JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1995, p. 2699–2703 Vol. 33, No. 10
0095-1137/95/$04.00+0

Diagnostic Value of an Amplification Method (Gen-Probe) Compared with That of Culture for Diagnosis of Tuberculosis

F. VLASPOLDER,[1]* P. SINGER,[1] AND C. ROGGEVEEN[2]

Department of Medical Microbiology, Medical Center Alkmaar, 1815 JD Alkmaar,[1] and Regional Public Health Laboratory, 2003 BR Haarlem,[2] The Netherlands

Received 14 March 1995/Returned for modification 10 May 1995/Accepted 10 July 1995

Five hundred fifty respiratory and nonrespiratory specimens from 340 patients were analyzed by comparing the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (MTD) with conventional culture, which was the method of reference, for the detection of the *Mycobacterium tuberculosis* complex. After resolution of discrepant results by retesting the samples and reviewing the patients' clinical histories, a total of 60 respiratory specimens were MTD and culture positive, 347 were MTD and culture negative, 4 were MTD positive and culture negative, and 1 was MTD negative and culture positive. This results in a sensitivity of 98.4%, a specificity of 98.9%, and positive and negative predictive values of 93.8 and 99.7%, respectively. Repeatedly, clinicians asked to test specimens of nonpulmonary origin by MTD. Although, MTD is not approved for use with nonrespiratory specimens, the following results were shown. Sixty-one pleural exudate specimens showed disappointing results (sensitivity, 20%). However, MTD performed well with another 77 nonrespiratory specimens; 17 samples were positive and 57 samples were negative by both MTD and culture. No false-negative results were found by MTD. Three MTD-positive, culture-negative specimens had high sample relative light unit/cutoff relative light unit ratios, strongly suggesting true tuberculosis. Positive microscopy and positive culture with MTD-negative results occurred 12 times. Those cultures showed atypical mycobacteria 11 times and *Actinomyces* species once. The stability of the reagents in the MTD kit was also assessed by testing reagents, including the enzyme mixture, kept at −70°C for at least 6 months. No loss of activity was seen.

The increased incidence of tuberculosis and the recent outbreak of multidrug-resistant tuberculosis have increasing public health significance (3, 4, 18). The diagnosis of tuberculosis on the basis of clinical and radiological findings, the presence of acid-fast bacilli in clinical smears, and other laboratory results are presumptive. Detection of *Mycobacterium tuberculosis* in culture is required for the definitive diagnosis of tuberculosis. Technological advances in amplifying and detecting specific regions of bacterial DNA or RNA have provided the methods necessary to make improvements in the laboratory diagnosis of tuberculosis. In recent years, PCR was reported to be useful for the direct diagnosis of tuberculosis infections from a variety of clinical specimens (1, 5, 7–9, 14) by using a diversity of genetic elements as the target template. Nevertheless, reports published on this subject vary greatly with respect to the ability of PCR to detect *M. tuberculosis* in clinical material (16). Another problem is that the PCR protocol does not easily fit into routine clinical laboratories because they usually lack the necessary technical equipment and expertise and because of its high costs.

In the study described here, we compared the diagnostic value of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test (MTD) for the detection of *M. tuberculosis* directly in specimens with conventional culture, which was used as the "gold standard."

A second goal of the study was to investigate how the MTD kit could be used in a cost-efficient way by testing the activities of reconstituted reagents, including the enzyme mixture, to determine if the shelf-lives of these reagents could be prolonged.

* Corresponding author. Mailing address: Department of Medical Microbiology, Medical Center Alkmaar, Wilhelminalaan 12, 1815 JD Alkmaar, The Netherlands.

MATERIALS AND METHODS

Patients and clinical specimens. The 550 specimens tested were collected from 340 patients admitted to the Medical Center Alkmaar, Alkmaar, The Netherlands, and two other hospitals in the north of the province of North-Holland. Later in the study we received specimens from patients highly suspected of having tuberculosis admitted to hospitals in Haarlem, The Netherlands. The patients were selected if their specimens gave a positive smear result or there was a high degree of suspicion that a patient with negative smear results have tuberculosis.

