Comparative Evaluation of the New Gen-Probe Mycobacterium tuberculosis Amplified Direct Test and the Semiautomated Abbott LCx Mycobacterium tuberculosis Assay for Direct Detection of Mycobacterium tuberculosis Complex in Respiratory and Extrapulmonary Specimens

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Received 27 May 1998/Returned for modification 23 July 1998/Accepted 16 September 1998

Two commercial assays that detect Mycobacterium tuberculosis complex (MTB) in clinical specimens by rRNA target amplification (AMTDII) and ligase chain reaction (LCx) were evaluated. The tests were applied to 457 respiratory (n = 273) and extrapulmonary (n = 184) specimens collected from 357 patients. The results were compared with those of acid-fast staining and culture. The combination of culture and clinical diagnosis was considered to be the “gold standard.” Seventy specimens were from patients with pulmonary tuberculosis and 28 specimens were from patients with extrapulmonary tuberculosis. After resolution of discrepant results, the overall sensitivities, specificities, and positive and negative predictive values for respiratory specimens were 92.8, 99.4, 98.5, and 97%, respectively, for AMTDII and 75.7, 98.8, 96.4, and 90.5%, respectively, for LCx. With extrapulmonary specimens, the overall sensitivities, specificities, and positive and negative predictive values were 78.6, 99.3, 95.6, and 96.2%, respectively, for AMTDII and 53.6, 99.3, 93.7, and 92.1%, respectively, for LCx. The level of agreement between AMTDII and LCx assay results was 78.2%. We conclude that although both nucleic acid amplification methods are rapid and specific for the detection of MTB in clinical specimens, AMTDII is significantly more sensitive than LCx with both respiratory (P = 0.005) and extrapulmonary (P = 0.048) specimens.

Amplification techniques have attracted considerable interest since they offer the opportunity to shorten the time required to detect and identify Mycobacterium tuberculosis complex (MTB) organisms in both respiratory and extrapulmonary specimens. A number of amplification systems have been described recently; besides in-house assays, commercial systems have been developed with the aim of providing better standardization and reducing contamination. Two systems among those developed by the industry are familiar to those working in mycobacteriology: the Amplified M. tuberculosis Direct Test (Gen-Probe Inc., San Diego, Calif.), which was recently upgraded by the manufacturer (AMTDII), and the ligase chain reaction (Abbott LCx Probe System [LCx]), a semiautomated amplification assay that was recently introduced by Abbott Laboratories Diagnostic Division (Abbott Park, Ill.).

Since the introduction of nucleic acid amplification assays into the field of diagnostic mycobacteriology, many papers describing investigations that have evaluated the performances of in-house or commercial assays in comparison with those of microscopy and culture have been published, but only a few have carried out head-to-head comparisons of different assays. Bearing this in mind, we undertook the present study with the aim of comparing the AMTDII and LCx amplification methods.

MATERIALS AND METHODS

Study design. Four hundred fifty-seven clinical specimens consecutively received for culture for acid-fast bacilli (AFB) by four Italian microbiology laboratories (center 1, Ancona; center 2, Pordenone; center 3, Vicenza; center 4, Milan) were entered into this study. All laboratories received an average of 150 to 400 specimens per month for mycobacterial culture. The specimens were almost entirely obtained from patients admitted to hospitals.

Specimen collection and processing. The investigated specimens collected from 357 patients included 170 sputum specimens, 7 bronchoalveolar lavage (BAL) specimens, 96 bronchial washings, 13 gastric aspirates, 54 urine specimens, 68 normally sterile body fluid (pleural, pericardial, and synovial fluids), 36 cerebrospinal fluid (CSF), and ascos fluid) specimens, and 49 miscellaneous samples such as pus and biopsy specimens.

Respiratory specimens were liquefied and decontaminated with an equal volume of N-acetyl-L-cysteine and 3% NaOH (NALC-NaOH) for 15 min at room temperature. Extrapulmonary specimens like urine, gastric aspirates (which were neutralized upon receipt with 0.067 M phosphate-buffered saline [PBS; pH 6.8], pleural, and other similar body fluids (pericardial, synovial, and ascites fluids) were centrifuged at 3,300 × g for 15 min at 4°C. The supernatant was discarded and the sediment was resuspended in 10 ml of sterile water and decontaminated with NALC-NaOH. After decontamination, all the specimens were added to an equal volume of PBS and the mixture was centrifuged at 3,300 × g for 15 min at 4°C. Then the sediment was resuspended in 2 ml of PBS and neutralized with 0.5 M HCl. Part of the sediment from each specimen was inoculated onto the culture media and used for acid-fast staining, while the remaining was aliquoted and stored at −80°C until the amplification techniques were performed. CSF specimens were cultured without prior decontamination.

