Turkish Journal of Medical Sciences

Volume 32 | Number 6 Article 8

1-1-2002

Clinical Evaluation of the FASTPlaqueTB for the Rapid Diagnosis of Pulmonary Tuberculosis

CENGİZ ÇAVUŞOĞLU

SEVİNÇ GÜNERİ

MUTAY SUNTUR

ALTINAY BİLGİÇ

Turk J Med Sci
32 (2002) 487-492

Cengiz ÇAVUŞOĞLU[1]
Sevinç GÜNERİ[2]
Mutay SUNTUR[3]
Altınay BİLGİÇ[1]

Clinical Evaluation of the *FASTPlaque*TB for the Rapid Diagnosis of Pulmonary Tuberculosis

Received: April 19, 2002

Departments of [1]Clinical Microbiology and [3]Infectious Diseases, Faculty of Medicine, Ege University, Bornova, İzmir.
[2]Regional Tuberculosis Laboratory, Kahramanlar, İzmir - Turkey

Abstract: The reliability of the *FASTPlaque*TB (FPTB) test (Biotec Ltd.) for the rapid diagnosis of pulmonary tuberculosis was evaluated and its diagnostic performance was compared with the Amplified Mycobacterium Tuberculosis Direct (MTD) test (Gen-Probe) by testing 80 sputum samples obtained from 77 patients.

The results were compared to those of mycobacterial culture, smear and clinical course. After a chart review 14 culture-negative patients who had been on antituberculosis therapy before the study began were excluded from the final analysis. Of the remaining 63 patients, 33 were considered to have tuberculosis; 29 of them were both smear and culture positive, for whom FPTB gave a sensitivity of 27% and a specificity of 97%. MTD exhibited a sensitivity and a specificity of 91% and 93%, respectively. In patients with a specimen storage and antituberculosis therapy period of ≤ 7 days, FPTB sensitivity increased up to 53% (8/15).

These data suggest that even under optimal conditions the sensitivity of FPTB is lower than that of MTD, smear and culture tests. It could be said, therefore, that FPTB for the direct detection of *M. tuberculosis* complex in respiratory specimens did not add an adjunct value to smears and culture. Thus, we conclude that the sensitivity of FPTB needs to be improved in order to be used for a rapid diagnosis of pulmonary tuberculosis.

Key Words: Phage amplification, transcription mediated amplification, and pulmonary tuberculosis

Introduction

Tuberculosis is the leading cause of death due to an infectious agent. It affects one third of the world's population, and 95% of the disease burden was caused by those born in developing countries (1). This situation is likely to deteriorate in the future, with annual disease rates expected to rise from 8.8 million in 1995 to 11.9 milllion per year in 2005 (2). According to the WHO Global Tuberculosis Control Report 2000, Turkey is among the countries with tuberculosis incidence rates of 25-49 per 100,000 (3), with an incidence rate of 30.3 per 100,000 (4).

Currently in Turkey, most laboratory diagnoses of *Mycobacterium tuberculosis* are performed by acid-fast staining and culture of decontaminated samples on solid or liquid culture. Staining has low sensitivity, but is considered a rapid and specific screening test of primary samples. Culture on solid media has acceptable sensitivity and specificity but is a very slow method, requiring up to 6 weeks to detect positive specimens. The use of automated culture systems has considerably quickened the diagnosis of *M. tuberculosis*, but even these techniques require an average of 2 weeks to detect positive specimens (5).

Recently, new products for the rapid diagnosis of tuberculosis have become commercially available. One of them is the Amplified *Mycobacterium tuberculosis* Direct (MTD) test (Gen-Probe, San Diego, California, USA), a system which uses the transcription mediated amplification method to amplify *M. tuberculosis* complex 16S rRNA via DNA intermediates, followed by chemiluminescence detection of the amplicon with an acridinum ester-labelled DNA probe (6). The newly developed *FASTPlaque*TB (FPTB) test (Biotec Ltd.) utilizes phage amplification technology (7) and clinical studies have been carried out.

The purpose of the present study was to eveluate the performance of the FPTB, in the diagnosis of pulmonary tuberculosis.

Materials and Methods

Study design: Clinical samples were collected from the microbiology laboratories of the Regional Tuberculosis Laboratory, İzmir, between December 2000 and December 2001. This laboratory serves around 8 million people living in western Turkey. The Regional Tuberculosis Laboratory receives an average of 100 specimens per day for mycobacterial culture which are obtained from patients of its 28 peripheral dispenseries.

