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Abstract

Seven hundred thirty-seven clinical samples from 460 patients were processed for direct detection of Mycobacterium tuberculosis complex by a semiautomated ligase chain reaction commercial assay, the LCx Mycobacterium tuberculosis Assay (LCx assay) from Abbott Laboratories. Results were compared to those of direct microscopy and standard microbiological culture. Of 26 patients (5.7%) with a culture positive for M. tuberculosis, 22 (84.6%) were found positive by the LCx assay. The sensitivity of the LCx assay was 98% for smear-positive samples and 27% for smear-negative samples. With an overall culture positivity rate for M. tuberculosis of 8.3% (61 of 737 samples) and after resolution of discrepant results according to clinical data, the sensitivity, specificity, and positive and negative predictive values of the LCx assay were 78, 100, 95, and 98%, respectively, compared to 85, 100, 100, and 98%, respectively, for culture and 67, 99, 87, and 97%, respectively, for acid-fast staining....

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Evaluation of the Abbott LCx Mycobacterium tuberculosis Assay for Direct Detection of Mycobacterium tuberculosis Complex in Human Samples

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Seven hundred thirty-seven clinical samples from 460 patients were processed for direct detection of Mycobacterium tuberculosis complex by a semiautomated ligase chain reaction commercial assay, the LCx Mycobacterium tuberculosis Assay (LCx assay) from Abbott Laboratories. Results were compared to those of direct microscopy and standard microbiological culture. Of 26 patients (5.7%) with a culture positive for M. tuberculosis, 22 (84.6%) were found positive by the LCx assay. The sensitivity of the LCx assay was 98% for smear-positive samples and 27% for smear-negative samples. With an overall culture positivity rate for M. tuberculosis of 8.3% (61 of 737 samples) and after resolution of discrepant results according to clinical data, the sensitivity, specificity, and positive and negative predictive values of the LCx assay were 78, 100, 95, and 98%, respectively, compared to 85, 100, 100, and 98%, respectively, for culture and 67, 99, 87, and 97%, respectively, for acid-fast staining. In conclusion, the LCx assay proved satisfactory and appears to be an easy-to-use 1-day test which must be used with standard culture methods but can considerably reduce diagnosis time versus culture. However, its clinical interest appears to be limited in our population with low mycobacterial prevalence because of its cost considering the small gain in sensitivity versus direct microscopy.

Rapid diagnosis of Mycobacterium tuberculosis infection remains a challenge for every medical laboratory. According to the current Centers for Disease Control and Prevention recommendations, identification and antimicrobial susceptibility testing should be available to clinicians within 2 weeks (25). Direct microscopic examination is straightforward but has poor sensitivity and does not allow differentiation between tuberculous and nontuberculous mycobacteria (NTM). Culture is sensitive and specific but very slow. In such a setting, direct amplification tests (DAT) have been actively developed and evaluated for the rapid diagnosis of mycobacterial diseases, and two commercial assays, the Gen-Probe Mycobacterium tuberculosis Direct Test (Gen-Probe, San Diego, Calif.) (1, 6, 15, 17, 21, 27) and the Amplicor M. tuberculosis test (Roche Diagnostic Systems, Basel, Switzerland) (4, 5, 8, 10, 18, 23, 28), have already been approved by the Food and Drug Administration for smear-positive specimens, after extensive evaluations of their comparable performance characteristics.

Another amplification assay has been recently introduced, the LCx Mycobacterium tuberculosis Assay (LCx assay) (Abbott Laboratories, Chicago, Ill.), which is based on the ligase chain reaction. This amplification assay uses a semiautomated system which allows direct detection of M. tuberculosis in clinical specimens (3, 13, 16, 19, 26).

The aim of this study was to assess prospectively the performance of the LCx assay and compare its results with those of microscopic examination and culture for a large number of clinical specimens submitted to the laboratory for the diagnosis of mycobacterial infections.

Materials and methods. From February to August 1997, we prospectively investigated routine clinical specimens submitted for diagnosis of mycobacterial disease from two university hospitals. Specimens from patients under antituberculous treatment were not included.

All specimens were processed by the N-acetyl-L-cysteine–NaOH digestion-decontamination procedure (9). In all cases, half of the resuspended sediment was stored at −20°C for the LCx assay, while the rest was used for acid-fast staining and inoculation onto solid and liquid culture media. Smears were stained with auramine-rhodamine fluorochrome as a screening method, and positive smears were confirmed by Ziehl-Neelsen staining. Two solid slants were inoculated per sample: Lowenstein-Jensen and Coletssos (bioMérieux, Marcy l’Étoile, France). For the liquid medium, BACTEC 12B medium (Becton Dickinson, Aalst, Belgium) was used in one hospital, while the oxygen-sensitive fluorescent medium Mycobacterium Growth Indicator Tube (Becton Dickinson) was employed at the other hospital. Liquid and solid media were incubated at 37°C for 6 and 8 weeks, respectively, and were read twice a week. A culture was considered positive if at least one of the media grew mycobacteria. In addition to conventional biochemical tests, thin-layer chromatography and gas-liquid chromatography were used for isolate identification (20). The LCx assay was performed in accordance with the manufacturer’s recommendations on a weekly basis (3).

