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Evaluation of FAST*Plaque*TB-RIF™ for determination of rifampicin resistance in *Mycobacterium tuberculosis* complex isolates

O. Kisa, A. Albay, O. Bedir, O. Baylan, L. Doganci

Department of Microbiology and Clinical Microbiology, Gulhane Military Medical Academy, Ankara, Turkey

SUMMARY

SETTING: The Microbiology and Clinical Microbiology Department, Gulhane Military Medical Academy, Ankara, Turkey, a tertiary referral hospital in a region endemic for tuberculosis.

OBJECTIVE: To evaluate rifampicin resistance of *Mycobacterium tuberculosis* complex strains using FAST*Plaque*TB-RIF™, a rapid and novel bacteriophage-based susceptibility technique.

DESIGN: Results of isolates tested with the BACTEC 460 TB system were compared with FAST*Plaque*TB-RIF™.

RESULTS: Susceptibility to rifampicin of *M. tuberculosis* complex isolates was tested for 88 isolates using FAST*Plaque*TB-RIF™. Sixty-seven isolates were susceptible and 21 were resistant to rifampicin using the BACTEC 460 TB system. Overall accuracy for FAST*Plaque*TB-RIF™ was 94.3% (95%CI 87.3–97.5) for the detection of rifampicin susceptibility. The sensitivity and specificity of FAST*Plaque*TB-RIF™ were respectively 100.0% (95%CI 84.5–100) and 92.5% (95%CI 83.6–96.7).

CONCLUSION: This study demonstrates that FAST*Plaque*TB-RIF™ is a rapid and inexpensive test which has a good correlation with the BACTEC 460 TB system.

KEY WORDS: tuberculosis; rifampicin resistance; mycobacteriophage; susceptibility test

TUBERCULOSIS (TB) has re-emerged in almost all high- and low-income countries, and it has been estimated that one-third of the world's population is infected by *Mycobacterium tuberculosis*, the causative organism, leading to 3 million deaths annually.[1] The global spread of the disease is further complicated by the ubiquitous appearance of drug-resistant and especially multiple drug-resistant (MDR) strains.[2] Rapid and accurate techniques for the detection of *M. tuberculosis* strains resistant to anti-tuberculosis drugs are probably one of the most important factors in taking suitable measures to minimise the spread of infection.[3] Resistance to rifampicin is of particular importance, as it is a marker for MDR-TB strains, defined as resistance to isoniazid and rifampicin with or without resistance to other drugs.[4–7] Determination of rifampicin resistance identifies those resistant tuberculosis cases who do not respond to standard treatment regimens.[5]

Current methods of susceptibility testing of *M. tuberculosis* include the absolute concentration method, the resistance ratio method, the agar proportion method and the radiometric method.[8] Several well-automated liquid culture-based systems have been introduced, such as MB Bac/T (Organon Teknika, Puteaux, France), BACTEC 460 TB, BACTEC MGIT 960 TB system and BACTEC 9000 MB (Becton Dickinson, Sparks, MD, USA). The average detection time for antimycobacterial susceptibility tests was 5–11 days with the MB Bac/T system, 3–15 days with the BACTEC 460 TB system, and 3–14 days with the BACTEC MGIT 960 TB system.[9,10] Therefore, fast and valuable methods for the detection of *M. tuberculosis* and drug susceptibility are urgently needed to instigate appropriate therapeutic measures.[11]

FAST*Plaque*TB-RIF™ was recently developed by Biotec Laboratories Ltd (Ipswich, UK) for the rapid (within 48 hours) detection of rifampicin resistance in *M. tuberculosis* cultures.[5,6] This method utilises mycobacteriophage technology for the detection of rifampicin resistance, and can detect low numbers of organisms.[5–7,12,13] Viable target TB bacilli are infected with phage. The sample is then treated with a virucide, which destroys any phage remaining outside the bacilli. The new phage are amplified by the addition of a nonpathogenic *M. smegmatis* to support phage replication. The sample is incorporated into an agar mixture and incubated overnight. Plaques in the cell lawn

Correspondence to: Ozgul Kisa, Department of Microbiology and Clinical Microbiology, Gulhane Military Medical Academy, Ankara, 06018 Turkey. Tel: (+90) 312 304 34 15. Fax: (+90) 312 304 34 02. e-mail: okisa@gata.edu.tr

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indicate the presence of viable TB bacilli in the original sample.[5,6]

The FAST*Plaque*TB-RIF™ test can be performed in any laboratory that has access to basic microbiological equipment, as it requires no sophisticated equipment. This and its low cost are the main advantages of the FAST*Plaque*TB-RIF™ test over the semi-automated systems.[5,6]

The aim of the present study was to evaluate the performance of the FAST*Plaque*TB-RIF™ test (Biotec Laboratories Ltd, UK) on clinical isolates of *M. tuberculosis* complex by comparing it with the BACTEC 460 TB system (Becton Dickinson, USA).

