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Evaluation of Three Nucleic Acid Amplification Methods for Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

S. X. WANG AND L. TAY*

Central Tuberculosis Laboratory, Department of Pathology, Singapore General Hospital, Singapore, Republic of Singapore

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Two hundred thirty respiratory specimens from 230 patients were analyzed by using COBAS AMPLICOR PCR, Amplified *Mycobacterium tuberculosis* Direct Test, and ligase chain reaction methods. Results were compared with those of smear microscopy and radiometric culture (Bactec) methods. No significant differences were observed among the results of the three methods, which are acceptable for direct detection of *M. tuberculosis* complex in respiratory specimens.

Tuberculosis has recently reemerged as a public health concern. The World Health Organization (WHO) declared it to be a global emergency in 1993. According to a WHO report, there are 20 million cases of tuberculosis worldwide, with 8 million new cases and 3 million deaths each year. The problem of tuberculosis management and control has been compounded by the emergence of multiple-drug-resistant strains of *M. tuberculosis* and the human immunodeficiency virus epidemic. One of the main obstacles to the effective control of tuberculosis is the long time taken for laboratory diagnosis by culture (1). On the other hand, acid-fast microscopy, although rapid enough to provide a result within 24 h, lacks sensitivity and is unable to distinguish tubercle bacilli from other mycobacteria. Recently, some new products for rapid diagnosis of tuberculosis have become commercially available. These include the PCR-based COBAS AMPLICOR *Mycobacterium tuberculosis* Test (Roche Diagnostic Systems, Branchburg, N.J.); the Amplified *M. tuberculosis* Direct Test (MTD) (Gen-Probe, San Diego, Calif.), which is based on transcription-mediated amplification; and the ligase chain reaction-based LCx test (LCx *M. tuberculosis*; Abbott Diagnostics Division, Abbott Park, Ill.) (3, 9, 24). We evaluated all three methods by comparison with the microscopic and culture results for 230 clinical respiratory specimens.

MATERIALS AND METHODS

Specimen processing. Two hundred thirty respiratory specimens (222 sputum specimens, 4 bronchoalveolar lavage fluid specimens, 2 laryngeal swabs, and 2 endotracheal aspirates) were collected from 230 patients. Specimens were processed by an NaOH digestion-decontamination procedure (16), using 4% NaOH. After centrifugation, one or two drops of phenol red indicator solution was added to each sediment, which was neutralized by adding 2 N HCl dropwise until the sediment turned from red to yellow. The sediment was resuspended in 1 ml of phosphate buffer (pH 6.8), and the suspension was used for acid-fast bacillus smear, culture, and nucleic acid amplification assays (50 μl for MTD, 100 μl for COBAS AMPLICOR PCR, and 100 μl for LCx).

Acid-fast microscopy. Auramine O fluorescent stain was used to detect the presence of acid-fast bacilli in patients' specimens (10).

Mycobacterial culture and identification. A 0.5-ml portion of the processed specimen was inoculated into a BACTEC 12B culture vial. The BACTEC 460 instrument (Becton Dickinson Diagnostic Instrument System, Sparks, Md.) was used to detect the presence of growth in BACTEC vials twice a week for the first 2 weeks and weekly thereafter. The vials were incubated for a total of 6 weeks at 37°C. The BACTEC NAP (*p*-nitro-α-acetylamino-β-hydroxypropiophenone) test was performed to identify *M. tuberculosis* complex isolates (22).

Nucleic acid amplification test. All three nucleic acid amplification methods were performed by using micropipettes with aerosol barrier tips.

COBAS AMPLICOR PCR. The COBAS AMPLICOR test was performed according to the manufacturer's instructions (9). The internal control used in the COBAS AMPLICOR assay is a sequence of plasmid DNA with primer-binding regions identical to those of the *M. tuberculosis* target sequence. A unique probe-binding region differentiates the internal control from the target amplicon. The internal control is introduced into each amplification reaction and is coamplified with the possible target DNA from the clinical specimen. Specimens for which A_{660} is <0.35 and internal controls for which it is ≥0.35 should be interpreted as negative. For a valid run, specimens with A_{660} of ≥0.35 are interpreted as positive for *M. tuberculosis* regardless of the internal control results. Specimens with A_{660} of <0.35 and internal controls with A_{660} of <0.35 should be interpreted as having an invalid result.

LCx. LCx *M. tuberculosis* test was performed according to the manufacturer's instructions (8, 24). Each series of tests (no more than 20 specimens) was run along with a negative control and a calibrator in duplicate. Amplified tubes were transferred unopened to the carousel of the analyzer, which directly detects the amplification products by a microparticle enzyme immunoassay and displays the results as fluorescence rates, which are compared to the calibrator rate. If the rates exceeded 30% of the average calibrator rate, the results were considered positive.

MTD. The MTD procedure was carried out according to the instructions of the manufacturer (3, 11). The specimen results were read in a Leader 450 luminometer (Gen-Probe); a cutoff value of 30,000 relative light units or more was used for diagnosing positive specimens. Positive and negative controls were included in every run.

