

The Clinical Utility of Polymerase Chain Reaction for the Diagnosis of Pleural Tuberculosis

Jin Wook Moon,¹ Yoon Soo Chang,¹ Se Kyu Kim,^{1,2,3} Young Sam Kim,^{1,3} Hyuk Min Lee,⁴ Sung Kyu Kim,^{1,3} and Joon Chang^{1,3}

¹Department of Internal Medicine, ²Brain Korea 21 Project for Medical Sciences, ³Institute of Chest Diseases, and ⁴Department of Laboratory Medicine, Yonsei University College of Medicine, Seoul, Korea

Background. There is no exact consensus about the usefulness of the *Mycobacterium tuberculosis* polymerase chain reaction (PCR) testing for the diagnosis of tuberculous pleural effusion because of the diverse PCR methods and the different diagnostic criteria that are described in other studies.

Methods. We analyzed pleural effusion specimens obtained from 111 patients for whom the exclusion of the possibility of tuberculous pleural effusion was necessary. We performed *M. tuberculosis* PCR testing using the Cobas Amplicor MTB test (Roche Diagnostic Systems), which is fully automated and commercially available.

Results. Results of the *M. tuberculosis* PCR test of pleural effusion specimens were positive for 7 (17.1%) of the 41 patients with confirmed pleural tuberculosis and for 3 (18.8%) of the 16 patients with probable pleural tuberculosis. The overall sensitivity and specificity of *M. tuberculosis* PCR testing of pleural effusion were 17.5% and 98.1%, respectively. The sensitivity of *M. tuberculosis* PCR testing for each group of patients with tuberculous pleural effusion detected by smear-positive results, smear-negative and culture-positive results, and culture-negative and pleural biopsy-positive results, was 100.0%, 33.3%, and 3.7%, respectively. Of the 57 patients with pleural tuberculosis, only 3 (5.3%) had positive results of *M. tuberculosis* PCR testing along with negative results of smearing, negative results of pleural pathological analysis, and a low level of adenosine deaminase.

Conclusion. For specimens such as pleural effusion, in which the bacillary load is very low, the clinical utility of PCR testing seems highly limited.

Tuberculosis is still a major health problem worldwide. It is estimated that 8–9 million new cases develop every year, of which 3–4 million cases have smear-positive results [1]. The recent increase in tuberculosis in the world and the emergence of multidrug-resistant strains have prompted more rapid and sensitive methods in the laboratory diagnosis than previous conventional diagnostic techniques. The diagnosis of tuberculosis at an earlier stage would be advantageous, because it is less contagious and is associated with a lower morbidity and mortality during the early stage [2–4]. Although culture of *Mycobacterium tuberculosis* continues to be the gold standard in the diagnosis of tuberculosis, the results are neither satisfactorily sensitive nor rapid. My-

cobacterial culture takes at least 2 weeks to perform, depending on the culture media that is used [5]. Nucleic acid amplification tests (NATs), including PCR, have improved the accuracy of and the time for diagnosis of tuberculosis made on the basis of examination of respiratory specimens. For nonrespiratory specimens, such as pleural effusion specimens, however, previous studies have shown highly variable results regarding the usefulness of NATs because of the use of different in-house NAT methods, small study populations, and diverse criteria for pleural tuberculosis.

The Cobas Amplicor MTB test (Roche Diagnostic Systems) is a well established and commercially available PCR test commonly used for the direct detection of *M. tuberculosis* in clinical samples. The test uses biotinylated genus-specific primers (KY18 and KY75) to amplify the 584–base-pair sequence within the 1500–base-pair region encoding 16s rRNA of MTB. The test combines 5 instruments into 1 (a thermal cycler, an automatic pipettor, an incubator, a washer, and a reader). Because it is fully automated for amplification and detection and is consolidated into a single instru-

Received 1 February 2005; accepted 11 April 2005; electronically published 26 July 2005.

Reprints or correspondence: Dr. Joon Chang, Dept. of Internal Medicine, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul, Korea (chang@yumc.yonsei.ac.kr).

Clinical Infectious Diseases 2005;41:660–6

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1058-4838/2005/4105-0012\$15.00

ment, it is thought to be able to minimize interindividual variabilities. Furthermore, because it binds *M. tuberculosis*-specific oligonucleotide probes to the amplified sequences, it can increase the overall specificity.

