# Is the detection of *Mycobacterium tuberculosis* DNA by ligase chain reaction worth the cost: experiences from an inner London teaching hospital

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## Abstract

*Aims*—To evaluate the clinical usefulness and the costs of using a rapid, commercial ligase chain reaction test (LCx) to detect *Mycobacterium tuberculosis* directly from clinical samples.

*Methods*—A prospective study of 2120 routine clinical specimens from 1161 patients over a 13 month period. Investigations for mycobacterial disease by microscopy, culture, and the Abbott LCx assay were performed. Sequential LCx assays were monitored in a cohort of patients undergoing treatment. The costs of the assay were calculated using the WELCAN system. Sensitivity, specificity, and positive and negative predictive values of the LCx assay were compared with conventional tests. The performance of the assay in patients undergoing treatment and cost in terms of WELCAN units converted to pounds/annum was studied.

*Results*—The assay was 85%/88% sensitive and 98%/100% specific in culture confirmed/clinically confirmed cases of tuberculosis, respectively. The assay was not useful for the measurement of treatment outcomes. The test cost approximately £42 500/annum.

*Conclusions*—The assay is a rapid, sensitive, and specific adjunct to clinical diagnosis, especially in differentiating nontuberculous mycobacteria. However, it does not differentiate old and treated tuberculosis from reactivated disease, it is not useful to monitor adherence to treatment, and it is expensive.

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One third of the world's population is infected with *Mycobacterium tuberculosis*. Active disease develops in eight to 10 million people/year, three million of whom die, making it the leading infectious cause of death in the world.[1–3] Notification rates for tuberculosis continue to rise in London, with some inner London boroughs experiencing three to 10 times the national notification rate.[4] Social deprivation, homelessness, immigration, the AIDS epidemic, and the failure of tuberculosis control all play a part in this increase.[5]

Tuberculosis control without improvements in socioeconomic conditions relies on case finding and chemotherapy.[6] Delays in diagnosis and failure to isolate cases have been impli-cated in the transmission of tuberculosis in the health care setting.[7 8] Serious hospital outbreaks of tuberculosis have occurred in wards with immunosuppressed patients.[9] Clinical diagnosis is confirmed by microscopy and culture. Microscopy for acid fast bacilli is simple and rapid but is not sensitive and does not differentiate between *M tuberculosis* and other species, such as *Mycobacterium avium* complex (MAC). Culture is specific and more sensitive but takes several weeks. Rapid differentiation between the mycobacterium species is important for treatment and infection control.

To speed up the process of the specific identification of mycobacteria, new methods based on DNA amplification have been developed. Polymerase chain reaction (PCR) based methods are rapid and sensitive[10]; however, they are labour intensive and require special facilities to prevent laboratory contamination. In house assays vary widely in their specificity and sensitivity.[11 12] The LCx *M tuberculosis* assay (Abbott Laboratories, Diagnostic Division, Chicago, Illinois, USA) is based on the ligase chain reaction and is the first commercial, semiautomated nucleic acid amplification test method developed for use in the routine diagnostic laboratory for the direct detection of *M tuberculosis* DNA from respiratory specimens.[13 14] The aim of our study was to compare the performance of the assay with conventional methods in a busy diagnostic laboratory, serving a population with a relatively high risk of tuberculosis.[15] In addition, we studied the clinical usefulness of the LCx assay, compared with smear and culture, in specimens collected sequentially over a period after onset of treatment from 15 patients with confirmed tuberculosis.

The cost of the assay was calculated in terms of WELCAN units.[16] We present the cost implication of such a test during one year's unrestricted use.

