

Evaluation of a Commercial Ligase Chain Reaction Kit (Abbott LCx) for Direct Detection of *Mycobacterium tuberculosis* in Pulmonary and Extrapulmonary Specimens

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Direct detection of *Mycobacterium tuberculosis* by means of a commercial ligase chain reaction DNA amplification method (LCx *M. tuberculosis*; Abbott Diagnostics Division, Abbott Park, Ill.) was investigated with 511 (including 147 extrapulmonary) specimens collected from 358 patients. LCx results were compared with standard microbiological data, and conflicting cases were resolved according to the final clinical diagnosis. *M. tuberculosis* was detected in 45 of 358 subjects by means of the LCx test. The test was negative for all 30 specimens with mycobacteria other than *M. tuberculosis*. The sensitivity, specificity, and positive and negative predictive values for the LCx test, compared with culture results, were 93.90, 92.31, 70.00, and 98.75%, respectively; these values rose in resolved cases to 95.53, 99.25, 97.27, and 98.75%, respectively. With respiratory specimens, for which the LCx system is licensed, the sensitivity reached 98.97%. In patients with a final clinical diagnosis of tuberculosis the sensitivity of the LCx system was 89.36% compared to 82.98% for cultures and 78.72% for microscopy. We conclude that the LCx test is user friendly, rapid, fairly sensitive, and highly specific. It can also be effectively used on extrapulmonary specimens provided possible false-negative results are taken into account. However, the use of LCx test appears to be less appropriate for the monitoring of antituberculosis therapy, as the majority of samples from treated tuberculosis patients gave consistently positive results, despite the sterilization of cultures.

As was recently stressed by the Centers for Disease Control and Prevention (18), the main obstacle to the effective control of tuberculosis is represented by the long time required for laboratory diagnosis; 10 to 14 days for the isolation and identification of *Mycobacterium tuberculosis* has been set as a goal to be reached. However, this is hardly achievable even in laboratories which use liquid culture media. On the other hand, microscopy, although rapid, lacks sensitivity and is unable to distinguish tubercle bacilli from other mycobacteria.

Investigators have resorted to biomolecular methods for the rapid and sensitive detection of specific nucleic acids in clinical samples. Two such commercial methods for the *M. tuberculosis* complex have shared the market so far: the PCR-based AmpliCor *M. tuberculosis* (Roche Diagnostic Systems, Branchburg, N.J.) test and the Amplified *M. tuberculosis* Direct Test (Gen-Probe, San Diego, Calif.), which is based on transcriptase-mediated amplification. Both methods have performed well in terms of rapidity, sensitivity, and specificity (1-3, 5, 13, 14, 20).

Recently, ligase chain reaction technology (7) has become commercially available, and a kit has been developed for the detection of *M. tuberculosis* complex-specific DNA in clinical samples (LCx *M. tuberculosis*, Abbott Diagnostics Division, Abbott Park, Ill.). We investigated the reliability of this new method in comparison with microscopy and culture in over 500 clinical samples from different sources.

Materials and methods. A total of 511 specimens from 358 patients were investigated. Specimens were selected from among those sent to our laboratory for standard mycobacteriological investigation during the second half of 1996, with particular attention to samples coming from patients with sus-

pected or established tuberculosis. Our survey included, along with a majority of respiratory specimens (256 sputa and 108 bronchial aspirates), a number of extrapulmonary samples: 66 urine specimens, 30 pleural fluid specimens, 22 cerebrospinal fluid specimens, 15 biopsy specimens from various body sites, 11 lymph nodal pus specimens, and 3 ascites specimens. Apart from samples from sterile sites (pleural fluid, cerebrospinal fluid, ascites) all specimens were processed according to the conventional NALC-NaOH digestion-decontamination procedure (12); tissues were mechanically digested prior to decontamination. Their centrifuged sediments, resuspended in distilled water, were examined as auramine-stained smears and were seeded both on Lowenstein-Jensen medium and in radiometric broth (Bactec; Becton Dickinson, Towson, Md.); the medium and the broth were incubated for 8 and 6 weeks, respectively. The LCx test was performed with part of the suspension, either within 2 days or after several days of storage at -20°C.

