Clinical utility of the Gen-Probe amplified *Mycobacterium tuberculosis* direct test compared with smear and culture for the diagnosis of pulmonary tuberculosis

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Objective: To evaluate the clinical efficacy of the Gen-Probe amplified *Mycobacterium tuberculosis* direct test (AMTD), a recently developed amplification test for the detection of *M. tuberculosis* complex directly from clinical specimens, for the diagnosis of pulmonary tuberculosis and its suitability for use in a routine microbiology laboratory.

Methods: Sequential respiratory specimens were tested with AMTD and results were compared with those of acid-fast stain and culture. Performance of AMTD was tested over a 13-month period, using 278 respiratory specimens, from 219 patients, submitted to the microbiology laboratory of our hospital. AMTD's sensitivity, specificity and positive and negative predictive values were determined, with the combination of culture and clinical diagnosis being taken as the standard.

Results: Thirty-three specimens were collected from 23 patients with a conclusive diagnosis of pulmonary tuberculosis. Of these specimens, 13 were smear positive, 22 culture positive and 30 AMTD positive. AMTD was more sensitive in detecting pulmonary tuberculosis in patients partially treated but with undiagnosed disease (100%), and in smear-positive disease (100%). The overall sensitivities, specificities and positive and negative predictive values were: 39.4%, 100%, 100%, and 92.4% for staining; 66.7%, 100%, 100% and 95.7% for culture; and 90.9%, 100%, 100%, and 98.8% for AMTD.

Conclusions: AMTD is a rapid, reliable and accurate test for the detection of *M. tuberculosis* complex in respiratory specimens. Repeat testing of those samples whose results fall between 30 000 and 300 000 relative light units, increases test specificity by preventing the majority of false positives.

Key words: rRNA amplification assay, direct detection, pulmonary tuberculosis, diagnosis of partially treated patients

INTRODUCTION

Over the past two decades, available therapies for the management of pulmonary tuberculosis have improved considerably. The introduction of new regimens (short-course and intermittent chemotherapy) has proved effective in reducing the load and cost of drugs [1], ensuring patient compliance [2] and lowering failure/relapse rates [3]. In addition, direct observation of therapy has been shown to reduce non-compliance rates [4]. Despite this, tuberculosis is on the increase throughout the world [5] and remains one of the few infectious diseases whose diagnosis often relies on clinical suspicion.

The majority of routine clinical laboratories still depend on acid-fast bacilli (AFB) smear and culture, whose limitations are well known. Microscopy,

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although quick and easy, has poor sensitivity [6,7], while culture on solid media is more specific and sensitive, but can take several weeks to be carried out. More recently, the use of liquid media in conjunction with nucleic acid probes (Accuprobe, Gen-Probe Inc., San Diego, USA) has considerably shortened detection time [6,7], but even these procedures require a minimum of 2 weeks before a definitive laboratory diagnosis of tuberculosis can be made. Recent studies have focused on the rapid, direct detection of *Mycobacterium tuberculosis* for initial diagnosis of pulmonary tuberculosis [8,9]. Different assays which amplify either DNA or RNA have been used for this purpose. Some commercially available kits, employing standardized nucleic acid-based amplification techniques, can yield reliable results within 5–7 h of sample processing [10–13]. The Gen-Probe amplified *M. tuberculosis* direct test (AMTD) (Gen-Probe Inc., San Diego, USA) is a diagnostic test based on the isothermal amplification of rRNA via DNA intermediates, with detection of the amplified product by an acridinium-ester-labeled DNA probe. The aim of the present study was to evaluate the utility of this test for the diagnosis of pulmonary tuberculosis.