The 550 specimens could be divided into two major groups: 412 respiratory specimens (sputa, bronchial and tracheal aspirates, and bronchoalveolar lavages) and 138 nonrespiratory specimens (61 pleural exudates, 77 samples of different origin [Table 1]). First, the specimens were divided into two portions before digestion and decontamination: one part was used for routine culture and one part was used for MTD plus control culture. This second portion was digested and decontaminated by different regimens. The first series of specimens was decontaminated with sodium triphosphate overnight and was thereafter neutralized to pH 7.0 with 1 N HCl. Later, we used the *N*-acetyl-L-cysteine (NALC)–3% NaOH method and concentrated the specimens by centrifugation at 3,000 × *g* for 15 min (12, 15). The sediment was resuspended in approximately 1.5 ml of phosphate buffer (0.067 M; pH 6.8) containing 0.5% Tween 80. Tween 80 was added to achieve better homogenization of the sediment. One-half milliliter of the sediment was directly used for the amplification, 0.5 ml was inoculated onto the control culture media, and the remaining amount was kept at −70°C.

Culture. The processed sediment was inoculated onto three Löwenstein-Jensen solid media (medium I with pyruvate and glycerol, medium II without pyruvate and with glycerol, and medium III without pyruvate and without glycerol), and the media were incubated at 37°C for 8 weeks. The reason for using three different solid media is as follows: (i) to differentiate between human and bovine mycobacteria, (ii) to bring more patient material into culture, and (iii) to lessen the risk of contamination.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome as a screening method (11). Positive slides were confirmed to be positive by Ziehl-Neelsen staining.

Identification of mycobacteria. In our laboratory, the Accuprobe culture confirmation kits (Gen-Probe, Inc., San Diego, Calif.) were used to identify the isolates. All specimens with culture-positive results were sent to the National Institute of Public Health and Environmental Protection (Rijksinstituut voor Volkgezondheid en Milieuhygiëne) in Bilthoven, The Netherlands, for confirmation (Accuprobe and restricted fragment length polymorphism analysis) and susceptibility testing.

MTD. MTD uses a proprietary isothermal enzymatic amplification of target rRNA via DNA intermediates (10). Detection of the amplicon is achieved with

TABLE 1. Performance of Gen-Probe MTD in nonrespiratory samples[a]

Sample no.	Source	Microscopy (Aur/ZN)	Culture (no. of colonies)	RLU ratio (range)
1	Urine	ND	*M. tb* (+)	97.1
2	Urine	ND	*M. tb* (+)	88.0
3	Urine	ND	*M. tb* (++)	89.0
4	Urine	Pos	*M. tb* (+++)	85.7
5–16	Urine	ND	Neg	(0.1–0.3)
17	Lymph node	Neg	*M. tb* (+)	25.9
18	Lymph node	Pos	*M. tb* (+)	69.2
19	Lymph node	Pos	*M. tb* (+)	33.9
20	Lymph node	Pos	*M. tb* (+)	55.2
21	Lymph node	Pos	Neg	41.1
22–27	Lymph node	Neg	Neg	(0.1–0.2)
28	Endometrium	ND, granuloma	*M. bov* (++)	13.4
29	Gastric fluid	ND	Neg	74.0
30	Gastric fluid	ND	*M. tb* (+)	79.4
31	Gastric fluid	ND	*M. tb* (+)	24.4
32	Gastric fluid	ND	Neg	19.2
33	Gastric fluid	ND	Neg	(0.1)
34	Scrotum	Neg	*M. tb* (+)	56.1
35–40	CSF	Neg	Neg	(0.1–0.2)
41	CSF	Neg	*M. tb* (+)	32.1
42–49	Ascites	Neg	Neg	(0.1–0.9)
50	Sacrum	Neg	*M. tb* (+)	65.0
51–65	Pus	Neg	Neg	(0.1–0.3)
66	Pus (abdomen)	Pos	*M. tb* (+)	88.9
67	Biopsy specimen (lung)	Pos	*M. tb* (++)	48.1
68	Biopsy specimen (pleura)	Neg	*M. tb* (+)	3.0
69–75	Biopsy specimen	Neg	Neg	(0.2–0.3)
76–77	Vertebrae	Neg	Neg	(0.1)

[a] ND, not done; pos, positive; neg, negative; *M. tb*, *M. tuberculosis*; *M. bov*, *Mycobacterium bovis*; CSF, cerebrospinal fluid; Aur/ZN, auramine-rhodamine fluorochrome/Ziehl-Neelsen staining; number of colonies: +, ≤10; ++, ≤100; +++, >100.

an acridinium ester-labeled DNA probe specific for *M. tuberculosis* complex rRNA (2). For lysis, 50 μl of a decontaminated sample was used for the target-amplified test. A portion was used for a control culture in cases in which the results of routine culture were already known. The remaining portion was kept at −70°C for later retesting if necessary. All reactions were performed according to the manufacturer's procedures. 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.