Statistical analysis. Statistical comparisons of the three methods were calculated by using the chi-square test; a P value of <0.05 was considered significant.

RESULTS

A total of 230 respiratory specimens collected from 230 patients were included in this study. All these specimens were examined by use of fluorescence microscopy and BACTEC460 TB system as routinely performed in our laboratory. Of the 230 specimens, 66 were smear positive and culture positive, 6 were smear negative and culture positive, and the remaining 158 specimens were both smear and culture negative. As summarized in Table 1, the LCx assay detected all smear-positive, culture-positive specimens (100%) and smear-negative, culture-positive specimens (100%). There were seven (4.4%) smear-negative, culture-negative specimens that were positive by LCx. COBAS AMPLICOR detected 64 smear-positive, culture-positive specimens (96.9%) and five smear-negative, culture-positive specimens (83.3%). There were six (3.8%) smear-negative, culture-negative specimens that were positive by COBAS AMPLICOR. MTD assay detected 66 smear-positive, culture-positive specimens (100%) and 5 smear-negative, cul-

* Corresponding author. Mailing address: Central Tuberculosis Laboratory, Department of Pathology, Singapore General Hospital, Outram Rd., Singapore 169608, Republic of Singapore. Phone: (65)-3214906. Fax: (65)-2226826. E-mail: gpttay@sgh.gov.sg.

TABLE 1. Detection of *M. tuberculosis* complex infection by amplification assays, smear, and culture

Method	No. of patients with positive result			Sensitivity[a] (%)	Specificity[a] (%)	Positive predictive value[a] (%)	Negative predictive value[a] (%)
	Smear-positive culture-positive group (n = 66)	Smear-negative culture-positive group (n = 6)	Smear-negative culture-negative group (n = 158)				
COBAS AMPLICOR	64	5	6	96.1 [95.8]	100 [96.2]	100 [92]	98.1 [98.1]
LCx	66	6	7[b]	100 [100]	99.3 [95.6]	98.7 [91.1]	100 [100]
MTD	66	5	2	98.6 [98.6]	99.4 [98.7]	98.6 [97.3]	99.4 [99.4]

[a] Unresolved data are shown in brackets.
[b] After resolution, the results showed that LCx produced one false-positive result and MTD produced one false-positive result plus one false-negative result whereas COBAS AMPLICOR produced three false-negative results. No significant differences were obtained among the three methods for the smear-positive, culture-positive group (P = 0.133); smear-negative, culture-positive group (P = 0.570); and smear-negative, culture-negative group (P = 0.236).

ture-positive specimens (83.3%). There were two (1.3%) smear-negative, culture-negative specimens that were positive by MTD. For those specimens with positive amplification results but negative smears and cultures, the patients' data were retrieved to check relevant tuberculosis history. Of the six patients with positive results detected by COBAS AMPLICOR, four and two had records indicating that positive smear and culture had been obtained within the previous 6 months and within the previous 12 months, respectively. Of the two patients with positive results detected by MTD, only one had records indicating that positive smear and culture had been obtained within the previous 12 months. Of the seven patients with positive results detected by LCx, five and one had records indicating that positive smear and culture had been obtained within the previous 6 months and within the previous 12 months, respectively. After resolution, the results showed that LCx produced one false-positive result and MTD produced one false-positive result plus one false-negative result (all these specimens were smear negative), whereas COBAS AMPLICOR produced three false-negative results (two specimens were smear positive and one specimen was smear negative). The COBAS *M. tuberculosis* and internal control readings for these three specimens were 0.005 (*M. tuberculosis* negative) and 2.343 (internal control positive) with positive smear result, 0.008 (*M. tuberculosis* negative) and 0.010 (internal control negative) with negative smear result, and 0.030 (*M. tuberculosis* negative) and 0.316 (internal control negative) with positive smear result, respectively. All three specimens were positive by LCx and MTD. The analysis of the first two specimens was repeated and showed reproducible results. We were unable to repeat the analysis of the third specimen due to insufficient volume. Although the second and third specimens gave invalid negative results according to the manufacturer's criteria for interpretation of results, since this study was an evaluation of the three methods and the other two methods were able to detect *M. tuberculosis* complex in the same specimens, we considered these two specimens to be negative in the analysis of results in this study.

The resolved results showed that the overall (smear-positive plus smear-negative specimens) sensitivities and specificities were 100 and 99.3% for LCx, 96.1 and 100% for COBAS AMPLICOR, and 98.6 and 99.4% for MTD, respectively. The positive and negative predictive values were 98.7 and 100% for LCx, 100 and 98.1% for COBAS AMPLICOR, and 98.6 and 99.4% for MTD, respectively. Statistical analysis showed that there were no statistically significant differences among the three methods. The P values were 0.133 for smear-positive plus culture-positive specimens, 0.570 for smear-negative but culture-positive specimens, and 0.236 for smear-negative plus culture-negative specimens (Table 1).