## Materials and methods

Routine clinical specimens submitted to the laboratory for investigation of mycobacterial disease from December 1997 to December 1998 were studied prospectively. The LCx assay was done in parallel with auramine smear examination and culture on Lowenstein-Jensen medium. All specimens other than urine and cerebrospinal fluid (CSF) were processed by the *N*-acetyl-L-cysteine-NaOH digestion/decontamination procedure,[17] before carrying out the LCx assay, smear, and culture. Cultures were held at 35°C for 12 weeks. All positive

*Table 1  Evaluation of the ligase chain reaction (LCx) assay in culture confirmed Mycobacterium tuberculosis infection*

| Specimens (no.) | Culture positive for M tuberculosis (n = 131) | | Culture negative for M tuberculosis (n = 1989) | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|---|
| | LCx positive | LCx negative | LCx positive | LCx negative | | | | |
| All (2120) | 109 | 22 | 33 | 1956* | 85.6 | 98.4 | 79.9 | 98.9 |
| Smear positive (114) | 91 | 1 | 7 | 15** | 98.9 | 75.9 | 92.9 | 95.7 |
| Smear negative (2006) | 18 | 21 | 26 | 1941 | 65.0 | 98.7 | 60.0 | 98.9 |
| Respiratory (2058) | 91 | 21 | 33 | 1913 | 84.2 | 98.3 | 77.2 | 98.9 |
| Non-respiratory (49) | 5 | 1 | 0 | 43 | 85.7 | 100.0 | 100.0 | 97.7 |
| Quality control (13) | 10 | 0 | 0 | 3 | 100.0 | 100.0 | 100.0 | 100.0 |

*Sixty eight were contaminated cultures.
**Nine were positive by culture for non-tuberculous mycobacteria; six were culture negative.
NPV, negative predictive value; PPV, positive predictive value.

cultures were sent to the Mycobacterial Reference Unit for confirmation, speciation, and antimicrobial susceptibility testing. A specimen was considered positive if culture yielded *M tuberculosis* as confirmed at the reference unit, or if the patient's clinical history, including chest roentgenograms and clinical presentation, was sufficiently indicative of tuberculosis to warrant empirical antituberculosis treatment.[14]

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LCx assay were calculated and compared with smear and culture results and relevant clinical data. The prognostic value of sequential LCx assays together with smear and culture investigations were monitored in a cohort of 15 patients receiving antituberculosis treatment over a period of one to nine months.

The costs of introducing the LCx assay into a routine diagnostic laboratory were calculated using the WELCAN unit system.[16]

STATISTICAL ANALYSIS
Sensitivity, specificity, PPV, and NPV were calculated by standard methods. The $\chi^2$ test was used to compare rates.

**Results**
In all, 2120 specimens (1700 sputum, 358 bronchial aspirates/lavages, 49 non-pulmonary specimens, and 13 quality control samples) yielded 131 *M tuberculosis* positive cultures (6.2%) from 48 (4.1%) patients out of 1161 investigated. Nine patients were diagnosed with non-tuberculous mycobacteria (NTM), including three *M avium* complex isolates, three *M xenopi*, one *M. kansasii*, and two unspeciated environmental mycobacteria. All quality control specimens (n = 13) yielded the correct test result.
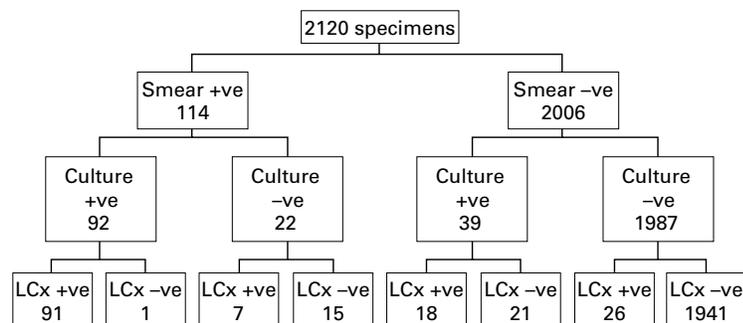
The performance of the LCx assay, evaluated using culture confirmed tuberculosis as the gold standard, is shown in table 1 and represented schematically in fig 1. One hundred and fourteen specimens were smear positive (5.4%), 92 of which (80.7%) were culture positive for *M tuberculosis*. Seven specimens were smear positive and LCx positive but culture negative for *M tuberculosis*. All seven patients were treated for tuberculosis on clinical grounds.

