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Detection and Identification of *Mycobacterium tuberculosis* Directly from Sputum Sediments by Amplification of rRNA

VIVIAN JONAS,[1] MARTHA J. ALDEN,[1] JANIS I. CURRY,[2] KEICHII KAMISANGO,[1] CAROLINE A. KNOTT,[1] ROGER LANKFORD,[1] JULIA M. WOLFE,[2] AND DOUGLAS F. MOORE[2]*

Public Health Laboratory, Orange County Health Care Agency, Santa Ana, California 92706,[2] and Gen-Probe Incorporated, San Diego, California 92121[1]

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Seven hundred fifty-eight processed sputum sediments received for the diagnosis of tuberculosis or other mycobacterial infections were tested by utilizing a rRNA target amplification assay and traditional culture techniques. The results from the rRNA target amplification assay (Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test), available in 5 h, were compared with the results from standard culture techniques held for 6 weeks. A total of 119 specimens (16%) were culture positive for *Mycobacterium tuberculosis*. Overall sensitivity, specificity, positive predictive value, and negative predictive value were 82, 99, 97, and 96%, respectively, for the Gen-Probe assay; 88, 100, 100, and 97%, respectively, for culture; and 53, 99.8, 99.6, and 91%, respectively, for fluorochrome stain. The Gen-Probe assay employs the isothermal enzymatic amplification of *M. tuberculosis* complex rRNA followed by detection of the amplicon with an acridinium ester-labeled DNA probe. This assay has the potential of reducing the time for diagnosis of tuberculosis to 1 day.

Laboratory diagnosis of *Mycobacterium tuberculosis* utilizing acid-fast staining and culture of processed sputum specimens has been utilized for decades in the United States. During this time the techniques have been continuously refined and improved (18), but even today they have severe limitations. Culture on solid media, considered the most accurate test because of high sensitivity and specificity, is labor intensive and requires up to 8 weeks of incubation to achieve the maximum sensitivity (18). Radiometric liquid culture (BACTEC), the most rapid culture technique widely utilized, requires an average of 13 days to become positive (1). Microscopic examination of acid-fast smears, while rapid and fairly specific, has a sensitivity low enough to be useful only as a presumptive screening test (18). Even today, the most sensitive and rapid culture and staining techniques available are not utilized by all laboratories because of funding, staffing, and training difficulties (16). The recent increase in tuberculosis cases in the United States and the emergence of multidrug-resistant strains have demonstrated the weaknesses in the currently utilized techniques and underscored the need for more-rapid and -accurate methods of laboratory diagnosis (8).

Technological advances in amplifying and detecting specific regions of bacterial DNA or RNA have provided the methods necessary to make improvements in laboratory diagnosis of tuberculosis. The polymerase chain reaction (PCR) has recently been utilized to detect *M. tuberculosis* in respiratory (5, 6, 9, 10, 13, 15, 19–21, 23, 24, 26) and other clinical (10, 11, 17, 22) samples. The clinical sensitivity compared with that of culture has been reported to be from 74% to greater than 100%, with actual detection limits of 1 to 100 cells (5, 6, 9, 13–15, 19–21, 23, 24, 26). Other nucleic acid amplification techniques, such as the ligase chain reaction, have also been utilized to detect other bacteria or purified nucleic acid (12, 28). While these reports have been promising, the techniques reported are not yet suitable for use in clinical laboratories. Several researchers have addressed the problem and have developed simplified PCR techniques for detecting *M. tuberculosis* (24–26). While these techniques are an improvement, the clinical laboratories that perform the majority of *M. tuberculosis* work need a simple, rapid technique which fits in with the work flow. Described here is an evaluation of an *M. tuberculosis* rRNA nucleic acid amplification assay which is formatted for use in a large-volume clinical microbiology laboratory.

