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Evaluation of the Semiautomated Abbott LCx *Mycobacterium tuberculosis* Assay for Direct Detection of *Mycobacterium tuberculosis* in Respiratory Specimens

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Five hundred twenty processed respiratory specimens from 326 patients received for the diagnosis of tuberculosis or other mycobacterial infections were tested by means of the LCx *Mycobacterium tuberculosis* Assay from Abbott Laboratories, which uses ligase chain reaction technology for the direct detection of *M. tuberculosis* complex in respiratory specimens. The results of the LCx *M. tuberculosis* Assay were compared with the results of culture and staining techniques. After a combination of culture results and the patient's clinical data, a total of 195 specimens were collected from 110 patients who were positively diagnosed as having pulmonary tuberculosis. Twenty-three of these 195 specimens which corresponded to 10 patients with a history of pulmonary tuberculosis (TB) and anti-TB treatment ranging from 1 to 6 months were culture negative. The other 172 specimens were culture positive for *M. tuberculosis*. With an overall positivity rate of 37.5% (195 of 520 specimens), the sensitivity, specificity, and positive and negative predictive values were 90.8, 100, 100, and 94.7%, respectively, for the LCx *M. tuberculosis* Assay; 88.2, 100, 100, and 93.4%, respectively, for culture; and 82.6, 92, 72.9, and 97.6%, respectively, for acid-fast staining. For 161 specimens (82.6%) from patients smear positive for the disease and 34 specimens (17.4%) from patients smear negative for the disease, the sensitivity values for the LCx *M. tuberculosis* Assay were 98.8 and 53%, respectively. There were no statistically significant differences in the sensitivities and specificities between the LCx *M. tuberculosis* Assay and culture ($P > 0.05$). Conclusively, the LCx *M. tuberculosis* Assay has proved to have an acceptable sensitivity and a high specificity in detecting *M. tuberculosis* and has the potential of reducing the diagnosis time to an 8-h working day.

At present, the laboratory diagnosis of pulmonary tuberculosis (TB) is based on acid-fast staining and culture on solid and/or liquid media. Detection by microscopy is useful as a rapid screening test, but its sensitivity is low (10). Culture on solid medium can take up to 8 weeks to yield a negative result (1, 13). The radiometric BACTEC 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) has been an important addition to culture methods; however, this technique requires an average of 13 to 15 days to detect positive specimens (1, 13, 19).

New diagnostic methods that use gene technology based on amplification and detection of the bacterial DNA or RNA are expected to improve the speed, sensitivity, and specificity of mycobacterial detection (29). PCR is a well-developed technique extensively used for the diagnosis of numerous infectious diseases, including TB (2, 5, 11, 14–16). Nevertheless, recent reports on quality control of in-house PCR applications resulted in an unexpectedly high variation in sensitivity (9, 11, 17, 23, 27). In addition, the PCR protocol does not easily fit into routine clinical laboratory practice because such laboratories usually lack the necessary technical equipment and expertise and because of the high cost of PCR (14–16, 23, 28). There are still problems, including the presence of inhibitors in clinical samples, which may cause false-negative results, and contamination with amplicons, which gives false-positive results (5, 11, 23, 28). Therefore, the search for rapid, standardized, and reliable commercially available detection systems for *Mycobacterium tuberculosis* continues (6, 29).

The LCx *M. tuberculosis* Assay (Abbott Laboratories, Diagnostic Division, Chicago, Ill.) uses ligase chain reaction (LCR) amplification technology for the direct detection of *M. tuberculosis* complex in respiratory specimens. The LCR amplification methods have previously been evaluated for their ability to detect other infectious agents (4, 8, 9, 24). The target sequence of the LCx *M. tuberculosis* Assay is found within the chromosomal gene of *M. tuberculosis* which encodes for protein antigen b (3). This gene sequence appears to be specific to the *M. tuberculosis* complex and has been detected in all *M. tuberculosis* complex strains examined to date (33). The four oligonucleotide probes in the amplification reaction of the LCx *M. tuberculosis* Assay, designed in pairs, recognize and hybridize to complementary single-strand *M. tuberculosis* target sequences exposed in the sample preparation. When a pair of probes has hybridized to the protein antigen b gene target sequence on a single strand of DNA, there is a gap of a few nucleotides between the probes. Polymerase acts to fill in this gap with the nucleotides. Once the gap is filled, ligase can covalently join the pair of probes to form an amplification product which is complementary to the original target sequence and which can itself serve as a target in subsequent rounds of amplification. The product of the LCR is detected on the Abbott LCx Analyzer (7).