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TABLE 1. Comparison of AMTDII and LCx amplification assays with smear and culture results and clinical data for respiratory specimens

<table>
<thead>
<tr>
<th>Patient status</th>
<th>No. of specimens</th>
<th>No. of specimens positive by AMTDII</th>
<th>No. of specimens positive by LCx</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear and culture positive</td>
<td>36</td>
<td>36</td>
<td>33</td>
<td>NS</td>
</tr>
<tr>
<td>Smear negative, culture positive</td>
<td>25</td>
<td>24</td>
<td>16</td>
<td>0.004</td>
</tr>
<tr>
<td>Smear and culture negative with</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>a final diagnosis of TBb</td>
<td>All positive for TB</td>
<td>70</td>
<td>65</td>
<td>53</td>
</tr>
<tr>
<td>Smear and/or culture positive</td>
<td>25</td>
<td>14</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>previously, negative by therapy</td>
<td>Smear and culture negative, non-TB pulmonary disease</td>
<td>164</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Isolation of NTM</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

* NS, not significant; NA, not applicable.

a TB, tuberculosis.

RESULTS

Analytical performance of Gen-Probe AMTDII and Abbott LCx assays. Positive and negative results could be clearly distinguished by the magnitudes of both the RLU values and the fluorescence rates. The majority of samples with positive results had values of >1,500,000 RLUs for AMTDII and fluorescence rates of >1,000 (showing S/CO ratios of >2.5) for LCx. Negative samples exhibited results approximately 20-fold lower than the CO values, which were 30,000 RLUs for AMTDII and fluorescence rates ranging from 300 to 450 for LCx.

Clinical results. (i) Identification of NTM. Twenty-one specimens (six were smear positive) from 19 patients yielded non-tuberculous mycobacteria (NTM). The mycobacterial species identified from these specimens were M. avium (n = 12), M. gordonae (n = 4), M. kansasi (n = 2), M. xenopi (n = 2), and M. fortuitum (n = 1).

(ii) Respiratory specimens. A total of 273 respiratory specimens collected from 205 patients were tested. Altogether, 75 specimens were culture positive for AFB; 61 isolates were found to belong to MTB (51 were from sputum specimens, 9 were from bronchial washings, and 1 was from a BAL specimen), while the remaining 14 strains (11 from sputum specimens, 2 from bronchial washings, and 1 from a BAL specimen) were identified as nontuberculous species. A comparison of the amplification results with the smear and culture results and clinical data is summarized in Table 1. A total of 95 specimens were from patients with a diagnosis of tuberculosis, and 178 were from patients with nontuberculous pulmonary disease on the basis of clinical and microbiological findings. Of the 36 samples which were smear and culture positive, all were AMTDII positive and 33 were LCx positive (the difference was statistically not significant). Twenty-five samples were smear negative for AFB but culture positive; 24 were AMTDII positive, but only 16 were LCx positive. The difference was statistically significant (P = 0.004). Nine samples collected from patients in whom tuberculosis was strongly suspected at that moment were smear and culture negative but were later confirmed to be positive by additional cultures. Of these, five were AMTDII positive and four were LCx positive (the difference was statistically not significant). The cumulative difference for all M. tuberculosis-positive specimens (65 positive by AMTDII and 53 positive by LCx) was statistically significant (P = 0.005).

Twenty-five specimens were collected from patients with pulmonary tuberculosis who were receiving drug therapy. All of these specimens were smear and/or culture positive before therapy but were both smear and culture negative at the time that the sample was taken for amplification. Fourteen of these specimens were positive by both assays. Of the 164 samples from patients with nontuberculous pulmonary disease that were smear and culture negative for AFB, 1 was AMTDII positive and 2 were LCx positive. After resolution of discrepant results on the basis of the patients’ clinical histories, these samples were considered to be false positive. NTM were grown from 14 specimens; all these specimens were negative by both amplification assays. Table 2 presents the sensitivity, specificity, and predictive values of both amplification methods for smear-positive and smear-negative specimens compared with the results of AFB smear and culture, assuming that the combination of culture and clinical diagnosis is the gold standard. Results for samples from patients with pulmonary tuberculosis receiving drug therapy were not considered in the analysis whose results are presented in Table 2.

