JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 4138–4142 Vol. 40, No. 11
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.11.4138–4142.2002

Performance Assessment of Two Commercial Amplification Assays for Direct Detection of *Mycobacterium tuberculosis* Complex from Respiratory and Extrapulmonary Specimens

Claudio Piersimoni,[1]* Claudio Scarparo,[2] Paola Piccoli,[2] Alessandra Rigon,[2] Giuliana Ruggiero,[2] Domenico Nista,[1] and Stefano Bornigia[1]

Department of Clinical Microbiology, General Hospital Umberto I-Torrette, Ancona,[1] and Regional Mycobacteria Reference Centre, San Bortolo Hospital, Vicenza,[2] Italy

Received 11 February 2002/Returned for modification 30 June 2002/Accepted 16 August 2002

The new BDProbeTec ET *Mycobacterium tuberculosis* Complex Direct Detection Assay (DTB) was compared with the enhanced *M. tuberculosis* Amplified Direct Test (AMTDII). The system is an automated walkaway system characterized by simultaneous DNA amplification (strand displacement amplification) and real-time fluorometric detection. It also contains an internal amplification control (IAC) designed to identify inhibition from the processed samples. The AMTDII assay amplifies rRNA by transcription-mediated amplification; it uses hybridization with a chemoluminescent probe as a detection system and is entirely manual. A total of 515 *N*-acetyl-L-cysteine–sodium hydroxide-decontaminated respiratory ($n = 331$) and extrapulmonary ($n = 184$) sediments (from 402 patients) were tested in parallel by both assays. The results were compared with those of acid-fast staining and culture (solid plus liquid media), setting the combination of culture and clinical diagnosis as the "gold standard." Culture results from the tested specimens were as follows: 121 *Mycobacterium tuberculosis* complex (MTB) (98 smear-positive), 46 nontuberculous mycobacteria (38 smear-positive), and 338 culture-negative results. After resolution of the discrepant results, the percent sensitivity, percent specificity, and positive and negative likelihood ratios for AMTDII were 88%, 99.2%, 110, and 0.11 for respiratory specimens and 74.3%, 100%, 740, and 0.26 for extrapulmonary specimens, respectively. The corresponding values for DTB were 94.5%, 99.6%, 235, and 0.05 for respiratory specimens and 92.3%, 100%, 920, and 0.07 for extrapulmonary specimens, respectively. The cumulative difference for all tuberculosis-positive extrapulmonary specimens was significant ($P = 0.03$). The overall inhibition rate for DTB was 5% (26 specimens). We conclude that both amplification assays proved to be rapid and specific for the detection of MTB in clinical samples and particularly feasible for a routine laboratory work flow. DTB combines a labor-intensive specimen preparation procedure with a completely automated amplification and detection. Finally, differences between AMTDII and DTB sensitivities were associated with the presence of inhibitory samples that the former assay, lacking IAC, could not detect.

Tuberculosis (TB) continues to be a global public health problem. In industrialized countries, four main conditions are currently referred to as promoting the spread of TB: human immunodeficiency virus pandemic, immigration from high-TB-prevalence areas, the worsening of economic and social conditions (including an increase in homelessness), and the emergence of multidrug-resistant strains. Successful TB control depends on effective case finding and rapid detection of *Mycobacterium tuberculosis* complex (MTB). Conventional methods include staining smears for acid-fast bacilli (AFB) and culture by liquid and solid media. However, AFB staining lacks sensitivity and specificity, whereas culture results are usually not available earlier than 2 to 3 weeks.

Because of their theoretical ability to detect a single organism, nucleic acid amplification (NAA) methods have the potential to reduce detection and identification time to within hours of sample collection. In order to address these problems and to enhance gradual application of amplification technologies into clinical microbiology laboratories, companies have developed kit-based NAA tests specific for MTB. They include PCR (Cobas Amplicor *Mycobacterium tuberculosis* System; Roche Diagnostic Systems, Inc., Branchburg, N.J.) and rRNA-based transcription-mediated amplification (Amplified *Mycobacterium tuberculosis* Direct Test [AMTDII]; Gen-Probe, Inc., San Diego, Calif.), both of which have been approved by the U.S. Food and Drug Administration, ligase chain reaction (LCx *Mycobacterium tuberculosis* assay; Abbott Diagnostic Division, Abbott Park, Ill.) and, more recently, strand displacement amplification (SDA; BDProbeTec ET *Mycobacterium tuberculosis* Complex Direct Detection Assay [DTB]; Becton Dickinson Biosciences, Sparks, Md.).