The purpose to the present study was to evaluate the per-

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formance of the LCx *M. tuberculosis* Assay for the direct detection of *M. tuberculosis* complex in respiratory specimens and to compare this method with standard culture and staining techniques.

MATERIALS AND METHODS

Patients and clinical specimens. From June to October 1996, we investigated a total of 520 respiratory specimens collected from 326 patients at the Hospital Universitario "Germans Trias i Pujol," Barcelona, Spain. Only clinical samples from patients suspected of having pulmonary TB and specimens from patients with pulmonary TB who were being monitored for treatment with antituberculosis drugs were selected for inclusion in the study. The patients were selected if their specimens gave a positive smear result or there was a high degree of suspicion that a patient with a negative smear result had tuberculosis. The 520 respiratory specimens included 449 expectorated sputum specimens, 60 bronchial or tracheal aspirates, 8 bronchoalveolar lavage specimens, and 3 gastric juice aspirates. Once collected, the specimens were kept at 4°C prior to processing. Gastric juice aspirates were immediately neutralized with trisodium phosphate buffer after retrieval.

Decontamination procedures. The samples were digested and decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH as described previously (32). The concentrated specimen pellet (approximately 0.2 ml) was washed with 30 ml of sterile distilled water and was centrifuged at 3,300 × *g* for 20 min, and the supernatant was discarded. All specimen sediment was finally resuspended in 2.2 ml of 0.067 M phosphate buffer (pH 6.8). For all specimens, half of the sediment was kept at −80°C for the LCx *M. tuberculosis* Assay and the other half was inoculated onto the culture medium and used for acid-fast staining.

Microscopy. Smears were stained with auramine-rhodamine fluorochrome as a screening method. Positive slides were confirmed to be positive by Ziehl-Neelsen staining (19).

Culture. Equal aliquots (approximately 250 μl) of the processed sediment were inoculated onto two solid slants, Löwenstein-Jensen and Coletsos slants. The Coletsos medium is an egg-based medium containing pyruvate, osein, inorganic salts solution, asparagine, glutamate, glycerol, and malachite green; this medium has a faster detection time for *M. bovis* and disgonic strains of *M. tuberculosis* (12). Slants were incubated at 37°C for 8 weeks in a 6% CO_2 atmosphere. In addition, 500 μl of the sediment was cultivated into BACTEC 12B medium supplemented with 0.1 ml of antimicrobial mixture (polymyxin B, azlocillin, nalidixic acid, trimethoprim, and amphotericin B), and the medium was incubated at 37°C for up to 8 weeks. Solid media were read weekly, and BACTEC cultures were read twice weekly for the first 2 weeks and once weekly thereafter. A growth index of >100 on BACTEC medium was considered positive, and smears stained with Ziehl-Neelsen stain and culture on solid media were used to detect acid-fast bacilli.

Identification of mycobacteria. Routine biochemical methods (19), gas-liquid chromatography (25), and the Accuprobe culture confirmation tests (Gen-Probe Inc., San Diego, Calif.) (18) were used for isolate identification.

LCx *M. tuberculosis* Assay protocol. The LCx *M. tuberculosis* Assay flow is outlined in Fig. 1. The LCx *M. tuberculosis* Assay consists of three steps (specimen preparation, amplification, and detection) and was performed according to the manufacturer's package insert.

The removal of specimen inhibitors was carried out by adding 500 μl of pretreated (SDS-NaOH) specimen into an LCx Respiratory Specimen Tube and centrifuging the tube at 1,500 × *g* for 10 min. The supernatant was aspirated, and 1 ml of LCx Respiratory Specimen Resuspension Buffer was added to the specimen tube. Once again, the specimen tube was centrifuged at 1,500 × *g* for 10 min. The supernatant was removed, and 0.5 ml of LCx Respiratory Specimen Resuspension Buffer was pipetted into the specimen tube and the tube was vortexed for 5 s. The suspension was placed into an LCx Covered Dry Bath at 95°C for 20 min. Finally, mycobacterial DNA was released by mechanical lysis in the LCx Lysor for 10 min.

One hundred microliters of lysed specimen was added to the appropriately labeled LCx Tuberculosis Amplification Vial containing 100 μl of the LCR mixture for the amplification reaction. The LCR mixture contained thermostable DNA ligase and DNA polymerase, deoxyribonucleoside triphosphates, and four oligonucleotide probes labeled with haptens. Two calibrators and two negative controls were included in each test run. The specimens and controls were placed in the LCx Thermal Cycler and were amplified for 37 incubation cycles of incubation for 1 s at 94°C, 1 s at 55°C, and 40 s at 69°C. During thermal cycling, sufficient numbers of target amplification product are accumulated and can be detected by microparticle enzyme immunoassay (MEIA) on the Abbott LCx Analyzer.

