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Rapid Diagnosis of Extrapulmonary Tuberculosis by Ligase Chain Reaction Amplification

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A rapid amplification-based test for the diagnosis of extrapulmonary tuberculosis, the LCx *Mycobacterium tuberculosis* Assay from Abbott Laboratories, was evaluated. Results from the LCx *M. tuberculosis* Assay were compared with those from culture and the final clinical diagnosis for each patient. A total of 526 nonrespiratory specimens from 492 patients were tested. The specimens included urine; feces; lymph node exudates; pleural, cerebrospinal, articular, and ascitic fluids; tissue biopsies; gastric aspirates; purulent exudates; blood; and bone marrow aspirates. After combination of the culture results and the patient's clinical data, a total of 135 specimens were collected from 122 patients with a diagnosis of extrapulmonary tuberculosis. The sensitivity, specificity, and positive and negative predictive values for the LCx *M. tuberculosis* Assay were 77.7, 98.7, 95.2, and 93.1%, respectively; these values rose in resolved cases of TB to 78.5, 100, 100, and 93.1%, respectively. For 37 (27.4%) specimens from patients smear positive for the disease and 98 (72.6%) specimens from patients smear negative for the disease, the sensitivities of the LCx *M. tuberculosis* Assay were 100 and 71.1%, respectively. Statistically significant differences ($P < 0.01$) in sensitivities were found between culture and the LCx *M. tuberculosis* Assay. These differences were even greater among smear-negative specimens. The results demonstrate that the LCx *M. tuberculosis* Assay will provide rapid and valuable information for the diagnosis of extrapulmonary tuberculosis.

Disease caused by *Mycobacterium tuberculosis* has been always a serious world health problem. Important unresolved questions concerning tuberculosis (TB) are the need for rapid laboratory diagnosis, the requirement for prolonged treatment, and the absence of a reliable vaccine (23, 25, 43).

Bacteriological diagnosis of extrapulmonary TB is currently based on acid-fast staining and culture on solid and/or liquid media. Detection by microscopy is useful as a rapid screening test, but lacks sensitivity (11). Culture on solid media can take up to 8 weeks to yield a positive result (1, 14). The radiometric BACTEC 460 TB system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) has been an important addition to culture methods, although this technique requires an average of 13 to 15 days to detect positive specimens (1, 14, 23). Technological advances in amplifying and detecting specific regions of bacterial DNA or RNA have provided the methods necessary to make improvements in the laboratory diagnosis of TB. The use of nucleic acid amplification technologies for the detection of *M. tuberculosis* in respiratory and other clinical samples has been reported (2, 7, 12, 15–17) with promising results. However, most laboratories that use these technologies have developed their own tests with a wide variety of primers and probes and extraction, amplification, and detection techniques. This has led to unexpectedly wide variations in sensitivity and specificity (6, 7, 12, 20, 27, 34). To overcome these difficulties, two important techniques have been introduced in a kit-based format: PCR (31, 37) and transcription-mediated amplification (2, 18, 19, 42). These techniques supply all of the necessary reagents for sample preparation and for amplification and detection. Recently, ligase chain reaction (LCR) technology has become commercially available for the detection of *M. tuberculosis* in clinical specimens (3, 41). However, there are still problems, including the presence of inhibitors of enzymatic amplification reactions in clinical specimens, which may cause false-negative results, and contamination with amplicons, which gives false-positive results (6, 12, 28, 33). Moreover, the amplification techniques for detection of *M. tuberculosis* described in most reports have mainly been applied to clinical samples of respiratory origin, and experience with nonrespiratory specimens is still limited. Paradoxically, however, it is precisely extrapulmonary TB (tuberculous pleuritis, peritonitis, meningitis, lymph node TB, etc.) for which a rapid and accurate laboratory diagnosis is of prime importance, since the traditional techniques for detecting acid-fast bacilli have important well-known limitations (1, 11, 23).