When discrepancies were observed between the results of direct staining, culture, and the LCx assay, the same decontaminated portion of the specimen was retested by the LCx assay. If the discrepancy persisted, clinical data and results obtained with additional samples from the patient were analyzed. A specimen was considered truly positive for M. tuberculosis when a culture positive for M. tuberculosis was obtained or if a culture negative for M. tuberculosis and a positive LCx assay result were obtained if other concomitant material from
the patient was culture positive or if the patient’s clinical history, including chest roentgenograms and actual clinical presentation, was sufficiently indicative of tuberculosis for empirical antituberculosis therapy. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LCx assay were calculated and compared with culture results and with culture results plus the patient’s clinical data.

**Results.** A total of 737 samples were collected from 460 patients suspected of mycobacterial disease and processed by staining methods, standard cultures, and the LCx assay to evaluate the assay’s ability to detect *M. tuberculosis* complex organisms. Six hundred eighty-two were respiratory specimens from 22 patients, including 280 sputum, 312 bronchial aspirate, 68 bronchoalveolar lavage, 4 endotracheal aspiration, and 18 gastric juice aspirate samples. The remaining 55 were nonrespiratory specimens from 35 patients, including 7 cerebrospinal fluid, 8 urine, and 40 exudate samples.

Seventy-one (9.6%) specimens of 737 from 32 (7%) of 460 patients were positive for mycobacteria by culture. Isolates were distributed as follows: 61 (8.3%) *M. tuberculosis* isolates from 26 patients, 57% of 10 NTM isolates from 6 patients (1.3%), which included 7 *M. avium* complex isolates, 2 *M. simiae* isolates, and 1 *M. gordonae* isolate.

Fifty-five samples were positive by fluorochrome staining, and all of these were confirmed by acid-fast staining. Forty-six (75.4%) of 61 samples whose culture yielded *M. tuberculosis* were positive for acid-fast bacilli, as were 7 (70%) of 10 samples which were positive by culture for NTM. The two remaining smear-positive samples were culture negative.

Seventy-two specimens from 27 patients gave results consistent with the clinical resolved results (culture plus clinical data). For an overall sensitivity of 78%, there was still a significant difference in sensitivity between smear-positive and smear-negative specimens (98 versus 38%), while the difference between respiratory and nonrespiratory specimens was reduced (79 versus 70%).

The results of the LCx assay, culture, and microscopic examination were then compared with the clinical resolved results for the 737 specimens (Table 3) from the 460 patients. Seventy-two specimens from 27 patients gave results consistent with a clinical diagnosis of tuberculosis. Sixty-one (85%) of 72 were positive for *M. tuberculosis* by culture, 56 (78%) of 72 were positive by LCx assay, and 48 (67%) of 72 were positive for acid-fast bacilli. Twenty-three (85%) patients of 27 with a

### TABLE 1. Comparison of LCx assay results with culture results

<table>
<thead>
<tr>
<th>Specimens (no.)</th>
<th>No. <em>M. tuberculosis</em> positive by culture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>No. <em>M. tuberculosis</em> negative by culture&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCx positive</td>
<td>LCx negative</td>
<td>LCx positive</td>
<td>LCx negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (737)</td>
<td>49</td>
<td>12</td>
<td>10</td>
<td>666</td>
<td>80.3</td>
<td>98.5</td>
</tr>
<tr>
<td>Smear positive  (55)</td>
<td>45</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>97.8</td>
<td>77.8</td>
</tr>
<tr>
<td>Smear negative (682)</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>659</td>
<td>26.7</td>
<td>98.8</td>
</tr>
<tr>
<td>Respiratory (682)</td>
<td>48</td>
<td>9</td>
<td>4</td>
<td>621</td>
<td>84.2</td>
<td>99.4</td>
</tr>
<tr>
<td>Nonrespiratory (55)</td>
<td>1</td>
<td>3</td>
<td>6</td>
<td>45</td>
<td>25.0</td>
<td>88.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Total n = 61.