The LCx Tuberculosis Amplification Vials were removed from the LCx Thermal Cycler, transferred to the LCx Reaction Cell in the carousel, and then locked into the LCx Analyzer for detection purposes. Each individual oligonucleotide probe has either a capture hapten or a detection hapten. Thus, the amplification product has the capture hapten at one end and the detection hapten at the other. An amplification product sample is automatically transferred to an incubation well, in which the microparticles coated with anti-capture hapten bind the amplification product. The reaction mixture is then automatically transferred to a glass fiber matrix to which the microparticle complexes bind irreversibly. A wash step removes the unligated probe with only the detection hapten. The bound microparticle complexes are then incubated with antihapten-alkaline phosphatase conjugate, which binds to the detection haptens. This antibody conjugate can then be detected by addition of the substrate 4-methylumbelliferyl phosphate, which is dephosphorylated by alkaline phosphatase to produce a fluorescent molecule, 4-methylumbelliferona, that is measured by the MEIA optical assembly (7). The presence or absence of *M. tuberculosis* was determined by relating the LCx *M. tuberculosis* Assay results for the specimen to the cutoff value (CO). The CO value is the mean rate of LCx Calibrator duplicates multiplied by 0.30. The S/CO value (sample rate/CO) was determined by calculating a ratio of the sample rate to the CO value. An S/CO ratio of >1.0 indicates a positive LCx *M. tuberculosis* Assay result.

Analysis of discrepant results. In those cases in which results from the culture and the LCx *M. tuberculosis* Assay were discrepant, clinical data and other results obtained with additional specimens from the patient were analyzed. Moreover, all specimens with discrepant results were retested by the LCx *M. tuberculosis* Assay with 500 μl of the same decontaminated sample. Assessment of each patient's clinical picture included the patient's history, signs, symptoms, chest X ray, cytological and histological results for patient specimens, tuberculin skin test result, history of drugs administered, and response to empirical treatment for TB.

Statistical analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LCx *M. tuberculosis* Assay were calculated by contrasting the results with the culture results. A further comparison was then made with culture results together with the patient's clinical data. Statistical comparison was performed by using chi-square analysis.

RESULTS

The LCx *M. tuberculosis* Assay was evaluated for its ability to detect *M. tuberculosis* complex organisms in 520 respiratory specimens from 326 patients received in the mycobacteriology laboratory for initial diagnosis or follow-up of respiratory mycobacterial infections. The clinical performance of the LCx *M. tuberculosis* Assay was determined by comparison of the results of the assay with those of standard culture and staining methods.

Of the 520 specimens, 172 specimens were culture positive for *M. tuberculosis*. Of these, 141 specimens (80%) were smear positive and 31 specimens (20%) were smear negative. Thirty-two specimens (six smear-negative specimens) were culture positive for nontuberculous mycobacteria (NTM). The species of NTM identified from these specimens were *M. kansasii* (17 specimens), *M. avium-M. intracellulare* complex (7 specimens), *M. xenopi* (6 specimens), *M. gordonae* (1 specimen), and *M. fortuitum* (1 specimen). Three hundred sixteen specimens (20 smear-positive specimens) were culture negative.

A comparison of the amplification results with smear and culture results is summarized in Table 1. One hundred fifty-four specimens (139 smear-positive and 15 smear-negative specimens) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*, and 293 specimens (all smear-negative and culture-negative specimens) were LCx *M. tuberculosis* Assay negative. The 32 specimens with NTM isolates were LCx *M. tuberculosis* Assay negative.

In total, there were 41 discrepant results. Eighteen specimens (16 smear-negative specimens) were LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis* (Table 1). Of these, two specimens (smear positive) had positive culture results on BACTEC 12B and solid media, with ≤100 colonies. Five smear-negative specimens exhibited only positive radiometric culture results within a period of between 25 and 50 days (average time, 36 days), and the other 11 specimens (also smear negative) had positive culture results on BACTEC 12B and solid media, which had 2 to 10 colonies (average time, 45 days) (Table 2). These 18 specimens with discrepant results were retested with a new aliquot (500 μl) of the same processed specimen. The results were confirmed and were considered false negative, although two specimens showed a signal 20 times greater than that of the negative