MATERIALS AND METHODS

Specimen collection and processing

Two hundred and seventy-eight sequential respiratory tract specimens from 219 patients, including expectorated or induced sputa, gastric aspirates and bronchoscopy specimens (bronchial aspirates and bronchoalveolar lavages), collected by the Clinical Microbiology Department of the Umberto I°-Torrette Hospital between October 1995 and November 1996, were included in the study. All specimens were liquefied with *N*-acetyl-L-cysteine and decontaminated with NaOH (final concentration 1.5%). After decontamination, an equal volume of phosphate buffered saline (PBS) of pH 6.8 was added, and the specimens were centrifuged at 3500*g* for 20 min at 4°C. The sediment was resuspended in 2 mL of PBS and neutralized with 1 M HCl. Part of the sediment from each specimen was inoculated on the culture medium and used for acid-fast staining, while the remainder was stored at 4°C for no more than 48–72 h until the amplification assay was performed.

Culture

The processed sediment (0.5 mL) was cultivated by a radiometric Bactec technique (Becton-Dickinson Diagnostic Instrument Systems, USA) and by MB-Check AFB culture bottles (Becton Dickinson Microbiology Systems, USA) [6,7]. In addition, 0.2 mL of the sediment was inoculated on Lowenstein–Jensen (LJ) medium. The LJ tubes and MB-Check AFB bottles were incubated at 35–37°C for 8 weeks and inspected for growth twice a week for the first 4 weeks and weekly thereafter. The radiometric growth index of cultures grown in the Bactec instrument was automatically recorded twice a week for 6 weeks. A growth index of >10 was considered to be positive, and smears were made to confirm the presence of acid-fast bacilli (AFB).

Microscopy

Smears were stained by the Ziehl–Neelsen method and examined under the oil-immersion objective lens of the microscope (×1000).

Identification of mycobacteria

Isolates were identified by specific DNA probes (Accuprobe Gen-Probe Inc., San Diego, USA) and by standard procedures [14].

Gen-Probe AMTD procedure

The amplification assay was run in three separate areas, set up in two different rooms to avoid the possibility of contamination. The Gen-Probe AMTD assay (Gen-Probe Inc., San Diego, USA) was performed in accordance with instructions supplied by the manufacturer. Briefly, a 50-μL aliquot of sediment was added to a tube containing glass beads and sample buffer and sonicated for 15 min in a water-bath sonicator at room temperature. A 50-μL aliquot of lysate was added to a tube containing 25 μL of amplification reagent and 200 μL of oil. The tube was incubated at 95°C for 15 min and then cooled to 42°C for 5 min. An enzyme reagent mix was added, and the mixture was incubated at 42°C for 2 h. Termination reagent was added, and the reaction mixture was further incubated at 42°C for 10 min. For detection, a specific labeled hybridization probe was added to the tube and incubated at 60°C for 15 min, followed by addition of a selection reagent and incubation at 60°C for a further 10 min. Samples were read in a Leader 50 luminometer (Gen-Probe, Inc., San Diego, USA), and a cut-off value of 30 000 relative light units (RLU), corresponding to approximately 40 mycobacteria, was used for positive specimens [15]. Each run included positive and negative amplification controls as well as positive and negative hybridization controls. Moreover, two smear-positive sediment samples of clinical origin, previously collected and stored at −80°C, were also included as controls in each run.

Patient clinical evaluation

Clinical assessment included the patient's medical history,

signs, symptoms, chest X-ray, microbiological results, and follow-up observations, as well as the results obtained from additional specimens collected prior to patient admission into hospital or during the follow-up. All patient records were reviewed by a tuberculosis expert and each was classified according to the American Thoracic Society (ATS) tuberculosis classification [16]. Thus, a combination of culture and clinical diagnosis was set as the standard. Following this analysis, AMTD discrepant results were reclassified as appropriate.

RESULTS

Clinical and microbiological data

Two hundred and seventy-eight specimens from 219 patients were collected during the study period. The specimens included 142 bronchial aspirates, 110 sputa, 20 gastric aspirates and six bronchoalveolar lavages. All patients except two were white, and the majority of them (151) were male. About 10% of the patients had identified risk factors for tuberculosis, such as HIV-positive status, intravenous drug use or homelessness.