SDA is a novel DNA amplification method in which segments of the insertion sequence IS*6110* specific to MTB, together with a sequence of the 16S rRNA gene common to most mycobacterial species, are amplified isothermally (11). DTB is a fully automated walkaway system which couples SDA to a fluorescent-energy transfer (hence the "ET" definition) detection system in a closed, high-throughput assay format. An internal amplification control (IAC), designed to detect the presence of inhibiting substances, is run with each sample.

The purpose of the present study was to carry out a com-

* Corresponding author. Mailing address: Department of Clinical Microbiology, General Hospital Umberto I-Torrette, Via Conca, Ancona I-60020, Italy. Phone: 39-071-596-3049. Fax: 39-071-596-4184. E-mail: piersim@tin.it.

parative evaluation of the DTB assay and the U.S. Food and Drug Administration-approved AMTDII.

MATERIALS AND METHODS

Study design. A total of 515 clinical specimens, consecutively received for AFB culture by two Italian microbiology laboratories (Center 1, Vicenza; Center 2, Ancona) entered the study. Specimens, which were almost entirely collected from in-patients screened for TB on the basis of different levels of clinical suspicion, were submitted to both laboratories from different hospitals within the respective regions.

Specimen collection and processing. Investigated specimens collected from 402 patients included 211 sputum samples, 14 bronchoalveolar lavage samples (BAL), 106 bronchial washings, 16 gastric aspirates, 46 urine samples, 57 samples from normally sterile body fluid (pleural, pericardial, synovial, and cerebrospinal [CSF] fluids and ascites), and 65 miscellaneous samples such as pus and biopsy specimens.

Respiratory specimens were liquefied and decontaminated by the standard *N*-acetyl-L-cysteine–sodium hydroxide (NALC-NaOH) method (13). Extrapulmonary specimens such as urine, gastric aspirates (which were neutralized upon receipt with 0.067 M phosphate-buffered saline [pH 6.8; PBS]), and pleural and other similar body fluids (pericardial fluid, synovial fluid, and ascites) were centrifuged at 3,500 × *g* for 15 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 10 ml of sterile water and decontaminated by NALC-NaOH. Part of the sediment from each specimen was inoculated onto the culture media and used for acid-fast staining, whereas the remaining portion was aliquoted and stored at −80°C until the amplification techniques were performed. CSF specimens were cultured without prior decontamination.

Pretreatment of selected clinical specimens for amplification. (i) Pretreatment of CSF. CSF was treated by NALC-NaOH and centrifuged at 12,000 × *g* for 10 min. The pellet was resuspended in PBS and frozen in aliquots until the amplification techniques were performed.

(ii) Pretreatment of pleural and other sterile fluids. After decontamination by NALC-NaOH, the sediment was washed twice with sterile distilled water before it was stocked for amplification assays.

Culture. A 0.5-ml portion of the processed sediment was cultivated by radiometric Bactec technique (Becton-Dickinson Diagnostic Instrument Systems) and by Löwenstein-Jensen solid medium. All media were incubated for 6 weeks at 36 ± 1°C. Bactec 12B culture vials were tested for growth twice a week for the first 3 weeks and weekly thereafter. The radiometric growth index was recorded by the Bactec instrument; a radiometric growth index of >50 was considered suspect, and smears were made daily to confirm the presence of AFB.

Löwenstein-Jensen slants were inspected weekly for growth, and acid fastness from suspect colonies was confirmed by the Ziehl-Neelsen staining.

Microscopy. To detect AFB, smears were stained with the auramine-rhodamine fluorescent stain.