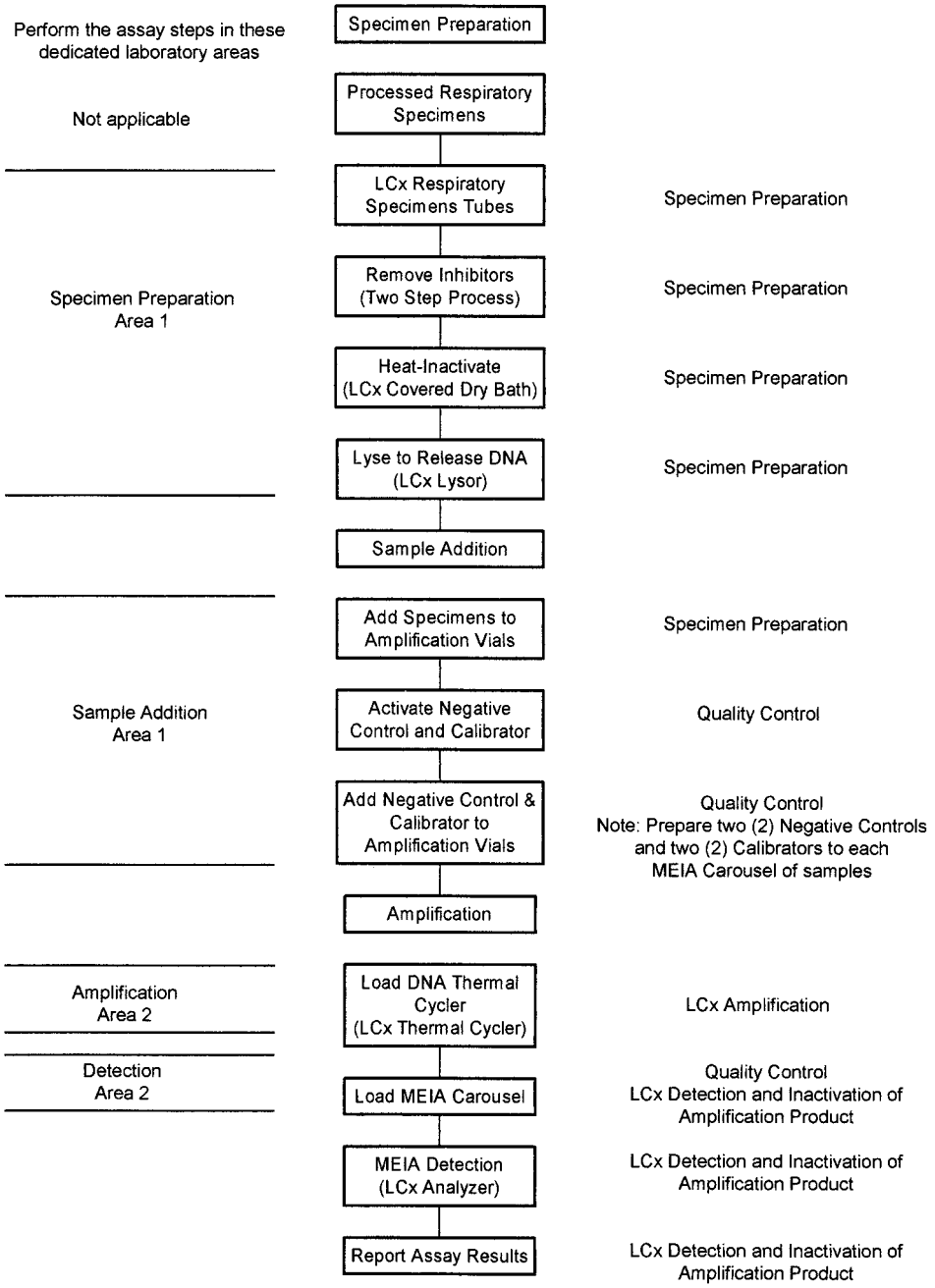

FIG. 1. Description of the LCx *M. tuberculosis* Assay flow.

control (S/CO, 0.77 in both cases). These 18 specimens were from 14 patients, including 2 patients with multidrug-resistant TB.

The other 23 specimens with discrepant results (20 smear-positive specimens) were LCx *M. tuberculosis* Assay positive and culture negative (Table 1). These specimens were further investigated by repeat testing with a new aliquot of the same processed specimen and reviewing laboratory and patient clinical data (Table 2). The specimens were from 10 patients, all of whom were diagnosed with pulmonary TB; all of them had previous positive cultures for *M. tuberculosis* and had been on anti-TB treatment for a period of 1 to 6 months at the time that they were enrolled in the study. A positive result on repeat testing, a patient history of concurrent therapy for TB, and a history of a previous or a subsequent isolation of an *M. tuberculosis* isolate serve as criteria to consider the 23 specimens as culture misses and true positives by the LCx *M. tuberculosis* Assay.