Nine of 15 samples that were smear positive, LCx negative, and culture negative for *M tuberculosis* yielded NTM. The remaining six samples in this group were negative for tuberculosis by clinical evaluation and were not treated with antimycobacterial drugs. The sensitivity of the assay was higher in smear positive samples than smear negative samples (98.9% *v* 65%; table 1).

The performance of the LCx assay with clinically resolved cases of tuberculosis (culture plus clinical and radiological data) is shown in table 2 and represented schematically in fig 2. The overall sensitivity was 88%, with significant differences in sensitivity between the smear positive and smear negative specimens (99% *v* 76%). All samples that were LCx positive were either from culture confirmed cases of tuberculosis or clinically resolved cases of tuberculosis. There were no false positives.

Results of the LCx assay, microscopy, and culture for *M tuberculosis* were compared in parallel using clinical criteria for the diagnosis of tuberculosis as the reference standard. Table 3 shows these data. The LCx assay was 88.2% sensitive and 100% specific. The sensitivity of auramine staining was significantly lower at 60.4% (p < 0.01).

Fifteen patients had a series of tests done after initial presentation and the initiation of treatment. There was only one patient (JK) with a sustained false negative result, which on repeated assay was culture positive for *M tuberculosis* but remained LCx negative. Table 4 presents the effect of treatment on test results over a period of two to nine months. In nine of 15 patients, the LCx result remained positive after treatment, and in four patients the LCx remained positive after cultures were negative. A brief case study is presented for each of these patients.

For specimens requiring decontamination and digestion each assay was costed at 15 WELCAN units costing £22.25 (~ $36.00)/



*Figure 1  Evaluation of the ligase chain reaction (LCx) assay in culture confirmed tuberculosis.*

2120 specimens
 — Smear +ve 114
   — Culture +ve 92
     — LCx +ve 91
     — LCx –ve 1
   — Culture –ve 22
     — LCx +ve 7
     — LCx –ve 15
 — Smear –ve 2006
   — Culture +ve 39
     — LCx +ve 18
     — LCx –ve 21
   — Culture –ve 1987
     — LCx +ve 26
     — LCx –ve 1941

*Table 2   Evaluation of the ligase chain reaction (LCx) assay in clinically resolved cases of Mycobacterium tuberculosis infection (culture results and clinical data)*

| Specimens (n = 2120) | No. resolved as positive for M tuberculosis (n = 164) | | No. resolved as negative for M tuberculosis (n = 1956) | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|---|
| | LCx positive | LCx negative | LCx positive | LCx negative | | | | |
| All (2120) | 142 | 22 | 0 | 1956* | 88.2 | 100.0 | 100.0 | 98.9 |
| Smear positive (114) | 98 | 1 | 0 | 15** | 99.0 | 100.0 | 100.0 | 93.8 |
| Smear negative (2006) | 44 | 21 | 0 | 1941 | 75.6 | 100.0 | 100.0 | 98.9 |
| Respiratory (2058) | 124 | 22 | 0 | 1913* | 86.9 | 100.0 | 100.0 | 98.9 |
| Non-respiratory (49) | 5 | 1 | 0 | 43 | 85.7 | 100.0 | 100.0 | 97.7 |
| Quality control (13) | 10 | 0 | 0 | 3 | 100.0 | 100.0 | 100.0 | 100.0 |

*Sixty eight were contaminated cultures.
**Nine were positive by culture for non-tuberculous mycobacteria; six were culture negative.
NPV, negative predictive value; PPV, positive predictive value.

test; for sterile fluids the assay was worth 10 WELCAN units at £10.68 (~ $16.00)/test. The total cost of this test was £46 658.25 (~ $70 000.00) over 13 months or approximately £42 000/year.