(iii) Extrapulmonary specimens. A total of 184 extrapulmonary specimens collected from 152 patients were tested. Altogether, 71 specimens were culture positive for AFB; 24 isolates were found to belong to the MTB (2 were from gastric aspirates, 11 were from urine, 4 were from sterile body fluids, and 10 were from miscellaneous specimens), while the remaining 17 strains (3 from urine, 3 from sterile body fluids, and 1 from a...
miscellaneous specimen) were identified as nontuberculous species. A comparison of the amplification results with the smear and culture results and clinical data for various methods with respiratory specimens is summarized in Table 3. A total of 32 specimens were from patients with a diagnosis of extrapulmonary tuberculosis and 152 were from nontuberculous patients on the basis of clinical and microbiological findings. Of the 11 samples which were smear and culture positive, all were AMTDII positive and 9 were LCx positive (the difference was statistically not significant). Thirteen samples were smear negative for AFB but culture positive; 10 were AMTDII positive, but only 5 were LCx positive. The difference was statistically significant ($P = 0.047$). Four samples collected from patients for whom tuberculosis was strongly suspected clinically were smear and culture negative. Of these, only one was positive by both assays. The cumulative difference for all $M. tuberculosis$-positive specimens (22 by AMTDII and 15 by LCx) was statistically significant ($P = 0.048$). Four specimens were collected from patients with extrapulmonary tuberculosis who were receiving drug therapy. All of these were smear and/or culture positive before therapy but were both smear and culture negative at the time that the sample was taken for amplification. Two of them were AMTDII positive and one was LCx positive. Of the 145 samples from patients with nontuberculous disease that were smear and culture negative for AFB, 1 was positive by both assays. After resolution of the discrepant results as reported above, these samples were considered to be false positive. NTM were grown from seven specimens; all these specimens were negative by both amplification assays. Table 4 presents the sensitivity, specificity, and predictive values of both amplification methods for smear-positive and smear-negative specimens compared with the results of AFB smear and culture, assuming that the combination of culture and clinical diagnosis is the gold standard. Results for samples from patients receiving antituberculous chemotherapy were not considered in the analysis whose results are presented in Table 4.

### DISCUSSION

Rapid diagnostic methods, such as amplification methods, significantly decrease the time required for the diagnosis of $M. tuberculosis$ infections. Two of these test methods, the Gen-Probe AMTDII assay and the Abbott LCx assay, were compared in this study. The kits contain all the reagents needed for sample amplification and detection and appeared to fit well in a routine microbiology laboratory’s work flow. Both amplification assays exhibited good analytical performances; in particular, the reproducibility of AMTDII seemed to be considerably upgraded compared with that of its former release (2, 3). We admit that a correct evaluation of the reproducibility of AMTDII compared to that of the first version (AMTDII versus AMTD) can be assessed only in comparative evaluations. However, on average, we observed a drastic reduction in the number of weakly positive results, which represented a considerable drawback in the earlier release (11, 12). Generally, differences between CO values, values for positive and negative controls, and values for samples were broad enough to permit easy discrimination. The turnaround time of AMTDII is shorter than that of LCx (<3 versus 6 h), and the AMTDII procedure, although entirely manual, is easier to perform.