After a combination of culture results and the patient's clinical data, a total of 195 specimens were collected from 110 patients with a diagnosis of pulmonary TB, including the 23 specimens corresponding to 10 patients who were receiving anti-TB treatment. In summary, given that the overall positivity rate was 37.5% (195 of 520 specimens), the sensitivities, specificities, PPVs, and NPVs were 90.8, 100, 100, and 94.7%, respectively, for the LCx *M. tuberculosis* Assay; 88.2, 100, 100,

TABLE 1. Detection of *M. tuberculosis* in respiratory specimens by LCx *M. tuberculosis* Assay

Microscopy result	LCx *M. tuberculosis* Assay result	No. of specimens with the following culture result:		
		Positive[a]	Negative	Total
Positive	Positive	139	20[b]	159
	Negative	2	26[c]	28
	Total	141	46	187
Negative	Positive	15	3[d]	18
	Negative	16	299[e]	315
	Total	31	302	333
Total	Positive	154	23[f]	177
	Negative	18	325[g]	343
	Total	172	348	520

[a] Only data for specimens with positive culture results for *M. tuberculosis* are included.
[b] Twenty specimens from eight patients with active TB and who had been receiving anti-TB treatment for 1 to 6 months.
[c] Twenty-six specimens were LCx *M. tuberculosis* Assay negative and culture positive for NTM.
[d] Three specimens from two patients with active TB and who had been receiving anti-TB treatment for 1 to 2 months.
[e] Six specimens were LCx *M. tuberculosis* Assay negative and culture positive for NTM.
[f] Twenty-three specimens from 10 patients with active TB and who had been receiving anti-TB treatment for 1 to 6 months.
[g] Thirty-two specimens were LCx *M. tuberculosis* Assay negative and culture positive for NTM.

and 93.4%, respectively, for culture; and 82.6, 92, 72.9, and 97.6%, respectively, for acid-fast staining (Table 3). For 161 specimens (82.6%) from patients smear positive for the disease and 34 specimens (17.4%) from patients smear negative for the disease, the sensitivities of the LCx *M. tuberculosis* Assay were 98.8 and 53%, respectively. There were no statistically significant differences in the sensitivity and specificity between the LCx *M. tuberculosis* Assay and culture ($P > 0.05$).

DISCUSSION

Definitive diagnosis of pulmonary TB depends upon the isolation of *M. tuberculosis*, a process which can take up to 8 weeks to yield a negative result due to the slowly growing nature of the microorganism (36). The ability to detect *M. tuberculosis* directly from respiratory specimens can yield a rapid diagnosis of TB. The most recent advances in the diagnosis of TB have been concentrated in the field of the direct detection of *M. tuberculosis* in clinical specimens by PCR (2, 11, 14–16, 27). However, although simplified PCR procedures were investigated, all these methods remain too complex and too long and are not reliable enough to be used in routine clinical practice (14–16, 17, 27). Recent developments in commercially available nucleic acid amplification systems will perhaps solve these problems (29).

The LCx *M. tuberculosis* Assay is the first semiautomated nucleic acid amplification test which has been developed for use in clinical laboratories. In this system, sample preparation is performed manually, and the amplification is realized in the LCx Thermal Cycler. The detection of the amplification product is fully automated in the LCx Analyzer.

The LCx *M. tuberculosis* Assay is a rapid and specific test that can be done directly with respiratory specimens, with the potential to obtain results from the laboratory on the same day that the sample is submitted for testing. The LCx *M. tuberculosis* Assay uses specific ready-to-use reagents, which are needed for specimen preparation, amplification, and amplicon detection. The analytical sensitivity of this system is 56.5 CFU, with 27 of 30 isolates being detected at or below 35 CFU per assay.

The analytical performance of the LCx *M. tuberculosis* Assay was monitored by including negative and positive sediment samples in each run. This method had a good reproducibility, and the differences between the signal for the negative control, the cutoff value, and the signal for the positive control were broad enough to allow for a good resolution of the results.

The diagnostic utility of any laboratory test is influenced by factors related to the composition and performance of the test itself, as well as the distribution of positive and negative specimens in the sample population. Our study population had a disease prevalence rate of 33.7% and a positivity rate of 37.5%. The sensitivity of the staining technique (82.6%) was higher than is commonly reported (10) and was probably a reflection of the patient population studied. Many of the specimens submitted to the mycobacteriology laboratory were from AIDS patients with pulmonary TB, many of whom exhibited high bacillary loads in their respiratory secretions (34). It is important to point out that the overall sensitivity obtained in our study (90.8%) was probably influenced by this fact.