Tuberculous pleural effusion occurs in up to 30% of patients with tuberculosis [6] and accounts for the major portion of the morbidity associated with extrapulmonary tuberculosis [7]. However, the number of organisms in pleural effusion specimens obtained from most patients with tuberculous pleuritis is relatively low, with positive culture results in <25% of cases. Even pleural biopsy reveals granulomatous inflammation only in ~60% of patients [8]. PCR has been used to detect *M. tuberculosis* in pleural fluid samples, with highly variable sensitivities (11%–81%) in previous studies with different in-house PCR methods [9–14]. This is why we investigated the clinical utility of *M. tuberculosis* PCR testing with the Cobas Amplicor MTB Analyzer with regard to the rapid and accurate diagnosis of pleural tuberculosis.

METHODS

Study population and samples. One hundred eleven patients >18 years of age, for whom the exclusion of the possibility of tuberculous pleural effusion was necessary, were prospectively analyzed over a 2-year period (August 2002–August 2004) at the Yonsei University Medical Center (Seoul, Korea). Suspicion of pleural tuberculosis was based on unilateral pleural effusion with clinical manifestations suggestive of tuberculosis, such as productive cough, chronic low-grade fever, weight loss, anorexia, and night sweating. Pleural effusion specimens obtained from all 111 patients were subjected to routine analysis, acid-fast bacilli (AFB) smear, mycobacterial culture, *M. tuberculosis* PCR with the Cobas Amplicor MTB Analyzer, measurement of the adenosine deaminase (ADA) level, Gram staining, bacterial culture, and cytologic examination. Sputum samples were collected in the morning from each patient and were used for AFB smear and mycobacterial culture. Pleural biopsy and pathologic examination were not performed for 32 of the 111 patients because of the small amount of pleural effusion. Informed consent was obtained from each patient. The use of the samples and the study protocols were reviewed and approved by our institutional review board.

Diagnostic criteria of pleural tuberculosis. A case of confirmed pleural tuberculosis was defined as having one of the following characteristics: a pleural fluid sample that was culture-positive for *M. tuberculosis* and/or a histopathologic finding consistent with tuberculosis on pleural biopsy. A case of probable pleural tuberculosis was defined as having one of the following characteristics: a sputum specimen that was culture-positive for *M. tuberculosis* and/or other biologic specimens that were culture-positive for *M. tuberculosis* and/or a positive re-

sponse to antituberculosis medication without other possible causes of pleural effusion.

AFB smear and mycobacterial culture. For microscopic examination, Ziehl-Neelsen staining was performed. After each of the collected sputum and pleural effusion samples was decontaminated by an equal volume of 4% sodium hydroxide (NaOH) solution, 2 slopes of Ogawa media containing 3% potassium dihydrogen phosphate (KH₂PO₄) (3% Ogawa media) were inoculated with them [15]. The inoculated medium was incubated at 35°C–37°C until the growth was observed or was discarded as negative after 8 weeks.

Determination of ADA activity in pleural effusion. ADA activity was determined with 2 mL of pleural fluid, according to the colorimetric method described by Giusti and Galanti [16]. An ADA level of <45 IU/L was considered to be a negative finding.

Tuberculin skin test. Tuberculin skin testing was not performed in this study because there were difficulties in interpreting the results in Korea, where a *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) vaccination program covers >90% of the population.

Nucleic acid amplification and detection techniques. Specimen preparation was as follows: 10 mL of each pleural fluid specimen was decontaminated with an equal amount of 4% NaOH solution and was centrifuged at 3000 g for 20 min to collect the sediment. One hundred μ L of sediment from each pleural effusion sample was transferred to a microcentrifuge tube containing 500 μ L of washing solution and centrifuged at 12,500 g for 10 min. The supernatant was discarded, and 100 μ L of specimen lysis reagent was added to extract a DNA template. The mixture was vortexed and incubated at 60°C in a dry-heat block for 45 min, after which 100 μ L of specimen neutralization reagent was added. Then, a 50- μ L aliquot of DNA extract was transferred to a PCR tube containing 50 μ L of amplification mixture. Amplification, hybridization, detection, and interpretation were performed according to the manufacturer's instructions.