MATERIALS AND METHODS

Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. The assay utilizes a proprietary isothermal enzymatic amplification of target rRNA via DNA intermediates. Detection of amplicon is achieved by using an acridinium ester-labeled DNA probe (2). All reactions were performed following instructions in the package insert. Fifty microliters of sample was added to 200 μl of sample buffer in a lysing tube and sonicated for 15 min in a water bath sonicator at room temperature. Twenty-five microliters of reconstituted amplification reagent and 200 μl of oil were placed in a reaction tube, and 50 μl of lysate was added below the oil layer. The tubes were incubated at 95°C for 15 min and 42°C for 5 min. Twenty-five microliters of enzyme mix was added, and the reaction mixture was incubated at 42°C for 2 h. Twenty microliters of termination reagent was added, and the mixture was incubated at 42°C for 10 min. One hundred microliters of reconstituted probe was added, and the mixture was incubated at 60°C for 15 min. This was followed by the addition of 300 μl of selection reagent and incubation at 60°C for 5 min to hydrolyze the acridinium ester on the unhybridized probe. The tubes were cooled at room temperature for 10 min and read in a luminometer. All runs included an amplification-positive and -negative control and a hybridization-positive and -negative control.

For experiments in which purified rRNA was used, 50 μl of an rRNA dilution was placed directly into a reaction tube with the amplification reagent and oil. Specificity experiments were performed with bacterial and yeast strains from the American Type Culture Collection (see Tables 2 and 3)

* Corresponding author.

TABLE 1. Amplification of 25 fg of *M. tuberculosis* rRNA in the presence and absence of two concentrations of other bacteria

Bacterial species	ATCC no.[a]	Gen-Probe result (RLU)[b]		
		290,000 CFU/ reaction	2,900 CFU/ reaction	0 CFU/ reaction
Haemophilus influenzae	19418	1,352,456	2,149,025	2,001,148
Streptococcus pneumoniae	33400	1,814,873	2,119,640	2,065,117
Legionella pneumophila	33152	899,639	2,206,019	2,033,331
Pseudomonas aeruginosa	9027	213,624	1,865,116	2,073,948
Mycobacterium gordonae	14470	546,980	2,102,524	2,154,300
Mycobacterium avium	25291	697,861	1,901,832	2,070,336

[a] ATCC, American Type Culture Collection.
[b] Cutoff, ≥30,000 RLU.

by placing a 1-μl loopful of a microbial colony and 50 μl of distilled water into 200 μl of sample buffer in the lysing tube. The availability of rRNA for amplification was verified by hybridizing an aliquot of each lysate with a proprietary probe which detects all bacteria. Interference studies were done by diluting bacterial or yeast colonies in sample buffer to an approximate concentration of 3×10^8 CFU/ml and sonicating the mixture as above. Fifty microliters of lysate dilutions and 10 μl of an appropriate concentration of rRNA were then added to the reaction tube. All other steps were performed as described above.

Aliquots (50 μl) of sediments from processed clinical specimens, which had been stored at −70°C for up to 1 year, were tested as described above after being thawed at room temperature and vortexed. During the trial a total of three different lots of the testing system were utilized. Before beginning the clinical trial, all microbiologists performing the assay were qualified by correctly testing two sets of 20 unknowns on two successive runs.

PCR assay. The PCR assay utilized amplification of a portion of the insertion sequence IS*6110* as described by Eisenach et al. (13), with the following modifications. The probe was an acridinium ester-labeled sequence (2) directed against a portion of the amplicon. The final reaction mixture contained 26 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.8 mM EDTA, 4 mM *N*-acetyl-L-cysteine, 200 μM each deoxynucleotide triphosphate, 2.5 U of *Thermus aquaticus* (*Taq*) polymerase (Perkin-Elmer), and 1 μM each primer. The reaction mixture was overlayed with 100 μl of silicon oil, to which 50 μl of specimen was added. The reaction was performed in a Perkin-Elmer thermocycler with denaturation at 94°C for 5 min followed by 30 amplification cycles. Each cycle consisted of a 94°C denaturation for 2 min, a 68°C annealing for 2 min, and a 72°C primer extension for 2 min. The extension time was increased 5 s with each subsequent cycle. After amplification the samples were incubated at 95°C for 5 min in a ice-water bath for 3 min and then transferred into a tube containing 100 μl of probe solution. The reaction was incubated at 60°C for 15 min, 300 μl of selection reagent was added, and the mixture was incubated at 60°C for 5 min. The tubes were cooled at room temperature for 5 min and then read in the luminometer by using a 2-s read time.