## Discussion

The rapid and reliable detection of *M tuberculosis* in clinical specimens is an important aspect of the management of the disease. Fluorochrome staining and microscopy is simple and rapid but requires a skilled microscopist, is tedious to perform if the work load is heavy, and does not lend itself to automation. Apart from being relatively insensitive, it cannot differentiate between *M tuberculosis* and NTM. Culture is specific and more sensitive but takes weeks. The LCx assay is a semiautomated assay; the initial sample preparation and DNA extraction is performed manually and constitutes the major portion of assay time.
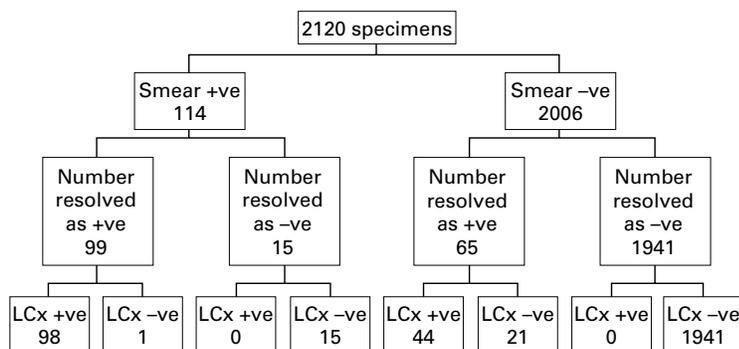


*Figure 2   Evaluation of the ligase chain reaction (LCx) assay in clinically resolved cases of tuberculosis (culture results and clinical data).*

*Table 3   Performance of the ligase chain reaction (LCx) assay compared with culture and microscopy in clinically resolved cases of tuberculosis (TB)*

| Test method and outcome (no.)* | Clinical diagnosis | | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|
| | TB+ (n = 164) | TB− (n = 1956) | | | | |
| *LCx assay* | | | | | | |
| Positive (142) | 142 | 0 | 88.2** | 100.00 | 100.00 | 98.9 |
| Negative (1978) | 22 | 1956 | | | | |
| *Auramine staining* | | | | | | |
| Positive (114) | 99 | 15 | 60.4 | 99.2 | 91.6 | 96.8 |
| Negative (2006) | 65 | 1941 | | | | |
| *Culture* | | | | | | |
| Positive (131) | 131 | 0 | 83.2** | 100.00 | 100.00 | 98.3 |
| Negative (1989) | 33 | 1956 | | | | |

*Includes specimens subject to repeat testing after diagnosis and initiation of treatment.
**LCx is more sensitive than culture because patients on treatment who become culture negative remain LCx positive.
NPV, negative predictive value; PPV, positive predictive value.

The amplification, hybridisation, and detection steps are automated and the assay can be completed from receipt of sample to reporting of results in five to six hours, making same day reporting feasible. Although the test is not validated for non-respiratory specimens, we included a selection of these specimens in our study. We also used the assay to confirm early culture growth from solid media, with 100% sensitivity and specificity compared with reference methods of identification.

The sensitivity, specificity, and ease of use of the LCx assay has been corroborated by many studies.[13] [14] [18] [19] Most of the studies evaluating the performance of the LCx assay have focused on patients with a high suspicion of tuberculosis; this has a bearing on the sensitivity, specificity, and the PPV of the test.[14] [20] In our study, all respiratory and a selection of non-respiratory specimens that were submitted to the mycobacteriology laboratory were examined serially. The test results are hence a more realistic representation of performance in a relatively high risk area for tuberculosis. The LCx yielded no false positives in our hands; other workers have reported false positives, suggesting contamination in the laboratory as a possible explanation.[14]