Statistical analysis. The relationship between the bacillary load and the rate of positive *M. tuberculosis* PCR test results was assessed with Fisher's exact test by partitioning patients with confirmed pleural tuberculosis into 3 groups. SPSS software, version 11.0 (SPSS), was used.

RESULTS

Subjects' characteristics. Initially, 113 patients were enrolled in our study, but pleural effusion specimens obtained from 2 patients (1.8%) repeatedly showed an inhibition to the amplification reaction. Therefore, the data for these 2 patients were not included in the statistical analysis. Tuberculous pleural effusion was diagnosed in one of the patients, and parapneumonic effusion was diagnosed in the other.

Of the 111 remaining patients, 57 received diagnoses of pleural tuberculosis, including confirmed and probable pleural tuberculosis. Patients with confirmed pleural tuberculosis (41 patients) had pleural fluid that was culture-positive for *M. tuberculosis* (14 patients) and/or a histopathologic finding consistent with tuberculosis on pleural biopsy (32 patients). Patients with probable pleural tuberculosis (16 patients) had sputum that was culture-positive for *M. tuberculosis* (5 patients), a paravertebral abscess that was culture-positive for *M. tuberculosis* (1 patient), or a positive response to antituberculosis medication (10 patients) without other possible causes of pleural effusion. The mean age was 43.3 years for the tuberculous-pleural-effusion group and 55.9 years for the nontuberculous-pleural-effusion group. There were 17 women (29.8%) in the tuberculous-pleural effusion group and 12 women (22.2%) in the nontuberculous-pleural effusion group (table 1). The etiologies of nontuberculous pleural effusion included malignancy (in 27 [50.0%] of 54 patients with nontuberculous pleural effusion), bacterial pneumonia (in 16 patients [29.6%]), intra-abdominal infection (in 3 patients [5.6%]), liver cirrhosis (in 2 patients [3.7%]), and sepsis (in 1 patient [1.9%]), and 5 patients (9.3%) had nontuberculous pleural effusion of an undetermined origin.

Sensitivity and specificity of *M. tuberculosis* PCR testing, AFB smear, *M. tuberculosis* culture, measurement of ADA activity, and pleural biopsy pathology. Of the 57 tuberculous pleural effusion specimens, 10 had positive *M. tuberculosis* PCR test results, 2 had positive AFB smear results, 14 had positive *M. tuberculosis* culture results, and 46 were determined to be positive by measurement of ADA activity (defined as an ADA level of >45 IU/L). Considering the combination of *M. tuberculosis* culture, pleural biopsy pathology, and clinical finding as the reference method of diagnosing pleural tuberculosis, the

sensitivities of *M. tuberculosis* PCR testing of pleural effusion specimen, AFB smearing of pleural effusion specimen, *M. tuberculosis* culture of pleural effusion specimen, and measurement of ADA were 17.5%, 3.5%, 24.6%, and 80.7%, respectively. Of the 57 patients with pleural tuberculosis, 39 underwent pleural biopsy, and 32 of those patients had a histologic finding consistent with pleural tuberculosis. Of the 57 patients with pleural tuberculosis, 21 had a sputum culture result that was positive for *M. tuberculosis*. The sensitivity of pleural biopsy and sputum culture was 82.1% and 36.8%, respectively. Of the 54 nontuberculous pleural effusion specimens, 1 was PCR-positive for *M. tuberculosis* and 3 were ADA-positive. The specificity of *M. tuberculosis* PCR testing and measurement of ADA activity, therefore, was 98.1% and 94.4%, respectively (table 1). We also separately evaluated the sensitivity of *M. tuberculosis* PCR testing for patients with confirmed and probable pleural tuberculosis. These sensitivities were almost equivalent to the overall sensitivity (17.1% for the group with confirmed pleural tuberculosis and 18.8% for the group with probable pleural tuberculosis) (table 2).

Sensitivity of *M. tuberculosis* PCR testing according to the bacillary load. Patients with confirmed pleural tuberculosis were separated into 3 groups with respect to the assumed bacillary load in the pleural effusion. Results of *M. tuberculosis* PCR tests of pleural effusion specimens were positive for 2 of 2 patients with positive pleural effusion AFB smear results, for 4 of 12 patients with negative pleural effusion AFB smear results and positive culture results, and for 1 of 27 patients with negative culture results and positive pleural biopsy results. The sensitivity of *M. tuberculosis* PCR testing for each group was 100.0%, 33.3%, and 3.7%, respectively ($P < .05$, by Fisher's exact test).

