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The evaluation of FAST*Plaque*TB™ test for the rapid diagnosis of tuberculosis

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Abstract

FAST*Plaque*TB™ (Biotec Laboratories Ltd., Ipswich, UK) is a rapid test which utilizes bacteriophage amplification technology for the detection of viable *Mycobacterium tuberculosis* in clinical specimens. We evaluated performance of the FAST*Plaque*TB™ test by comparing with BACTEC 460 TB culture system (Becton Dickinson Co., Maryland, USA), polymerase chain reaction (PCR) and acid fast bacilli (AFB) smear methods. We investigated 192 sputum specimens collected from the patients suspected of having pulmonary TB by AFB smear, BACTEC 460 TB culture system, PCR and FAST*Plaque*TB™ test. The sensitivity of AFB smear, PCR and FAST*Plaque*TB™ test were 57.8%, 84.4% and 87.5% respectively when we accepted BACTEC 460 TB culture system as gold standard. We conclude that FAST*Plaque*TB™ test has a good potential for rapid diagnosis of *Mycobacterium tuberculosis* as a result of the evaluation of these three tests by comparison to the BACTEC 460 TB culture system.

1. Introduction

During the last decade, tuberculosis has been one of the most important health problems not only in developing countries but also in industrialized countries. The World Health Organization (WHO) estimates that 20 million people are infected with *M. tuberculosis*, more than 8 million new cases of tuberculosis occur each year and approximately 3 million people die from this disease (Wang and Tay, 1999). In this view, the increased incidence of tuberculosis has stimulated the development of sensitive, rapid and direct detection methods for the laboratory diagnosis of *M. tuberculosis*. Rapid detection of *M. tuberculosis* strains is one of the most important factors in taking suitable measures to minimize the spread of contagion. Culture methods have been considered as gold standard for the detection of *M. tuberculosis*. BACTEC 460 TB culture system (Becton Dickinson Co., Maryland, USA) which shortens the duration of time required for the detection of mycobacteria has been recommended as a valuable system with its high sensitivity (Somoskovi and Magyar, 1999; Brunello et al., 1999; Tokars et al., 1996). Recently, nucleic acid amplification techniques such as PCR provide an alternative approach in the rapid detection of *M. tuberculosis* (Badak et al., 1999; Scarparo et al., 2000; Bergman et al., 1999; Woods, 1999).

FAST*Plaque*TB™ (Biotec Laboratories Ltd., Ipswich, UK) is a rapid test which utilizes bacteriophage amplification technology for the detection of viable *M. tuberculosis* in clinical specimens (FAST*Plaque*TB: Product Insert, 2000; Albert et al., 2002a; Albert et al., 2001; Albert et al., 2002b; Trollip et al., 2001; Eltringham et al., 1999). Bacteriophages replicate hundreds of times faster than bacteria; if amplified in a suitable bacterial host a single bacteriophage will reach detectable levels in 3-4 h. By adding target specific bacteriophage to a decontaminated sputum sample, all the target bacteria are rapidly infected (FAST*Plaque*TB: Product Insert, 2000). After phage infection, a virucidal solution is added which destroys all phage that have not infected the tubercle bacilli. The new phage are amplified by the addition of a non-pathogenic rapid-growing mycobacterial host (*M. smegmatis*), and can be visualized as plaques (FAST*Plaque*TB: Product Insert, 2000; Albert et al., 2002a; Albert et al., 2001; Albert et al., 2002b).

In our study, we evaluated the performance of FAST*Plaque*TB™ test for the detection of *M. tuberculosis* from

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sputum specimens by comparing it with BACTEC 460 TB culture system, PCR and acid fast bacilli (AFB) smear methods.

2. Materials and methods

2.1. Specimens

The patients whose sputum specimens sent to the laboratory were either outpatients or hospitalized at the Department of Chest Diseases of Gulhane Military Medical Academy, Ankara, Turkey. In this study, 192 sputum specimens collected from these patients suspected of having pulmonary TB who have not previously been treated for TB were included. Only one specimen from each patient was studied. Specimens were kept at 2-8°C and processed on the day of collection.

2.2. Decontamination of sputum specimens

All clinical specimens likely to contain bacteria other than mycobacteria were digested and decontaminated by N-acetyl-L-cysteine-NaOH method, as described by Kent and Kubica (Kent and Kubica, 1985). Each specimen was concentrated by refrigerated centrifugation for 15-20 min after adding phosphate buffer (pH:6.8). Following centrifugation, the sediments were resuspended in 2 mL of phosphate buffer. Smears were prepared from the sedimented specimens for staining and used for inoculation into Middlebrook 7H12 medium (BACTEC 12B vials) (Siddiqi, 1996). The 0.5 mL of the sediments were used for PCR, and 0.1 mL was used to inoculate Lowenstein Jensen (LJ) medium slants.