The LCx *M. tuberculosis* Assay (Abbott Laboratories, Diagnostic Division, Chicago, Ill.) uses LCR amplification technology for the direct detection of *M. tuberculosis* in clinical samples. The LCR amplification methods have been evaluated previously for the detection of other infectious agents (5, 9, 10, 28). The target sequence of the LCx *M. tuberculosis* Assay is found within the chromosomal gene of *M. tuberculosis* which codes for protein antigen b (4). This gene sequence appears to be specific to the *M. tuberculosis* complex (MTBC) and has been detected in all of the MTBC strains examined to date (39).

The LCx *M. tuberculosis* Assay is the first commercial semiautomated nucleic acid amplification test developed for use

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TABLE 1. Initial comparison of the LCx *M. tuberculosis* Assay with culture for the detection of *M. tuberculosis* in 526 nonrespiratory specimens

Specimen type (no.)	No. of specimens with result				% Sensitivity	% Specificity	% PPV	% NPV
	Culture positive		Culture negative					
	LCx positive	LCx negative	LCx positive	LCx negative				
Smear positive (61)	33	0	4[a]	24[b]	100	85.7	89.1	100
Smear negative (465)	68	29	1[c]	367[d]	70.1	99.7	98.5	92.6
All (526)	101	29	5	391	77.7	98.7	95.2	93.1

[a] From four patients with active TB treated with anti-TB drugs for 1 to 7 months.
[b] Twenty-four specimens with culture positive for NTM.
[c] From one patient with active cervical tuberculous lymphadenitis.
[d] Fourteen specimens with culture positive for NTM.

with respiratory specimens, and limited experience has been reported for nonrespiratory specimens (41). In this system, sample preparation is performed manually, and the amplification is carried out in the LCx Thermal Cycler. The detection of the amplified product is fully automated in the LCx Analyzer.

The purpose of the present study was to evaluate the performance and clinical usefulness of the LCx *M. tuberculosis* Assay in the detection of *M. tuberculosis* in nonrespiratory specimens and to compare this method with standard culture and staining techniques.

MATERIALS AND METHODS

Subjects and clinical specimens. From July through December 1996, we investigated 526 nonrespiratory specimens collected from 492 patients suspected of having extrapulmonary TB. The 526 nonrespiratory specimens comprised 69 urine specimens, 35 fecal specimens, 175 organic fluid specimens (55 pleural fluid specimens, 43 cerebrospinal fluid specimens, 45 ascitic fluid specimens, and 32 articular fluid specimens), 5 gastric juice aspirate specimens, 36 tissue biopsy specimens, 10 purulent exudate specimens, 62 lymph node exudate specimens, 39 bone marrow aspirate specimens, and 95 blood specimens. Upon receipt, specimens were kept at 4°C prior to processing. Gastric juice aspirates were immediately neutralized with trisodium phosphate buffer after retrieval.

Sample processing. Tissue specimens were sliced and homogenized in a mortar with 2 ml of 0.9% NaCl under sterile conditions before processing. Urine and other fluid samples had been previously centrifuged at 3,300 × *g* for 20 min. All samples, with the exception of bone marrow aspirates and blood samples, were digested and decontaminated with sodium dodecyl (lauryl) sulfate (SDS)-NaOH as previously described (38). The concentrated specimen pellet was resuspended in 30 ml of sterile distilled water and centrifuged for 20 min at 3,300 × *g*, and the supernatant fluid was removed. Bone marrow aspirates were pretreated with 100 μl of 10% SDS. Blood samples (5 ml) collected in EDTA-anticoagulated tubes were processed immediately with 500 μl of 10% SDS. After being vortexed for 10 min at room temperature, bone marrow aspirates and blood samples were washed with 30 ml of distilled water and centrifuged for 20 min at 3,300 × *g*, and the supernatant was removed. The sediment from all specimens was finally resuspended in 2.2 ml of 0.067 M phosphate buffer (pH 6.8). For all specimens, half of the sediment was stored at −80°C for the LCx *M. tuberculosis* Assay, and the other half was inoculated onto the culture media and used for acid-fast staining.

