dooley, M. D. Hutton, J. Otten, A. Breeden, J. T. Crawford, A. E. Pitchenik, C. Woodley, G. Cauthen, and W. R. Jarvis.** 1992. Hospital outbreak of multidrug-resistant *Mycobacterium tuberculosis* infections. JAMA **268:**1280–1286.
2. **Bloom, B. R., and C. J. L. Murray.** 1992. Tuberculosis: commentary on a reemergent killer. Science **257:**1055–1064.
3. **Brisson-Noël, A., C. Aznar, C. Chureau, S. Nguyen, C. Pierre, M. Bartoli, R. Bonete, G. Pialoux, B. Gicquel, and G. Garrigue.** 1991. Diagnosis of tuberculosis by DNA amplification in clinical practice evaluation. Lancet **338:**364–366.
4. **Brisson-Noël, A., B. Gicquel, D. Lecossier, V. Lévy-Frébault, X. Nassif, and A. J. Hance.** 1989. Rapid diagnosis of tuberculosis by amplification of mycobacterial DNA in clinical samples. Lancet **ii:**1069–1071.
5. **Centers for Disease Control.** 1992. Meeting the challenge of multidrug-resistant tuberculosis: summary of a conference. Morbid. Mortal. Weekly Rep. **41:**51–57.
6. **Centers for Disease Control.** 1992. Management of persons exposed to multidrug-resistant tuberculosis. Morbid. Mortal. Weekly Rep. **41:**61–70.
7. **Clarridge, J. E., III, R. M. Shawar, T. M. Shinnick, and B. B. Plikaytis.** 1993. Large-scale use of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in a routine mycobacteriology laboratory. J. Clin. Microbiol. **31:**2049–2056.
8. **Desmond, E. P.** 1992. Molecular approaches to the identification of mycobacteria. Clin. Microbiol. Newsl. **14:**145–152.
9. **Dooley, S. W., W. R. Jarvis, W. J. Martone, and D. E. Snider.** 1992. Multidrug-resistant tuberculosis. Ann. Intern. Med. **117:**257–259.
10. **Eisenach, K. D., M. D. Sifford, M. D. Cave, J. H. Bates, and J. T. Crawford.** 1991. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. Am. Rev. Respir. Dis. **144:**1160–1163.
11. **Fauville-Dufaux, M., B. Vanfleteren, L. De Wit, J. P. Vincke, J. P. Van Vooren, M. D. Yates, E. Serruys, and J. Content.** 1992. Rapid identification of tuberculous and non-tuberculous mycobacteria by polymerase chain reaction amplification of a 162 bp DNA fragment from antigen 85. Eur. J. Clin. Microbiol. Infect. Dis. **11:**797–803.
12. **Forbes, B. A., and K. E. S. Hicks.** 1993. Direct detection of *Mycobacterium tuberculosis* in respiratory specimens in a clinical laboratory by polymerase chain reaction. J. Clin. Microbiol. **31:**1688–1694.
13. **French, G. L., C. Y. Chan, S. W. Cheung, and K. T. Oo.** 1987. Diagnosis of pulmonary tuberculosis by detection of tuberculostearic acid in sputum by using gas chromatography-mass spectrometry with selected ion-monitoring. J. Infect. Dis. **156:**356–362.
14. **Frieden, T. R., T. Sterling, A. Pablos-Mendez, J. O. Kilburn, G. M. Cauthen, and S. W. Dooley.** 1993. The emergence of drug-resistant tuberculosis in New York City. N. Engl. J. Med. **32:**521–526.
15. **Jonas, V., M. J. Alden, J. I. Curry, K. Kamisango, C. A. Knott, R. Lankford, J. M. Wolfe, and D. F. Moore.** 1993. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by amplification of rRNA. J. Clin. Microbiol. **31:**2410–2416.
16. **Kent, P. T., and G. Kubica.** 1985. Public health mycobacteriology. A guide for the level III laboratory. Public Health Service, U.S. Department of Health and Human Services. Centers for Disease Control, Atlanta.
17. **Morgan, M. A., C. D. Horstmeier, D. R. DeYoung, and G. D. Roberts.** 1983. Comparison of a radiometric method (BACTEC) and conventional culture media for recovery of mycobacteria from smear-negative specimens. J. Clin. Microbiol. **18:**384–388.
18. **Muranishi, H., M. Nakashima, R. Isobe, T. Ando, and N. Shigematsu.** 1990. Measurement of tuberculostearic acid in sputa, pleural effusions, and bronchial washings. Diagn. Microbiol. Infect. Dis. **13:**235–240.
19. **Nolte, F. S., B. Metchock, J. E. McGowan, Jr., A. Edwards, O. Okwumabua, C. Thurmond, P. S. Mitchell, B. Plikaytis, and T. Shinnick.** 1993. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridization. J. Clin. Microbiol. **31:**1777–1782.
20. **Roberts, G. D., E. W. Koneman, and Y. K. Kim.** 1991. Mycobacterium, p. 304–339. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
21. **Salfinger, M., and F. M. Kafader.** 1987. Comparison of two pretreatment methods for the detection of mycobacteria of BACTEC and Lowenstein-Jensen slants. J. Microbiol. Methods **6:**315–321.
22. **Shawar, R. M., F. A. K. El-Zaatari, A. Nataraj, and J. E. Clarridge.** 1993. Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridization methods. J. Clin. Microbiol. **31:**61–65.
23. **Soini, H., M. Skurnik, K. Liippo, E. Tala, and M. K. Viljanen.** 1992. Detection and identification of mycobacteria by amplification of a segment of the gene coding for the 32-kilodalton protein. J. Clin. Microbiol. **30:**2025–2028.
24. **Wolinsky, E.** 1993. Statement of the Tuberculosis Committee of the Infectious Diseases Society of America. Clin. Infect. Dis. **16:**627–628.