Patient selection: Seventy-seven patients were investigated. Patients included in the study were separated into two groups: tuberculosis positive (Group 1), and tuberculosis negative (Group 2). For patients to be considered to have tuberculosis (Group 1), they are expected to meet one of the following criteria: (i) their cultures are positive for *M. tuberculosis*; (ii) culture-negative patients' acid-fast bacilli (AFB) smears are positive and their clinical history and chest roentgenograms sufficiently indicate tuberculosis; iii) patients are diagnosed with either (i) or (ii) and are receiving therapy for tuberculosis. Patients categorized as Group 2 were excluded from being diagnosed as having tuberculosis as (i) they were old tuberculosis patients with negative AFB smears and cultures who were clinically resolved; ii) they had nonspecific respiratory symptoms and were culture negative and AFB smears negative.

Clinical specimens, culture and microscopy: A total of 80 sputum samples obtained from 77 patients were investigated. Specimens were processed by NaOH digestion and decontaminated by the N-acetyl-L-cysteine NaOH method for culture (8). Each sample was divided into two portions. While one portion was immediately processed for culture, one half of the second portion was stored at 4 °C for FPTB for 1-28 days and the other half of the second portion was stored at –80 °C for MTD for 1-4 months. The processed sediments (0.5 ml) were inoculated into Löwenstein-Jensen (L-J) slants. Mycobacterial cultures were incubated at 37 °C for 8 weeks. L-J slants were examined weekly for positive culture. The standard biochemical tests were performed to identify *M. tuberculosis* isolates (8). Smears were stained by the Zeihl-Neelsen method.

MTD: Gene-Probe RNA amplification was carried out as described by the manufacturer. The samples were read by a Leader 450 luminometer and a cut- off value of 500,000 RLU was used for positive specimens. Samples that read between 30,000 and 500,000 RLU were retested, and specimens above 30,000 RLU were considered positive. In each run, negative and positive controls were included.

FPTB: The mycobacteriophage assay was performed as described by the manufacturer. A sample was considered positive if the number of plaques on the plate was greater than 20. When the number was less than 20, the sample was considered negative. Samples were evaluated only if 20-300 plaques were present on the control plates including organisms, and if less than 10 plaques were present on the control plates without organisms.

Statistical analysis: The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MTD and FPTB were calculated in comparison with culture results plus patients' clinical data. The differences between MTD and FPTB were calculated by paired sample chi-square test. The effects of the duration of antituberculosis treatment and storage period to prior processing on the sensitivity of FPTB were calculated by logistic regression. $p < 0.05$ indicated significance. Data were evaluated by using SPSS 8.0.

Results

Seventy-seven patients (49 males and 28 females) made up the patient population. While 33 of the 77 patients were considered to have tuberculosis (Group 1), 30 of the 77 patients were tuberculosis negative (Group 2). The remaining 14 patients were culture negative and were receiving therapy (38 days to 236 days) for tuberculosis and their former culture had been positive for *M. tuberculosis*. Therefore they were excluded. Thirty of the 33 tuberculosis patients were culture positive. The remaning three patients were culture negative but smear positive. In addition, their clinical history and chest roentgenogram were sufficiently indicative of tuberculosis. Ten of the 30 tuberculosis-negative patients (Group 2), were old tuberculosis patients who were culture negative and clinically resolved. Twenty patients were culture and smear negative and had nonspecific respiratory symptoms. For culture-positive specimens, the average turnaround time was 4 weeks. The patient groups included in the study are shown in Figure 1.

Susceptibility testing was performed for 26 of the 30 culture-positive patients. Nineteen of these 26 patients