Smear examination and culture. Smears were stained by auramine-rhodamine fluorochrome as a screening method. Positive slides were confirmed to be positive by Ziehl-Neelsen stain (23). Equal aliquots of the processed sediment were inoculated onto two solid slants containing Löwenstein-Jensen and Coletsos media (13). Slants were incubated at 37°C for 8 weeks in a 6% CO_2 atmosphere. In addition, 500 μl of the sediments was inoculated into BACTEC 12B medium and then incubated at 37°C for up to 8 weeks. Furthermore, 5 ml of blood samples was inoculated into BACTEC 13A bottles and incubated at 37°C for 8 weeks. Solid media were read weekly, and BACTEC cultures were read twice weekly for the first 2 weeks and once weekly thereafter. A BACTEC growth index of >100 was considered positive, and a smear for Ziehl-Neelsen staining and culture on solid media were prepared to detect acid-fast bacilli.

Identification. The identification of isolates was performed by routine biochemical methods (23), by gas-liquid chromatography (29), and with the Accuprobe culture confirmation tests (Gen-Probe, Inc., San Diego, Calif.) (22).

Detection of *M. tuberculosis* by the LCx *M. tuberculosis* Assay. The LCx *M. tuberculosis* Assay consists of three steps—specimen preparation, amplification, and detection—and was performed according to the manufacturer's recommendations.

Specimens were prepared by addition of 500 μl of pretreated (SDS-NaOH) specimen to an LCx Respiratory Specimen Tube and then were centrifuged 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. After vortexing, the suspension was placed into LCx Covered Dry Bath for 20 min at 95°C. Finally, mycobacterial DNA was released by mechanical lysis in the LCx lysor for 10 min.

For the amplification reaction, 100 μl of the specimen lysed was added to the appropriately labeled LCx Tuberculosis Amplification Vial containing 100 μl of the LCR mixture. The specimens and controls were placed in the LCx Thermal Cycler and amplified for 37 cycles of incubation for 1 s at 94°C, 1 s at 55°C, and 40 s at 69°C.

Amplified tubes were transferred unopened to the carousel of the LCx Analyzer, which directly detects the amplification products and displays the results as fluorescence rates, which are compared to the calibrator rate (8). A sample rate/cutoff value ratio of >1.0 indicates an LCx *M. tuberculosis* Assay-positive result.

Clinical diagnosis of extrapulmonary tuberculosis. For the clinical diagnosis of extrapulmonary TB, each patient's chart was reviewed. Clinical assessment included the patient's history, signs, symptoms, chest X ray, cytological and histological results for specimens, result of the tuberculin skin test, history of drugs administered, and response to empirical TB treatment. In 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 with the LCx *M. tuberculosis* Assay.

Statistical analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LCx *M. tuberculosis* Assay were calculated in comparison with culture results and, separately, in comparisons with culture results plus the patient's clinical data. Statistical comparisons were performed by chi-square analysis.

RESULTS

The LCx *M. tuberculosis* Assay was evaluated for its ability to detect MTBC organisms in 526 specimens from 492 patients with clinical signs or symptoms of extrapulmonary TB. Of the 526 specimens examined, 130 were culture positive for *M. tuberculosis*. Of these, 33 (25.4%) specimens were smear positive, and 97 (74.6%) specimens were smear negative. Thirty-eight specimens (24 smear positive) were culture positive for nontuberculous mycobacteria (NTM); the species of NTM identified from these specimens were *Mycobacterium avium-Mycobacterium intracellulare* complex (17 specimens), *Mycobacterium kansasii* (7 specimens), *Mycobacterium genavense* (11 specimens), *Mycobacterium gordonae* (1 specimen), and *Mycobacterium xenopi* (2 specimens). All NTM isolates corresponded to patients with clinical pictures compatible with extrapulmonary or disseminated mycobacteriosis. Three hundred fifty-eight specimens (4 smear positive) were culture negative.