### TABLE 2. Comparison of LCx assay results with culture results plus clinical data (resolved results)

<table>
<thead>
<tr>
<th>Specimens (no.)</th>
<th>No. resolved as <em>M. tuberculosis</em> positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. resolved as <em>M. tuberculosis</em> negative&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LCx positive</td>
<td>LCx negative</td>
<td>LCx positive</td>
<td>LCx negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All (737)</td>
<td>56</td>
<td>16</td>
<td>3</td>
<td>662</td>
<td>77.8</td>
<td>99.5</td>
</tr>
<tr>
<td>Smear positive  (55)</td>
<td>47</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>97.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Smear negative (682)</td>
<td>9</td>
<td>15</td>
<td>3</td>
<td>655</td>
<td>37.5</td>
<td>99.5</td>
</tr>
<tr>
<td>Respiratory (682)</td>
<td>49</td>
<td>13</td>
<td>3</td>
<td>617</td>
<td>79.0</td>
<td>99.5</td>
</tr>
<tr>
<td>Nonrespiratory (55)</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>45</td>
<td>70.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

<sup>b</sup>Total n = 665.
tests, along with the Roche Cobas Amplicor acid amplification test, and it is among the first semiautomated tuberculosis assay (11, 22, 30). Although DNA extraction is still nonuniform distribution in the clinical samples. Indeed, six specimens were from patients with previous LCx-positive samples. On the other hand, for two patients with smear-negative specimens, an early diagnosis of tuberculosis could be made thanks to the positive LCx assay results. However, in the first case, we received additional samples 2 days later with smear-positive results so that, only 1 patient with smear-negative samples of the 460 patients tested really benefitted from this DAT in terms of rapidity of diagnosis and treatment instauration. The real advantage of this test appeared for smear-positive specimens because NTM and M. tuberculosis are both readily detected by fluorescence microscopy (29) and are almost impossible to distinguish by smear alone. A diagnosis of both tuberculosis and infection with NTM should initially be considered until a more definite diagnosis can be made, especially in selected populations, such as the AIDS patient population. We received samples from 15 human immunodeficiency virus-infected patients, 7 of whom had smear-positive samples. Only two patients were infected with NTM and were correctly diagnosed by the LCx assay. In our study, the PPV of the LCx assay for smear-positive samples were found to be significantly higher than that for smear-negative samples (96 versus 33%) (Table 1), as for the other approved DAT (2). Conversely, the PPVs of smear and LCx assay results were similar (87 and 95%, respectively) (Table 3) because, unfortunately, the proportion of specimens smear positive for NTM was low in the period chosen for the study. To really benefit from the rapidity afforded by a same-day automated method, testing should be performed every day. Although theoretically possible, this is not practical in most laboratories due to the cost. Hence, due to the four controls and calibrators for a run of a maximum of 20 specimens, the cost per test, including reagents and manpower, varies from about $30 if 20 specimens are processed together to $200 if a single specimen must be run alone. To perform this test on a daily basis would only be possible in reference or central laboratories. In our setting, it would be done only once a week, thus undermining a great part of the argument for rapidity.

We conclude that the LCx assay demonstrates very satisfactory performance in terms of sensitivity and specificity for smear-positive specimens, allowing rapid confirmation of positive smear results as true tuberculosis. Such features may be of considerable importance for laboratories dealing with a high proportion of infections caused by NTM, such as in the AIDS patient population. It offers the advantage of semiautomatation, which saves labor and allows a same-day result. On the other hand, the assay’s performance obtained with specimens negative by direct examination is not sufficient to warrant its use on a routine basis, and this test cannot replace standard culture and susceptibility testing for mycobacteria. Moreover, the cost linked to calibrators and controls is such that this technology is probably suitable only for reference laboratories processing large series of specimens and having good communication with clinicians because of the possibility of both false-positive and false-negative results.

### REFERENCES


### TABLE 3. Comparison of LCx assay, culture, and microscopy results with clinical diagnosis of tuberculosis

<table>
<thead>
<tr>
<th>Method and result (no. of samples)</th>
<th>No. of specimens</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TB</strong></td>
<td><strong>TB</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCx assay</td>
<td>Positive (59)</td>
<td>56</td>
<td>36</td>
<td>78</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative (678)</td>
<td>16</td>
<td>662</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture</td>
<td>Positive (61)</td>
<td>61</td>
<td>0</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative (676)</td>
<td>11</td>
<td>665</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microscopy</td>
<td>Positive (55)</td>
<td>48</td>
<td>76</td>
<td>67</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>Negative (682)</td>
<td>24</td>
<td>658</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* TB, tuberculosis positive (total n = 72); TB, tuberculosis negative (total n = 665).
* One sample was negative when reasayed.
* Including 10 samples positive for NTM.
* Seven specimens were culture positive for NTM and LCx assay negative.