Analytic performance of Gen-Probe AMTD assay and RLU values

Positive and negative results could be clearly distinguished by the magnitude of the RLU value. The majority of smear-positive, culture-positive samples for *M. tuberculosis* exceeded 1 700 000 RLU. Of the specimens collected from patients strongly suspected of having tuberculosis (smear negative, culture positive or smear and culture negative), 71% exhibited values with a greater magnitude, whereas 29% showed values <1 000 000 RLU (range 94 396–753 393 RLU). Samples with negative results had values far below the cut-off of 30 000 RLU, with 89.9% being <10 000 RLU. All negative values obtained upon AMTD repeat assay fell below 10 000 RLU.

Comparison of smear, culture and AMTD results

From the 278 respiratory specimens, four Bactec cultures grew nontuberculous mycobacteria, including *M. avium* (two isolates), *M. gordonae* (one isolate) and *M. terrae* (one isolate). In all these cases, AMTD remained negative.

From the clinical specimens tested, 20 were AMTD and culture positive and 221 were negative by both techniques. In total, there were 37 discrepant results: 31 were AMTD positive, but negative by culture, and for six specimens, all smear negative, the opposite was true. On the basis of these data, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of AMTD were 90.9%, 85.7%, 58.8%, and 98.6%, respectively.

Of the 31 results suspected to be false positive, 10 were from eight patients with pulmonary tuberculosis who had been partially treated, and still had undiagnosed disease (ATS class 5). In these cases, *M. tuberculosis* grew from samples collected prior to admission to our hospital and antibiotic therapy, or from additional sputum specimens. The remaining 21 specimens were from patients lacking any sign of active pulmonary tuberculosis. About 50% of these specimens showed RLU values of <100 000, ranging from 35 910 to 317 288 RLU (mean value 152 069 RLU). Upon repeat AMTD assay, all 21 samples were negative. This phenomenon has previously been reported in the literature by Pfyffer et al [17], who also suggested that repeat testing of specimens yielding weakly positive values helps to prevent the majority of false-positive results. According to this procedure, we decided to consider for final evaluation the results obtained upon repeat AMTD assay.

Of the six specimens from five patients suspected as being false negative, four grew non-tuberculous mycobacteria, so only two were considered to be true false negatives. A combination of three culture methods (Bactec 12B, MB-Check AFB bottles and LJ medium) was used in the comparison with AMTD. About 60% of samples grew on all three media: Bactec 12B performed the best, followed by MB-Check AFB and LJ medium. After the resolution of discrepancies, the sensitivity, specificity, PPV and NPV of AMTD increased to 90.9%, 100%, 100%, and 98.8%, respectively (Table 1). Distinguishing between smear-positive ($n=13$) and smear-negative ($n=265$) samples, uncorrected values for AMTD sensitivity were 100% and 86.9%, respectively. Specificities were 100% and 91.3%, respectively. For smear-negative samples, specificity increased to 100% after resolution of discrepant results.

The performances of smear, culture and AMTD for specimens collected from patients who entered the study with active tuberculosis (ATS class 3) or were strongly suspected of having tuberculosis (ATS class 5)

Table 1 Sensitivity, specificity and predictive values of various methods[a]

Method	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Smear	39.4	100	100	92.4
Culture	66.7	100	100	95.7
AMTD	90.9	100	100	98.8

[a]The combination of culture results and clinical diagnosis was taken as the standard.

Table 2 Comparison of smear, culture and AMTD results versus initial ATS classification

Specimens (n=278)	Smear				Culture				AMTD			
	0, 1, 2	3	4	5	0, 1, 2	3	4	5	0, 1, 2	3	4	5
Positive	0	13	0	0	4[a]	22	0	0	0[c] (21)[b]	20	0	10
Negative	235	9	10	11	231	0	10	11	235[c] (214)[b]	2	10	1

[a]These specimens grew MOTT. [b]Before discrepant results repeat. [c]After discrepant results repeat.