Clinical specimens and culture techniques. Specimens were collected from patients being screened for tuberculosis or other mycobacterial infections or being followed during antituberculosis therapy at the Orange County Health Care Agency Pulmonary Disease Clinic. Samples from patients from which at least three specimens had been taken on different days were included in the study. The charts of all patients were reviewed to determine clinical diagnosis, therapy, and outcome. All specimens were induced sputum samples taken by utilizing a Ultra-Neb 99 nebulizer (DeVilbiss, Somerset, Pa.) with a 0.45% NaCl solution and a 50-ml sterile conical tube collection kit (Sage Products, Crystal Lake, Ill.). Specimens were held at 4°C, received by the laboratory within 2 h, and processed by the standard laboratory procedures at the Orange County Public Health Laboratory. The majority of specimens were processed within 24 h by a standard *N*-acetyl-L-cysteine sodium hydroxide method (18), with the addition of 1.0 ml of 0.2% bovine serum albumin (BBL, Cockeysville, Md.)–45.5 U of penicillin G per ml–9% wide range indicator (LaMotte Chemical Company, Chestertown, Md.) to the final pellet followed by titration to pH 6.8 to 7.2 with 0.5 N HCl. For each specimen, two Lowenstein-Jensen tubes (BBL) were inoculated with 0.1 ml of specimen, and a smear was made for fluorochrome staining. The remainder of the sample was frozen at −70°C. Fluorochrome staining was performed by using standard procedures (18). Cultures were examined weekly for a total of 6 weeks. Positive cultures were quantitated, and acid-fast isolates were identified by standard biochemical techniques (18), DNA-RNA hybridization (Accu-Probe; Gen-Probe, San Diego, Calif.), or high-performance liquid chromatography (7).

Statistical methods. Statistical comparisons of sensitivity were performed by using χ^2 analysis.

RESULTS

Analytical sensitivity and specificity. The analytical sensitivity of the assay was determined by assaying serial dilutions of purified *M. tuberculosis* rRNA. The Gen-Probe test detects as little as 2.5 fg of purified *M. tuberculosis* rRNA. Since there is 3 to 5 fg of rRNA per *M. tuberculosis* cell (3, 27), the assay is capable of detecting the rRNA contained in a single cell. The signal for samples containing 2.5 to 250 fg of rRNA ranged from 1,841,772 to 2,088,432 relative light units (RLU). These signals are in the maximum range measurable by the luminometer and indicate that the assay is not quantitative. Results were still positive when 25 fg of *M. tuberculosis* rRNA was amplified in the presence of 2,900 or 290,000 CFU per reaction mixture of other mycobacteria and selected potential respiratory pathogens (Table 1).

Sixty-five mycobacterial strains representing 54 distinct mycobacterial species were tested to determine which species would be detected by the Gen-Probe assay (Table 2). Only members of the *M. tuberculosis* complex (*Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium bovis BCG*, and *M. tuberculosis* [avirulent strain H37R$_a$ and

TABLE 2. Amplification and detection of *Mycobacterium* species by the Gen-Probe assay

Mycobacterium sp.	ATCC no.[a]	RLU[b]
M. acapulcensis	14473	2,216
M. africanum	25420	2,320,296
M. agri	27406	2,606
M. aichiense	27280	4,712
M. asiaticum	25276	12,313
M. aurum	23366	10,037
M. avium	25291	5,084
M. austroafricanum	33464	3,336
M. bovis	19210	2,238,978
M. bovis BCG	35734	2,167,540
M. brunense	23434	4,258
M. chelonae	14472	2,226
M. chelonae subsp. *abscessus*	19977	2,683
M. chelonae subsp. *chelonae*	35752	3,311
M. chelonae chemovar *niacinogenes*	35750	3,074
M. chitae	19627	4,206
M. chubuense	27278	3,364
M. diernhoferi	19340	2,985
M. duvalii	43910	2,429
M. engbaekii	27353	3,872
M. farcinogenes	35753	3,431
M. fallax	35219	4,087
M. flavescens	14474	2,491
M. fortuitum	6841	3,153
M. fortuitum subsp. *acetamidolyticum*	35931	3,487
M. gadium	27726	5,607
M. gallinarum	19710	2,381
M. gastri	15754	15,141
M. gilvum	43909	3,320
M. gordonae	14470	5,565
M. haemophilum	29854	2,577
M. intracellulare	13950	3,404
M. kansasii	12478	9,950
M. komossense	33013	3,403
M. lactis	27356	3,589
M. malmoense	29571	3,228
M. marinum	927	10,983
M. moriokaense	43059	5,936
M. neoaurum	25795	3,657
M. nonchromogenicum	19530	3,093
M. novum	19619	4,822
M. obuense	27023	2,727
M. parafortuitum	19686	2,958
M. petroleophilum	21497	2,857
M. phlei	11758	2,304
M. porcinum	33776	2,889
M. poriferae	35087	3,882
M. pulveris	35154	2,518
M. rhodesiae	27024	3,778
M. scrofulaceum	19981	9,943
M. shimoidei	27962	4,186
M. simiae	25275	4,346
M. smegmatis	14468	2,716
M. sphagni	33027	2,848
M. szulgai	35799	3,542
M. terrae	15755	4,889
M. thermoresistibile	19527	2,891
M. tokaiense	27282	3,748
M. triviale	23292	2,744
M. tuberculosis ($H37R_a$)	25177	1,734,753
M. tuberculosis ($H37R_v$)	27294	2,007,432
M. ulcerans	19423	2,314
M. vaccae	15483	3,041
M. valentiae	29356	3,036
M. xenopi	19250	3,334