Identification of mycobacteria. Isolates were identified by specific DNA probes assays (Accuprobe [Gen-Probe] and INNO LiPA Mycobacteria [Innogenetics, Ghent, Belgium] [15]), by standard biochemical tests, and by high-pressure liquid chromatography (5).

Amplification procedures. Amplification assays were run in three separate areas which had been set up in two different rooms.

Gen-Probe AMTDII. The Gen-Probe AMTDII assay was performed according to the instructions supplied by the manufacturer. Each run included positive and negative amplification controls: the former being prepared from a 10^{-4} to 10^{-5} dilution of a 1 McFarland nephelometric standard suspension of *M. tuberculosis* ATCC 27294, whereas the latter was made from a similarly prepared suspension of *M. gordonae* ATCC 14470. Although a cutoff value of 30,000 relative light units (RLU) was set by the manufacturer for positive specimens, their mean values are usually greater than 2,000,000 RLU. Therefore, to tentatively detect inhibiting substances, the following categories of samples were retested after 1:5 dilution with PBS: (i) samples whose results fell between 30,000 and 500,000 RLU (within 48 h from the first run) and (ii) discrepant samples showing AMTDII-negative results (retrospectively).

DTB. The test was done according to the instructions supplied by the manufacturer. The procedure, starting from a 500-μl sediment sample portion, consisted of two steps: specimen preparation and combined, fully automated amplification and detection. During preparation, samples were added to sample wash buffer to remove possible inhibitors and then centrifuged and heated at 105°C for 30 min (BDProbetc ET oven). The pellet was then resuspended in sample lysis buffer and sonicated for 45 min at 65°C in a water sonic bath (Branson Ultrasonic Corp., Danbury, Conn.). After the addition of sample neutralization buffer, the samples were ready for assay. For each assay, one positive and one negative control were prepared. Corresponding numbers of priming and amplification microwells were placed into two different plates; the first ones contained amplification primers, fluorescence-labeled detector, and other reagents, whereas the second ones contained two enzymes (DNA polymerase and restriction endonuclease) necessary for SDA. By using a programmable eight-channel pipettor, samples and controls were dispensed into priming microwells that were incubated at room temperature for 20 min and then placed into a 71.5°C heating block. Meanwhile, amplification microwells were placed into a 53.5°C heating block for prewarming. After 10 min, samples from the priming microwells were transferred into the corresponding amplification microwells, which were promptly sealed and immediately placed in the DTB instrument. Once samples were introduced to the instrument, amplification and detection assay was completed within 1 h. An IAC built into the priming microwell and containing a DNA sequence identical to that of the MTB target sequence was run with each amplification reaction and coamplified with the possible DNA target from the clinical specimen. Samples showing an MTB MOTA ("metric other than acceleration" values greater than 3,400 were considered positive for MTB regardless of the IAC values. If the MTB MOTA was less than 3,400 and the corresponding IAC MOTA was greater than 5,000, the specimen was considered negative. Finally, if the MTB MOTA was less than 3,400 and the corresponding IAC MOTA was less than 5,000, the result was regarded as indeterminate and the processed sample was retested within 48 h after 1:5 dilution with PBS. In addition, a procedural control was also run weekly by testing a suspension of a 10^{-4} to 10^{-5} dilution of a 1 McFarland nephelometric standard suspension of *M. tuberculosis* ATCC 27294.

Patients' clinical evaluation. Clinical assessment included the patients' medical history, signs, symptoms, chest X-ray, pathology, and microbiology results, as well as follow-up observations. All of the records were carefully reviewed in order to set up the combination of culture and clinical diagnosis as the "gold standard." Two categories of samples were considered as true positives: (i) samples that were culture positive for MTB and (ii) samples that were culture negative for MTB but either they originated from a patient whose other samples were culture positive or else the patient's clinical history provided enough evidence of TB to initiate antituberculous chemotherapy. After this analysis, amplification results were reclassified as appropriate.