An initial comparison of amplification results with culture results is summarized in Table 1. One hundred one specimens (33 smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*, and 353 specimens (all smear and culture negative) were LCx *M. tuberculosis* Assay negative. The 38 specimens with NTM isolates were LCx *M. tu-*

TABLE 2. Discrepant results between culture and LCx *M. tuberculosis* Assay obtained for nonrespiratory specimens

Type of specimen	No. of specimens	Smear result	Culture result[a]	LCx result	Final interpretation of LCx result
Urine	6	Negative	Positive	Negative	False negative
Feces	1	Negative	Positive	Negative	False negative
Lymph node	3	Negative	Positive	Negative	False negative
Lymph node	3[b]	Positive	Negative	Positive	True positive
Lymph node	1[c]	Negative	Negative	Positive	True positive
Pleural exudate	4	Negative	Positive	Negative	False negative
Cerebrospinal fluid	3	Negative	Positive	Negative	False negative
Ascitic fluid	3	Negative	Positive	Negative	False negative
Articular fluid	3	Negative	Positive	Negative	False negative
Tissue biopsy	1	Negative	Positive	Negative	False negative
Tissue biopsy	1[d]	Positive	Negative	Positive	True positive
Bone marrow	2	Negative	Positive	Negative	False negative
Blood	3	Negative	Positive	Negative	False negative

[a] Culture result for *M. tuberculosis.*
[b] From three patients with active TB treated with anti-TB drugs for 1 to 7 months.
[c] From one patient with active cervical lymphadenitis and good response to TB treatment.
[d] From one patient with active TB treated with anti-TB drugs for 4 months.

berculosis Assay negative. There were 34 specimens with discrepant results. Twenty-nine of these specimens (all smear negative) were LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis*. The 29 specimens were retested with a new aliquot of the same processed specimen. The results were confirmed and were considered false negative (Table 2). The overall sensitivity, specificity, and PPVs and NPVs of the LCx *M. tuberculosis* Assay were, compared with culture results, 77.7, 98.7, 95.2, and 93.1%, respectively (Table 1).

Four smear-positive specimens were LCx *M. tuberculosis* Assay positive and culture negative. These specimens were further investigated by repeat testing with a new aliquot of the same processed specimen and review of laboratory and patient clinical data. These four specimens were from four patients with disseminated TB who had been receiving anti-TB treatment for 1 to 7 months at the time 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 subsequent isolation of an *M. tuberculosis* isolate serve as criteria to consider the four specimens as culture misses and true positives by the LCx *M. tuberculosis* Assay (Table 2). The last specimen with discrepant results was LCx *M. tuberculosis* Assay positive and smear and culture negative and corresponded to a patient with active cervical tuberculous lymphadenitis and a good response to TB treatment; this specimen was retested with the LCx *M. tuberculosis* Assay and other amplification techniques and was considered true positive (Table 2).

Of the 69 urine specimens tested, 14 (5 specimens smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. Forty-seven specimens were LCx *M. tuberculosis* Assay negative and culture negative. Two specimens (one smear positive) were LCx *M. tuberculosis* Assay negative and culture positive for NMT. Finally, six urine specimens (all smear negative) were LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis*; these specimens were considered false negative (Table 2). After review of the patients' clinical data, the sensitivity and specificity of the LCx *M. tuberculosis* Assay for urine specimens were 70 and 100%, respectively (Table 3).

Of 35 fecal specimens examined, 7 (all smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. There were 9 specimens LCx *M. tuberculosis* Assay negative and culture negative. Eighteen specimens (17 smear positive) were LCx *M. tuberculosis* Assay negative and culture positive for NTM. One specimen (smear negative) was LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis* and was considered LCx *M. tuberculosis* Assay false negative (Table 2). The sensitivity and specificity of the LCx *M. tuberculosis* Assay for fecal specimens were 87.5 and 100%, respectively (Table 3).

Of the 175 organic fluids tested, 26 (2 smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. There were 135 LCx *M. tuberculosis* Assay-negative and culture-negative specimens. One specimen (smear

TABLE 3. Specimen sources and comparison of LCx *M. tuberculosis* Assay results after resolution of discrepancies with clinical diagnosis of TB

Specimen	No. of specimens with TB result				% Sensitivity	% Specificity	% PPV	% NPV
	Clinical diagnosis positive		Clinical diagnosis negative					
	LCx positive	LCx negative	LCx positive	LCx negative				
Urine	14	6	0	49	70.0	100	100	89.1
Feces	7	1	0	27	87.5	100	100	96.4
Organic fluids	26	13	0	136	66.6	100	100	91.2
Blood and bone marrow aspirates	28	5	0	101	84.8	100	100	95.2
Lymph nodes, biopsies, and others	30[a]	4	0	79	88.2	100	100	95.1

[a] Four patients with anti-TB treatment and one patient with active cervical tuberculous lymphadenitis.