2.3. Examination for smears

Ziehl-Neelsen stained smears were examined microscopically for 5 to 10 min by an experienced microbiologist. All stained smears were stored till the study ending. All positive smears and smears from specimens giving discrepant results were reexamined by a different microbiologist. Smears were considered positive if any AFB were seen.

2.4. Cultures

Inoculated LJ slants were incubated at 37°C and growth was recorded at weekly intervals for up to 8 weeks. Specimens inoculated into the 7H12 medium were incubated at 37°C under 5 to 10% CO_2. 12B vials were tested three times per week for the first 3 weeks, and weekly for the next 3 weeks using the BACTEC 460 instrument. Any vial with a growth index (GI) ≥ 10 was accepted as growth positive. At the end of 6 weeks, vials with GI < 10 were considered negative. Differentiation of the *M. tuberculosis* complex and non tuberculous mycobacteria was achieved by selective inhibition of the *M. tuberculosis* complex in the presence of 5 μL/ml of p-nitro-α-acetyl-amino-β-hydroxypropiophenone (NAP) according to BACTEC manual (Siddiqi, 1996). Smears were stained from both positive LJ cultures and growth positive 12 B vials in order to confirm presence of AFB smear (Siddiqi, 1996).

2.5. PCR assay

The sputum specimens were lysed for PCR amplification according to the following protocol. Sediments were washed three times with an equal volume of Tris-EDTA buffer (10 mM Tris-HCL, 1 mM EDTA; pH:8.0) at 5000 $\times$ g for 5 min. The resulting pellet was resuspended in 0.25 mL of Tris-EDTA buffer and then boiled for 20 min. After centrifuged at 5000 $\times$ g for 5 min, 5μl of the supernatant was analyzed by PCR in a 50μl reaction mixture. The PCR reaction mixture contained 50 mM KCl, 10 mM Tris-HCl, pH:8.3, 1.5 mM $MgCl_2$, 200μM deoxynucleoside triphosphates (dNTP), 2.5 U Taq polymerase (Boehringer Mannheim, Germany) and 0.5μM (each) of the primers. The primer sets used to amplify the 123-bp IS 6110 gene fragment consisted of TBC_1 (CCT GCG AGC GTA GGC GTC GG) and TBC_2 (CTC GTC CAG GGC CGC TTC GG) (Eisenach et al., 1991; Bennedsen et al., 1996; Desjardin et al., 1998). The reaction mixture was subjected to 30 cycles of amplification (95°C, 30 sec; 68°C, 30 sec; 72°C, 30 sec) followed by a 5-min extension at 72°C (Thermocycler; MJ Research, Watertown, Massachusetts, USA). Fifteen μl of the amplification products were analyzed by electrophoresis in an ethidium bromide stained 2% agarose gel.

2.6. FASTPlaqueTB™ test procedure

FAST*Plaque*TB™ was performed daily, following receipt of specimens according to the manufacturer's protocol as follows. 15 mL FAST*Plaque*TB™ (FPTB) Medium Plus was added to remaining sediments of the processed specimen. All of the tubes were centrifuged at 3000 $\times$ g for 20 min. Supernatants were discarded and 1 mL FPTB Medium Plus added to each tube. After all of the specimens were incubated overnight at 37°C, viable target TB bacilli were infected with phage by adding Actiphage™ (mycobacteriophage reagent). The specimens were incubated at 37°C for 1 h and then treated with Virusol™ solution (a virucidal solution) which destroys phages remaining outside the bacilli. The specimens were incubated at room temperature for 5 min. Five milliliters of FPTB Medium Plus was added to neutralize the Virusol™ activity. As the last step, each specimen was mixed with rapid-growing non-pathogenic mycobacteria Sensor™ cells (*M. smegmatis*) and contents of the reaction tube were then added into 5 mL molten FAST*Plaque*TB™ Agar in a sterile petri dish and incubated overnight at 37°C. The resulting phage was observed as clear areas (plaques) (zones of clearing) in the lawn of Sensor™ cells. The number of plaques visualized from a

given sample was related to the number of viable tubercle bacilli in the original sample. A positive and a negative control were processed and tested for each study. Negative controls should have ≤10 plaques and positive controls should have 20 to 300 plaques for the batch of tests to be considered valid. Interpretation of the results was as follows; 0 to 19 plaques was considered as negative, ≥20 plaques was considered as positive (FAST*Plaque*TB: Product Insert, 2000; Albert et al., 2002a).

The sensitivity, specificity, positive and negative predictive value of all methods were calculated by comparison with the culture results. These parameters were determined according to the following equation: sensitivity = true positives/(true positives + false negatives), specificity = true negatives/(true negatives + false positive), positive predictive value (PPV) = true positives/(true positives + false positives), negative predictive value (NPV) = true negatives/(true negatives + false negatives) (Shultz, 1999).