Statistical analysis. Statistical comparisons were calculated by using the chi-square test; $P < 0.05$ was considered significant. AMTDII-negative specimens which turned out positive when frozen aliquots were tested retrospectively were considered as negatives according to their first assay, whereas DTB specimens showing IAC inhibition and a subsequent positive result upon repeat testing were considered as positives. Moreover, samples which remained inhibited by DTB assay despite dilution were considered uninterpretable and therefore excluded from calculations. Sensitivity, specificity, and likelihood ratios (LRs) were determined accordingly. Calculation of LRs involves answering the following two questions: (i) how likely is it to obtain a given test result among people with the target disorder (positive LR) and (ii) how likely is it to obtain the same test result among people without the target disorder (negative LR). LRs were adopted since they are known to provide the same information as predictive values without being subject to the bias of an uneven disease prevalence, as may occur in the population of a multicenter study (10).

RESULTS

Respiratory specimens. A total of 331 respiratory specimens collected from 253 patients were tested. Altogether, 126 specimens yielded culture-positive results for AFB; 91 isolates were found to belong to the MTB, whereas the remaining 35 strains were identified as nontuberculous species. A comparison of amplification results, with smears, cultures, and clinical data, is presented in summary form in Table 1. A total of 92 specimens were from patients with TB, and 239 specimens were from patients with nontuberculous pulmonary disease, as determined based on clinical and microbiological findings.

Of the 76 samples that were smear and culture positive, 71 were AMTDII positive and 75 were DTB positive. Fifteen samples were smear negative for AFB but culture positive; 10 were AMTDII positive and 11 were DTB positive. For both groups of tested samples the differences were statistically not significant. There was one sample both smear and culture

TABLE 1. Respiratory specimens: comparison of AMTDII and DTB amplification assays with smear and culture results and clinical data[a]

Patient status	No. of specimens	No. positive by AMTDII	No. positive by DTB	P
Smear and culture positive	76	71 [5]	75	NS
Smear negative, culture positive	15	10 [2]	11 (1)	NS
Smear and culture negative with TB final diagnosis	1	0 [1]	1	NS
All TB positive	92	81 [8]	87 (1)	NS
Smear and culture negative, non-TB pulmonary disease	207	0	1	NS
Isolation of NTM	32	2	0	NS

[a] Values in brackets represent the number of specimens that turned out to be positive when retrospectively tested after dilution. Values in parentheses represent the number of specimens showing a negative amplification control that turned out to be positive when retested after dilution.

TABLE 2. Extrapulmonary specimens: comparison of AMTDII and DTB amplification assays with smear and culture results and clinical data[a]

Patient status	No. of specimens	No. positive by AMTDII	No. positive by DTB	P
Smear and culture positive	22	21 [1]	22	NS
Smear negative, culture positive	8	3 [2]	7 (1)	NS
Smear and culture negative with TB final diagnosis	9	5 [3]	7	NS
All TB positive	39	29 [6]	36 (1)	0.03
Smear and culture negative, non-TB pulmonary disease	134	0	0	NS
Isolation of NTM	11	0	0	NS

[a] See Table 1, footnote *a*.

negative that was collected from a patient strongly suspected of pulmonary TB. This sample was AMTDII negative and DTB positive. The cumulative difference for all TB-positive specimens (81 positive by AMTDII and 87 positive by DTB) was statistically not significant. A total of 11 discrepant samples (AMTDII negative but collected from TB patients) were tested retrospectively after dilution. Eight samples were determined to be positive, whereas the others remained negative. On the other hand, one specimen tested by DTB assay showed IAC inhibition and was determined to be positive on repeat testing. Of the 207 samples from patients with nontuberculous pulmonary disease that were smear and culture negative for AFB, none was found to be positive by AMTDII and 1 was found to be positive by DTB assay. In all, 32 specimens (31 were smear positive) from 23 patients grew nontuberculous mycobacteria (NTM). The following species were identified: *M. chelonae* ($n = 10$), *M. avium* complex ($n = 7$), *M. malmoense* ($n = 6$), *M. gordonae* ($n = 5$), *M. fortuitum* ($n = 3$), and *M. kansasii* ($n = 1$). All of these specimens except two were found to be negative by both assays. These samples, collected from the same patient and growing *M. malmoense*, were found to be positive by AMTDII.