Clinical utility of *M. tuberculosis* PCR testing in terms of the rapid diagnosis of pleural tuberculosis. In only 3 of the

Table 1. Baseline characteristics of patients with tuberculous pleural effusion and patients with nontuberculous effusion and the sensitivity and specificity of *Mycobacterium tuberculosis* PCR testing, acid-fast bacilli (AFB) smear, pleural effusion culture, adenosine deaminase (ADA) activity, pleural biopsy pathology, and sputum culture.

Variable	Tuberculous pleural effusion group (n = 57)	Nontuberculous pleural effusion group (n = 54)	Sensitivity, %	Specificity, %
Female sex	17 (29.8)	12 (22.2)
Age, mean years (range)	43.3 (19–85)	55.9 (19–87)
Positive result of <i>M. tuberculosis</i> PCR test of pleural effusion specimen	10	1	17.5	98.1
Positive finding of AFB smear of pleural effusion specimen	2	0	3.5	...
Positive result of pleural effusion culture	14	0	24.6	...
ADA level >45 IU/L	46	3	80.7	94.4
Positive finding of pleural biopsy	32 ^a	0 ^b	82.1	...
Positive result of sputum culture	21	0	36.8	...

NOTE. Data is no. (%) of patients, unless indicated otherwise.

^a Thirty-two of the 39 patients who underwent biopsy had a histologic finding consistent with pleural tuberculosis.

^b Forty patients underwent pleural biopsy.

Table 2. Sensitivities of pleural effusion *Mycobacterium tuberculosis* PCR, acid-fast bacilli (AFB) smear, *M. tuberculosis* culture, measurement of adenosine deaminase (ADA) activity, pleural biopsy pathology, and sputum *M. tuberculosis* culture in each group of confirmed and probable tuberculous pleural effusion.

Variable	Confirmed tuberculous pleural effusion group (n = 41)	Probable tuberculous pleural effusion group (n = 16)
Positive result of <i>M. tuberculosis</i> PCR test of pleural effusion specimen	7 (17.1)	3 (18.8)
Positive finding of AFB smear of pleural effusion specimen	2 (4.9)	...
Positive result of pleural effusion culture	14 (34.1)	...
ADA level >45 IU/L	34 (82.9)	12 (75.0)
Positive finding of pleural biopsy	32 (86.5) ^a	0 ^b
Positive result of sputum culture	16 (39.0)	5 (31.3)

^a Of the 37 patients who underwent biopsy, 32 had a histologic finding consistent with pleural tuberculosis.

^b Of the 2 patients who underwent biopsy, none had a histologic finding consistent with pleural tuberculosis.

57 patients with pleural tuberculosis, results of the rapid diagnostic modalities, including pleural effusion AFB smear, sputum AFB smear, pleural biopsy pathology, and measurement of ADA activity, were all negative, except results of *M. tuberculosis* PCR testing. Pleural tuberculosis in these 3 patients was later confirmed by pleural effusion culture and/or sputum culture. Therefore, as a rapid diagnostic laboratory method, *M. tuberculosis* PCR testing of pleural effusion specimen was useful only in 3 (5.3%) of the 57 patients with pleural tuberculosis.

DISCUSSION

With respect to tuberculous pleurisy, early in the course of a tuberculous infection, a few organisms may gain access to the pleura and cause a hypersensitivity response in the presence of cell-mediated immunity [17, 18]. Commonly, this form of tuberculous pleurisy goes unnoticed, and it resolves spontaneously. In some patients, however, the tuberculous involvement of the pleura is manifested as an acute illness with fever and pleuritic pain. The effusion is generally small and unilateral. In ~30% of patients, there is no radiographic evidence of involvement of the lung parenchyma, despite the presence of lung parenchymal lesions in most cases, as evidenced by findings of lung dissections [19]. In the absence of concurrent pulmonary tuberculosis, the diagnosis of pleural tuberculosis requires thoracentesis and, in most cases, even pleural biopsy [8]. For tuberculous pleural effusion, the number of organisms in the pleural fluid is very small, thus, conventional methods for the detection of *M. tuberculosis* are often of no use. Although the combination of microscopic examination and culture of pleural biopsy specimens was reported to increase the rate of diagnosis up to 90%, it is time-consuming [20]. Thus, many physicians request NATs, including PCR testing of pleural effusion specimens, to obtain a rapid and accurate diagnosis of pleural tuberculosis.