Preparation of frozen reagents and enzyme mixture. The amplification reagent, *Mycobacterium* enzyme mixture, and *M. tuberculosis* probe reagent provided with the MTD kit were reconstituted as described in the package insert. The total amount was divided into four equal aliquots and was kept at −70°C.

During a 6-month period, approximately every 4 weeks the reagents were thawed and used in the MTD with amplification and hybridization controls and two *M. tuberculosis* standards.

RESULTS

A total of 248 consecutive patients were enrolled in the study, and 412 respiratory specimens from these patients were evaluated for *M. tuberculosis* disease by a newly developed transcription-mediated amplification system for the detection of *M. tuberculosis*. MTD was also evaluated for its ability to detect *M. tuberculosis* complex organisms in nonrespiratory samples. These specimens were divided into two groups of 61 pleural and 77 other nonrespiratory samples (Table 2).

Positive and negative results could be clearly discriminated by the magnitudes of the RLUs. Seventy percent of all samples with positive results had values of >2,000,000 RLUs. Ten percent of samples had results of between 500,000 and 2,000,000 RLUs. Conversely, samples with negative results usually had values far below the cutoff value of 30,000 RLUs (94% had RLU sample/RLU cutoff [= RLU ratio] values of <0.5).

TABLE 2. Detection of *M. tuberculosis* in nonrespiratory specimens by MTD and culture

MTD result	No. of pleural specimens with the following culture result[a]:			No. of nonpleural specimens with the following culture result[b]:		
	Positive	Negative	Total	Positive	Negative	Total
Positive	1	2	3	17	3	20
Negative	4	54	58	0	57	57
Total	5	56	61	17	60	77

[a] Sensitivity, 20.0%; specificity, 96.4%; positive predictive value, 33.3%; negative predictive value, 93.1%.
[b] Sensitivity, 100%; specificity, 95%; positive predictive value, 85.0%; negative predictive value, 100%.

Respiratory samples. Fifty-nine specimens (80% smear positive) were MTD positive and culture positive, and 343 were negative by both methods. Results for 10 specimens were discrepant: 8 were positive by MTD and negative by culture and 2 were negative by MTD and positive by culture (Table 3).

On the basis of the results, the sensitivity, specificity, positive predictive value and negative predictive value were 96.7, 97.7, 88.1, and 99.4%, respectively.

Table 4 provides detailed results for discrepant samples. The bronchial aspirates from patients 1 and 5 were not retested, and no new specimens were received from these patients. The bronchial sample from patient 2 and the sputa from patients 6 and 7 were retested and had RLU ratios of 0.1. The bronchial sample from patient 3 was retested and again showed a positive result, with an RLU ratio of 1.8. Two sputum specimens from patient 8 were simultaneously sent for testing. One specimen which was positive by MTD with an RLU ratio of 1.1 was not retested, and the other was clearly negative (RLU ratio, 0.1). The bronchial aspirate from patient 4 was retested. The first result showed an RLU ratio of 0.1, but the second time a clearly positive result was found and showed an RLU ratio of 74.8. Two samples (from patients 9 and 10) were positive by MTD but were microscopy and culture negative. After a review of discrepant results (Table 4) the adjusted sensitivity, specificity, and positive and negative predictive values of the MTD were 98.4, 98.9, 93.8, and 99.7%, respectively (Table 5).

Data for 12 specimens with positive smear and positive culture results but with negative MTD findings are presented in Table 6. Atypical mycobacteria were cultured from 11 specimens, and an *Actinomyces* species was cultured from 1 specimen.