The LCx test was performed according to the manufacturer's recommendations. In short, 500 µl of each preprocessed and concentrated sample was put in a screw-cap microcentrifuge tube and centrifuged with respiratory specimen buffer and glass beads; the pellet was then resuspended in resuspension buffer, centrifuged, resuspended again, and vortexed. The suspension was then inactivated for 20 min at 95°C in the LCx covered dry bath (Abbott) and lysed for 10 min with Lysor sonicator (Abbott). One hundred microliters of supernatant was transferred to a ready-to-use tube containing the amplification mixture. The amplification was carried out in a separate area for 37 cycles in the LCx thermal cycler as follows: 94°C for 1 s, 64°C for 1 s, and 69°C for 40 s, with a holding period at 25°C at the end of the last cycle. Each series of tests (including no more than 20 samples) was run along with a negative control and a calibrator in duplicate. Amplified tubes were trans-

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TABLE 1. Initial comparison of the LCx test with culture for the detection of *M. tuberculosis*

| Specimen | No. of specimens for which <i>M. tuberculosis</i> culture results were: | | | | Sensitivity (%) | Specificity (%) | PPV ^a (%) | NPV ^b (%) |
|----------------|---|----------|-------------------------------------|----------|-----------------|-----------------|----------------------|----------------------|
| | Positive and LCx test results were: | | Negative and LCx test results were: | | | | | |
| | Positive | Negative | Positive | Negative | | | | |
| All | 77 | 5 | 33 | 396 | 93.90 | 92.31 | 70.00 | 98.75 |
| Smear positive | 69 | 2 | 20 | 24 | 97.18 | 54.54 | 77.53 | 92.31 |
| Smear negative | 8 | 3 | 13 | 372 | 72.72 | 96.62 | 38.09 | 99.20 |
| Respiratory | 67 | 1 | 32 | 264 | 98.53 | 89.19 | 67.68 | 99.62 |
| Nonrespiratory | 10 | 4 | 1 | 132 | 71.43 | 99.25 | 90.91 | 97.06 |

^a PPV, positive predictive value.

^b NPV, negative predictive value.

ferred unopened to the carousel of the LCx analyzer which directly detects the amplification products by a microparticle enzyme immunoassay (8) and displays the results as fluorescence rates, which are compared to the calibrator rate. If the rate exceeded 30% of the average calibrator rate, the results were considered positive.

Results. Mycobacteria grew in 112 of 511 specimens tested; 82 of them (39 patients) were identified as *M. tuberculosis*, while the remaining 30 (15 patients) were identified as nontuberculous species (11 *Mycobacterium xenopi* isolates, 8 *Mycobacterium kansasii* isolates, 6 *Mycobacterium avium* isolates, 2 *Mycobacterium intracellulare* isolates, and one isolate each of *Mycobacterium chelonae*, *Mycobacterium genavense*, and *Mycobacterium gordonae*). Acid-fast bacilli were seen in 115 specimens; of 88 mycobacteria isolated, 71 belonged to the species *M. tuberculosis*.

As summarized in Table 1, the LCx assay was positive for 110 specimens from 45 patients. For 77 of 110 specimens, *M. tuberculosis* grew in culture. Overall sensitivity, specificity, and positive and negative predictive values of the LCx test were, in comparison with culture results, 93.90, 92.31, 70.00, and 98.75%, respectively. As expected, sensitivity was higher (97.18%) for smear-positive than for smear-negative specimens (72.72%).

When pulmonary and nonpulmonary specimens were considered separately (Table 1), the sensitivity and specificity were found to be 98.53 and 89.19%, respectively, for respiratory tract specimens (for which the LCx kit is licensed) and 71.43 and 99.25, respectively, for extrapulmonary specimens as a whole.

None of the 30 specimens yielding mycobacteria other than *M. tuberculosis* in culture was LCx positive.

Thirty-three LCx-positive specimens remained negative in culture; 28 of them, however, were collected from patients in

whom tubercle bacilli had been previously isolated (the mean time elapsed from the last positive culture was 95 days; range, 20 days to 14 months). Microscopy was still positive in many instances (71%). The remaining five patients had neither previous nor subsequent *M. tuberculosis* growth in culture. Revision of medical records was possible for three of these patients; two of them had a clinical diagnosis of tuberculosis on the basis of X rays, signs, and symptoms, while no evidence of disease was found in the remaining three.

After resolution of the discrepancies (the 28 samples from patients with previous *M. tuberculosis*-positive cultures and the two samples from patients with clinical signs of tuberculosis were considered true positives) three false-positive results, including two from patients for whom no clinical information was available, and five false-negative results remained. Sensitivity, specificity, and positive and negative predictive values for LCx were thus 95.53, 99.25, 97.27, and 98.75%, respectively (Table 2).