After resolution of discrepant results and assuming the combination of culture and clinical diagnosis as the gold standard, the percent sensitivity, the percent specificity, and the positive and negative LRs (PLR and NLR) for AMTDII were 88%, 99.2%, 110, and 0.11, respectively, whereas the corresponding values for DTB were 94.5%, 99.6%, 235, and 0.05, respectively.

Extrapulmonary specimens. A total of 184 extrapulmonary specimens collected from 149 patients were tested. Altogether, 41 specimens were culture positive for AFB and 30 isolates were found to belong to the MTB, whereas the remaining 11 strains were identified as nontuberculous species. A comparison of amplification results with smears, cultures, and clinical data is summarized in Table 2. A total of 39 specimens were from patients with extrapulmonary TB and 134 were from nontuberculous patients based on the clinical and microbiological findings.

Of the 22 samples that were smear and culture positive, 21 were AMTDII positive and 22 were DTB positive (the difference was statistically not significant). Eight samples were smear negative for AFB but culture positive; three were AMTDII positive, and seven were DTB positive (the difference was statistically not significant). There were nine samples, both smear and culture negative, collected from patients in whom TB was strongly suspected clinically. Of these, five were AMTDII positive and seven were DTB positive. The cumulative difference for all TB-positive specimens was statistically significant ($P = 0.03$). As previously reported, a total of 10 discrepant samples (AMTDII negative but collected from TB patients) were tested retrospectively after dilution. Six samples turned out to be positive, whereas the others remained negative. On the other hand, one specimen tested by DTB assay showed IAC inhibition and turned out to be positive on repeat testing. Of the 134 samples from patients with nontuberculous disease that were smear and culture negative for AFB, none was found to be positive by both the assays. Eleven specimens (seven of which were smear positive) from nine patients grew NTM. The following species were identified: *M. avium* complex ($n = 5$), *M. gordonae* ($n = 5$), and *M. kansasii* ($n = 1$). All of these specimens were found to be negative by both amplification assays.

After resolution of discrepant results assuming the combination of culture and clinical diagnosis as the gold standard, the sensitivity, the specificity, the PLR, and the NLR for AMTDII were 74.3%, 100%, 740, and 0.26, resepctively, whereas the corresponding values for DTB were 92.3%, 100%, 920, and 0.07, respectively.

When the results of both of the assays were evaluated for each patient (Table 3), a significant difference was found ($P = 0.046$). AMTDII allowed a correct diagnosis in 72 of 85 TB patients, whereas 80 of 85 were correctly detected by DTB.

TABLE 3. Evaluation of both amplification assays according to the number of patients with a conclusive diagnosis of TB ($P = 0.046$)

Test and result	No. of patients with conclusive diagnosis of TB	
	Positive	Negative
AMTDII		
Positive	72	2
Negative	13	315
DTB		
Positive	80	1
Negative	5	316

Specimen inhibition. Since all specimens were not tested for inhibition, an accurate inhibition rate could not be calculated for AMTDII. Nevertheless, according to the criteria adopted for specimen selection, the AMTDII inhibition rate was 5.0%, ranging from 4.8 to 5.4% for respiratory and extrapulmonary specimens, respectively. The DTB overall inhibition rate was 5.0%, ranging from 4.2 to 6.5% for respiratory and extrapulmonary specimens, respectively. A proper dilution was able to overcome inhibition in 17 of 26 (65.4%) samples tested by the DTB assay.

DISCUSSION

The major difference between TB and other mycobacterial infections is that MTB is spread from person to person. For this reason, it is particularly important to diagnose TB as early as possible, and therefore new technological developments making this possible are of great importance. In this context, an ideal rapid diagnostic test should have some desirable attributes. Top priority should be given to sensitivity and specificity. However, in evaluating the suitability of such a new test, it is important to remember that the test also needs to be technically simple, automated, and easy to fit into the lab workflow, possibly with the ability to detect inhibiting substances. Additional attributes would include random access, data management capabilities, and other options such as differentiation between live and dead mycobacteria and simultaneous evaluation of possible drug resistance.

