

## Rapid Diagnosis of Pulmonary Tuberculosis with the LCx *Mycobacterium tuberculosis* Assay and Comparison with Conventional Diagnostic Techniques

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**The LCx MTB amplification assay is a nucleic acid amplification test intended for the direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. We evaluated its performance on 2,001 consecutive respiratory specimens; 78 were culture positive for *M. tuberculosis*. Sensitivity, specificity, and positive and negative predictive values of this assay for all specimens compared to culture results were 88.5, 97.7, 60.5, and 99.5%, respectively. When referred to resolved clinical diagnosis of active tuberculosis, these values improved to 90.2, 98.4, 72.8, and 99.5%, respectively.**

Nucleic acid amplification tests have contributed to a more rapid and reliable diagnosis of pulmonary tuberculosis. Two of these methods, based on transcription-mediated amplification (Mycobacterium Tuberculosis Direct Test MTD; Gen-Probe, San Diego, Calif.) and on PCR (Amplicor *M. tuberculosis*; Roche Diagnostic Systems, Somerville, N.J.) were approved in 1996 by the Food and Drug Administration for the detection of *Mycobacterium tuberculosis* complex in smear-positive respiratory specimens in conjunction with culture for untreated patients (2). Another amplification assay, the LCx MTB (Abbott Diagnostics Division, Abbott Park, Ill.) uses the ligase chain reaction to detect *Mycobacterium tuberculosis* complex directly in respiratory specimens. We prospectively evaluated its performance on consecutive, nonselected specimens.

A total of 2,001 clinical specimens (1,108 sputum, 540 bronchial aspirate, 320 bronchoalveolar lavage, 21 gastric fluid, and 12 tracheal aspirate specimens) obtained from 1,130 patients were analyzed by two clinical mycobacteriology laboratories. Smear samples were stained with fluorochrome dye. At site A (Geneva, Switzerland), specimens were digested and decontaminated with the 4% sodium hydroxide method (7, 10) before inoculating BACTEC 12B vials (Becton Dickinson, Sparks, Md.) and Coletsos (BioMérieux, Lyon, France) and Stonebrink egg-based slants (Becton Dickinson). All media were incubated at 36°C for 14 weeks. At site B (Zurich, Switzerland), the NALC-NaOH procedure (7, 8) was used and one BACTEC 12B vial, one Löwenstein-Jensen slant, and one Middlebrook 7H10/sel 7H11 agar (bi-plate; Becton Dickinson Microbiology Systems, Cockeysville, Md.) were inoculated per specimen. BACTEC 12B vials were incubated at 36°C for 8 weeks, and solid media were incubated at 36°C for 9 weeks. The LCx MTB assay, which consists three main steps (specimen preparation, amplification, and detection), was performed according to the manufacturer's instructions (1, 6). The remaining volumes of the decontaminated samples were frozen at -20°C so that the LCx assay could be repeated for specimens yielding discrepant

results by LCx assay and culture. For LCx MTB-positive specimens which remained culture negative, patient charts were reviewed or the treating physician was questioned as to whether clinical evidence of active pulmonary tuberculosis existed for these patients. Specimens which were LCx MTB positive but culture negative and which originated from patients with strong clinical evidence of tuberculosis were considered true positive in the resolved results.

From 117 (5.8%) of the 2,001 specimens, mycobacteria were cultured, 78 (3.9%) were identified as *M. tuberculosis* and 39 were identified as nontuberculous mycobacteria. Acid-fast bacilli could be detected by microscopy in 68 (3.4%) specimens. Of the 78 specimens from which *M. tuberculosis* could be cultured, 58 (74.4%) were smear positive. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LCx MTB assay for all specimens as well as for smear-positive and smear-negative specimens are given in Table 1. Table 2 shows the performance of the LCx MTB assay compared to clinical evidence of active pulmonary tuberculosis. Fourteen samples collected from patients with pulmonary tuberculosis were microscopy and culture negative; five of these patients had received antituberculous treatment when the samples were collected. Table 3 summarizes the results ob-

TABLE 1. Comparison of LCx MTB results with culture results for the detection of *M. tuberculosis* complex