TABLE 4. Comparison of confirmed results by LCx *M. tuberculosis* Assay and culture in 526 nonrespiratory specimens

Test and result	No. of specimens with extrapulmonary TB result			% Sensitivity	% Specificity	% PPV	% NPV
	Positive	Negative	Total				
LCx *M. tuberculosis* Assay				78.5	100	100	93.1
Positive	106	0	106				
Negative	29	391[a]	420				
Total	135	391	526				
Culture				96.2	100	100	98.7
Positive	130	0	130				
Negative	5[b]	391[a]	396				
Total	135	391	526				

[a] Thirty-eight specimens with culture positive for NTM.
[b] From four patients with anti-TB treatment and one patient with active cervical tuberculous lymphadenitis.

positive) was LCx *M. tuberculosis* Assay negative and culture positive for *M. kansasii*. Finally, 13 specimens (4 pleural exudates, 3 cerebrospinal fluids, 3 ascitic fluids, and 3 articular fluids) were LCx *M. tuberculosis* Assay negative, smear negative, and culture positive for *M. tuberculosis* and were considered LCx *M. tuberculosis* Assay false negative (Table 2). The sensitivity and specificity of the LCx *M. tuberculosis* Assay for organic fluid specimens were 66.6 and 100%, respectively (Table 3).

Of 134 blood-contained samples (95 blood specimens and 39 bone marrow aspirates), 28 were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. There were 88 LCx *M. tuberculosis* Assay-negative and culture-negative specimens. Thirteen specimens were LCx *M. tuberculosis* Assay negative and culture positive for NTM. Five specimens (two bone marrow aspirate specimens and three blood specimens) were LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis* and were considered LCx false negative (Table 2). The sensitivity and specificity of the LCx *M. tuberculosis* Assay for blood specimens and bone marrow aspirates were 84.8 and 100%, respectively (Table 3).

Of 62 lymph nodes tested, 16 (13 smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. Thirty-eight specimens were LCx *M. tuberculosis* Assay negative and culture negative. One specimen (smear positive) was LCx *M. tuberculosis* Assay negative and culture positive for *M. kansasii*. Three specimens (all smear negative) were LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis* and were considered LCx *M. tuberculosis* Assay false negative. Three specimens (all smear positive) were LCx *M. tuberculosis* Assay positive and culture negative. These three specimens originated from patients with a recent history of TB; all of them had been on anti-TB treatment for a period of 1 to 7 months at the time they were included in the study and were therefore considered true positive (Table 2). Finally, one specimen (smear negative) was LCx *M. tuberculosis* positive and culture negative. This specimen originated from a patient with active cervical tuberculous lymphadenitis, with compatible histology (granulomatous inflammation), and a good response to TB treatment. This specimen was positive upon retesting with the LCx *M. tuberculosis* Assay and other amplification techniques and was considered true positive. The sensitivity and specificity of the LCx *M. tuberculosis* Assay for lymph node specimens were 86.9 and 100%, respectively.

The majority of the other 51 nonrespiratory specimens (10 purulent exudates, 5 gastric juice aspirates, and 36 tissue biopsies) gave LCx *M. tuberculosis* Assay results which mainly agreed with those of the culture. Nine specimens (all smear positive) were LCx *M. tuberculosis* Assay positive and culture positive for *M. tuberculosis*. There were 37 LCx *M. tuberculosis* Assay-negative and culture-negative specimens. Three specimens were LCx *M. tuberculosis* Assay negative and culture positive for NTM. One specimen (tissue biopsy) was LCx *M. tuberculosis* Assay negative and culture positive for *M. tuberculosis* and was considered false negative. Finally, one specimen (smear positive) was LCx *M. tuberculosis* Assay positive and culture negative. This specimen (tissue biopsy) was derived from a patient on anti-TB treatment for 4 months. This LCx *M. tuberculosis* Assay-positive result was considered true positive. Thus, the sensitivity and specificity of the LCx *M. tuberculosis* Assay for these specimens were 90.9 and 100%, respectively.