3. Results

We evaluated 201 sputum specimens by using four different methods. Culture and/or FAST*Plaque*TB^{TM} results of a total of 9 specimens could not be interpreted due to contamination. These specimens were excluded from the analysis. Two specimens were contaminated on culture, five specimens were contaminated on FAST*Plaque*TB^{TM} and two specimens were contaminated on both tests. The specimens included in this study were detected positive by BACTEC 460 TB culture system and LJ culture medium. At least one sequential specimen of the same patients was also culture positive. Sixty-four sputum specimens were positive by the BACTEC 460 TB culture system. Of the specimens found positive by BACTEC 460 TB culture system, 56 (88%) were positive by FAST*Plaque*TB^{TM} test, 54 (84%) were positive PCR method and 37 (58%) were positive by AFB smear. Of 155 specimens detected as negative by microscopy, 27 (17%) were positive by BACTEC 460 TB culture system, 23 (15%) were positive by FAST*Plaque* TB^{TM} test and 19 (12%) were positive by PCR. Two specimen was positive only by PCR whereas four were positive only by FAST*Plaque*TB^{TM} test and one of the specimens was detected as positive by FAST*Plaque*TB^{TM} test and BACTEC 460 TB culture system. The photographs of positive and negative plates were shown in Figure 1 and 2. The sensitivity, specificity, positive predictive (PPV) and negative predictive values (NPV) of the methods calculated by considering BACTEC 460 TB culture system as gold standard were shown in Table 1. We found PPV and NPV of FAST*Plaque*TB^{TM} as % 67.5 and % 99.1 respectively according to the prevalence of tuberculosis (%6.84) of our patient population in our hospital.

Considering the results of 192 sputum specimens, the correlation of BACTEC 460 TB culture system with AFB smear method was 85.9%, 93.7% with PCR and 93.7% with FAST*Plaque*TB^{TM} test.

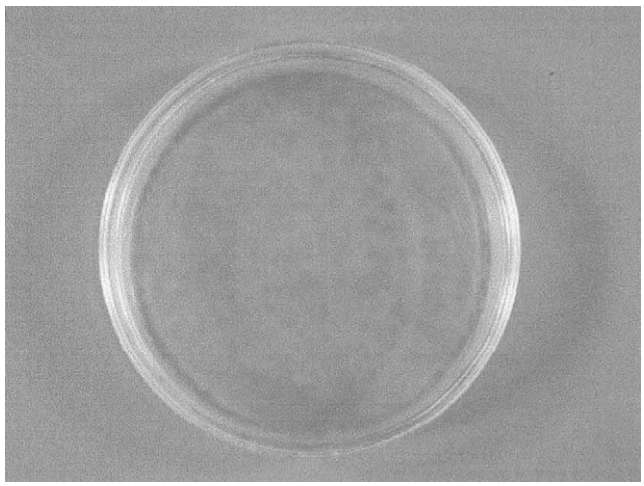

Fig. 1. Photograph of positive plate from FAST*Plaque* TB^{TM} test.

4. Discussion

Recently, paralleling the increase in the incidence of tuberculosis new and more rapid techniques for the diagnosis of tuberculosis are being developed to overcome the shortcomings of conventional methods. Currently, culture methods have been considered as gold standard for the detection of *M. tuberculosis.* BACTEC 460 TB culture system has been recommended as a valuable system with its high sensitivity and shortened duration of time required for the detection of mycobacteria 10 to 14 days. Thus, BACTEC 460 TB culture system has been considered as gold standard even in classic microbiology texts (Somoskovi and Magyar, 1999; Tokars et al., 1996). Nucleic acid amplification methods such as PCR provide an alternative approach in the detection of microorganisms and thus offer

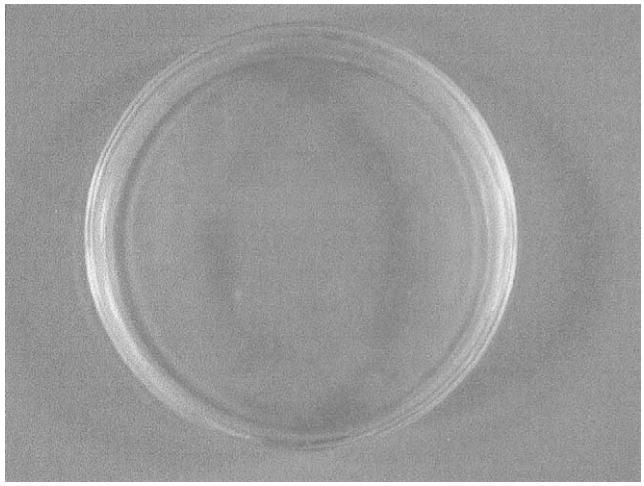

Fig. 2. Photograph of negative plate from FAST*Plaque* TB^{TM} test.