MATERIALS AND METHODS

M. tuberculosis *strains*

Eighty-eight *M. tuberculosis* strains isolated from various clinical samples in the Mycobacteriology Laboratory of the Department of Microbiology and Clinical Microbiology, Gulhane Military Medical Academy, Ankara, Turkey, were included in the study. Primary isolation of *M. tuberculosis* from clinical samples was performed using the N-acetyl L-cysteine sodium hydroxide method and the BACTEC 460 TB system.[14] Isolates grown in BACTEC 12B vials were also subcultured on Löwenstein-Jensen (LJ) medium at 37°C. Fresh cultures on LJ medium were used as a source of the organisms.

BACTEC 460 TB system

Rifampicin resistance was tested with the BACTEC 460 TB system. The final concentration of rifampicin was 2.0 μl/ml.[15]

*FAST*Plaque*TB-RIF*™

Rifampicin resistance testing was performed according to the manufacturer's instructions. Briefly, the test suspension was prepared from LJ medium. Approximately 1 μl *M. tuberculosis* culture was added to 5 ml FPTB Medium Plus in a sterile tube containing 6–8 glass beads. The suspension was homogenised using a vortex mixer for 15–20 s and 0.5 ml of supernatant was added to each of two separate plastic tubes containing a solution without RIF (drug-free control tube, RIF−) and with RIF (RIF+). The final concentration of rifampicin was unlikely to have been 5 μg/ml. Test suspensions were incubated overnight at 37°C. All of the specimens were then infected with phage by adding actiphage (mycobacteriophage), and incubated at 37°C for 90 min. Then the specimens were treated with a virucide which destroys extracellular phages. Each specimen was mixed with 5 ml FPTB Medium Plus and 1 ml sensor cells (non-pathogenic *M. smegmatis*). These contents were incorporated into 5 ml FAST*Plaque*TB™ Agar (Middlebrook 7H9-based agar, kept at 50–60°C in a water bath) in a sterile Petri dish and incubated overnight at 37°C. A positive and a negative control were prepared and tested for each study.[5,6,16]

Results were judged to be valid if 100 or more plaques were observed on the RIF– plates. The positive control had 20–300 plaques and the negative control had 10 plaques or less.[5,6,16] The interpretation of the results was as follows: if 10 plaques or less were counted on the RIF+ plate, the strain was considered to be susceptible to rifampicin; if 11–49 plaques were counted on the RIF+ plate, the strain was considered to be have intermediate susceptibility to rifampicin; if 50 plaques or more were counted on the RIF+ plate, the strain was considered to be resistant to rifampicin. Isolates giving discrepant results between FAST-*Plaque*TB-RIF™ and BACTEC 460 TB system were repeat tested.

The sensitivity, specificity and confidence intervals of the FAST*Plaque*TB-RIF™ test were calculated using the BACTEC 460 TB system as the gold standard. The ability to detect true resistance is accepted as sensitivity, and the ability to detect true susceptibility is accepted as specificity. Overall accuracy is the number of correct results over the total number of results.[6,17,18]

RESULTS

Although it depends on the experience of the personnel performing the test, approximately 5 hours were required to set up the FAST*Plaque*TB-RIF™. With the incubation periods the total test time was 48 hours. Testing the susceptibility of 88 isolates to rifampicin with the BACTEC 460 TB system took 7 days: 67 were susceptible and 21 were resistant to rifampicin. Of the 67 rifampicin-susceptible isolates, FAST*Plaque*TB-RIF™ found 62 to be susceptible to rifampicin, three resistant and two intermediate. All of the 21 rifampicin-resistant isolates were also found to be resistant by FAST*Plaque*TB-RIF™. Eight isolates had to be retested due to discrepant results between the two methods and insufficient plaque numbers in the RIF– plates. We retested the three rifampicin resistant and two rifampicin intermediate isolates and observed the same results. Sometimes, failure of plaque formation on control plates may result in difficulties in counting the plaques. When we retested three isolates with insufficient plaque numbers in the RIF– plates the test results obtained were valid. Our three plates had been contaminated by fungi during the study because of the manipulation steps of the assay. Overall accuracy for FAST*Plaque*TB-RIF™ was 94.3 (95% confidence interval [CI] 87.3–97.5) for the detection of rifampicin susceptibility. In comparing FAST*Plaque*TB-RIF™ with the BACTEC 460 TB system for testing the susceptibility of *M. tuberculosis* isolates to rifampicin, the sensitivity and specificity of FAST*Plaque*TB-RIF™ were respectively 100% (95%CI 84.5–100) and 92.5% (95%CI 83.6–96.7) (Table).