Several commercial and in-house NATs to detect *M. tuberculosis* in clinical specimens have been developed. These tests amplify various targets in DNA or RNA sequences that are genus-specific or species-specific, and this is followed by detection with gel electrophoresis or hybridization. Currently, there are 4 commercial NATs for the detection of *M. tuberculosis*: the Amplicor MTB test and its automated version, the Cobas Amplicor MTB test; the Enhanced Amplified *Mycobacterium Tuberculosis* Direct Test (E-AMTDT) (Gen-Probe); the BDProbe Tec ET test (Becton Dickinson); and the INNO-LiPA-Rif TB test (Innogenetics). Of these, the E-AMTDT was approved by the US Food and Drug Administration for the direct detection of *M. tuberculosis* in both smear-positive and smear-negative respiratory specimens obtained from patients suspected of having tuberculosis, and the Amplicor MTB test was approved only for use with smear-positive respiratory specimens [21–23]. With respect to in-house NATs, in previous studies, diverse methods with different primers have been used to detect *M. tuberculosis* in pleural fluid samples, with highly variable sensitivities (11%–81%) [9–14]. Sensitivities of commercial NATs have also been variable (20%–100%), because the number of the patients with pleural tuberculosis was rather small, and in some studies, only the culture-positive cases were included in the pleural tuberculosis group [24–30] (table 3). Because cases with positive culture results for *M. tuberculosis* represent a relatively small portion of all the cases of pleural tuberculosis, and because the sensitivity of *M. tuberculosis* PCR testing largely depends on the bacillary load, the sensitivity for the study group of patients with culture results positive for *M. tuberculosis* does not reflect the sensitivity for all patients with pleural tuberculosis. For these reasons, there has been no consensus regarding the usefulness of *M. tuberculosis* PCR testing in the diagnosis of tuberculous pleural effusion. In this study, we used the commercially available Cobas Amplicor MTB test,

Table 3. Comparison of previous studies of the clinical utility of *Mycobacterium tuberculosis* nucleic acid amplification testing of pleural effusion specimens.

Reference	NAT	Target sequence	Criteria for diagnosis of tuberculous pleural effusion	No. of		Sensitivity, %		NAT		
				tuberculous pleural effusion specimens	nontuberculous pleural effusion specimens	AFB <i>M. tuberculosis</i> smear	ADA activity	Biopsy	Sensitivity, %	Specificity, %
[10]	In-house PCR	336 bp of DNA	Culture and clinical findings	53	31	NA	NA	77.3	81	77.4
[11]	In-house PCR	IS6110	Culture and clinical findings	15	NA	0	NA	NA	60	NA
[12]	In-house PCR	IS6110	Culture and biopsy findings	21	86	14	86 ^a	72	81	100
[13]	In-house PCR	IS6110	Culture and clinical findings	61	70	0	86.9	64.3	60.7	90.0
[14]	In-house PCR	150 bp of DNA	Culture and clinical findings	20	40	20	55 ^b	NA	70	100
[23]	Gen-Probe E-AMTDT	rRNA	Culture and clinical findings	3	32	NA	NA	NA	100	100
[24]	Gen-Probe E-AMTDT	rRNA	Culture findings	4	61	0	100 ^c	NA	50.0	95.4
[25]	Gen-Probe E-AMTDT	rRNA	Culture findings	5	56	NA	100 ^c	NA	20.0	96.4
[26]	Amplicor MTB test	16s rRNA	Culture findings	ND ^d	ND ^d	NA	100 ^c	NA	83	98
[27]	Amplicor MTB test	16s rRNA	Culture findings	8	367	NA	100 ^c	NA	50	99.7
[28]	Amplicor MTB test	16s rRNA	Culture findings	3	257	0	100 ^c	NA	0	100
[29]	Amplicor MTB test	16s rRNA	Culture and clinical findings	ND ^d	ND ^d	NA	NA	NA	83.5 ^e	98.8 ^e

NOTE. AFB, acid-fast bacilli; E-AMTDT, Enhanced Amplified *Mycobacterium Tuberculosis* Direct Test; NA, not available; NAT, nucleic acid amplification test; ND, not defined.