[a] ATCC, American Type Culture Collection.
[b] Cutoff, ≥30,000 RLU.

virulent strain $H37R_v$]) were positive. The highest result for any negative organism was 15,141 RLU for *Mycobacterium gastri*. This value is well below the 30,000-RLU cutoff. Organisms representing both closely related and widely divergent microbial genera were tested in the same fashion (Table 3). No positive results were observed.

Clinical performance of assay. The clinical performance of the Gen-Probe assay was determined by comparing the Gen-Probe results to those of standard culture and fluorochrome stain. Included in the study were 758 specimens from 235 patients received by the laboratory for initial diagnosis or follow-up of respiratory mycobacterial infections. Each run of 50 specimens and controls was completed in approximately 5 h. The overall results presented in Table 4 indicate that compared with that of culture the Gen-Probe sensitivity was 79.8% and the specificity was 96.7%, while the fluorochrome stain had a sensitivity of 56.3% and a specificity of 98.9%. As indicated in Table 5, the culture-positive specimens ranged from low positive to high positive. Sensitivity for both the Gen-Probe and fluorochrome stain varied with the level of culture positivity of the specimens. Both techniques were more sensitive with specimens containing a greater number of viable organisms. The sensitivity of the Gen-Probe assay ranged from 54% for low-positive specimens with <100 CFU/ml to 100% for specimens with >1,000 CFU/ml. The fluorochrome stain sensitivity ranged from 26 to 90% for the low- and high-positive specimens, respectively (Table 5).

Eighteen of the 24 specimens which were Gen-Probe negative and culture positive were low positives containing ≤100 CFU of *M. tuberculosis* per ml. These 24 specimens were from 21 patients, including 9 being given therapy for tuberculosis. These 24 specimens were retested with the Gen-Probe test before and after the addition of 5 fg of *M. tuberculosis* rRNA to determine whether amplification inhibitors were present. Six specimens were positive on retest, indicating possible sampling error; 12 were negative on retest and did not inhibit amplification; and 6 were found to inhibit amplification.

The 21 specimens which were Gen-Probe assay positive and culture negative were further investigated. These 21 specimens also included 6 of the 7 specimens that were fluorochrome stain positive and culture negative. Each specimen was tested by utilizing a PCR specific for *M. tuberculosis*, and the clinical history, past laboratory results, and treatment regimen were reviewed. These results are presented in Table 6. The 21 specimens represented 15 different patients. Eight patients (13 specimens) had previous *M. tuberculosis* isolates and a positive PCR result. One patient (two specimens) had a positive history, a positive PCR result, and no previous isolate. Two patients (two specimens) were clinically diagnosed as having tuberculosis, were treated for the disease, and showed clinical improvement but never had a positive culture. Four patients (four specimens) had neither a history of tuberculosis, a previous isolate, nor a positive PCR result. The final results of the discrepant analysis were based on patient history and the presence of a previous isolate.