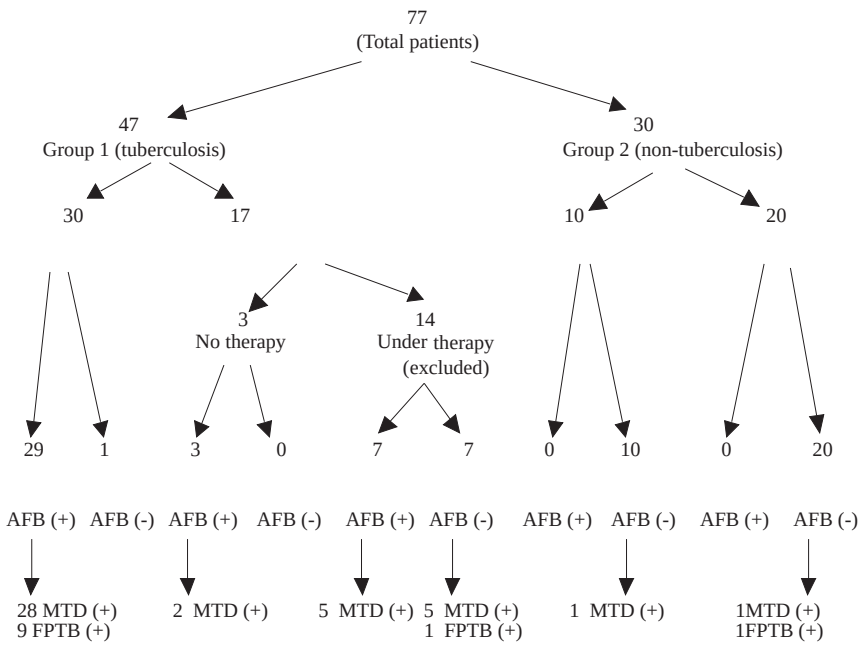

Figure 1. Results of conventional and diagnostic testing of patients including in the study

were infected with *M. tuberculosis* susceptible to all primary drugs. Two patients had strains resistant to a single drug (streptomycin one; rifampin one). Five patients had strains resistant to more than one drug (isoniazid, rifampin, streptomycin two; isoniazid, ethambutole one).

After a reevaluation of the results, 33 patients were considered to have tuberculosis, for which MTD and FPTB gave a sensitivity of 91% and 27%, and a NPV of 90% and 55%, respectively. Of the 30 culture-positive patients, 28 were positive by MTD (sensitivity 93%) and nine were positive by FPTB (sensitivity 27%). With the two false positive MTD and one false positive FPTB results, a specificity of 93% and 97%, and a PPV of 94% and 90% were calculated, respectively. Table 1 shows the comparison of FPTB with culture, smear and MTD.

Discussion

The increase in the incidence of tuberculosis has stimulated the development of rapid direct detection methods for the laboratory diagnosis of *M. tuberculosis*. The Gen-Probe MTD is a standardized commercial detection system and has already been used with a high degree of sensitivity and specificity for the diagnosis of pulmonary tuberculosis (9-12). Newly developed FPTB utilizes phage amplification technology and clinical studies have been carried out. The aims of this study were to compare the diagnostic performance of FPTB with MTD in pulmonary tuberculosis and to evaluate the effects of the factors in routine application– including storage period of the specimen prior to processing and duration of the antituberculosis treatment– on the performance of the FPTB.

Table1. Comparison of FPTB with MTD, smear and culture for detection of tuberculosis infection (n = 63).

Method	Smear-pos. culture-pos. TB group (n = 29)	Smear-neg. culture-pos. TB group (n = 1)	Smear-pos. culture-neg. TB group (n = 3)	Smear-neg. culture neg. non-TB group (n = 30)	Sen (%)	Spec (%)	NPV (%)	PPV (%)
MTD	28	-	2	2	91	93	90	94
FPTB	9	-	-	1	27	97	55	90

Fourteen culture-negative patients were receiving antituberculosis therapy. Seven of the 14 patients were smear positive, while the remaning seven were smear negative. Therapy duration varied from 38 to 157 days for smear-positive patients to 48 to 236 days for smear-negative patients. While 10 of these 14 patients were positive by MTD, FPTB gave a positive result in only one patient. MTD may be positive in culture-negative specimens during therapy. The non-cultivable organism shedding period can be extensive; the maximum time seen may be 254 days but is usually much shorter. The exact physical state of *M. tuberculosis* rRNA during the non-cultivable organism shedding period is not known. A non-cultivable organism shedding period detected by MTD when the AFB smear results are also positive suggests that intact organisms with a detectable rRNA sequence are present. They do not reproduce in culture because they are either totally nonviable or so damaged that they can not grow on laboratory media (13,14). Therefore, 14 culture-negative patients receiving therapy were excluded from the study due to uncertainty about the presence of bacilli (viable or not) and were not further evaluated.