Table Results of a comparison of the BACTEC 460 TB system and FAST*Plaque*TB-RIF™ for testing the susceptibility of *M. tuberculosis* isolates to rifampicin

	BACTEC 460 TB system		
FAST*Plaque*TB-RIF™	Resistant *n*	Susceptible *n*	Total *n*
Resistant	21	3	24
Intermediate	0	2	2
Susceptible	0	62	62
Total	21	67	88

The cost of FAST*Plaque*TB-RIF™ was US$8 per test, compared to US$15 for the BACTEC 460 TB system.

DISCUSSION

Although resistance to rifampicin is less common than to isoniazid and streptomycin, it is usually associated with resistance to isoniazid. Therefore, the rapid detection of rifampicin resistance could be an important tool for the efficient control of multidrug-resistant strains.[4] Although the period required for culture is shortened by the BACTEC 460 TB system, drug susceptibility testing in a liquid medium still requires 1 to 2 weeks for final determination and reporting to the clinicians.[10] With the FAST*Plaque*TB-RIF™ test, results are generally obtained within 2 days after primary isolation of *M. tuberculosis*.

FAST*Plaque*TB-RIF™ is a mycobacteriophage-based test for the rapid detection of the susceptibility of *M. tuberculosis* isolates to rifampicin. The application of mycobacteriophage-based tests offers new opportunities for clinical management of individual patients, efficient treatment and control of drug-resistant tuberculosis.

FAST*Plaque*TB-RIF™ is an alternative method for susceptibility testing in developing countries, where drug resistance is relatively low and susceptibility tests are mainly performed for surveillance rather than for patient management. Because of the recent rise in MDR-TB, drug susceptibility test results are important for proper patient management. Unfortunately, as rapid testing systems are expensive and the time needed for conventional tests by solid media is very long, susceptibility tests can not be performed in many areas where TB is endemic. Susceptibility results with solid medium testing are generally unavailable until 4 to 5 weeks after inoculation, even when done directly from sputum concentrates.[5,6,19,20]

According to our study, the sensitivity, specificity and overall accuracy for the FAST*Plaque*TB-RIF™ were respectively 100.0% (95CI, 84.5–100), 92.5% (95%CI 83.6–96.7) and 94.3% (95%CI, 87.3–97.5) for the detection of the susceptibility of *M. tuberculosis* isolates to rifampicin. The results from previous studies in which FAST*Plaque*TB-RIF™ testing was performed from solid cultures (LJ or Middlebrook 7H11 media) or from the BACTEC 460 TB system are very similar to ours.[5,6] The susceptibility results of five isolates were different with the BACTEC 460 TB system and FAST*Plaque*TB-RIF™. Three rifampicin-susceptible isolates were determined as resistant and two were detected to have intermediate susceptibility with FAST*Plaque*TB-RIF™. In a previous study, discrepant results between the BACTEC 460 TB system and FAST*Plaque*TB-RIF™ were found.[5] In this study, the results were confirmed by the gold standard proportion method on 7H11 medium. In each case, the 7H11 method agreed with the FAST*Plaque*TB-RIF™ results. Discrepancies were proposed to be due to slow growth of a low number of resistant organisms in the BACTEC 460 TB system. In a second FAST-*Plaque*TB-RIF™ study, a specimen was FAST*Plaque*TB-RIF™ resistant but BACTEC 460 TB system susceptible.[6] This isolate revealed a mutation at codon *rpoB* 533, proposed to be associated with low-grade rifampicin resistance. Intermediate results should be repeated by both the FAST*Plaque*TB-RIF™ and the BACTEC 460 TB system; they may be due to insufficient addition or mixing of the virucidal solution, resulting in failure to efficiently destroy the bacteriophages outside of the target cell. If an intermediate result is obtained consistently, it may be due to intermediate or partial resistance. If discrepant results persist between FAST*Plaque*TB-RIF™ and the BACTEC 460 TB system, ideally a third independent method, such as proportion method or *rpoB* sequencing, should be performed to confirm the results.