^a The cutoff value of ADA was 45 IU/L.

^b The cutoff value of ADA was 50 IU/L.

^c The *M. tuberculosis* culture of pleural effusion specimens was used as the diagnostic criteria.

^d The number of pleural effusion specimens out of all nonrespiratory samples was not described.

^e The sensitivity and specificity of *M. tuberculosis* PCR testing were calculated for all nonrespiratory specimens, not just for the pleural effusion specimens.

which can minimize the intertester variabilities and maximize the specificities because of its automated specimen processing and detection. And the combination of *M. tuberculosis* culture, pleural pathological analysis, and clinical diagnosis was employed as the reference method for diagnosing pleural tuberculosis.

In Korea, tuberculosis is still a major infectious disease. We examined the clinical utility of *M. tuberculosis* PCR testing in the diagnosis of pleural tuberculosis with respect to its sensitivity, specificity, and usefulness in terms of a rapid diagnostic method. Our study demonstrated that *M. tuberculosis* PCR testing of pleural effusion specimens has a lower sensitivity (17.5%) than do mycobacterial culture of pleural effusion specimens (24.6%) and pathologic examination of pleural biopsy specimens (82.1%). The sensitivity of *M. tuberculosis* PCR testing of pleural effusion specimens for each group of patients who had smear-positive, smear-negative and culture-positive, and culture-negative and pleural biopsy pathology-positive results was 100.0%, 33.3%, and 3.7%, respectively. These results indicate that the sensitivity of *M. tuberculosis* PCR testing is largely dependent on the bacillary load in the specimens, which is in accordance with the previous studies [30–33]. Therefore, for the specimens such as pleural effusion specimens, in which the bacillary load is very low, the clinical utility of *M. tuberculosis* PCR testing seems highly limited. Among the 57 patients with pleural tuberculosis, only 3 (5.3%) had a positive result of *M. tuberculosis* PCR testing and a negative smear result, a negative finding of pleural biopsy, and a low level of ADA activity. In terms of the rapid detection of *M. tuberculosis* in pleural effusion, therefore, *M. tuberculosis* PCR testing does not seem to be very helpful. Of the 11 patients with a positive result of *M. tuberculosis* PCR tests of pleural effusion specimens, 1 received a diagnosis of malignant pleural effusion due to metastatic breast cancer, which was confirmed by cytologic examination of a pleural effusion specimen. This patient was an 84-year-old woman who did not have any clinical or radiological evidence of previous tuberculosis. This false-positive result might have originated from cross-contamination during specimen processing, from cross-reaction with nonmycobacterial DNA, or from latent infection with tuberculosis [34–37]. Johansen et al. [37] suggested that the lung tissue of persons residing in an area where *M. tuberculosis* is endemic could be colonized with a small amount of *M. tuberculosis* without active infection that was detectable by PCR testing. Because the prevalence of tuberculosis is still high in Korea, this might explain the false-positive *M. tuberculosis* PCR result in our study. Therefore, the positive *M. tuberculosis* PCR result should be carefully interpreted in conjunction with results of mycobacterial culture, findings of histopathologic examination of pleural biopsy spec-

imens, and clinical findings to reach the diagnosis of tuberculous pleural effusion.

The sensitivity of NAT depends not only on the number of mycobacteria but also on their homogenous distribution in the specimen, the presence of the amplification inhibitor in the sample, and the type of the primers that are used [38]. When applying the *M. tuberculosis* PCR test to paucibacillary specimens, therefore, all of these aspects should also be considered.

In conclusion, although *M. tuberculosis* PCR testing provides a rapid result and has a potential role in confirming tuberculous pleuritis, it has limitations in itself. Our results suggest that *M. tuberculosis* PCR testing of pleural effusion specimens with the Cobas AmpliCor MTB test has a low sensitivity and thus does not seem to be useful in excluding the disease. Therefore, it cannot replace the conventional diagnostic methods, including culture techniques and histopathologic examinations. Furthermore, the results of *M. tuberculosis* PCR tests need to be interpreted in conjunction with those of conventional methods and clinical findings to reach the final diagnosis of pleural tuberculosis.

