INT J TUBERC LUNG DIS 11(8):904–908

Rapid detection of *Mycobacterium tuberculosis* from sputum specimens using the FAST*Plaque*TB test

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SUMMARY

OBJECTIVES: To determine the performance of the FAST*Plaque*TB test, based on bacteriophage amplification technology, by comparison with the BACTEC 460 TB culture system, the Löwenstein-Jensen (LJ) medium culture method and Ziehl-Neelsen (ZN) staining.

METHODS: Of 400 sputum specimens studied in our laboratory, 19 were excluded due to contaminant growth. The FAST*Plaque*TB test was performed according to the manufacturer's instructions.

RESULTS: Only 42 of the 381 specimens examined were positive on at least one test: 30 were positive with ZN staining, 34 with LJ medium, 36 with the FAST*Plaque*TB test and 39 with BACTEC 460 TB. The combination of BACTEC 460 TB and LJ medium culture was considered the gold standard. The sensitivity and specificity were 70.7% and 99.7% for ZN staining, 87.8% and 100% for the FAST*Plaque*TB test, 82.9% and 100% for LJ, and 95.1% and 100% for BACTEC 460 TB.

CONCLUSIONS: The FAST*Plaque*TB test is useful in the rapid diagnosis of TB.

KEY WORDS: FAST*Plaque*TB test; *Mycobacterium tuberculosis*; sputum

AS THE LEADING CAUSE of death due to a single infectious agent, not only has tuberculosis (TB) become more prevalent in recent years, but multidrug-resistant strains and mycobacteria other than tuberculosis (MOTT) have increased in variety. This has led to problems with treatment, which in turn have boosted research studies seeking early diagnosis of the disease.[1] The increasing incidence of TB and other mycobacterial diseases has made it essential for laboratories to quickly detect and identify mycobacteria from human clinical material. Ziehl-Neelsen (ZN) staining and culture of microorganisms are traditional methods in the laboratory diagnosis of mycobacterial infections. The FAST*Plaque*TB test, a rapid test (48 h) based on bacteriophage amplification technology to reflect the presence of viable *Mycobacterium tuberculosis*, is used in the diagnosis of TB in sputum specimens.[2,3]

The aim of our study was to determine the performance of the FAST*Plaque*TB test in comparison with the BACTEC 460 TB culture system, culture on Löwenstein-Jensen (LJ) medium and ZN staining.

MATERIALS AND METHODS

Patients

A total of 400 sputum specimens obtained from suspected TB cases were studied in the Microbiology Laboratory at the Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, Turkey. Of these, 19 were excluded due to contaminant growth. One sputum sample was included per patient.

Decontamination

Sputum specimens were decontaminated using the standard N-acetyl-L-cysteine-NaOH method and centrifuged. The sediment obtained was used to perform the FAST*Plaque*TB test. The decontaminated, concentrated sediment was resuspended in 2 ml sterile 0.67 M phosphate buffer (pH 6.8) and used for phage assay and cultures by the BACTEC 460 TB and LJ methods. Smears were made of all of the sputum specimens included in the study and stained with ZN for detection of acid-fast bacilli (AFB).[4]

*FAST*Plaque*TB assay*

The principle of the FAST*Plaque*TB assay is shown on the Biotec website (Biotec Laboratories, Ipswich, UK; www.biotec.com). The assay was carried out using the FAST*Plaque*TB kit. All sputum specimens were processed according to the instructions given by the manufacturer. Both positive and negative controls were included in the assay and tested according to the manufacturer's instructions. Negative controls contained 1 ml of FAST*Plaque*TB broth, and three positive controls were prepared by serial dilutions (10^{-2}, 10^{-4}, 10^{-6}) of *M. smegmatis*. These controls were included

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Article submitted 6 February 2007. Final version accepted 4 May 2007.

to assess the integrity of the phage and the effectiveness of the phagicidal agent. For the assay, 1 ml of decontaminated and concentrated sediment was mixed with 1 ml of FAST*Plaque*TB broth and incubated at 37°C overnight to enrich viable TB bacilli present in the sample. After enrichment, 100 μl of the mycobacteriophage solution was added and incubated for a further 1 h to allow infection to take place; 100 μl of virucide solution was then added to destroy all bacteriophages that had not infected host cells, and incubated at room temperature for 15 min. Then 5 ml FAST*Plaque*TB medium was added to neutralise excess virucide, followed by 1 ml of helper cells. After thorough mixing, it was added to the Petri dish and overlayered with 5 ml molten agar. The plates were rotated several times, clockwise and counterclockwise, after pouring. The plates were then allowed to set and were incubated at 37°C.