After resolution of the discrepant results (the 4 specimens from 4 patients with disseminated TB who were receiving anti-TB treatment and 1 specimen from a patient with active cervical tuberculous lymphadenitis were considered true positives), a total of 135 specimens were collected from 122 patients with a diagnosis of extrapulmonary TB. The sensitivity, specificity, and PPVs and NPVs were thus 78.5, 100, 100, and 93.1%, respectively, for the LCx *M. tuberculosis* Assay and 96.2, 100, 100, and 98.7%, respectively, for culture (Table 4). For 37 specimens (27.4%) from patients smear positive for the disease and 98 specimens (72.6%) from patients smear negative for the disease, the sensitivities the LCx *M. tuberculosis* Assay were 100 and 71.1%, respectively.

Statistically significant differences in sensitivities ($P < 0.01$) were found between culture and the LCx *M. tuberculosis* Assay. These differences were even greater among smear-negative specimens.

DISCUSSION

The laboratory diagnosis of mycobacterial infections usually requires 2 to 8 weeks. The recent increase in new cases of TB has shown that the need for rapid, specific diagnostic assays for *M. tuberculosis* is urgent (35).

Nucleic acid amplification procedures have had considerable impact on the rapid detection of *M. tuberculosis* directly from respiratory and nonrespiratory specimens (2, 12, 20, 28, 33–36).

In this paper, we have investigated whether the LCx *M. tuberculosis* Assay is suitable for rapid and direct detection of *M. tuberculosis* in a wide range of nonrespiratory specimens. The sensitivity and specificity results of the LCx *M. tuberculosis* Assay were shown to be as high as those reported by other

investigators for respiratory specimens (18, 24, 31, 37) with several commercial amplification tests.

One drawback to using commercial amplification systems in the daily laboratory routine is that the manufacturers do not recommend their use with nonrespiratory specimens. The performance of the LCx *M. tuberculosis* Assay with nonrespiratory specimens has been consistent. Of the 135 specimens from 120 patients with diagnosis of extrapulmonary TB, 106 specimens were LCx *M. tuberculosis* Assay positive. The 29 false-negative LCx *M. tuberculosis* assay results included 6 urine samples and 1 feces sample; 3 lymph node and 4 pleural exudates; 3 cerebrospinal, 3 ascitic, and 3 articular fluids; 1 tissue biopsy; 2 bone marrow aspirates; and 3 blood specimens. All of these specimens were smear negative and gave positive cultures in BACTEC 12B and solid media with <100 colonies. These false-negative results could be explained by a low number of microorganisms or a nonuniform distribution of the microorganisms in the clinical samples. These false-negative results also could be explained by the presence of possible amplification inhibitors in the sample.

The usefulness of the DNA amplification techniques is often limited by the presence of amplification inhibitors in clinical samples. In several studies, rates of occurrence of false-negative results of greater than 20% have been reported (12, 26, 32, 33, 40). In a prospective study (26), it was observed that nucleic acids prepared with the Amplicor Sputum Preparation Kit frequently showed the presence of inhibitors of the amplification reaction. Of a total of 252 samples prepared by this procedure, 21% revealed the presence of inhibitors (specimens obtained from the respiratory tract, 17.8%; those from cerebrospinal fluid, 54.5%; and those from urine, gastric fluid, peritoneal fluid, and pleural fluid, 25%). After the nucleic acids prepared by the Amplicor procedure were subjected to the guanidium isothiocyanate method, the amplification inhibitors were eliminated in 96% of the specimens. In another study (19) utilizing the Gen Probe Amplified *M. tuberculosis* Direct Test, 7.5% of nonrespiratory specimens revealed the presence of inhibitors of the amplification reaction. The importance of proper sample preparation for amplification procedures to eliminate inhibitors has been demonstrated in several studies (2, 6, 12, 30, 36). In this study, the nonrespiratory specimens were pretreated by a protocol involving SDS-NaOH (38), which eliminates most of the inhibitory compounds present in clinical specimens (21, 36). Although, the LCx *M. tuberculosis* Assay incorporates two washing steps, heat inactivation, and mechanical lysis in the specimen preparation, we believe that these procedures did not inactivate all of the inhibitory substances found in these specimens. Therefore, the LCx *M. tuberculosis* Assay should include internal controls in order to assess the efficacy of each amplification reaction and to ensure that the sample is free of interfering substances. The use of internal controls will identify those samples that are inappropriate for amplification or that require further manipulation to remove inhibitors, and their use will ultimately increase confidence in the reliability of negative results.