Nonrespiratory specimens. (i) Pleural exudates. For 61 pleural exudate specimens derived from 45 patients, results

TABLE 3. Detection of *M. tuberculosis* in respiratory specimens by MTD and culture (uncorrected data)

MTD result	No. of specimens with the following culture result[a]:		
	Positive	Negative	Total
Positive	59	8	67
Negative	2	343	345
Total	61	351	412

[a] Sensitivity, 96.7%; specificity, 97.7%; positive predictive value, 88.1%; negative predictive value, 99.4%.

TABLE 4. Analysis of discrepant results between MTD and culture[a]

Patient no.	Type of specimen	Microscopy result	MTD result	RLU ratio	Retest result	Culture result	Clinical diagnosis	Final interpretation
1	Br. asp	Neg	Pos	2.5	ND	Neg	No TB	False pos
2	Br. asp	Neg	Pos	1.2	0.1	Neg	No TB	True neg
3	Br. asp	Neg	Pos	2.4	1.8	Neg	No TB	False pos
4	Br. asp	Pos	Neg	0.1	74.3	Pos (++)	Active TB	True pos
5	Br. asp	Pos	Neg	0.2	ND	Pos (+++)	Active TB	False neg
6	Sputum	Neg	Pos	1.1	0.1	Neg	No TB	True neg
7	Sputum	Neg	Pos	1.1	0.1	Neg	No TB	True neg
8	Sputum	Neg	Pos	1.1	0.1	Neg	No TB	True neg
9	Br. asp	Neg	Pos	12.6	ND	Neg	Active TB	True pos
10	Br. asp	Neg	Pos	18.0	ND	Neg	Active TB	True pos

[a] Abbreviations: Br. asp, bronchial aspirate; pos, positive; neg, negative; ND, not done; TB, tuberculosis; RLU ratio, sample RLU/cutoff RLU; number of colonies: ++, ≤100 colonies; +++, >100 colonies.

were not comparable to those for the respiratory specimens. Only one of five specimens that were culture positive gave a positive result in MTD. Two culture-negative specimens were positive by MTD. The RLU ratios for these two specimens were 3.1 and 2.4, respectively. Fifty-four samples were negative by both methods.

Although the sample size was small, the sensitivity, specificity, and positive and negative predictive value were 20.0, 96.4, 33.3, and 93.1%, respectively (Table 2).

(ii) Other nonrespiratory specimens. We used MTD when extrapulmonary tuberculosis was suspected. Seventy-seven specimens from 47 patients were tested. The sources of these specimens are given in Table 1. Seventeen samples were positive by both methods. Three MTD-positive samples (samples 21, 29, and 32 in Table 1) had negative culture results. The smear results for these 20 positive samples were as follows: 7 were positive, 5 were negative, and 8 were not tested because of the kind of material. There were no false-negative results by MTD. Fifty-seven of the 60 samples that did not yield *M. tuberculosis* growth were also correctly identified by MTD. The sensitivity, specificity, and positive and negative predictive values were 100, 95.0, 85.0, and 100%, respectively (Table 2).

Frozen reagents and enzyme mixture. After we had thawed the reagents provided with the MTD kit, we tested two standard *M. tuberculosis* strain suspensions containing 5 and 50 CFU per sample. Figure 1 shows that the amplification control had high RLU ratios throughout the 29-week test period except during week 22 (RLU ratio, 8.1). The standard *M. tuberculosis* strain suspensions also had high positive RLUs (RLU ratio range, 40.8 to 93.0). The negative controls had only very low RLU ratios. The RLUs of the hybridization controls were as expected (RLU ratio range, 1.9 to 2.8) (Fig. 1).

DISCUSSION

The laboratory diagnosis of mycobacterial infections usually requires 2 to 8 weeks. The recent increase in new cases of tuberculosis has shown that the need for rapid, specific diagnostic assays for *M. tuberculosis* is urgent (3, 4, 18). Most of the nucleic acid amplification assays, which are predominantly based on the amplification of mycobacterial DNA by PCR, are rapid and specific; however, they are not yet suitable for use in routine clinical laboratories. MTD overcomes many of the difficulties of the PCR procedure. MTD is based on the same principles on which the culture confirmation method (Accuprobe) is based, including a preceding enzymatic rRNA amplification step that satisfies the urgent need for greater sensitivity. The Gen-Probe MTD detects rRNA, which is present at a level of approximately 2,000 copies per cell (17). This advantage enhances the sensitivity of the test compared with those of other tests, which detect the target sequences present in only a single or a very low copy number (7).