Group of specimens (no. of specimens analyzed) and LCx result	No. of cultures with <i>M. tuber- culosis</i>		Sensi- tivity (%)	Speci- ficity (%)	PPV (%)	NPV (%)
	Positive	Negative				
Total (n = 2,001)			88.5	97.7	60.5	99.5
Positive	69	45				
Negative	9	1,878				
Smear-positive (n = 68)			94.8	90.0	98.2	75.0
Positive	55	1				
Negative	3	9				
Smear-negative (n = 1,933)			70.0	97.7	24.1	99.7
Positive	14	44				
Negative	6	1,869				

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TABLE 2. Comparison of LCx MTB and microscopic results with resolved results for the clinical diagnosis of tuberculosis

Method (2,001 specimens and result)	No. of specimens obtained from patients with clinical diagnosis of tuberculosis:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
LCx						
Positive	83	31	90.2	98.4	72.8	99.5
Negative	9	1,878				
Smear			63.0	99.5	85.3	98.2
Positive	58	10				
Negative	34	1,899				

tained for specimens with initially discrepant results between LCx MTB assay and culture compared to the results of the repeated LCx test.

The results achieved with the LCx MTB assay on respiratory specimens pretreated with either the NaOH or NALC-NaOH method are similar and comparable to those obtained for the MTD and Amplicor *M. tuberculosis* nucleic acid amplification assays (4). Other evaluations of the LCx MTB assay have been published recently (1, 6, 12). These studies were performed on selected specimens in order to include a large number of specimens containing species of the *M. tuberculosis* complex. With selected specimens, high positivity rates ranging from 16 to 33% were obtained. Sensitivities, specificities, and NPVs were comparable in all LCx MTB evaluations (>88%), but the PPV was relatively low in one evaluation (70.0%) (6), which was close to the value we obtained (60.5%). The reason for this observation in the current study was the large number of culture-negative specimens ( $n = 1,923$ ), which is generally observed when analyzing nonselected samples. As reported for other nucleic acid amplification tests (1, 2, 4, 6, 11, 12), higher sensitivity was achieved with the LCx MTB assay on smear-positive specimens than on smear-negative specimens, i.e., 94.8 versus 70.0%, respectively (Table 1).

In our study, 14 specimens were obtained from patients with clinical evidence of tuberculosis and with LCx MTB assay-positive but culture-negative results. Culture media may not reveal all specimens containing *M. tuberculosis*. In this study, the BACTEC 12B medium alone detected 75 (96.7%) of the 78 *M. tuberculosis* strains as the most sensitive medium used in both participating laboratories. "False-positive" nucleic acid amplification test results have been reported for patients receiving adequate antituberculous treatment with residual *M. tuberculosis* detectable with nucleic acid amplification assays in their respiratory specimens collected up to 6 months after the onset of treatment (3, 9). In the present study, five

TABLE 3. Initial and repeat results of the LCx MTB test obtained for specimens with initially discrepant results<sup>a</sup>

Results compared	No. of specimens (with initial discrepant results)			
	True positive	True negative	False positive	False negative
First LCx versus culture	0	0	45	9
Repeat LCx versus culture	1	23	22	8
First LCx versus resolved	14	0	31	9
Repeat LCx versus resolved	13	23	8	10

<sup>a</sup> Results for 54 specimens with discrepancies between the initial LCx MTB and culture compared to culture and clinically resolved results.

specimens were collected from patients receiving antituberculous treatment which were LCx MTB positive but microscopy and culture negative. False-positive results have also been reported for specimens collected with sterilized bronchoscopes which may be contaminated with residual *M. tuberculosis* (5). In the current study, a relatively large number of bronchoalveolar lavage specimens ( $n = 320$ ) was included. Of these specimens, only two gave false positive results by the LCx MTB assay, which is negligible compared to the total number of 31 false-positive LCx results.

Commercially available automated amplification assays have advantages over in-house techniques, since automation reduces variations during the technical procedure and large-scale production of reagents limits lot-to-lot variations and inter-laboratory differences. In addition, external quality control data from different testing sites allow an objective validation of the performance of a test system as well as comparisons of the results. The main advantage of the LCx MTB test over other currently available nucleic acid amplification tests is the automated microparticle enzyme immunoassay detection of the amplified product with the LCx analyzer. Cost awareness is becoming an important issue in the management of diagnostic laboratories. The frequency of testing largely influences the cost efficiency of these tests. Testing once weekly allows batching and economic advantages, but the impact on patient care and the benefit of rapid testing are reduced. Therefore, such tests should be run at least twice weekly (11). Laboratories processing large numbers of specimens may need several instruments, since a maximum of 20 specimens can be processed per LCx MTB run.

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