The specimens were divided into two groups, smear positive and smear negative, for further analysis. The results are shown in Table 2. The sensitivities and specificities for smear-negative specimens were 100 and 99.3% for LCx, 91.7 and 100% for COBAS AMPLICOR, and 85.7 and 99.4% for MTD, respectively. Positive predictive values for smear-negative specimens were 92.3% for LCx, 85.7% for MTD, and 100% for COBAS AMPLICOR. Negative predictive values for smear-negative specimens for LCx, MTD, and COBAS AMPLICOR were 100, 99.4, and 99.3%, respectively. The sensitivities and specificities for smear-positive specimens were 100 and 100% for LCx, 96.9 and 100% for COBAS AMPLICOR, and 100 and 100% for MTD, respectively. Positive predictive values for smear-positive specimens were 100% for all three methods. Efficiency was 98.7% for COBAS AMPLICOR, 99.1% for MTD, and 99.6% for LCx.

DISCUSSION

The increased incidence of tuberculosis has stimulated the development of rapid and direct detection methods for the

TABLE 2. Evaluation of the three nucleic acid amplification systems for direct detection of *M. tuberculosis* complex in respiratory specimens

Nucleic acid amplification system	No. of specimens (no. confirmed positive for *M. tuberculosis*)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Efficiency (%)
MTD	All, 230 (72)	98.6	99.4	98.6	99.4	99.1
	SP,[a] 66 (66)	100	100	100	NA[b]	
	SN,[a] 162 (6)	85.7	99.4	85.7	99.4	
COBAS AMPLICOR	All, 230 (72)	96.1	100	100	98.1	98.7
	SP, 66 (66)	96.9	100	100	NA	
	SN, 162 (6)	91.7	100	100	99.3	
LCx	All, 230 (72)	100	99.3	98.7	100	99.6
	SP, 66 (66)	100	100	100	NA	
	SN, 162 (6)	100	99.3	92.3	100	

[a] SP, smear positive; SN, smear negative. Two specimens positive for nontuberculous mycobacteria were excluded from the analysis.
[b] NA, not applicable.

laboratory identification of *M. tuberculosis*. Newly developed, standardized, commercially available test kits include automated COBAS AMPLICOR PCR, Gen-Probe MTD, and LCx. The kits contain all the reagents needed for specimen amplification and detection, as well as controls. Generally, differences between cutoff values of positive and negative controls and specimens were broad enough to permit easy discrimination. Negative results obtained by amplification assays for culture-positive specimens may be explained by the presence of inhibitors of enzymatic amplification and/or by a small number of mycobacteria, unequally distributed in the test suspension (6, 19). Of the three specimens that were negative by COBAS AMPLICOR but positive by culture in our study, two of the internal controls showed negative results, indicating the existence of endogenous inhibitors. However, the internal control for the third one was positive. Hence, our results, to some extent, support the view that a single-sample PCR-negative result must be considered carefully because of the potential for false-negative results (6). Our results obtained from the analysis of all samples showed that the sensitivities of LCx, COBAS AMPLICOR, and MTD were 100, 96.1, and 98.6%, respectively. The specificities were 99.3, 100, and 99.4%, respectively. The positive and negative predictive values, derived by comparison with culture results, were 98.7 and 100% for LCx, 100 and 98.1% for COBAS AMPLICOR, and 98.6 and 99.4% for MTD, respectively. In general, data taken from the literature are in agreement with our findings. MTD sensitivities and specificities ranged from 91 to 98.4% and from 96.9 to 100%, respectively (1, 17, 19). However, the sensitivity of 96.1% and specificity of 100% obtained for the automated COBAS AMPLICOR PCR are higher than those obtained for the manual AMPLICOR MTB Test, which has sensitivities and specificities ranging from 66.7 to 86.5% and from 97 to 100%, respectively (4, 5, 11, 21). Published data (8, 24) showed that when the LCx test was used for direct detection of *M. tuberculosis* complex in pulmonary and extrapulmonary specimens, the sensitivity, specificity, and positive and negative predictive values, derived by comparison with culture results, were 95.5, 99.3, 97.3, and 98.8%, respectively. When the LCx test was used for respiratory specimens only, its sensitivity reached 99.0% (7, 8, 24). The data we obtained were in agreement with those findings for respiratory specimens. It should be noted that the LCx yielded the highest sensitivity among the three kits in our study. However, LCx also produced one false-positive result. As to this result produced by LCx and the false-positive result produced by MTD, neither of these specimens was analyzed following analysis of a positive specimen either in the carousel of the LCx instrument or in the reading rack of the Leader 450 luminometer, ruling out a carryover effect. These false-positive results might have been due to an accidental contamination of the specimens. Our results suggest that nucleic acid amplification methods for direct detection of *M. tuberculosis* complex in respiratory specimens should be applied as an adjunct to smears and culture. In conclusion, our results suggest that all the three assays are acceptable rapid diagnostic methods for pulmonary tuberculosis.

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