The selected study population (respiratory samples) had an infection prevalence rate of 26.2% and a positivity rate of 15.8%. The sensitivity, specificity, and positive and negative predictive values of MTD for respiratory specimens from the population examined (Table 5) are comparable to the values reported previously by Pfyffer et al. (13).

Of the 412 respiratory specimens tested, 8 were false positive by MTD (Table 4). Six of the eight samples had RLU ratios that were less than or equal to 2.5. Four of these samples were retested and exhibited RLU ratios of 0.1. The samples were considered true negatives on the basis of the RLU ratio and because these and other specimens from these patients were

TABLE 5. Detection of *M. tuberculosis* in respiratory specimens by MTD and culture (corrected data)

MTD result	No. of specimens with the following culture result[a]:		
	Positive	Negative	Total
Positive	60	4	64
Negative	1	347	348
Total	61	351	412

[a] Sensitivity, 98.4%; specificity, 98.9%; positive predictive value, 93.8%; negative predictive value, 99.7%.

TABLE 6. Positive microscopy and positive culture results with negative MTD findings

Patient	Type of specimen	Microscopy (no. of bacilli)[a]	RLU ratio by MTD	Culture result[b]
A	Sputum	Pos (+)	0.1	*Mycobacterium chelonae*
B	Br. asp[c]	Pos (+++)	0.2	*Mycobacterium avium*
C	Sputum	Pos (+)	0.1	*Mycobacterium avium*
D	Sputum	Pos (+)	0.1	*Mycobacterium kansasii*
E	Sputum	Pos (+)	0.1	*Mycobacterium malmoense*
F	Pus	Pos (+++)	0.1	*Actinomyces* species
G	Br. asp	Pos (+++)	0.2	*Mycobacterium chelonae*
H	Sputum ($n = 2$)	Pos (+,+++)	0.2	*Mycobacterium chelonae*
I	Sputum	Pos (+)	0.1	No *M. tb*, no MAI
J	Sputum ($n = 2$)	Pos (+++)	0.2	No *M. tb*, no MAI

[a] Pos, positive; number of bacilli: +, few bacilli; +++, many bacilli.
[b] *M. tb*, *M. tuberculosis* complex; MAI, *Mycobacterium avium-intracellulare.*
[c] Br. asp, bronchial aspirate.

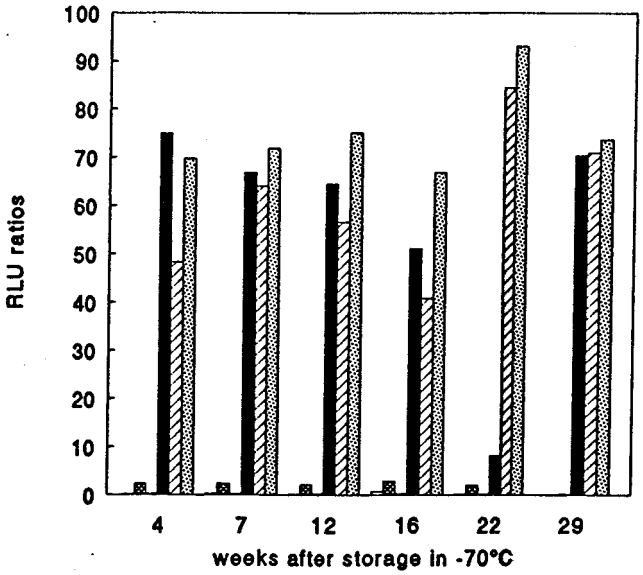

FIG. 1. RLU ratios. □, negative hybridization control; ▨, positive hybridization control; ▧, negative amplification control; ■, positive amplification control; ▨, *M. tuberculosis* standard (5 CFU); ⊡, *M. tuberculosis* standard (50 CFU).