Acknowledgments

We thank Dr. Sung Ho Kim for reviewing this article.

Financial support. This work was partly supported by the Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, Korea.

Potential conflicts of interest. All authors: no conflicts.

References

1. World Health Organization. Global tuberculosis control: surveillance, planning, financing: WHO report 2004. Geneva: World Health Organization, 2004.
2. Menzies D. Issues in the management of contacts of patients with active pulmonary tuberculosis. *Can J Public Health* 1997; 88:197–201.
3. Behr MA, Warren SA, Salamon H, et al. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet* 1999; 353:444–9.
4. Toman K. Tuberculosis case-finding and chemotherapy: questions and answers. Geneva: World Health Organization, 1979.
5. Al Zahrani K, Al Jahdali H, Poirier L, Rene P, Gennaro ML, Menzies D. Accuracy and utility of commercially available amplification and serologic tests for the diagnosis of minimal pulmonary tuberculosis. *Am J Respir Crit Care Med* 2000; 162:1323–9.
6. Ogawa K, Koga H, Hirakata Y, Tomono K, Tashiro T, Kohno S. Differential diagnosis of tuberculous pleurisy by measurement of cytokine concentrations in pleural effusion. *Tuber Lung Dis* 1997; 78:29–34.
7. Iseman MD. A clinician's guide to tuberculosis. Philadelphia: Lippincott Williams & Wilkins; 2000.
8. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000; 161:1376–95.
9. Kolk AH, Schuitema AR, Kuijper S, et al. Detection of *Mycobacterium tuberculosis* in clinical samples by using polymerase chain reaction and a nonradioactive detection system. *J Clin Microbiol* 1992; 30:2567–75.
10. de Wit D, Maartens G, Steyn L. A comparative study of the polymerase chain reaction and conventional procedures for the diagnosis of tuberculous pleural effusion. *Tuber Lung Dis* 1992; 73:262–7.
11. de Lassence A, Lecossier D, Pierre C, Cadranet J, Stern M, Hance AJ. Detection of mycobacterial DNA in pleural fluid from patients with