The present study demonstrates that both of the amplification tests considered here can detect MTB in clinical samples within a few hours, fulfilling some if not all of the above reported attributes. Although entirely manual, the AMTDII turnaround time is shorter than that of DTB (2.5 versus 3.5 to 4 h). The first part of the DTB procedure, which is variable in duration according to the number of specimens being prepared for amplification, is more labor-intensive; thereafter, the assay is nearly completely automated. Both kits, containing all of the reagents needed for sample amplification and detection, were easy to perform and appeared to fit well in the work flow of a reference laboratory performing amplifications two or three times a week. From an analytical point of view, differences among cutoff values, positive and negative controls, and samples were broad enough to allow easy discrimination by both assays. In fact, mean AMTDII values for negative and positive respiratory and positive extrapulmonary specimens were 4,316 RLU (range, 2,225 to 18,648), 2,419,922 RLU (range, 50,000 to 3,508,265), and 2,130,579 RLU (range, 30,881 to 3,553,685), whereas the corresponding values for DTB were 122 MTB MOTA (range, 0 to 442), 57,738 MTB MOTA (range, 17,116 to 101,622), and 50,456 MTB MOTA (range, 8,735 to 91,852), respectively.

In comparison with the gold standard, the sensitivity and specificity of AMTDII were 88 and 99.2% for respiratory specimens and 74.3 and 100% for extrapulmonary specimens, respectively. In agreement with our previous findings (14), negative results obtained from smear-positive and smear-negative, MTB-yielding samples were shown to depend almost entirely on the presence of inhibitors of enzymatic amplification. This held true also for four smear- and culture-negative samples collected from patients highly suspect for TB. In addition, nine smear-positive MTB-yielding samples (seven respiratory and two extrapulmonary), whose results fell between 30,000 and 500,000 RLU (mean, 241,280) were retested after dilution, showing a mean of 2,207,443 RLU. These samples were considered as true positives from the outset and therefore were not reported in brackets in Tables 1 and 2. Although specimen inhibition rates for both assays were found to be the same, it is likely that a regular search for inhibitors in all AMTDII tested samples would detect a considerably higher one. It is now well established that the larger the specimen volume (it was increased from 50 to 450 μl in the "enhanced" version of AMTD), the higher the test sensitivity, but this also means more frequent the exposure to inhibiting substances (9). Inhibition was shown to affect the majority of kit-based amplification assays (6, 12). Various attempts have been made to remove inhibitors (3, 4; P. Singer and F. Vlaspolder, Abstr. 20th Annu. Conf. Eur. Soc. Mycobacteriol., p. 76, 1999). Inexpensive methods such as dilution or the addition of bovine albumin have been shown to be partially effective, whereas other methods, although highly effective, are too expensive or do not appear to fit easily in the routine work flow. Moreover, inhibiting substances probably affect amplification techniques unevenly so that different procedures may be required according to the amplification technique adopted in each lab (8). In this context, some authors (14; P. Della Latta and M. Saragias, Abstr. 21st Annu. Cong. Eur. Soc. Mycobacteriol., p. 11, 2000) using AMTDII have suggested reducing the sample volume to 225 μl or performing a preliminary dilution. This approach has allowed them to reduce the inhibition rate especially in respiratory samples.

The sensitivity and specificity of DTB were 94.5 and 99.6% for respiratory specimens and 92.3 and 100% for extrapulmonary specimens, respectively. No significant differences in sensitivity between respiratory and extrapulmonary specimens were observed. Literature data about DTB sensitivity and specificity are in partial agreement with our findings (e.g., 92.4 and 96.4% [85.1 and 94.4% for smear-negative samples] [S. Rusch-Gerdes, Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000, abstr. U-68, p. 657, 2000], 93.8 and 99.8% [2], and 80 and 98.9%, respectively [P. Della Latta, M. Saragias, and L. Karapici, Abstr. 22nd Annu. Cong. Eur. Soc. Mycobacteriol., p. 26, 2001]). In the last of these studies, the authors, in a comparative evaluation between AMTDII and DTB on respiratory samples, reported a superior sensitivity with AMTDII despite a 10-fold-higher inhibition rate.