Vuorinen et al. (35) evaluated the Gen-Probe Amplified *M. tuberculosis* Direct Test (AMTDT) and Amplicor *M. tuberculosis* test (Roche Diagnostic Systems, Somerville, N.J.) with 256 respiratory specimens from 243 patients, with a positivity rate of 12.7% and a staining sensitivity of 76%. The sensitivities, specificities, PPVs, and NPVs were 86.2, 100, 100, and 98.2%, respectively, for AMTDT and 82.8, 100, 100, and 97.8%, respectively, for the Amplicor test. Lower sensitivities were obtained for smear-negative specimens by AMTDT (42.9%) and the Amplicor test (28.6%). In a further study (30), AMTDT was evaluated with 1,117 specimens from 988 patients, with a positivity rate of 12% and a staining sensitivity of 40%; the sensitivities values obtained with smear-positive and smear-negative specimens (100 and 81%, respectively) were higher than those reported in our study. A comparative evaluation between AMTDT and the Amplicor test was recently carried out with 327 specimens from 236 patients (31). With a prevalence of 15%, the sensitivities, specificities, PPVs, and NPVs were 95.9, 98.9, 94 and 99.2%, respectively, for AMTDT and 85.4, 99.6, 97.9 and 97.1%, respectively, for the Amplicor test. With a staining sensitivity of 70%, the sensitivities for smear-positive and smear-negative specimens were 100 and 85.7%, respectively, for AMTDT and 96.7 and 50%, respectively, for the Amplicor test.

Conclusively, the results obtained by the LCx *M. tuberculosis* Assay with smear-negative specimens (53%) are in good accordance those obtained by other DNA amplification methods (11, 27, 28). The higher sensitivity of AMTDT comes from detecting *M. tuberculosis* rRNA, which is present at approximately 2,000 copies per cell, which offers a good theoretical sensitivity for specimens with a small load of tubercle bacilli.

In view of the high-level sensitivity shown by the LCx *M. tuberculosis* Assay when applied exclusively to smear-positive specimens (98.8%), when the LCx *M. tuberculosis* Assay is found to be negative with a smear-positive specimen, it is highly unlikely that the patient from whom the specimen was obtained has TB. A negative result indicated an atypical mycobacteriosis; precautions aimed at diminishing the risk of transmission, such as protective isolation, would be unnecessary and would allow for the immediate initiation of treatment with another combination of antituberculosis drugs other than those usually used for the treatment of TB.

We obtained false-negative LCx *M. tuberculosis* Assay re-

TABLE 2. Analysis of discrepant results between LCx *M. tuberculosis* Assay, culture, and staining