Microscopic examination of an acid-fast smear is a simple and inexpensive method. The value of positive staining results from primary specimens is, however, limited because of the increasing frequency of NTM infections. The LCx *M. tuberculosis* Assay showed a sensitivity of 100% with smear-positive specimens; therefore, when the LCx *M. tuberculosis* Assay result is found to be negative with a smear-positive specimen, it is highly unlikely that the patient from whom the specimen was derived has TB. A smear-positive and LCx *M. tuberculosis* Assay-negative result indicated an atypical mycobacteriosis, and precautions aimed at diminishing the risk of transmission, such as protective isolation, would be unnecessary and would allow the immediate initiation of treatment with another combination of anti-TB drugs instead of the drugs usually used for TB treatment.

In our study, four of the five LCx *M. tuberculosis* Assay-positive and culture-negative specimens (four smear positive) were obtained from four patients with disseminated TB who had been receiving anti-TB treatment for between 1 and 7 months at the time they were enrolled in the study. These four specimens were considered culture misses and true positives by the LCx *M. tuberculosis* Assay. Successful therapy will kill the organisms and cause subsequent cultures to be negative; however, the DNA of these killed organisms can still be amplified and detected by the LCx *M. tuberculosis* Assay. Cultures are designed to detect viable organisms. The LCx *M. tuberculosis* Assay, however, is capable of amplifying DNA from viable as well as nonviable organisms. Therefore, the test would not be useful for tracking the efficacy of anti-TB therapy. However, before this can be considered, additional studies need to be done to corroborate the existing data for patients undergoing antimicrobial therapy.

The fifth specimen with discrepant results was LCx *M. tuberculosis* Assay positive and smear and culture negative and corresponded to a patient with active cervical tuberculous lymphadenitis, compatible histology (granulomatous inflammation), and a good response to TB treatment; this specimen was retested by the LCx *M. tuberculosis* Assay with a new aliquot of the same processed specimen. The result was confirmed and was considered true positive.

The LCx *M. tuberculosis* Assay contains all of the specific reagents needed for specimen lysis, amplification, and detection of amplified products. The assay was well suited for use in an experienced routine clinical microbiology laboratory, and there were no problems due to sample or amplicon contamination.

Recently, Ausina et al. (3) evaluated the LCx *M. tuberculosis* Assay with 520 respiratory specimens. With an overall positivity rate of 37.5%, the sensitivity and specificity obtained were 90.8 and 100%, respectively. In another report (41), the LCx *M. tuberculosis* Assay was evaluated with 511 respiratory and 147 nonrespiratory specimens. For respiratory specimens, the sensitivity and specificity obtained were 98.97 and 98.97%, respectively, and for nonrespiratory specimens, they were 73.33 and 100%, respectively. These results are in good accordance with those obtained in our study.

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 TB will change quickly. In our experience, the LCx *M. tuberculosis* Assay is suitable for high-volume testing (48 to 72 specimens) per 8-h working day. Despite progress in the molecular understanding and rapid detection of resistance to primary anti-TB agents (44), biomass of cultured organisms is still mandatory for routine susceptibility testing and species identification. Therefore, any amplification based-test for direct detection of *M. tuberculosis* in clinical specimens may only be used as an adjunct to conventional standard procedures (35).

In summary, our data indicate that the LCx *M. tuberculosis* Assay provides the clinician, laboratory technician, and infection control practitioner with very valuable, rapid, and clinically relevant information for the diagnosis and control of extrapulmonary TB.

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