The number of plaques was counted after overnight incubation. As recommended by the manufacturer, a cut-off of 20 plaques was used to interpret the results.[5] A negative result meant that the specimen did not contain live TB bacilli, while a positive result meant that live TB bacilli were present in the specimen. Examples of negative and positive results obtained in our study are shown in the Figure.

LJ culture

For LJ culture, 0.5 ml of processed specimen was inoculated onto two slants of LJ egg medium and incubated at 37°C. The slants were inspected every day during the first week, then once weekly for 10 weeks. All positive cultures were confirmed by ZN microscopy. Further identification was done using standard biochemical tests.[4]

BACTEC 460 TB culture system

For the BACTEC method (Becton Dickinson, Cockeysville, MD, USA), 0.5 ml of processed specimen was inoculated into the BACTEC 12B vial supplemented with PANTA (a mixture of five different antibiotics: polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) and incubated at 37°C. Readings were taken twice a week for the first 2 weeks and once a week thereafter for culture positivity for 6 weeks. AFB smears were made from vials with a growth index (GI) of ⩾50–100, and further identification of mycobacteria grown in BACTEC cultures was done using the para-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) test.[4]

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for the phage assay were calculated by comparing them with those for AFB smear microscopy, the BACTEC and LJ methods and with clinical evidence of disease.

RESULTS

The distribution of the 42 positive samples using the applied methods is shown in Table 1. Positive results were obtained by all of the methods in 28 of the 42 (66.6%) positive samples. In 14 of the 42 (33.3%) positive samples, we obtained distinct positive results with four different methods. Table 2 shows the positive results using the different methods.

According to the reports of various studies, the gold standard for the diagnosis of mycobacteria is determined by inoculating the samples into one solid medium and one broth medium.[5,6] In our study, we used the combination of Bactec 12B and LJ as the gold standard and evaluated and compared the sensitivity, specificity, PPV and NPV of other methods.

The results of the FAST*Plaque*TB test were compared with the results of the culture method (BACTEC 12B + LJ) in 381 sputum samples. In 36 of 41 sputum samples that were positive on culture (BACTEC 12B

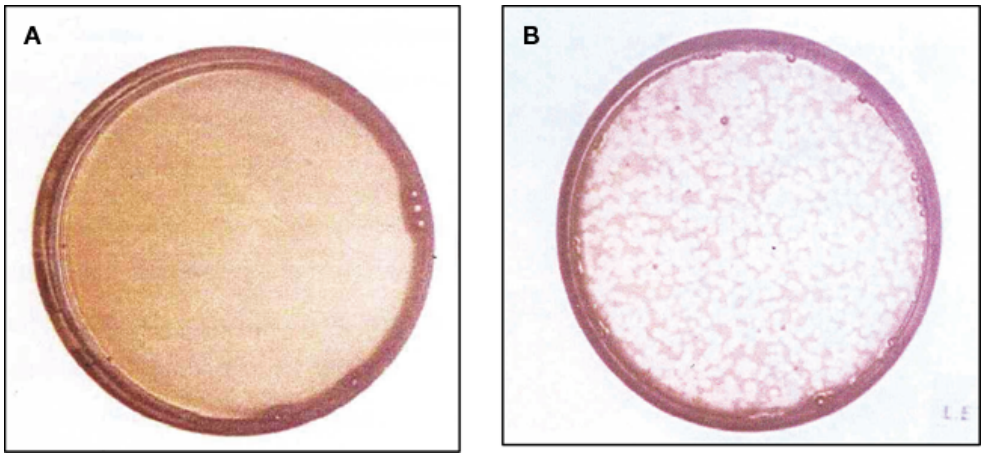

Figure Negative **A**) and positive **B**) results using the FAST*Plaque*TB test. This image can be viewed online in colour at http://www.ingentaconnect.com/content/iuatld/ijtld.