The diagnostic performances of the AMTDII and LCx assays for both respiratory and extrapulmonary specimens were noticeably different. In comparison with the gold standard, the sensitivity and specificity of AMTDII were 92.8 and 99.4%, respectively, for respiratory specimens and 78.6 and 99.3%, respectively, for extrapulmonary specimens. Our data agree with those from a recently published study by Gamboa and colleagues (4) showing sensitivities of 94.7% and 86.8% for respiratory and extrapulmonary specimens, respectively, and specificities of 100% for both categories of specimens. In our study, we found a lower sensitivity for extrapulmonary specimens. This can be explained by taking into account the fact that the AMTDII procedure has an increased specimen vol-
ume, from 50 to 450 μL. Such a change would lead to an increased number of positive results but may also enhance inhibition by those substances which are more likely to be contained in extrapulmonary specimens (4). AMTDII appeared to be significantly more sensitive than LCX for both respiratory (P = 0.005) and extrapulmonary (P = 0.048) specimens. The difference was found to be significant within the category of smear-negative and culture-positive samples (Tables 1 and 3). The sensitivity and specificity of LCX were 75.7 and 98.8%, respectively, for respiratory specimens and 53.6 and 99.3%, respectively, for extrapulmonary specimens. Data on the sensitivity of LCX presented in the literature are different: our data conflict with those of Ausina et al. (1), Lindbråthen et al. (7) (90.8 and 90.2% sensitivities for respiratory specimens, respectively), Tortoli et al. (13) (98.7 and 73.3% sensitivities for respiratory and extrapulmonary specimens respectively), and Gamboa et al. (5) (78.5% sensitivity for extrapulmonary specimens) but are very close to those of Moore and Curry (8) (77% sensitivity for respiratory specimens) and Yuen et al. (14) (36.8% sensitivity for smear-negative respiratory specimens). Discrepancies among sensitivities can be explained at least to some extent by analyzing the ratios of smear-positive specimens/smear-negative specimens for the MTB yielding specimens enrolled in those studies. We found higher ratios in papers from investigators reporting better sensitivities (2.9, 4.5, and 6.4 for Lindbråthen et al. (7), Ausina et al. (1), and Tortoli et al. (13), respectively), while ratios from investigators who detected less satisfactory sensitivities were somewhat or considerably lower (2.6, 0.6, and 1.2 for Yuen et al. (14), Moore and Curry (8), and our study, respectively).

Negative results obtained by the LCX amplification assay for smear-negative, MTB-yielding samples may be explained by the presence of inhibitors of enzymatic amplification. We did not search for the presence of inhibitory substances; however, all smear-positive, LCX-negative samples were retested, and negative results were obtained upon repeat assay. In this context, nucleic acid extraction from mycobacterial cells may also be as important as amplification itself in the performance of commercial assays (1, 14). This step has probably been underestimated, but it deserves further attention for the optimization of sensitivity. For smear-negative, culture-positive samples, a low number of mycobacteria that are unequally distributed in the test suspension is perhaps the more likely explanation. Furthermore, the LCX procedure includes during specimen preparation two washing steps that should remove and eliminate inhibitors. We agree with Ausina and colleagues (1) that these steps may represent a potential drawback, causing sampling errors and consequent false-negative results especially when the number of AFB is low. Of the 13 samples (9 respiratory specimens and 4 extrapulmonary specimens) that were both smear and culture negative and that were obtained from patients strongly suspected of having tuberculosis, 6 were AMTDII positive and 5 were LCX positive, suggesting that AMTDII also appears to be slightly more sensitive than LCX in tests with this category of specimens. During the follow-up, tuberculosis could be confirmed by additional cultures only for those patients from whom respiratory specimens were collected. A small number of samples collected from patients found to be negative for MTB by culture and clinical criteria showed discrepant results (Tables 1 and 3): two of them were positive by AMTDII and three were positive by LCX. We concluded that these specimens had true false-positive results because they gave positive results by one amplification assay only and additional specimens from the patients were also negative by any test.

Of the 29 samples (respiratory and extrapulmonary specimens) obtained from patients receiving standard antituberculous therapy, 16 were AMTDII positive and 15 were LCX positive. Data from the literature demonstrate that, despite differences in the amplification target, neither assay is validated for use in the monitoring of therapeutic efficacy (1, 6, 13). None of the 21 specimens yielding NTM in culture was positive by any assay.

In summary, although at present amplification assays cannot replace the conventional diagnostic techniques (6), AMTDII and LCX were found to be rapid and specific for the detection of MTB in clinical samples. The assay protocols were easy to perform and were suitable for a routine microbiology laboratory's workflow. On the basis of our data, the difference between the sensitivities of AMTDII and LCX was found to be statistically significant for both respiratory specimens (P = 0.005) and extrapulmonary specimens (P = 0.048). Moreover, to significantly increase the sensitivity of the latter assay, systematic control for sample inhibition is recommended.

ACKNOWLEDGMENT

We thank Abbott Diagnostici (Rome, Italy) for supporting this work and for technical assistance.

REFERENCES