Discussion. The ligase chain reaction is based on the joining, catalyzed sequentially by polymerase and ligase, of two performed oligonucleotide probes specific for adjacent sequences in the target DNA, once such segments are hybridized with the complementary sequences (7). Thermal cycles allow exponential generation of the bound oligonucleotides because of the continuous hybridization with the target sequence followed by denaturation. The two oligonucleotides used in the LCx reaction (16) are linked to different haptens, one for capture and the other for detection. Therefore, only bound products have both haptens and are revealed in a microparticle enzyme immunoassay. In the LCx automatic system an enzyme conjugate captured by the amplified ligase products causes the substrate to emit a fluorescence signal related to their concentration. A clear-cut distinction between negative and positive results was

TABLE 2. Assessment of the LCx test results after resolution of discrepancies with the clinical diagnosis of tuberculosis

| Specimen | No. of specimens for which clinical diagnosis of tuberculosis was: | | | | Sensitivity (%) | Specificity (%) | PPV ^a (%) | NPV ^b (%) |
|----------------|--|----------|-------------------------------------|----------|-----------------|-----------------|----------------------|----------------------|
| | Positive and LCx test results were: | | Negative and LCx test results were: | | | | | |
| | Positive | Negative | Positive | Negative | | | | |
| All | 107 | 5 | 3 | 396 | 95.53 | 99.25 | 97.27 | 98.75 |
| Smear positive | 89 | 2 | 0 | 24 | 97.80 | 100 | 100 | 92.31 |
| Smear negative | 18 | 3 | 3 | 372 | 85.71 | 99.20 | 85.71 | 99.20 |
| Respiratory | 96 | 1 | 3 | 264 | 98.97 | 98.97 | 96.96 | 99.62 |
| Nonrespiratory | 11 | 4 | 0 | 132 | 73.33 | 100 | 100 | 97.06 |

^a PPV, positive predictive value.

^b NPV, negative predictive value.

achieved in the overwhelming majority of tests. Ninety-two percent of negative results were more than 10-fold lower than the cutoff level, and 95% of the positive results were more than twice the cutoff level.

The usefulness of implementing the LCx system for the detection of *M. tuberculosis* in clinical specimens is substantiated by its rapidity (20 samples can be tested in less than 5 h) and sensitivity. In fact, it allowed a rapid diagnosis for 89.36% of tuberculosis-positive patients investigated in this study versus 82.98% by culture and 78.72% by microscopy.

No evaluation of the LCx system has been published so far. Our data appear comparable to those reported for other commercially available amplification systems. Our overall resolved sensitivity of 95.53% is within the range (91.0 to 98.4%) reported for the Gen-Probe assay (1, 9, 14, 15, 19) and better than the 67.6 to 86.0% reported for the Amplicor Direct test (4–6, 10, 17).

The low bacterial charge appears to be the cause of false negatives in four of five cases. In fact, four samples were negative or weakly positive (one case) by microscopy and grew scanty colonies in culture, while only one (a biopsy specimen) was characterized by heavy microscopic positivity, and its radiometric broth was already strongly positive at the day-7 reading. The presence of inhibitors, which resisted the removal procedure included in the LCx assay, or a possible inhibition due to excess target, seem to be the most plausible mechanism responsible for the latter case.

Although the LCx system is presently licensed only for respiratory specimens, we believe that its cautious use may be rewarding also with samples from other areas of the body; in our study it scored positive with 5 of 6 culture-positive urine specimens (66 tested), with the only culture-positive pleural fluid specimen (30 tested), with 2 of 3 culture-positive biopsy specimens (15 tested), and with 3 of 4 culture-positive samples of pus (11 tested). At present, the results on cerebrospinal fluid cannot be interpreted, as LCx failed for the only culture-positive sample of the 22 specimens tested.

The resolved specificity of LCx assay (99.25%) ranks high within the range (96.9 to 100%) reported for other commercial amplification kits (1, 4–6, 9, 10, 14, 15, 17, 19). At worst, we had three false-positive results, including the two patients on whom we lacked information; none of these samples followed a positive specimen in the carousel of the LCx instrument, ruling out a carryover. If they were in fact falsely positive, they might be due to an accidental contamination of the samples.

Among true-positive LCx results, most samples came from tuberculous patients under treatment. Such an occurrence is well documented both for DNA- and RNA-based amplification systems (2, 10, 11, 15); the long-lasting detectability of nucleic acids after the culture becomes negative makes LCx unsuitable for the monitoring of therapeutic efficacy.

Automation, which saves labor, is a major asset of the LCx system. Since the system is completely enclosed, the risk of contamination, which is the most serious problem for all amplification techniques, is minimized.

The dilemma as to whether smear-positive or smear-negative samples are the most appropriate target of amplification is still unresolved. Despite the different sensitivities (Tables 1 and 2), we consider the information provided by the LCx to be of interest in both groups: in the former, it allows a clinically relevant distinction of tuberculous from nontuberculous mycobacteria, and in the latter it shows a unique rapidity of diagnosis (eight patients in this study). In this respect, it should be emphasized that the negative predictive value of the LCx on smear-negative respiratory samples in our study was 99.62%, which gives confidence in reporting a negative result for *M.*

tuberculosis for this group, which includes the vast majority of samples routinely tested for mycobacteria.