Table 1 Sensitivity, specificity, PPV and NPV of the different methods used in tuberculosis diagnosis (n = 381)

Method	Total positive *n*	True-positive* *n*	True-negative *n*	False-positive *n*	False-negative *n*	Sensitivity %	Specificity %	PPV %	NPV %
AFB smear	30	29	339	1	12	70.7	99.7	96.7	96.6
Bactec 12B	39	39	340	0	2	95.1	100	100	99.4
LJ	34	34	340	0	7	82.9	100	100	98.0
FAST*Plaque* TB	36	36	340	0	5	87.8	100	100	98.6

* True positive standard number is 41 (41/42 positive samples were positive on culture and one was positive only on AFB smear).
PPV = positive predictive value; NPV = negative predictive value; AFB = acid-fast bacilli; LJ = Löwenstein-Jensen.

and/or LJ), the FAST*Plaque*TB test was positive, while in 5 of the 41 positive samples the FAST*Plaque*TB test failed. (Forty-two sputum samples were positive by at least one test; 41/42 positive samples were positive on culture [Bactec 12B and/or LJ] and 1/42 positive samples was positive only on AFB smear.) None of the culture-negative samples were positive with the FAST*Plaque*TB test. Three hundred and forty sputum samples were negative for both culture methods and the FAST*Plaque*TB test. The sensitivity and specificity of the FAST*Plaque*TB test for all samples were determined at 87.8% and 100%, respectively. Table 1 shows the sensitivity, specificity, PPV and NPV of the other methods with respect to the culture method (BACTEC 12B + LJ) used as the gold standard.

DISCUSSION

The most frequently used diagnostic methods for TB are culture and smear microscopy with acid-fast staining. Although smear microscopy is an easy, rapid and cheap diagnostic method for mycobacteria, its sensitivity is low.[7] For a definite diagnosis of TB, isolation of bacilli is needed. For culture, however, which is defined as the gold standard in the bacteriological diagnosis of *M. tuberculosis*, a period of 4–6 weeks is required.[7] Interest has recently increased in new techniques that provide rapid diagnosis of *M. tuberculosis*. The FAST*Plaque*TB test, developed for rapid diagnosis of live tubercle bacilli, is a method that is based on bacteriophage amplification technology. This method is simple, and the results are obtained in 48 h and interpreted easily.[1–3]

In a study conducted in Cape Town, South Africa, Albert et al. compared the FAST*Plaque*TB test and culture method (LJ) in 1618 sputum samples.[8] They found the sensitivity, specificity, PPV and NPV of the FAST*Plaque*TB test to be 72.5%, 99%, 91% and 96%, respectively. Muzaffar et al. compared the FAST*Plaque*TB test and culture method (LJ) in 514 sputum samples and reported a sensitivity, specificity, PPV and NPV for the FAST*Plaque*TB test of 81.6%, 97.7%, 97% and 85%, respectively.[3] Albay et al., in a study of 192 sputum samples, compared the FAST*Plaque*TB test with the BACTEC 460 TB culture system.[9] They found the sensitivity, specificity, PPV and NPV of the test to be 87.5%, 96.9%, 93.3% and 93.3%, respectively. Cavusoglu et al., however, reported a lower sensitivity (27%).[10]

Table 2 Analysis of positive results with four different methods used in tuberculosis diagnosis (n = 14)

Methods applied	*n* (%)
Bactec 12B+FAST*Plaque*TB	4 (28.58)
Bactec 12B+FAST*Plaque*TB + LJ	4 (28.58)
Bactec 12B	3 (21.42)
AFB smear + Bactec 12B + LJ	1 (7.14)
LJ	1 (7.14)
AFB smear	1 (7.14)
Total	14 (100)

AFB = acid-fast bacilli; LJ = Löwenstein-Jensen.

In our study, we compared the FAST*Plaque*TB test with culture (BACTEC 12B + LJ) and found the sensitivity, specificity, PPV and NPV of the method to be 87.8%, 100%, 100% and 98.5%, respectively. The results of our study show that the FAST*Plaque*TB test can be a beneficial detector of TB with high specificity, as reported by other authors.[3,8,9]

A total of 351 (92.1%) samples tested in our study were negative for AFB smear. When comparing the results of the FAST*Plaque*TB test and culture (BACTEC 12B + LJ) in AFB-negative samples, the FAST*Plaque*TB test plays an important role in early diagnosis, with high sensitivity (66.6%) and specificity (100%). In addition, the results are obtained rapidly, within 48 h, compared to 2–8 weeks for culture. Our results were comparable to those obtained from studies conducted in other high TB prevalence areas. Using the FAST*Plaque*TB test in AFB smear-negative sputum samples, Muzaffar et al. found the sensitivity and specificity to be 67% and 98%, respectively,[3] while Albert et al. reported a sensitivity and specificity of respectively 48% and 98%.[8]

When comparing the results of the FAST*Plaque*TB test and culture methods, we obtained false-negative results (FAST*Plaque*TB-negative, culture-positive) in one AFB smear-positive sample (n = 30) and in four AFB smear-negative samples (n = 351). The false-negative ratio was 12.2%. In similar studies by Muzaffar et al.,[3] Albert et al.[8] and Albay et al.,[9] false-negative ratios of respectively 8.7%, 3.5% and 4.1% were reported.