Thirty-two samples obtained from 30 patients were culture positive. All patients positive with FPTB were also culture and AFB smear positive. Of the nine FPTB-positive samples eight were stored prior to processing ≤ 7 days and FPTB sensitivity was 50% (8/16) in specimens stored ≤ 7 days. The difference was statistically significant (R^2 = 0.50, $p < 0.05$ y = 0.116-2.9 storage period). Five culture-positive samples were obtained from four patients who had been receiving antituberculosis therapy > 7 days (15 to 58 days) and FPTB did not detect any of them. Moreover, one of these patients' (patient no. 1) former sample (patient no. 6) obtained before starting the antituberculosis therapy was positive by FPTB. In patients with antituberculosis treatment and specimen storage period ≤ 7 days, FPTB sensitivity increased to 53% (8/15) (Tables 2 and 3). These findings demonstrate that the performance of FPTB is affected by the duration of specimen storage and of the antituberculosis treatment. It is argued that these factors can decrease the viable bacilli in the specimens. In the light of our findings, for a maximum performance by FPTB, specimens must be obtained before starting antituberculosis therapy and must be studied as soon as possible. A preliminary clinical study carried out shows that under optimal conditions FPTB sensitivity in smear-positive samples is 81%, while in smear-negative samples it is 25% (7). The sensitivity rates are in disagreement with our findings. Although the number of patients we studied was not very large, our sensitivity rate in optimal conditions was still lower compared to the clinical study above. MTD gave a sensitivity of 91%, and a specificity of 93%. The difference in sensitivity for the detection of *M. tuberculosis* complex in sputum samples between MTD and FPTB was statistically significant ($x^2 = 21$ $p < 0.05$).

Microscopic examination of acid-fast staining smears is a simple, rapid and inexpensive method; however, its sensitivity is limited (5). Smear is a highly specific method for populations such as ours where the prevalance of nontuberculosis mycobacteria infections is low. In AFB smear-positive patients with typical radiological and clinical findings, antituberculosis therapy is begun after the smear results are reported and generally no further diagnostic efforts are necessary. Therefore, the rapid diagnostic test is most beneficial in patient populations where a reasonable proportion of the smear-positive specimens contain nontuberculosis mycobacteria and in smear-negative patients suspected of having tuberculosis. However, FPTB can also give false positive results with certain strains of some nontuberculosis mycobacteria. Furthermore, the turnaround time of FPTB is around 48 h, which makes it slower than the smear. In addition, in our study the sensitivity of FPTB was lower than that of the smear.

Our results suggested that FPTB for direct detection of *M. tuberculosis* complex in respiratory specimens did not add any significant adjunct value to smears and culture. In conclusion, it seems that FPTB needs to be improved in terms of its sensitivity so that it becomes an effective diagnostic tool for the rapid diagnosis of pulmonary tuberculosis.

Acknowledgements

We thank Onur Ugur, for his excellent technical assistance, and Mestan Emek, for his statistical analysis. We also want to thank Organon Teknika, İzmir, and Makro Corp., İzmir, for providing us with *FASTPlaque*TB and MTD kits for analysis of sputum specimens. Last but not least, we want to extend our gratitude to the Regional Tuberculosis Laboratory, İzmir, which kindly provided the sputum samples.

Table 2. Effects of the duration of antituberculosis treatment and storage period to prior processing on sensitivity of FPTB in culture-positive specimens.

Patient no.	Storage period	Duration of the therapy	Susceptibility testing	MTD	FPTB
4	1 day	-	S	+	+
66	1 day	-	NA	+	+
11	1 day	-	S	+	-
13	1 day	-	R (SM)	+	-
67	2 days	4 days	S	+	+
33	2 days	-	S	+	+
63	3 days	-	S	+	-
14	4 days	3 days	S	+	+
15	4 days	2 days	S	-	-
29	5 days	-	R (INH, RIF, SM)	+	-
47	5 days		R (INH, RIF, SM)	+	-
12	7 days	-	S	+	+
26	7 days	-	R (RIF)	+	+
32	7 days	-	S	+	+
7[a]	7 days	15 days	S	+	-
48[a]	7 days	58 days	S	+	-
6[b]	8 days	30 days	R (INH, RIF)	+	-
3	9 days	-	S	+	-
24	9 days	-	NA	+	-
18	13 days	-	S	+	-
19	13 days	41 days	S	+	-
20	15 days	38 days	NA	+	-
17	15 days	-	S	+	-
36[c]	17 days	-	R (INH, ETB)	-	-
9	19 days	-	S	+	-
10	19 days	-	S	+	-
8	25 days	-	S	+	-
1[b]	27 days	-	R (INH, RIF)	+	+
68	27 days	-	S	+	-
2	27 days	-	R (INH, RIF)	+	-
22	27 days	2 days	NA	+	-
31	28 days	-	S	+	-