As a result of the discrepant analysis, 17 specimens from patients with a positive history of tuberculosis were considered true positives which were missed by culture, while 4 specimens were considered false positives. By utilizing these confirmed results, the initial results presented in Table 4 were recalculated and presented in Table 7. The sensitivity and specificity were 87.5 and 100%, respectively, for culture; 82.4 and 99.4%, respectively, for the Gen-Probe assay; and

TABLE 3. Amplification and identification of organisms representing a cross-section of microbial phylogeny

Organism	ATCC no.[a]	RLU[b]
Acinetobacter calcoaceticus	33604	1,973
Actinomadura madurae	19425	2,866
Actinomyces pyogenes	19411	2,036
Actinoplanes italicus	27366	2,453
Arthrobacter oxydans	14358	2,084
Bacillus subtilis	6051	2,570
Bordetella bronchiseptica	10580	2,076
Branhamella catarrhalis	25238	2,246
Brevibacterium linens	9172	2,068
Campylobacter jejeuni	33560	2,333
Candida albicans	18804	2,386
Chromobacter violaceum	29094	2,306
Clostridium perfringens	13124	2,719
Corynebacterium aquaticum	14665	2,792
Corynebacterium diphtheriae	11913	2,685
Corynebacterium genitalium	33030	2,792
Corynebacterium haemolyticum	9345	2,153
Corynebacterium matruchotii	33806	2,272
Corynebacterium minutissimum	23347	2,660
Corynebacterium pseudodiphtheriticum	10700	1,855
Corynebacterium pseudogenitalium	33035	2,295
Corynebacterium pseudotuberculosis	19410	2,362
Corynebacterium renale	19412	3,408
Corynebacterium striatum	6940	3,525
Corynebacterium xerosis	373	2,367
Deinococcus radiodurans	35073	2,412
Dermatophilus congolenis	14637	2,212
Derxia gummosa	15994	6,646
Erysipelothrix rhusiopathiae	19414	2,321
Escherichia coli	10798	2,158
Flavobacterium meningosepticum	13253	2,180
Haemophilus influenzae	19418	2,213
Klebsiella pneumoniae	23357	2,076
Lactobacillus acidophilus	4356	2,466
Legionella pneumophila	33152	12,323
Microbacterium lacticum	8180	2,721
Mycoplasma hominis	14027	1,898
Mycoplasma pneumoniae	15531	1,384
Neisseria meningitidis	13077	2,377
Nocardia asteroides	19247	3,651
Nocardia brasiliensis	19296	2,529
Nocardia otitidis-caviarum	14629	3,084
Nocardiopsis dassonvillei	23218	2,731
Oerskovia turbata	33225	2,566
Oerskovia xanthineolytica	27402	2,294
Paracoccus denitrificans	17741	2,481
Propionibacterium acnes	6919	2,266
Proteus mirabilis	25933	2,017
Pseudomonas aeruginosa	25330	2,249
Rahnella aquatilis	33071	2,010
Rhodococcus aichiensis	33611	2,698
Rhodococcus chubuensis	33609	2,318
Rhodococcus equi	6939	1,887
Rhodococcus obuensis	33610	2,072
Rhodococcus sputi	29627	14,203
Rhodospirillum rubrum	11170	2,504
Staphylococcus aureus	12598	1,983
Staphylococcus epidermidis	12228	1,837
Streptococcus mitis	9811	2,439
Streptococcus pneumoniae	6303	2,227
Streptococcus pyogenes	19615	3,315
Streptomyces griseus	23345	2,721
Vibrio parahaemolyticus	1802	1,908
Yersinia enterocolitica	9610	2,066

[a] ATCC, American Type Culture Collection.
[b] Cutoff, ≥30,000 RLU.

TABLE 4. Initial comparison of culture results with those of the Gen-Probe assay and fluorochrome stain

Test and result	No. of culture results		Sensitivity (%)	Specificity (%)
	Positive	Negative		
Gen-Probe				
Positive	95	21	79.8	96.7
Negative	24	618		
Fluorochrome stain				
Positive	67	7[a]	56.3	98.9
Negative	52	628		

[a] Four specimens which were fluorochrome stain positive and culture positive for *M. intracellulare* or *M. fortuitum* and rRNA amplification negative were removed from this part of the comparison.

57.3 and 99.8%, respectively for fluorochrome stain. The difference in the sensitivity between the Gen-Probe test and culture was not significant ($P > 0.1$), while the difference between culture and fluorescent stain was significant ($P < 0.001$).

DISCUSSION

The Gen-Probe assay is the first nucleic acid amplification test which has been developed for use in the clinical laboratory. The assay procedure is composed entirely of transfers, additions, and incubations and is not technically demanding. However, to avoid potential carryover contamination, careful adherence to the protocol, including bleach decontamination of reaction tubes and work surfaces, is required. In our public health laboratory, a moderately sized run (50 specimens and controls) can be completed in 5 h. Since processing a batch of specimens requires 2 h, it is possible to incorporate this assay into the normal work flow, with the ability to report results within one 8-h shift.