We did not observe any false-positive (FAST*Plaque*TB-positive, culture-negative) results in our

study. This indicates that the assay has a very high specificity for the *M. tuberculosis* strains present in our population. In similar studies by Albert et al.,[8] Muzaffar et al.[3] and Albay et al.,[9] false-positive ratios of respectively 0.8%, 2.9% and 2% were reported. Investigators have explained the false-positive results in the FAST*Plaque*TB test by the addition of low virucidal solution to samples or inadequate mixing and insufficient inactivation of the exogenous phage and by contamination of the sample with Sensor cell.[8,9]

We found a sensitivity of the FAST*Plaque*TB test of 87.8% and that of AFB smear microscopy of 70.7% in our study. AFB smear is positive if $>10^4$/ml bacilli are present in the sample, while the FAST*Plaque*TB test is positive if 100–300 bacilli/ml are present.[9] The FAST*Plaque*TB test therefore has a higher sensitivity than AFB microscopy.

We found a high specificity (99.7%) of AFB smear microscopy for *M. tuberculosis*. A false-positive AFB smear (FAST*Plaque*TB-negative, culture-negative) was detected in only one (0.2%) sample. This may be explained by microscopy artefacts due to non-specific staining of other microorganisms. The unexplained false-positive rate for smear microscopy varies between 10% and 20%.[1,3] Muzaffar et al.[3] and Albert et al.[8] reported false-positive AFB smear microscopy results of 2.9% and 2.1%, respectively, while Albay et al. reported no false-positive smear results.[9]

Our data showed that when combining the AFB smear and FAST*Plaque*TB results for the diagnosis of TB, we obtained rapid identification in 90.2% of the samples evaluated by culture (BACTEC 12B + LJ), with high specificity (99.7%). On combining AFB smear and FAST*Plaque*TB results, Muzaffar et al. reported that identification could be performed rapidly, with a specificity of 92.1%, in 90.6% of samples evaluated by culture (LJ).[3] Our results are comparable to those of other studies.

In our study, 34 (80.9%) of the 42 positive samples were positive on LJ, and 36 (85.7%) of 42 positive samples were positive using the FAST*Plaque*TB test. The much larger volume (1 ml) of specimen used in the FAST*Plaque*TB test as compared to the LJ culture inocula (0.5 ml) is likely to improve the sensitivity of the assay when testing pancibacillary specimens. These data demonstrate that the FAST*Plaque*TB method is better than LJ culture in detecting bacilli.

The concordance of the FAST*Plaque*TB test with the gold standard culture method (BACTEC 12B + LJ) was 98.6% in the present study. In a study of 192 sputum samples, Albay et al. reported a concordance of the FAST*Plaque*TB test with their gold standard method, the Bactec 460 TB culture method, of 93.7%.[9]

In conclusion, the sensitivity and specificity of the FAST*Plaque* TB test were higher than those for AFB smear microscopy in the rapid diagnosis of *M. tuberculosis*. Important advantages of this test are that it is rapid and inexpensive. Using this test in sputum samples in developing countries where TB incidence is higher is therefore logical. However, further studies are needed to investigate the effectiveness of the FAST*Plaque*TB test in samples other than sputum and in AFB smear-negative samples.

Acknowledgement

This study was supported by Eskisehir Osmangazi University Scientific Investigation Project Commission (Project No: 2002/11045).