For smear-negative, culture-positive noninhibitory samples (four respiratory samples and one CSF sample), a low number of mycobacteria, unequally distributed in the test suspension or a suboptimal target extraction, are perhaps the most likely explanations for a negative DTB result. The problem of smear-negative pulmonary TB is worth particular attention. In acute-care settings, as many as 8 to 10 patients are suspected to have TB for every one confirmed case (16). Moreover, patients with smear-negative, culture-positive TB have been reported to be responsible for ca. 17% of TB transmission (1). In addition, although many investigators have used NAA to test extrapulmonary specimens with encouraging results (17), the sensitivity of this method is still far from ideal when routinely applied to clinical settings whose proper evaluation may be crucial for a patient's outcome. Of the 10 samples (9 respiratory and 1

extrapulmonary) that were both smear and culture negative and were obtained from patients strongly suspected of having TB, 5 were found to be AMTDII positive and 8 were found to be DTB positive. Finally, we observed an overall inhibition rate of 5.0%, and no significant difference between respiratory (4.2%) and extrapulmonary (6.5%) specimens was found. False-negative inhibitory samples were easily detected and were soon reclassified as true positive or, when repeatedly inhibitory, as noninterpretable. In this context, none of these samples was shown to be positive for MTB by culture or clinical diagnosis. The negative result obtained by DTB assay for one smear-positive, MTB-yielding, noninhibitory sample remains unexplained.

The LRs indicate by how much a diagnostic test result will raise or lower the pretest probability of the target disease. Therefore, LRs of >1 increase this probability: the higher the LR, the greater the increase. Conversely, LRs of <1 decrease the probability of the target disease: the smaller the LR, the greater the decrease in probability and the smaller its final value. In this context, PLRs of both tests seem to generate conclusive changes from pretest to posttest probabilities (LRs of >10 or <0.1). NLRs appear to generate conclusive changes from pretest to posttest probabilities for DTB assay only, whereas with AMTDII only moderate shifts in pre- to posttest probabilities are shown (LRs of 5 to 10 or of 0.1 to 0.2). In other words, positive results obtained by both assays are fully reliable, whereas negative ones are more trustworthy for DTB than for AMTDII.

We can conclude that, although at present amplification assays cannot replace culture techniques, AMTDII and DTB proved to be rapid and specific for the detection of MTB in clinical samples. The protocols were easy to perform and were suitable for a routine microbiology laboratory's work flow. In agreement with our previously reported data, the difference between DTB and AMTDII sensitivities was found to depend mainly on the availability of IAC lacking in the latter assay. Finally, selection of patients on the basis of clinical judgment (7), proper number and quality of specimens, staff training, and quality control procedures, including IAC are currently referred to as the best ways to improve the sensitivity and specificity of commercial NAA tests.

ACKNOWLEDGMENT

We thank Carlo Costa (Diagnostic Systems Microbiology, Becton Dickinson Italia, S.p.A.) for kindly providing reagents and instrumentation.