- tuberculous pleurisy by means of the polymerase chain reaction: comparison of two protocols. *Thorax* **1992**;47:265–9.
12. Querol JM, Minguez J, Garcia-Sanchez E, Farga MA, Gimeno C, Garcia-de-Lomas J. Rapid diagnosis of pleural tuberculosis by polymerase chain reaction. *Am J Respir Crit Care Med* **1995**;152:1977–81.
 13. Villegas MV, Labrada LA, Saravia NG. Evaluation of polymerase chain reaction, adenosine deaminase, and interferon-gamma in pleural fluid for the differential diagnosis of pleural tuberculosis. *Chest* **2000**;118:1355–64.
 14. Nagesh BS, Sehgal S, Jindal SK, Arora SK. Evaluation of polymerase chain reaction for detection of *Mycobacterium tuberculosis* in pleural fluid. *Chest* **2001**;119:1737–41.
 15. World Health Organization. Laboratory services in tuberculosis control. Part III: Culture WHO/TB/98.258. Geneva: World Health Organization, **1998**. Available at: www.phppo.cdc.gov/dls/ILA/documents/lstc3.pdf. Accessed on 22 July 2005.
 16. Giusti G, Galanti B. Methods of enzyme analysis. New York: Academic Press, **1983**.
 17. Berger HW, Mejia E. Tuberculous pleurisy. *Chest* **1973**;63:88–92.
 18. Ellner JJ. Pleural fluid and peripheral blood lymphocyte function in tuberculosis. *Ann Intern Med* **1978**;89:932–3.
 19. Stead WW, Eichenholz A, Stauss HK. Operative and pathologic findings in twenty-four patients with syndrome of idiopathic pleurisy with effusion, presumably tuberculous. *Am Rev Tuberc* **1955**;71:473–502.
 20. Light RW. Pleural diseases. Philadelphia: Lea & Febiger, **1983**.
 21. Nightingale SL. From the Food and Drug Administration. *JAMA* **1996**;275:585.
 22. Centers for Disease Control and Prevention. From the Centers for Disease Control and Prevention. Update: Nucleic acid amplification tests for tuberculosis. *JAMA* **2000**;284:826.
 23. Shamputa IC, Rigouts L, Portaels F. Molecular genetic methods for diagnosis and antibiotic resistance detection of mycobacteria from clinical specimens. *APMIS* **2004**;112:728–52.
 24. Ehlers S, Ignatius R, Regnath T, Hahn H. Diagnosis of extrapulmonary tuberculosis by Gen-Probe amplified *Mycobacterium tuberculosis* direct test. *J Clin Microbiol* **1996**;34:2275–9.
 25. Pfyyffer GE, Kissling P, Jahn EM, Welscher HM, Salfinger M, Weber R. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other nonrespiratory, and respiratory specimens. *J Clin Microbiol* **1996**;34:834–41.
 26. Vlaspolder F, Singer P, Roggeveen C. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. *J Clin Microbiol* **1995**;33:2699–703.
 27. Carpentier E, Drouillard B, Dailloux M, *et al*. Diagnosis of tuberculosis by Amplicor *Mycobacterium tuberculosis* test: a multicenter study. *J Clin Microbiol* **1995**;33:3106–10.
 28. Shah S, Miller A, Mastellone A, *et al*. Rapid diagnosis of tuberculosis in various biopsy and body fluid specimens by the AMPLICOR *Mycobacterium tuberculosis* polymerase chain reaction test. *Chest* **1998**;113:1190–4.
 29. Levdiotou S, Vroni G, Galanakis E, Gesouli E, Pappa C, Stefanou D. Four-year experience of use of the Cobas Amplicor system for rapid detection of *Mycobacterium tuberculosis* complex in respiratory and nonrespiratory specimens in Greece. *Eur J Clin Microbiol Infect Dis* **2003**;22:349–56.
 30. Reischl U, Lehn N, Wolf H, Naumann L. Clinical evaluation of the automated COBAS AMPLICOR MTB assay for testing respiratory and nonrespiratory specimens. *J Clin Microbiol* **1998**;36:2853–60.
 31. Oh EJ, Park YJ, Chang CL, Kim BK, Kim SM. Improved detection and differentiation of mycobacteria with combination of *Mycobacterium* Growth Indicator Tube and Roche COBAS AMPLICOR system in conjunction with duplex PCR. *J Microbiol Methods* **2001**;46:29–36.
 32. Tortoli E, Tronci M, Tosi CP, *et al*. Multicenter evaluation of two commercial amplification kits (Amplicor, Roche and LCx, Abbott) for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens. *Diagn Microbiol Infect Dis* **1999**;33:173–9.
 33. Scarparo C, Piccoli P, Rigon A, Ruggiero G, Scagnelli M, Piersimoni C. Comparison of enhanced *Mycobacterium tuberculosis* amplified direct test with COBAS AMPLICOR *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extrapulmonary specimens. *J Clin Microbiol* **2000**;38:1559–62.
 34. Brienze VM, Tonon AP, Pereira FJ, *et al*. Low sensitivity of polymerase chain reaction for diagnosis of tuberculous meningitis in southeastern Brazil. *Rev Soc Bras Med Trop* **2001**;34:389–93.
 35. Bergmann JS, Keating WE, Woods GL. Clinical evaluation of the BDProbeTec ET system for rapid detection of *Mycobacterium tuberculosis*. *J Clin Microbiol* **2000**;38:863–5.
 36. Mazzarelli G, Rindi L, Piccoli P, Scarparo C, Garzelli C, Tortoli E. Evaluation of the BDProbeTec ET system for direct detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary samples: a multicenter study. *J Clin Microbiol* **2003**;41:1779–82.
 37. Johansen IS, Thomsen VO, Johansen A, Andersen P, Lundgren B. Evaluation of a new commercial assay for diagnosis of pulmonary and nonpulmonary tuberculosis. *Eur J Clin Microbiol Infect Dis* **2002**;21:455–60.
 38. Ruiz-Manzano J, Manterola JM, Gamboa F, *et al*. Detection of *Mycobacterium tuberculosis* in paraffin-embedded pleural biopsy specimens by commercial ribosomal RNA and DNA amplification kits. *Chest* **2000**;118:648–55.