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REFERENCES

1. Abe, C., K. Hirano, M. Wada, Y. Kazumi, M. Takahashi, Y. Fukasawa, T. Yoshimura, C. Miyagi, and S. Goto. 1993. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. *J. Clin. Microbiol.* **31**: 3270–3274.
2. Beavis, K. G., M. B. Lichty, D. L. Jungkind, and O. Giger. 1995. Evaluation of Amplicor PCR for direct detection of *Mycobacterium tuberculosis* from sputum specimens. *J. Clin. Microbiol.* **33**:2582–2586.
3. Bodmer, T., A. Gurtner, K. Schopfer, and L. Matter. 1994. Screening of respiratory tract specimens for the presence of *Mycobacterium tuberculosis* by using the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. *J. Clin. Microbiol.* **32**:1483–1487.
4. Carpentier, E., B. Drouillard, M. Dailloux, D. Moinard, E. Vallee, B. Dutilh, J. Maugein, E. Bergogne-Berezin, and B. Carbonnelle. 1995. Diagnosis of tuberculosis by Amplicor *Mycobacterium tuberculosis* test: a multicenter study. *J. Clin. Microbiol.* **33**:3106–3110.
5. D'Amato, R. F., A. A. Wallman, L. H. Hochstein, P. M. Colaninno, M. Scardamaglia, E. Ardila, M. Ghouri, K. Kim, R. C. Patel, and A. Miller. 1995. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. *J. Clin. Microbiol.* **33**:1832–1834.
6. Devallois, A., E. Legrand, and N. Rastogi. 1996. Evaluation of Amplicor MTB as adjunct to smears and culture for direct detection of *Mycobacterium tuberculosis* in the French Caribbean. *J. Clin. Microbiol.* **34**:1065–1068.
7. Dille, B. J., C. C. Butzen, and L. G. Birkenmeyer. 1993. Amplification of *Chlamydia trachomatis* DNA by ligase chain reaction. *J. Clin. Microbiol.* **31**:729–731.
8. Fiore, M., J. Mitchell, T. Doan, R. Nelson, G. Winter, C. Grandone, K. Zeng, R. Haraden, J. Smith, K. Harris, J. Leszczynald, D. Berry, G. Safford, A. Barnes, A. Scholnick, and K. Ludington. 1988. The Abbott IMx automated benchtop immunochemistry analyzer system. *Clin. Chem.* **34**:1726–1732.
9. Miller, N., S. G. Hernández, and T. J. Cleary. 1994. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and PCR for direct detection of *Mycobacterium tuberculosis* in clinical specimens. *J. Clin. Microbiol.* **32**:393–397.
10. Moore, D. F., and J. I. Curry. 1995. Detection and identification of *Mycobacterium tuberculosis* directly from sputum sediments by Amplicor PCR. *J. Clin. Microbiol.* **33**:2686–2691.
11. Moore, D. F., J. I. Curry, C. A. Knott, and V. Jonas. 1996. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. *J. Clin. Microbiol.* **34**:1745–1749.
12. Nolte, F. S., and B. Metchock. 1995. *Mycobacterium*, p. 400–437. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. ASM Press, Washington, D.C.
13. Pfyffer, G. E., P. Kissling, E. M. I. Jahn, H. M. Welsker, M. Salfinger, and R. Weber. 1996. Diagnostic performance of Amplified *Mycobacterium tuberculosis* Direct Test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. *J. Clin. Microbiol.* **34**:834–841.
14. Pfyffer, G. E., P. Kissling, R. Wirth, and R. Weber. 1994. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by a target-amplified test system. *J. Clin. Microbiol.* **32**:918–923.
15. Piersimoni, C., A. Callegaro, D. Nista, S. Bornigia, F. De Conti, G. Santini, and G. De Sio. 1997. Comparative evaluation of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens. *J. Clin. Microbiol.* **35**:193–196.
16. Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, J. D. Burezak, and H. H. Lee. 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. *J. Clin. Microbiol.* **32**:2540–2543.
17. Schirm, J., L. A. B. Oostendorp, and J. G. Mulder. 1995. Comparison of Amplicor, in-house PCR, and conventional culture for detection of *Mycobacterium tuberculosis* in clinical samples. *J. Clin. Microbiol.* **33**:3221–3224.
18. Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, and R. C. Good. 1993. The resurgence of tuberculosis. Is your laboratory ready? *J. Clin. Microbiol.* **31**:767–770.
19. Vlasopolder, F., P. Singer, and C. Roggeveen. 1995. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. *J. Clin. Microbiol.* **33**:2699–2703.
20. Vuorinen, P., A. Miettinen, R. Vuento, and O. Hällstrom. 1995. Direct detection of *Mycobacterium tuberculosis* complex in respiratory specimens by Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test and Roche Amplicor *Mycobacterium tuberculosis* test. *J. Clin. Microbiol.* **33**:1856–1859.