Although FAST*Plaque*TB-RIF™ has shortened the time to results, we feel, like Albert et al., that the use of samples with a positive growth index in BACTEC liquid medium to detect the rifampicin-susceptibility of *M. tuberculosis* isolates in FAST*Plaque*TB-RIF™ would give the results in a very short length of time.[6]

FAST*Plaque*TB-RIF™ is an inexpensive method that relies on basic microbiological techniques. Specialised equipment is not needed to perform the test or to evaluate the results. It is easy to perform in any laboratory, and can be helpful in the laboratories that use conventional manual culturing methods. However, because it requires more manipulation steps than the BACTEC 460 TB system, the time needed for the FAST*Plaque*TB-RIF™ procedure is longer, and may result in increased risk of contamination. Furthermore, as the evaluation of the results was performed by counting the plaques, the cut-off value for plaque count causes difficulties during the interpretation. The contamination of specimens by sensor cells may affect the plaque count during the study. If the amount of inoculum is not sufficient it may result in insufficient plaque count, and in addition, the results of retested isolates would be delayed for 2 more days.

The present study shows that FAST*Plaque*TB-RIF™ is a rapid, reliable and cost-effective test, and especially appropriate for developing countries with its

good performance that correlates with the BACTEC 460 TB system.

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RÉSUMÉ

CONTEXTE : Département de Microbiologie et de Microbiologie Clinique de l'Académie Militaire Médicale de Gulhane, à Ankara, Turquie. Il s'agit d'un hôpital de référence tertiaire dans une région où la tuberculose est endémique.

OBJECTIF : Evaluer la résistance à la rifampicine des souches du complexe *Mycobacterium tuberculosis* par l'utilisation de la FAST*Plaque*TB-RIF™, une technique rapide et innovatrice de détermination de la sensibilité basée sur les bactériophages.

SCHÉMA : Comparaison des résultats des isolats traités par le système BACTEC 460 TB et par la FAST-*Plaque*TB-RIF™.

RÉSULTATS : La sensibilité du complexe *M. tuberculosis* à la rifampicine a été testée dans 88 isolats par l'utilisation de la FAST*Plaque*TB-RIF™. Soixante-sept isolats sont sensibles à la rifampicine et 21 sont résistants selon le système BACTEC 460 TB. Dans l'ensemble, la précision de la FAST*Plaque*TB-RIF™ est de 94,3% (IC95% 87,3–97,5) pour la détection de la sensibilité à la rifampicine. La sensibilité et la spécificité de FAST-*Plaque* TB-RIF™ sont respectivement de 100% (IC95% 84,5–100) et de 92,5% (IC95% 83,6–96,7).

CONCLUSION : Cette étude démontre que FAST-*Plaque*TB-RIF™ est un test rapide et peu coûteux qui est en bonne corrélation avec le système BACTEC 460 TB.

RESUMEN

MARCO DE REFERENCIA : Departamento de Microbiología y de Microbiología Clínica de la Academia Militar Medical de Gulhane, Ankara, Turquía, un hospital terciario de referencia en una región donde la tuberculosis es endémica.

OBJETIVO : Evaluar la resistencia a la rifampicina de

cepas del complejo *Mycobacterium tuberculosis* utilizando FAST*Plaque*TB-RIF™, una técnica rápida e innovadora para determinar la sensibilidad basada en los bacteriófagos.

DISEÑO : Se compararon los resultados en aislados tratados con el sistema BACTEC 460 TB y con la técnica FAST*Plaque* TB-RIF™.

RESULTADOS : Se determinó la sensibilidad a la rifampicina en 88 aislados del complejo *M. tuberculosis* con la técnica FAST*Plaque*TB-RIF™. Sesenta y siete aislados eran sensibles a la rifampicina y 21 aislados eran resistentes con el sistema BACTEC 460 TB. La precisión global de FAST*Plaque*TB-RIF™ fue de 94,3% (IC95% 87,3–97,5) para la detección de la sensibilidad a la rifampicina. La sensibilidad y la especificidad de la técnica FAST*Plaque*TB-RIF™ eran 100% (IC95% 84,5–100) y 92,5% (IC95% 83,6–96,7), respectivamente.

CONCLUSIÓN : Este estudio demuestra que FAST-*Plaque*TB-RIF™ es una técnica rápida y de bajo costo, con buena correlación con el sistema BACTEC 460 TB.