negative on culture. The sample from patient 3 was retested and was shown to have an RLU ratio of 1.8. The sample was extremely contaminated with blood, and this could explain the false-positive results. The two samples yielding a positive result in culture and a negative result in the MTD might be explained by the storage of the samples at −20°C for several weeks. The sample from patient 4 had a clearly negative result (RLU ratio, 0.1). This sample was retested, because we found that another specimen (sputum) from this patient had a positive result, with an RLU ratio of 69.3. The retested sample was shown to have an RLU ratio of 74.3, and thus, the result for this specimen was considered a true positive. The specimen from patient 5 was not retested, and we did not obtain another specimen because of the patient's typical history, signs, symptoms, chest X ray, and positive culture. The result for this patient's sample was considered false negative. Two samples derived from patients 9 and 10 were MTD positive and smear and culture negative. Both patients had developed active tuberculosis. The sample derived from patient 9 was taken before starting therapy, but the sample from patient 10 was taken during therapy. We have already seen more samples with MTD-positive, smear-positive, culture-negative results during therapy, suggesting detection of stable rRNA from nonviable bacilli. On the other hand, we have also seen samples with smear-positive, MTD-negative, and culture-negative results. The MTD could probably be used as a test of cure (data not shown).

The performance of MTD with pleural exudates has been inconsistent. Possible inhibitory substances may interfere with the amplification process. Ehlers et al. (6) tried to avoid false-negative results by using as a control for possible inhibitory activity in assayed specimens artificial inoculation of low numbers of *M. tuberculosis* into a duplicate sample. Others recommend the use of sodium dodecyl (lauryl) sulfate-NaOH decontamination for pleural exudates (12a). Further investigations are required to determine which procedure for pleural exudate sample preparation is needed before performing the amplification step. One should take into account the fact that MTD is not recommended for use with nonrespiratory specimens.

Seventy-seven nonrespiratory (nonpleural exudate) samples derived from 47 patients showed good results. Urine, lymph node aspirate, gastric fluid, cerebrospinal fluid, and tissue biopsy specimens did not present a problem with MTD. Of the 77 specimens tested, we found three false-positive results by MTD. Sample 21 in Table 1 had a positive smear result and a high RLU ratio of 41.1, and the patient from whom the sample was obtained presented with a clear clinical picture. We consider this MTD result a true positive. Another one of these three samples (sample 29, Table 1) had a high RLU ratio of 74.0 but had a negative culture result. Sample 30 was derived 3 days later from the same patient from whom sample 29 was obtained, and the sample had positive results by both methods. The same explanation can be given for samples 31 and 32.

The low RLU ratios of the last mentioned samples (24.4 and 19.2, respectively) could be explained by the transport of the specimens to the laboratory in decontamination fluid containing sodium triphosphate. The samples were washed twice in saline and were then treated by the NALC-NaOH method. Excessive exposure to alkali actually destroys RNA, which is the amplification target in this test (this is in contrast to DNA, which is the target in PCR). These two consecutive procedures could harm the rRNAs of *M. tuberculosis* bacilli to such an extent that low RLU results might occur. Smear-positive results and a vague clinical picture for tuberculosis initiated specific anti-tuberculosis therapy in several patients. The results in Table 6 indicate that smear-positive, MTD-negative findings, indicating an atypical mycobacteriosis, can more rapidly result in the initiation of treatment with another combination of antituberculosis drugs than is usual for tuberculosis treatment.

A second goal was to find out how to use the MTD kits in the most efficient way by testing frozen, preprepared reagents and enzyme to save costs. This was done because after the enzyme mixture and probe reagent were reconstituted, according to the manufacturer's instructions, the enzyme mixture and probe reagent could only be maintained for a maximum of 2 weeks. As we have shown no loss of activity could be proven by keeping the preprepared reagents and enzyme mixture at −70°C for 29 weeks. During the 29-week test period, we once saw, during week 22, a low RLU ratio of 8.1 for the amplification control. A pipetting error was probably the cause of this low value: in the same run we found high RLUs for the *M. tuberculosis* standards (Fig. 1). Laboratories which perform small numbers of tests, for instance, on smear-negative, highly suspected samples for *M. tuberculosis*, could save substantial costs by using (deep) frozen preprepared reagents and enzyme mixture.