Table 3 AMTD compared with the standard (culture and clinical diagnosis)[a]

AMTD results	No. of patients with conclusive diagnosis of tuberculosis	
	Positive	Negative
Positive	21	0
Negative	2	196

[a]Data in the table were calculated after resolution of discrepant results.
Sensitivity, 91.3%; specificity, 100%; PPV, 100%; NPV, 98.9%.

are compared in Table 2. Twenty-two samples from 15 class 3 patients were obtained. Smear was positive in 13 samples, culture in 22 and AMTD in 20. There were 11 specimens from eight patients with suspected tuberculosis (ATS class 5) who ultimately were confirmed as having active disease. Surprisingly, AMTD was positive in 10 specimens, while smear and culture were both negative in all. When all samples were evaluated for each individual patient, taking the combination of culture and clinical diagnosis as the standard, AMTD performed very well, detecting 21 of 23 patients (91.3%) with active tuberculosis (Table 3).

DISCUSSION

This study evaluated the clinical utility of a commercially available amplification assay (Gen-Probe AMTD) for the rapid diagnosis of pulmonary tuberculosis. In our experience, 100% of patients with both AMTD- and smear-positive results had pulmonary tuberculosis. Similarly, when both tests were negative, tuberculosis could be excluded with 98.9% certainty. Nevertheless, amplification methods for smear-positive sputum specimens are still regarded as unnecessary. Recent studies have shown that even when *M. avium* is prevalent in the clinical setting owing to a large number of HIV-infected subjects, the presence of AFB in the sputum smear is almost entirely due to *M. tuberculosis* [18].

In this study, the overall sensitivity of AMTD equalled and sometimes exceeded that of culture. Unlike culture, however, AMTD results were available within a few hours. When amplification and smear results were in disagreement, AMTD diagnostic accuracy proved superlative. In smear-negative samples, AMTD sensitivity and PPV value were 86.9% and 100%, respectively. In particular, of the 11 smear- and culture-negative samples, obtained from eight patients strongly suspected of having tuberculosis (ATS class 5), 10 were AMTD positive. As these patients had been partially treated based exclusively on clinical suspicion, only AMTD-positive results, due to the shedding of non-culturable mycobacteria, were able to establish the correct diagnosis. Pulmonary tuberculosis could be confirmed in all the patients who grew *M. tuberculosis* from samples obtained prior to admission into our hospital, when they were still untreated, or from follow-up sputum specimens.

False negatives may be almost entirely due to the low number of mycobacteria unequally distributed in the test specimen, to the presence of amplification inhibitors or to low levels of rRNA amplification targets depending upon poor mycobacterial viability. There were 31 specimens with positive AMTD results, but with negative cultures. Of these, 10 turned out to be positive (Table 2), while the other 21 exhibited RLU values slightly above the cut-off and were true negatives upon retesting. In our opinion, all the samples showing positive results within a 10-fold limit of the proposed cut-off value (30 000 RLU) should be retested. This procedure did not increase the false-negative rate, because weakly positive results obtained from ATS class 3 patients were all confirmed as positive upon retesting. Such an empirical laboratory practice permitted us to avoid all the false positive results. These phenomena remain to be explained.

AMTD sensitivity, specificity, PPV and NPV were 90.9%, 100%, 100% and 98.8%, respectively. Our findings are in agreement with the literature (sensitivity and specificity ranging from 91% to 98.4% and from 96.9% to 100%, respectively) [10,19–23]. The specificity of

100% is slightly higher than what we have observed previously (98.9%). This can be attributed to our experience-guided practice of testing all the samples whose RLU values fell within the 'gray zone' (between >30 000 and ~300 000 RLU).

In conclusion, AMTD is demonstrated to be a useful tool for the diagnosis of pulmonary tuberculosis and suitable for a clinical microbiology laboratory's workflow. However, amplification techniques can at present only complement conventional microbiological procedures, not replace them.

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