References

1 Shenai S, Rodrigues C, Mehta A P. Evaluation of a new phage amplification technology for rapid diagnosis of tuberculosis. Indian J Med Microbiol 2002; 20: 194–199.
2 Eltringham L J, Wilson S M, Drobniewski F A. Evaluation of bacteriophage-based assay (phage amplified biological assay) as a rapid screen for resistance to isoniazid, ethambutol, streptomycin, pyrazinamide, and ciprofloxacin among clinical isolates of *Mycobacterium tuberculosis*. J Clin Microbiol 1999; 37: 3528–3532.
3 Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of the FAST*Plaque*TB assay for direct detection of *Mycobacterium tuberculosis* in sputum specimens. Int J Tuberc Lung Dis 2002; 6: 635–640.
4 Della-Latta P. Mycobacteriology and antimycobacterial susceptibility testing. In: Isenberg H D, ed. Clinical microbiology procedures handbook, 2nd ed, Vol 2. Washington, DC, USA: American Society for Microbiology, 2004: pp 7111–7883.
5 Oh E J, Park Y J, Chang C L, Kim B K, Kim S M. 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.
6 Palacios J J, Ferro J, Ruiz Palma N, et al. Fully automated liquid culture system compared with Lowenstein-Jensen solid medium for rapid recovery of mycobacteria from clinical samples. Eur J Clin Microbiol Infect Dis 1999; 18: 265–273.
7 Metchock B G, Nolte F S, Wallace R J. *Mycobacterium*. In: Murray P R, Baron E J, Pfaller M A, Tenover F C, Yolken R H, eds. Manual of clinical microbiology. 7th ed. Washington, DC, USA: ASM Press, 1999: pp 399–437.
8 Albert H, Heydenrych A, Brookes R, et al. Performance of a rapid phage-based test, FAST*Plaque*TB™, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. Int J Tuberc Lung Dis 2002; 6: 529–537.
9 Albay A, Kısa O, Baylan O, Dogancı L. The evaluation of FAST*Plaque*TB test for the rapid diagnosis of tuberculosis. Diagn Microbiol Infect Dis 2003; 46: 211–215.
10 Cavusoglu C, Guneri S, Suntur M, Bilgic A. Clinical evaluation of FAST*Plaque*TB for the rapid diagnosis of pulmonary tuberculosis. Turk J Med Sci 2002; 32: 487–492.

RÉSUMÉ

OBJECTIFS : Déterminer la performance du test FAST-*Plaque*TB basé sur une technologie d'amplification des bactériophages en comparaison avec le système de culture BACTEC 460 TB, la méthode de culture sur milieu de Löwenstein-Jensen (LJ) et la coloration de Ziehl-Neelsen (ZN).

MÉTHODES : Au total, 400 échantillons de crachats ont été étudiés dans notre laboratoire. On a exclu 19 échantillons en raison du développement de contaminants. Le test FAST*Plaque*TB a été exécuté selon les indications du fabricant.

RÉSULTATS : Des 381 échantillons examinés, seulement 42 ont été positifs à au moins un test. Trente des 42 ont été positifs à la coloration de ZN, 34 sur milieu de culture de LJ, 36 au test FAST*Plaque*TB et 39 après BACTEC 460 TB. La combinaison de BACTEC 460 TB et du milieu LJ a été considérée comme le *gold standard*. La coloration de ZN a une sensibilité de 70,7% et une spécificité de 99,7% ; le test FAST*Plaque*TB respectivement de 87,8% et de 100% ; le milieu de LJ respectivement de 82,9% et 100% et le BACTEC 460 TB respectivement de 95,1% et 100%.

CONCLUSION : Nous concluons que parmi les autres tests appliqués, le test FAST*Plaque*TB est valable pour un diagnostic rapide de la TB.

RESUMEN

OBJETIVOS : Determinar el rendimiento diagnóstico de la prueba FAST*Plaque*TB™, basada en una técnica de amplificación por bacteriófagos y compararlo con el rendimiento del sistema de cultivo BACTEC 460 TB, el método de cultivo en medio Löwenstein-Jensen (LJ) y con la coloración de Ziehl-Neelsen (ZN).

MÉTODOS : Se estudiaron en el laboratorio 400 muestras de esputo. Se excluyeron 19 muestras por contaminación del cultivo. La prueba FAST*Plaque*TB se realizó en conformidad con las instrucciones del fabricante.

RESULTADOS : Solo 42 de las 381 muestras examinadas fueron positivas por lo menos en una de las pruebas. De las 42 muestras, 30 fueron positivas con la coloración de ZN, 34 en cultivos con medio LJ, 36 con la prueba FAST-*Plaque*TB y 39 fueron positivas con el método BACTEC 460 TB. Considerando como método de referencia la combinación BACTEC 460 TB y cultivo en medio LJ, la sensibilidad y la especificidad observadas fueron de 70,7% y 99,7% con la coloración de ZN ; de 87,8% y 100% con la prueba FAST*Plaque*TB ; de 82,9% y 100% con el cultivo en LJ ; y de 95,1% y 100% con el método BACTEC 460 TB.

CONCLUSIÓN : Entre los diferentes métodos diagnósticos, la prueba FAST*Plaque*TB constituye un valioso sistema rápido de diagnóstico de la TB.