In all, we documented 16 of 164 patients with clinically suspicious tuberculosis in whom, at initial presentation, the LCx assay was the earliest test method to yield a positive result. In seven patients, smear examination was consistently negative; in the remainder, smear positivity appeared on repeat testing some weeks later. These patients would have experienced delays in diagnosis had it not been for the LCx assay; however, treatment was not delayed because clinically the index of suspicion was high. In six patients, the LCx assay was positive and the culture consistently failed to grow the organism or was contaminated throughout the course of their treatment. In only one patient did the LCx assay hasten both the diagnosis and the onset of treatment. One patient's specimen was consistently false negative even on repeat testing (JK; table 4).

As in the case of smear and culture analyses it is recommended that more than one sample should be tested at initial presentation to allow sufficient accuracy in the detection of *M tuberculosis* DNA.[14]

From table 4, it is apparent that the LCx assay remains positive after the institution of

*Table 4  Effect of treatment on test result over nine months in a cohort of 15 patients*

| Patient (sex) | Test | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MA (male) | LCx | - - - - | | | | - | | | - - + + + - | + + | Alcoholic liver disease, pulmonary TB, 12 months treatment, non-adherent, clinical resolution slow |
| | Aur | - - + - | | | | - | | | - - - - - | + + | |
| | Cul | - + - - | | | | + | | | - - - - - - | + + | |
| SB (male) | LCx | + + + + | | | | | | - | | | Alcoholic, pulmonary TB non-adherent to treatment, at 7 months erratic treatment but tests negative |
| | Aur | + + + + | | | | - | | - | | | |
| | Cul | c + + + | | | | - | | - | | | |
| JC (male) | LCx | + + | + + + + | + | | | | | | | Pulmonary TB. Uncomplicated treatment course, lasting 6 months, fully compliant |
| | Aur | + + | + + + + | + | | | | | | | |
| | Cul | + + | + + + + | + | | | | | | | |
| SF (male) | LCx | + | + | + | | | | | | | HIV+, pulmonary TB, failed supervised treatment, continues on regimen of 1st and 2nd line anti-TB drugs in combination |
| | Aur | + | + | - | | | | | | | |
| | Cul | + | + | + | | | | | | | |
| AH (male) | LCx | - - - - - | - - - | | + | | | | | | Reactivated pulmonary TB, psoas abscess with *B pseudomallei*. Non-adherent, slow resolution |
| | Aur | - - - - - | - - - | | - | | | | | | |
| | Cul | - - - | - + c | + | - | | | | | | |
| HJ (female) | LCx | - + - | + - - | - | | - | | | | | Pulmonary TB, non-adherent, poor tolerance to anti-TB drugs, 9 months' treatment, slow resolution |
| | Aur | - - | - - - | - | | - | | | | | |
| | Cul | + + + | + + - | + | | + | | | | | |
| MK (female) | LCx | - - | + - - | - | | | | | | | Miliary TB and cerebral cryptococcosis, refused HIV test. 6 months' treatment, TB resolved |
| | Aur | - - | - - - | - | | | | | | | |
| | Cul | + + + | + + - | + | | | | | | | |
| JK (male) | LCx | - | - - | - - - | | | - | | - - | | HIV+, strain resistant to INH, on quadruple treatment >1 year, no resolution |
| | Aur | - | + - | - - - | | | - | | - - | | |
| | Cul | + | + + | - - + | | | - | | - - | | |
| DL (male) | LCx | - - | + - | + + | | | - | | | | HIV+, Kaposi's sarcoma on chemotherapy, reactivated pulmonary TB 9 months' treatment resolved |
| | Aur | - - | - - | + + | | | - | | | | |
| | Cul | - - | + + | + + | | | - | | | | |
| MM (male) | LCx | - | - | + + - | | | | | | | Post renal transplant, pulmonary and metacarpal joint TB, 9 months' treatment resolved |
| | Aur | - | - | - - - | | | | | | | |
| | Cul | - | - | + + - | | | | | | | |
| JM (male) | LCx | + + + | | | + | | | | | | Alcoholic, non-adherent, needed supervised treatment, 9 months course for pulmonary TB |
| | Aur | + + + | | | - | | | | | | |
| | Cul | + + + | | | + | | | | | | |
| AP (male) | LCx | + + - | - - - | | | | | | | | Alcoholic myopathy, previous pulmonary TB, reactivation, completed 8 months course of treatment. |
| | Aur | - - - | - - - | | | | | | | | |
| | Cul | - - - | - - - | | | | | | | | |
| KR (male) | LCx | - + | | | + + + | | + + + | | | | Miliary TB, cavitating lung lesions, hepatitis, poor tolerance to anti-TB drugs, slow resolution |
| | Aur | - - | | | - - - | | - - - | | | | |
| | Cul | - c | | | - - - | | - - - | | | | |
| FS (male) | LCx | + + + | | | + + + | | - - - | | | | IVDU, presented with lung abscess positive for TB, 9 months of treatment, adherent, slow to resolve |
| | Aur | - - | | | - - - | | - - - | | | | |
| | Cul | + + - | | | - - - | | - - - | | | | |
| AW (male) | LCx | + | | + + + | + + + | | | | | | HIV+, pulmonary TB, non-adherent after 3 months' treatment, progressive disease. Died |
| | Aur | + | | - - - | - - - | | | | | | |
| | Cul | + | | + - - | - - - | | | | | | |
| WZ (male) | LCx | - | + + + | | | | | - | | | Homeless, pulmonary TB, non-adherent to treatment for 2 months, re-housed, supervised 6 months' treatment |
| | Aur | - | - - - | | | | | - | | | |
| | Cul | - | + - - | | | | | - | | | |