Results obtained by testing dilutions of purified *M. tuberculosis* rRNA indicate an analytical sensitivity of less than one organism. Other researchers have reported a range of sensitivity of 1 to 100 cells for PCR (5, 6, 9, 13–15, 19–24, 26). The increased sensitivity seen here may be due to several factors. (i) The Gen-Probe assay detects rRNA which is present at a level of approximately 2,000 copies per cell (27). This may enhance the sensitivity of the assay relative to that of others which detect target sequences present in only a single or very low copy number (10 to 16 copies for insertion sequence IS*6110*) (13). (ii) The test is performed directly from processed respiratory samples without the need for extensive nucleic acid purification. (iii) The detection step is hybridization with a sensitive acridinium ester-labeled DNA probe. The Gen-Probe assay was specific for members of the *M. tuberculosis* complex; 124 other species of bacteria and yeasts were negative when tested.

The clinical sensitivity was 82.4% compared with 87.5% for culture and 53.7% for fluorochrome stain. This compares with reported sensitivities ranging from 74% to greater than 100% by PCR. (5, 6, 9, 13, 15, 19–24, 26). In a study of this type, the clinical sensitivity of a test is dependent on four major factors: (i) The analytical sensitivity of the assay, (ii) the sensitivity of the standard (culture technique), (iii) the distribution of positive specimens, from low positive to high positive, included in the study, and (iv) the effects of sample heterogeneity, especially in samples with low-positive results. It is common for a commercial direct detection assay

TABLE 5. Sensitivity of Gen-Probe and fluorochrome stain by quantitative culture result

Quantitative culture result (CFU/ml)	No. of culture-positive specimens (%)	Gen-Probe		Fluorochrome stain	
		No. of true-positive specimens	Sensitivity (%)	No. of true-positive specimens	Sensitivity (%)
≤100	39 (33)	21	54	10	26
>100–≤500	31 (26)	26	84	14	45
>500–≤1,000	15 (13)	14	93	12	80
>1,000	31 (26)	31	100	28	90
NA[a]	3 (2.5)	3	100	3	100
Total	119 (100)	95	80	67	56

[a] NA, not available.

to have different sensitivities in different laboratories. Unless there is a technical error in performing the assay, the difference in sensitivity is usually due to factors ii and iii above. The clinical sensitivity of the Gen-Probe assay in this study may be less than that in studies performed by utilizing the PCR (6, 9, 15, 19–21, 24) because of any of the four reasons. However, because the analytical sensitivity is as high as or higher than that reported for other nucleic acid amplification assays, the difference is probably due to the relative sensitivity of our culture technique and the number of low positives included in the study. All samples in this study were induced, had optimum transport, were processed within a short time, and were neutralized in the presence of a pH indicator, all of which are factors known to enhance the sensitivity of culture. In addition, 33% of the culture-positive specimens contained <100 CFU/ml and thus were low positives. As shown in Table 5, the sensitivity of the Gen-Probe assay and fluorochrome stain is much less for these specimens. Another report that compared the PCR with quantitative culture also demonstrated a lower sensitivity with specimens containing <100 CFU/ml (21). In laboratories that do not receive low-positive specimens or whose collection, transport, processing, and culture protocols do not allow detection of *M. tuberculosis* from these specimens, it is very possible that the Gen-Probe assay will prove to be more sensitive than culture.

Nucleic acid amplification assays and direct antigen detection assays share the ability to detect noncultivable organisms. When culture is utilized as the standard technique in a comparison study, specimens containing noncultivable organisms, which are positive by the test assay, are initially identified as false-positive specimens. Lacking a perfect "gold standard," a rigorous discrepant analysis must be performed on these specimens to determine what portion of the specimens with initial false-positive results contain noncultivable organisms that culture cannot detect. The results from this analysis can then be utilized to correct the initial results and to calculate final performance values. As seen in other studies comparing PCR and culture, there were specimens in this study which were Gen-Probe positive and culture negative. The discrepant analysis was done by a thorough analysis of the patient history and previous laboratory results. Retesting the sample with a PCR assay, which detected another sequence in the *M. tuberculosis* genome (IS*6110*), was also carried out to determine the presence of *M. tuberculosis* DNA. As a result of the discrepant analysis all but 4 of the initial 21 false-positive samples were considered to be due to noncultivable organisms. The final sensi-