[a,b] Patients numbered 7 and 48, and patients numbered 1 and 6 are the same patients

[c] Smear negative

S: Susceptible, R: Resistant, NA: Not applied

INH: Isoniazid, RIF: Rifampin, SM: Streptomycin, ETB: Ethambutole

Table 3. Under optimal conditions*, comparison of FPTB with smear and culture for detection of tuberculosis infection (n = 45).

Method	Smear-pos. Culture pos. TB group (n = 14)	Smear-pos. Culture-neg. TB group (n = 1)	Smear-neg. culture neg. non-TB group (n = 30)	Sen (%)	Spec (%)	NPV (%)	PPV (%)
FPTB	8	-	1	53	97	81	89

* Patients with duration of antituberculosis treatment and storage period to prior processing ≤ 7 days

Corresponding author:
Cengiz ÇAVUŞOĞLU
Faculty of Medicine, Ege University
Department of Clinical Microbiology
Bornova, 35100, İzmir - TURKEY

References

1. Narain, JP, Raviglione MC, Kochi A. HIV associated tuberculosis in devoloping countries: epidemiology and strategies for prevention. Tuber Lung Dis 73: 311-21, 1992.
2. Raviglione, MC, Snider Jr DE, Kochi A. Global epidemiology of tuberculosis. JAMA 273: 220-6, 1995.
3. WHO, WHO Global Tuberculosis Programme, Geneva. Drug resistance in the world. The WHO/IUATLD Global Project on Drug Resistance Surveillance 1994-1997. (http://www.who.org; cited 2001 Sept 27)
4. Turkish National Society of State Tuberculosis Dispensaries. Tuberculosis of epidemiology and state of our country. Turkish Ministry of Health report. Tuberculosis incidence rates in Turkey. 2000. (http://www.saglik.gov.tr; cited 2001 Sept 27): (2 screens).
5. Vuorinen P, Miettinen A, Vuento R, Halström Q. 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: 1856-9, 1995.
6. Gamboa F, Fernandez G, Padilla E et al. Comparative evaluation of initial and new versions of the Gen-Probe amplified Mycobacterium tuberculosis direct test for direct detection of Mycobacterium tuberculosis in respiratory and non-respiratory specimens. J Clin Microbiol 36: 684-9, 1998.
7. Mole R, Heydenrych A, Linley K, Albert H. Evaluation of *FASTPlaqueTB* for the rapid diagnosis of TB. In: Program and Abstract of the International Colloquium on Tuberculosis: The Real Millennium Bug, Antwerp, Belgium, 3, 1999.
8. Kent PT, Kubica GP. Public health mycobacteriology. A guide for the level III laboratory. Centers for Disease Control. U.S. Department of Health and Human Services. Atlanta, Ga., 1985.
9. Soini H, Musser JM. Molecular diagnosis of Mycobacteria. Clin Chem 47: 809-14, 2001.
10. Woods GL. Molecular techniques in mycobacterial detection. Arch Pathol Lab Med, 125: 122-6, 2001.
11. Pfyffer GE, Kissling P, Jahn EMI, 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 34: 834-41, 1996.
12. Piersimoni C, Callegaro A, Scarparo A et al. Comparative evaluation of the new Gen-Probe Mycobacterium tuberculosis amplified direct test and semiautomated Abbott LCx Mycobacterium tuberculosis assay for direct detection of Mycobacterium tuberculosis complex in respiratory and extrapulmonary specimens. J Clin Microbiol 36: 3601-4, 1998.
13. Moore DF, Curry JI, Knott CA, Jonas V. Amplification of rRNA for assessment of treatment response of pulmonary tuberculosis patients during antimicrobial therapy. J Clin Microbiol. 34: 1745-9, 1996.
14. Vlaspolder F, Singer P, Roggeven C. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. J Clin Microbiol 33: 2699-703, 1995.