In conclusion, (i) MTD is useful as a rapid (4 to 5 h), suitable diagnostic test for *M. tuberculosis* complex in pulmonary specimens. (ii) MTD might also be suitable for testing specimens from nonrespiratory sites (with the exception of pleural exudate specimens). (iii) A positive smear result in combination with negative MTD results can lead to the detection of an atypical mycobacteriosis faster than waiting for culture results. (iv) The preprepared reagents and the enzyme mixture kept at −70°C for at least 6 months did not show any loss of activity.

ACKNOWLEDGMENT

We owe a great debt of gratitude to A. van der Wiel for the clerical work on the manuscript.

REFERENCES

1. **Anderson, A. B., S. Thybo, P. Godfrey-Faussett, and N. G. Stoker.** 1993. Polymerase chain reaction for detection of *Mycobacterium tuberculosis* in sputum. Eur. J. Clin. Microbiol. Infect. Dis. **12:**922–927.
2. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay

formats involving acridinium-ester-labeled DNA probes. Clin. Chem. **35:** 1588–1594.
3. **Centers for Disease Control.** 1992. Management of persons exposed to multidrug-resistant tuberculosis. Morbid. Mortal. Weekly Rep. **41:**61–70.
4. **Centers for Disease Control.** 1992. Meeting the challenge of multidrug-resistent tuberculosis: summary of a conference. Morbid. Mortal. Weekly Rep. **41:**51–57.
5. **De Wit, D., L. Steyn, S. Shoemaker, and M. Sogin.** 1990. Direct detection of *Mycobacterium tuberculosis* in clinical specimens by DNA amplification. J. Clin. Microbiol. **28:**2437–2441.
6. **Ehlers, S., M. Pirmann, W. Zaki, and H. Hahn.** 1994. Evaluation of a commercial rRNA target amplification assay for detection of *Mycobacterium tuberculosis* complex in respiratory specimens. Eur. J. Clin. Microbiol. Infect. Dis. **13:**827–829.
7. **Eisenach, K. D., M. D. Cave, J. H. Bates, and J. T. Crawford.** 1990. Polymerase chain reaction amplification of a repetitive DNA sequence specific for *Mycobacterium tuberculosis*. J. Infect. Dis. **161:**977–981.
8. **Hance, A. J., B. Grandchamp, V. Levy-Frebault, D. Lecossier, J. Rauzier, D. Bocart, and B. Gloquel.** 1989. Detection and identification of mycobacteria by amplification of mycobacterial DNA. Mol. Microbiol. **3:**843–849.
9. **Hermans, P. W., A. R. Schuitema, D. van Soolingen, C. P. Versteynen, E. M. Bik, J. E. Thole, A. H. Kolk, and J. D. van Emden.** 1990. Specific detection of *Mycobacterium tuberculosis* complex strains by polymerase chain reaction. J. Clin. Microbiol. **28:**1204–1213.
10. **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.
11. **Kent, P. T., and G. Kubica.** 1985. Public health mycobacteriology. A guide for level III laboratory. Centers for Disease Control, Atlanta.
12. **Kubica, G. P., E. Dye, M. L. Cohn, and G. Middlebrook.** 1963. Sputum digestion and decontamination with *N*-acetyl-L-cystein sodium hydroxide for culture of mycobacteria. Am. Rev. Respir. Dis. **87:**775–779.

12a. **Pfyffer, G. E.** Personal communication.

13. **Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber.** 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. J. Clin. Microbiol. **32:**918–923.
14. **Pierre, D., D. Lecossier, Y. Boussougant, D. Bocart, V. Joly, P. Yeni, and A. J. Hance.** 1991. Use of a reamplification protocol improves sensitivity of detection of *Mycobacterium tuberculosis* in clinical samples by amplification of DNA. J. Clin. Microbiol. **29:**712–717.
15. **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.
16. **Soini, H., M. Skurnik, K. Lioppo, 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.
17. **Winder, F. G.** 1982. Mode of action of the antimycobacterial agents and associated aspects of the moleculair biology of the mycobacteria, p. 353–438. *In* C. Ratledge and J. Stanford (ed.), The biology of the mycobacteria, vol. 1. Academic Press, Inc., New York.
18. **Wolinsky, E.** 1992. Statement of the Tuberculosis Committee of the Infectious Diseases Society of America. Clin. Infect. Dis. **16:**627–628.