Laboratory reference	Type of specimen[a]	Microscopy result[b]	Culture results[c]		Culture detection time (days)	LCx *M. tuberculosis* Assay		Clinical diagnosis	Period of anti-TB treatment (mo)	Comments[d]	Final interpretation of LCx *M. tuberculosis* Assay
			Solid media[e]	BACTEC 12B		Result	Rate (S/CO)				
3978	SP	2+	2+	Positive	13	Negative	441 (0.77)	Pulmonary TB	None		False negative
2193	SP	1+	2+	Positive	15	Negative	441 (0.77)	Pulmonary TB	None		False negative
3849	SP	0	7 colonies	Positive	48	Negative	29 (0.06)	Pulmonary TB	None		False negative
0002	SP	0	6 colonies	Positive	45	Negative	16 (0.04)	Disseminated TB	None	HIV positive	False negative
0022	SP	0	Negative	Positive	40	Negative	16 (0.04)	Pulmonary TB	None		False negative
0183	SP	0	8 colonies	Positive	40	Negative	18 (0.04)	Disseminated TB	None	HIV positive	False negative
0482	SP	0	3 colonies	Positive	50	Negative	17 (0.04)	Pulmonary TB	None		False negative
0489	BA	0	Negative	Positive	25	Negative	17 (0.04)	Pulmonary TB	None		False negative
0559	SP	0	2 colonies	Positive	50	Negative	17 (0.04)	Pulmonary TB	None		False negative
0538	SP	0	8 colonies	Positive	42	Negative	26 (0.05)	Disseminated TB	None	HIV positive MDR TB	False negative
0588	BA	0	10 colonies	Positive	40	Negative	19 (0.04)	Disseminated TB	None	HIV positive MDR TB	False negative
1011	BA	0	Negative	Positive	50	Negative	16 (0.04)	Pulmonary TB	None		False negative
1849	SP	0	7 colonies	Positive	44	Negative	19 (0.05)	Pulmonary TB	None		False negative
1850	SP	0	Negative	Positive	32	Negative	9 (0.06)	Pulmonary TB	None		False negative
1966	SP	0	Negative	Positive	33	Negative	23 (0.06)	Disseminated TB	None	HIV positive	False negative
1859	SP	0	8 colonies	Positive	44	Negative	17 (0.04)	Disseminated TB	None	HIV positive	False negative
1616	SP	0	10 colonies	Positive	42	Negative	23 (0.06)	Pulmonary TB	None		False negative
2144	BA	0	6 colonies	Positive	50	Negative	27 (0.06)	Pulmonary TB	None		False negative
2228	SP	1+	Negative	Negative		Positive	2,051 (4.77)	Pulmonary TB	2		True positive
2191	BA	1+	Negative	Negative		Positive	2,263 (5.26)	Pulmonary TB	2		True positive
2198	SP	2+	Negative	Negative		Positive	2,284 (5.31)	Disseminated TB	3.5	HIV positive	True positive
1970	SP	2+	Negative	Negative		Positive	556 (1.17)	Pulmonary TB	2		True positive
0516	SP	2+	Negative	Negative		Positive	1,649 (3.48)	Pulmonary TB	4		True positive
1214	SP	1+	Negative	Negative		Positive	1,863 (3.93)	Pulmonary TB	6		True positive
1213	SP	1+	Negative	Negative		Positive	1,513 (3.19)	Pulmonary TB	6		True positive
1945	SP	3+	Negative	Negative		Positive	1,669 (3.59)	Pulmonary TB	2		True positive
2066	SP	3+	Negative	Negative		Positive	2,405 (5.38)	Disseminated TB	3	HIV positive	True positive
0542	SP	1+	Negative	Negative		Positive	1,018 (2.12)	Pulmonary TB	4		True positive
0502	SP	1+	Negative	Negative		Positive	923 (1.92)	Pulmonary TB	4		True positive
0532	SP	2+	Negative	Negative		Positive	1,900 (3.95)	Pulmonary TB	4		True positive
0540	SP	1+	Negative	Negative		Positive	979 (2.04)	Pulmonary TB	4		True positive
0541	SP	1+	Negative	Negative		Positive	216 (4.92)	Pulmonary TB	4		True positive
1661	SP	3+	Negative	Negative		Positive	1,320 (2.91)	Disseminated TB	2	HIV positive	True positive
1342	SP	1+	Negative	Negative		Positive	1,766 (4.38)	Pulmonary TB	3		True positive
1154	SP	1+	Negative	Negative		Positive	2,425 (4.45)	Pulmonary TB	6		True positive
1153	SP	1+	Negative	Negative		Positive	1,732 (3.55)	Pulmonary TB	6		True positive
3896	SP	2+	Negative	Negative		Positive	2,524 (4.88)	Pulmonary TB	3.5		True positive
3792	SP	2+	Negative	Negative		Positive	2,305 (5.36)	Pulmonary TB	3.5		True positive
3906	SP	0	Negative	Negative		Positive	828 (1.60)	Pulmonary TB	4		True positive
3950	GA	0	Negative	Negative		Positive	837 (1.38)	Pulmonary TB	1		True positive
3946	SP	0	Negative	Negative		Positive	1,311 (2.15)	Pulmonary TB	4		True positive

[a] SP, sputum; BA, bronchial aspirate; GA, gastric aspirate.
[b] Semiquantitative reporting modified from a previous report (22); 0, no acid-fast bacilli seen; 1+, a few acid-fast bacilli seen; 2+, moderate numbers of acid-fast bacilli seen; 3+, many acid-fast bacilli seen.
[c] Only data for specimens with positive culture results for *M. tuberculosis* are included.
[d] HIV, human immunodeficiency virus; MDR, multidrug resistant.
[e] Modified from a previous report (22); 2+, 10 to 100 colonies on slant.