REFERENCES

1. **Behr, M. A., S. A. Warren, H. Salomon, P. C. Hopewell, A. Ponce de Leon, C. D. Daley, and P. M. Small.** 1999. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. Lancet **353:**444–449.
2. **Bergmann, J. S., W. E. Keating, and G. L. Woods.** 2000. Clinical evaluation of the BDProbeTec ET System for rapid detection of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **38:**863–865.
3. **Böddinghaus, B., T. A. Wichelhaus, V. Brade, and T. Bittner.** 2001. Removal of PCR inhibitors by silica membranes: evaluating the Amplicor *Mycobacterium tuberculosis* kit. J. Clin. Microbiol. **39:**3750–3752.
4. **Burkardt, H. J.** 2000. Standardization and quality control of PCR analyses. Clin. Chem. Lab. Med. **38:**87–91.
5. **Butler, W. R., and L. S. Guthertz.** 2001. Mycolic acid analysis by high-performance liquid chromatography for identification of *Mycobacterium* species. Clin. Microbiol. Rev. **14:**704–726.
6. **Carpentier, E., B. Drouillard, M. Dailloux, D. Moinard, E. Vallee, B. Dutilh, J. Maugein, E. Bergogne-Berezin, and B. Carbonelle.** 1995. Diagnosis of tuberculosis by Amplicor *Mycobacterium tuberculosis* test: a multicenter study. J. Clin. Microbiol. **33:**3106–3110.
7. **Catanzaro, A., S. Perry, J. E. Clarridge, S. Dumbar, S. Goodnight-White, P. A. LoBue, C. Peters, G. E. Pfyffer, M. F. Sierra, R. Weber, G. Woods, G. Mathews, V. Jonas, K. Smith, and P. Della-Latta.** 2000. The role of clinical suspicion in evaluating a new diagnostic test for active tuberculosis: result of a multicenter prospective trial. JAMA **283:**639–645.
8. **Della Latta, P.** 1999. The mycobacteriology milestones: journeying to the new millennium. Lab. Med. **30:**408–417.
9. **Gamboa, F., G. Fernandez, E. Padilla, J. M. Manterola, J. Lonca, P. J. Cardona, L. Matas, and V. Ausina.** 1998. Comparative evaluation of initial and new versions of the Gen-Probe amplified *Mycobacterium tuberculosis* direct test for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J. Clin. Microbiol. **36:**684–689.
10. **Giocoli, G.** 2000. Evidence-based clinical microbiology. J. Clin. Microbiol. **38:**3520–3521.
11. **Little, M. C., J. Andrews, R. Moore, S. Bustos, L. Jones, C. Embres, G. Durmovicz, J. Harris, D. Berger, K. Yanson, C. Rostkowsky, D. Yursis, J. Price, T. Fort, A. Walters, M. Collis, O. Llorin, J. Wood, F. Failing, C. O'Keefe, B. Scrivens, B. Pope, T. Hansen, K. Marino, K. Williams, and M. Boenisch.** 1999. Strand displacement amplification and homogeneous real-time detection incorporated in a second-generation DNA probe system, BDProbeTecET. Clin. Chem. **45:**777–784.
12. **Lumb, R., K. Davies, D. Dawson, R. Gibb, T. Gottlieb, C. Kershaw, K. Kociuba, G. Nimmo, N. Sangster, M. Worthington, and I. Batian.** 1999. Multicenter evaluation of the Abbott LCx *Mycobacterium tuberculosis* ligase chain reaction assay. J. Clin. Microbiol. **37:**3102–3107.
13. **Nolte, F., and B. Metchock.** 1995. *Mycobacterium*, p. 400–437. *In* P. R. Murray, E. J. O. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology. ASM Press, Washington, D.C.
14. **Scarparo, C., P. Piccoli, A. Rigon, G. Ruggiero, M. Scagnelli, and C. Piersimoni.** 2000. Comparison of enhanced *Mycobacterium tuberculosis* amplified direct test with COBAS AMPLICOR *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extrapulmonary specimens. J. Clin. Microbiol. **38:**1559–1562.
15. **Tortoli, E., A. Nanetti, C. Piersimoni, P. Cichero, C. Farina, G. Mucignat, C. Scarparo, L. Bartolini, R. Valentini, D. Nista, G., Gesu, C. Passerini-Tosi, M. Crovatto, and G. Brusarosco.** 2001. Performance assessment of a new multiplex probe assay for identification of mycobacteria. J. Clin. Microbiol. **39:**1079–1084.
16. **Wisnivesky, J. P., J. Kaplan, C. Henschke, T. G. McGinn, and R. G. Crystal.** 2000. Evaluation of clinical parameters to predict *Mycobacterium tuberculosis* in inpatients. Arch. Intern. Med. **160:**2471–2476.
17. **Woods, G. L., J. S. Bergmann, and N. Williams-Bouyer.** 2001. Clinical evaluation of the Gen-Probe amplified *Mycobacterium tuberculosis* direct test for rapid detection of *Mycobacterium tuberculosis* in select nonrespiratory specimens. J. Clin. Microbiol. **39:**747–749.