TABLE 6. Analysis of culture-negative, Gen-Probe-positive discrepant specimens

Patient no.	Specimen no.	Smear result	Gen-Probe result (RLU)[a]	PCR result	Tuberculosis case	Presence of previous isolate	Final result
1	1089	1+	4,433,054	+	+	–	+
1	1113	–	2,258,638	+	+	–	+
2	454	–	159,045	–	–	–	–
3	2163	–	2,098,546	–	+	–	+
4	9649	1+	2,354,100	+	+	+	+
4	9799	2+	2,188,372	+	+	+	+
4	32	1+	2,348,567	+	+	+	+
4	536	–	740,229	+	+	+	+
4	880	–	330,101	+	+	+	+
5	9808	–	452,569	–	–	–	–
6	55	1+	2,354,894	+	+	+	+
7	1013	–	39,278	+	+	+	+
8	9651	–	2,364,364	+	+	+	+
9	278	–	242,896	+	+	+	+
10	291	–	1,353,846	+	+	+	+
10	534	1+	2,440,957	+	+	+	+
11	289	–	1,333,644	+	+	+	+
12	369	–	536,035	–	–	–	–
13	375	–	2,481,164	+	+	+	+
14	2075	–	64,261	–	+	–	+
15	1000	–	287,822	–	–	–	–

[a] Cutoff, ≥30,000 RLU.

TABLE 7. Comparison of confirmed results by Gen-Probe, culture, and fluorochrome stain[a]

Test and result	No. of confirmed results		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
	Positive	Negative				
Gen-Probe						
Positive	112	4	82.4	99.4	96.6	96.3
Negative	24	618				
Culture						
Positive	119	0	87.5	100	100	97.3
Negative	17	622				
Fluorochrome stain						
Positive	73	1[b]	53.7	99.8	99.6	90.7
Negative	63	617				

[a] Results from Table 4 were modified by utilizing the final result presented in Table 6.
[b] Four specimens which were fluorochrome stain positive and culture positive for *M. intracellulare* or *M. fortuitum* and rRNA amplification negative were removed from this part of the comparison.

tivities of the Gen-Probe test (82.4%), culture (87.5%), and fluorochrome stain (53.7%) were determined by counting 17 specimens as noncultivable culture misses and 4 specimens as Gen-Probe false positive (Table 7).

With the commercial availability of an assay that can reliably detect and identify *M. tuberculosis* within 1 working day, the methodology of laboratory diagnosis of tuberculosis will change rapidly. However, the exact role of the Gen-Probe test and other similar tests remains to be determined and is the subject of ongoing studies. The data presented here show the excellent clinical sensitivity and specificity of an easily performed assay. However, for the foreseeable future, a culture will be required to insure that an isolate is available for antimicrobial testing. Is it possible to perform culture only on specimens which are positive by a direct test? In laboratories in which the sensitivity of a direct assay is equal to or greater than that of culture, it would be possible to perform culture only on positive specimens with no loss of overall sensitivity. The effort diverted from culturing and screening large quantities of negative specimens could be utilized to intensify efforts to rapidly isolate and perform sensitivity testing on positive specimens. While the Gen-Probe test in this study had a slightly lower sensitivity than culture, the difference was slight. Minor changes in assay format and laboratory protocols may increase the sensitivity to equal or exceed that of culture. At that time we will be able to explore the possibilities of the scenario described above.

Another possible use of a direct test for *M. tuberculosis* is the followup of patients on treatment. Physicians and public health personnel utilize acid-fast stain and culture results to monitor patient response to therapy and to guide decisions concerning infectivity. Acid-fast stain results, because they are available quickly, are utilized to determine the infectivity of a patient and the need for isolation or other public health measures (4). The possible use of a more-sensitive direct test to replace acid-fast stain and culture in this usage will require detailed long-term studies because of the ability of a direct test such as the Gen-Probe test to detect small numbers of noncultivable organisms. While this ability may make studies complicated, it may be very useful as an aid in the diagnosis of partially treated patients, especially those with acid-fast-stain-negative results. Even when utilized in conjunction with culture, a direct test with the performance characteristics of the Gen-Probe assay is a major achievement in the application of molecular biology to clinical microbiology.

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