TABLE 3. Comparison of confirmed results by LCx *M. tuberculosis* Assay, culture, and staining for detection of *M. tuberculosis*

Test and result	No. of specimens with the following confirmed results			Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative	Total				
LCx *M. tuberculosis* Assay							
Positive	177		177	90.8	100	100	94.7
Negative	18	325[a]	343				
Total	195	325	520				
Culture							
Positive	172		172	88.2	100	100	93.4
Negative	23	325[a]	348				
Total	195	325	520				
Microscopy							
Positive	161	26[b]	187	82.6	92	72.9	97.6
Negative	34	299	333				
Total	195	325	520				

[a] Thirty-two specimens were culture positive for NTM.
[b] Twenty-six specimens were culture positive for NTM and LCx *M. tuberculosis* Assay negative.

sults for 18 specimens. These specimens contained less than an estimated 100 CFU/ml in culture. These results illustrate the sampling problem commonly encountered with small loads of tubercle bacilli as a result of their tendency to clump together (nonuniform distribution of microorganisms). These false-negative results could also be explained by the presence of inhibitors of enzymatic amplification reactions in the sample. The importance of proper sample preparation for amplification procedures to eliminate inhibitors has been demonstrated in several studies (2, 5, 11, 30). In this study, the respiratory specimens were pretreated by a protocol with SDS-NaOH (32). Pfyffer et al. (30), using AMTDT, have reported success in pretreating samples with SDS, a detergent which denatures proteins and enzymes and which eliminates most of the inhibitory compounds present in clinical specimens. Moreover, our pretreatment protocol with SDS uses extensive washing to remove any traces of detergent which might interact with the amplification enzymes (26). Furthermore, the LCx *M. tuberculosis* Assay incorporates two washing steps (heat inactivation and mechanical lysis) during specimen preparation to remove and eliminate inhibitors which might interact with the assay system. We believe that in the present study the false-negative results were not due to sample inhibition but were due to either a sampling error because of a low number of microorganisms or a nonuniform distribution of these in the clinical sample.

Physicians and public health personnel use acid-fast staining and culture results to monitor patient response to therapy and to guide decisions concerning infectivity (21). Acid-fast staining results, because they are quickly available, are used to determine the infectivity of the patient and the need for patient isolation and other public health measures (19). The most important limitation of microscopy for these purposes is the possibility of detecting nonviable mycobacteria (10). In a recent follow-up study of patients with TB during the course of their treatment, the persistence of *M. tuberculosis* DNA in sputum was demonstrated >12 months after the start of treatment and >6 months after culture conversion in some patients (20), implying that the assessment of mycobacterial DNA content is not reflective of mycobacterial viability during treatment. In this study, the 23 LCx *M. tuberculosis* Assay-positive and *M. tuberculosis* culture-negative specimens were from 10 patients with a history of anti-TB therapy for periods ranging from 1 to 6 months. Successful therapy will kill the organisms and cause subsequent cultures to be negative. However, the DNA belonging to these killed organisms can still be amplified and detected by the LCx *M. tuberculosis* Assay. Cultures are designed to detect viable organisms by providing necessary nutrients for metabolism, survival, and replication. The LCx *M. tuberculosis* Assay, however, is capable of amplifying DNA from viable as well as nonviable organisms. It will be important to decide when to perform the LCx *M. tuberculosis* Assay in addition to routine cultures; both systems have different objectives, and each system has its own limitations. The results presented here indicate that the possible use of an accurate and more sensitive direct test to replace acid-fast staining and culture to monitor a patient's response to therapy will require long-term studies because of the ability of the LCx *M. tuberculosis* Assay to detect DNA from noncultivable organisms. Despite this, we believe that the LCx *M. tuberculosis* Assay can be useful for the detection of TB in partially treated patients who arrive with unknown clinical data, who are often culture negative for TB, and who may be positive or negative for TB by acid-fast staining.

With the commercial availability of an assay that can reliably detect and identify *M. tuberculosis* within 1 working day, the methodology of the laboratory diagnosis of TB will quickly change. In our experience, this assay is suitable for high-volume tests (48 to 72 specimens) in an 8-h workday. Despite progress in the molecular understanding and fast detection of resistance to primary anti-TB agents (37), a biomass of cultured organisms is still mandatory for routine susceptibility testing and species identification. Therefore, any amplification-based test for the direct detection of *M. tuberculosis* in clinical specimens may only be used as an adjunct to conventional standard procedures (29).

In conclusion, (i) the LCx *M. tuberculosis* Assay is an admissibly sensitive and highly specific technique for the rapid detection of *M. tuberculosis* complex organisms in respiratory specimens, (ii) a positive smear result in combination with a negative LCx *M. tuberculosis* Assay result can lead to the detection of an atypical mycobacteriosis faster than waiting for culture results, (iii) for optimum results by the LCx *M. tuberculosis* Assay, close cooperation between the clinician and the

laboratory personnel is needed to define those patients for whom the clinical suitability of this test is justified, and (iv) the LCx *M. tuberculosis* Assay is able to detect DNA from nonviable bacilli and is not useful for patient treatment follow-up.

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