Table 1
The sensitivity, specificity, positive predictive and negative predictive values of the methods (n = 192)

Method	True positive (n)	True negative (n)	False positive (n)	False negative (n)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
AFB smear	37	128	–	27	57.8	100.0	100.0	82.6
PCR	54	126	2	10	84.4	98.4	96.4	92.6
Fast*Plaque*TB™	56	124	4	8	87.5	96.9	93.3	93.9

new possibilities for a more rapid and accurate diagnosis of tuberculosis (Brisson-Noel et al., 1989).

The number of false negative results were 10 with PCR method and 8 with FAST*Plaque*TB™ test whereas we detected 2 false positive results with PCR and 4 with FAST*Plaque*TB™ test. False positive results in PCR might have been due to an accidental contamination of the specimens during or before the PCR assay. False negative results obtained by PCR for culture-positive specimens may be explained by the presence of inhibitors of enzymatic amplification or by small number of *M. tuberculosis* strains unequally distributed in the test suspension (Wang and Tay, 1999; Gamboa et al., 1998).

The results of FAST*Plaque*TB™ test were evaluated by counting the plaques formed in negative and positive control plates performed at the same time. Since the evaluation of the results was performed by counting the plaques, the cut-off value for plaque count causes difficulties during the interpretation. The number of plaques detected (20-25) was close to the cut-off value of test procedure (≥20 plaques = positive) in the specimens false positive by FAST*Plaque* TB™ test. Besides, false positive results may be due to insufficient addition or mixing procedure of virucidal solution resulting in failing to destroy the bacteriophages outside of the target cell and also it may be due to the contamination of the specimens by Sensor™ cells. However, when false positive results detected, these may be due to incomplete destruction of exogenous phage by the virucidal solution possibly because of the protective effect of sputum components on the phage (Albert et al., 2002a). FAST*Plaque*TB™ test requires viable bacilli (FAST*Plaque*TB: Product Insert, 2000). So, there may also be false negative results due to long time interval between specimen collection and the beginning of the test or low number of live cells in the specimens. Besides, FAST*Plaque*TB™ test needs intact phage receptors on the viable cell surface for the phage attachment and replication. Phage inhibitory substances may be present in the specimens in different concentrations. If they are in high concentrations they may inhibit the phage-TB interaction (Albert et al., 2002a).

AFB smear sensitivity has been reported to vary from 30% to more than 70% (Albert et al., 2002a). In our study, it was found as 57.8% and was within the range of the sensitivity reported. Of the 155 AFB smear negative specimens, 17%, 15%, 12% were positive by BACTEC 460 TB culture system, FAST*Plaque*TB™ test and PCR respectively. The sensitivity of the FAST*Plaque*TB™ test was found as 87.5%. AFB smear can detect $>10^4$/ml bacteria in a specimen whereas FAST*Plaque*TB™ test is able to detect 100-300 bacilli/ml. So, the sensitivity of FAST*Plaque*TB™ test is higher than AFB smear method. The sensitivity and specificity of FAST*Plaque*TB™ test were in agreement with PCR. The correlation between FAST*Plaque*TB™ test and BACTEC 460 TB culture system was 93.7% and the correlation between the BACTEC 460 TB culture system and PCR was found as 93.7%. When we take into consideration the prevalence of tuberculosis (%6.84) of our patient population in our hospital, we found PPV and NPV % 67.5, % 99.1 respectively. These data suggest that FAST*Plaque*-TB™ test can be used as a diagnostic test for *M. tuberculosis.* The test may be used in conjunction with sputum smear microscopy to detect additional cases that would be missed by smear alone.

FAST*Plaque* TB™ relies on basic microbiologic techniques. Specialized equipment is not needed to perform the test and to evaluate the results. It is easy to perform in any laboratory, and actually helps the laboratories that use conventional manual culturing methods. In addition, FAST-*Plaque* TB™ is used for determination of rifampicin resistance within 48 h in *M. tuberculosis* complex isolates (Albert et al., 2001; Albert et al., 2002b). The cost of FAST*Plaque*TB™ test was 4$ while 27$ for BACTEC 460 TB culture system per diagnosis approximately.

Rapid diagnosis, high sensitivity and no special instrument requirement are the advantages of FAST*Plaque*TB™ test. However risk of contamination during the test and the problems with the counting the plaques are disadvantages of this test.

In conclusion, FAST*Plaque*TB™ test has a good potential for rapid diagnosis of *M. tuberculosis* as a result of the evaluation of these three test results. But, this new technique should be evaluated with sufficient number of sputum specimens and the sensitivity of the method should be tested in AFB smear negative specimens other than sputum.

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