Aur, auramine staining; c, contaminated culture; Cul, culture for *Mycobacterium tuberculosis*; HIV, human immunodeficiency virus; INH, isonicotinic acid hydrazide; IVDU, intravenous drug user; TB, tuberculosis.

treatment, often when smears and later cultures are negative. Among the patients who were slow to resolve or succumbed to their illness, an equal number were LCx positive (MA, SF, KR, AW) as were LCx negative (SB, AH, JK, FS) on serial testing. AW and SF remained LCx positive long after the study period. Therefore, it is recommended that the LCx is not used to monitor adherence to treatment or as a prognostic test.

The obvious advantage of the LCx assay is the rapid differentiation of tuberculosis from cases of NTM in smear positive samples. The LCx did not crossreact with any of the NTM, both in patient specimens and in quality control samples. Given the absence of false positives it is apparent that an LCx positive specimen would strongly support the diagnosis of tuberculosis in clinically suspicious cases.

The cost of the assay, over 13 months unrestricted use, was £46 658.25 (~ $70 000) or approximately £42 500/year. As with all semi-automated tests, the more tests that are done on a daily basis the greater the cost benefit, both in terms of the laboratory budget and the cost implications in patient care. If the assay is only performed on stored samples once or twice a week, it would undermine the definite advantage of speed in achieving a diagnosis. The investment in an additional assay for the rapid diagnosis of tuberculosis is considerable and should be guided by the local rate of positive cultures for *M tuberculosis*.

In conclusion, the LCx assay, as an initial diagnostic test, has > 80% sensitivity and 100% specificity, making it a useful although expensive adjunct to confirm clinical suspicion of tuberculosis. LCx on non-respiratory specimens compares well with smear and culture in detecting *M tuberculosis*. The assay is not useful as a prognostic test and should not be used to evaluate adherence to treatment. It is particularly useful when rapid differentiation between *M tuberculosis* and NTM is needed. It has been used with 100% accuracy to confirm *M tuberculosis* from growth on solid media. Because the onset of treatment was influenced by the test in only one patient, the cost benefit of the assay remains questionable.

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