INT J TUBERC LUNG DIS 13(6):791–793

SHORT COMMUNICATION

Performance of *FASTPlaque*TB™ and a modified protocol in a high HIV prevalence community in South Africa

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SUMMARY

Modifications in the *FASTPlaque*TB™ test protocol have resulted in an increase in the analytical limits of detection. This study investigated whether the performance of a modified prototype was able to increase the detection of smear-negative, culture-positive sputum samples as compared to the first generation *FASTPlaque*TB test. Modifications to the *FASTPlaque*TB did result in increased detection of smear-negative samples, but this was associated with a decrease in the specificity of the test. Before the *FASTPlaque*TB can be considered as a viable replacement for smear microscopy and culture for the identification of tuberculosis, further work is required to resolve the performance issues identified in this study.

KEY WORDS: mycobacteriophages; *FASTPlaque*TB; HIV; diagnostic tests; South Africa; pulmonary tuberculosis

A NUMBER of rapid diagnostic tests are currently available for the diagnosis of tuberculosis (TB), but their performance compared to culture is variable. In particular, the diagnosis of smear-negative TB is a pressing challenge in settings of high human immunodeficiency virus (HIV) prevalence, where the proportion of cases of smear-negative pulmonary TB can be as high as 61%.[1]

The first generation *FASTPlaque*TB™ (Biotec Laboratories, Ipswich, UK) test detects approximately 87% of smear-positive cases and 48–67% of smear-negative, culture-positive cases within 2 days, and has >97% specificity.[2,3] A modified *FASTPlaque*TB protocol has been developed that allows up to a 10-fold increase in the analytical limit of detection in the test in vitro. The modified *FASTPlaque*TB protocol was not designed for the sake of cost, time effectiveness or practicality, but rather as an attempt to improve the sensitivity of the first generation *FASTPlaque*TB. This study compared the performance of the *FASTPlaque*TB test with a modified prototype protocol for the detection of smear-negative, culture-positive sputum samples in a high HIV prevalence community.

METHODS

The study site was an integrated TB-HIV clinic run by Médecins Sans Frontières and the provincial government of the Western Cape in Khayelitsha Township, South Africa, where TB-HIV co-infection is 73%. Sputum samples (n = 196) were collected between 10 October 2005 and 11 January 2006 from individuals presenting with symptoms of pulmonary TB. Smears were prepared and stained using auramine-O and examined by fluorescent microscopy (×450 magnification). Samples were processed further after transfer to a sealable sterile tube to which 5–10 3 mm glass beads and 0.5% w/v *N*-acetyl-L-cysteine (NALC) were added. After vortexing for 15 s, the tube was left for 15 min at room temperature to allow liquefaction. Samples were then transferred to a sterile centrifuge tube to which an equal volume of sodium hydroxide-citrate was added, and this was left at room temperature for another 15 min before neutralisation with 67 mM phosphate buffer (pH 6.8). The samples were then concentrated by centrifugation (3000 × g for 20 min), the supernatant removed and the pellet re-suspended in 1.3 ml of phosphate buffer.

Smears were prepared from 50 μl of the suspension; 0.1 ml was used to inoculate Löwenstein-Jensen (LJ) and Middlebrook 7H11 medium slants supplemented with nystatin, oxacillin and aztreonam (NOA) antimicrobial supplement (Biotec).[4] Inoculated LJ and Middlebrook 7H11 slants were incubated at 37°C and growth was recorded at weekly intervals for up to 8 weeks. Smears of positive cultures were prepared and stained by the Ziehl-Neelsen method. Confirmation of *Mycobacterium tuberculosis* complex was made by *p*-nitrobenzoic acid testing.[5]

Two 0.5 ml aliquots of suspension were transferred to separate sterile centrifuge tubes, 15 ml *FASTPlaque*-TB Medium Plus was added and the tubes were

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Article submitted 1 May 2008. Final version accepted 8 January 2009.

Table Performance of the *FASTPlaque*TB and modified protocol tests as compared to the combined culture result in smear-positive and smear-negative samples

	Overall (n = 188) % (95%CI)	Smear-positive (n = 51) % (95%CI)	Smear-negative (n = 137) % (95%CI)
*FASTPlaque*TB			
Sensitivity	44.3 (31.8–56.8)	57.6 (40.7–74.5)	28.6 (11.9–45.3)
Specificity	95.8 (91.7–99.8)	91 (74.1–100)	96.4 (92.4–100)
Negative predictive value	0.73 (0.64–0.8)	0.42 (0.27–0.57)	0.8 (0.73–0.87)
Positive predictive value	0.87 (0.82–0.92)	0.95 (0.89–1.0)	0.73 (0.65–0.81)
Positive likelihood ratio	10.5 (3.9–28.6)	6.33 (0.96–42)	8.0 (2.3–28.1)
Negative likelihood ratio	0.58 (0.46–0.73)	0.47 (0.3–0.7)	0.74 (0.58–0.94)
Modified protocol			
Sensitivity	56.7 (45.8–69.7)	57.9 (42.2–73.6)	57.1 (38.8–75.4)
Specificity	81.1 (73.2–89)	100	78.8 (70.1–87.5)
Negative predictive value	0.73 (0.64–0.8)	0.39 (0.65–0.8)	0.85 (0.78–0.92)
Positive predictive value	0.68 (0.61–0.75)	1.0	0.47 (0.39–0.56)
Positive likelihood ratio	3.0 (1.9–3.8)	∞ (0.77–∞)	2.7 (1.6–4.5)
Negative likelihood ratio	0.52 (0.39–0.71)	0.42 (0.29–0.61)	0.54 (0.35–0.85)

CI = confidence interval.

centrifuged at 3000 × g for 20 min. Supernatants were discarded and the resulting pellets suspended in 1 ml fresh *FASTPlaque*TB Medium Plus; 1 ml of sample was removed from each tube for processing by either the *FASTPlaque*TB test or the modified protocol.

The *FASTPlaque*TB test was performed according to the manufacturers' instructions.[6] The modified protocol was based on the *FASTPlaque*TB test, with the following changes: all FPTB Medium Plus was supplemented with NOA antimicrobial supplement to control contamination;[7] samples were incubated at 37ºC for 3 days before testing (to allow more time for resuscitation of the mycobacteria); a post-virucide amplification step was included by neutralising the Virusol in 45 ml *FASTPlaque*TB Medium Plus then incubating at 37°C for 4 h to permit increased phage infection and release, after which 3 ml of Sensor cells were added and the mixture plated in 30 ml of *FASTPlaque*TB agar.

Results were recorded and analysed using Microsoft® Office Excel (Microsoft, Redmond, WA, USA) and AcaStat™ software* (AcaStat, Ashburn, GA, USA).

RESULTS

Of 196 sputum samples collected, eight were discarded (three sample containers were empty, two samples were from patients aged <16 years, one smear result could not be retrieved and two samples were from patients on TB treatment). Smear microscopy indicated that 72.9% (137/188) of the samples were smear-negative, 6% (11/188) were scanty positive, 3.7% (7/188) were 1+ positive, 3.7% (7/188) were 2+ positive and 13.8% (26/188) were 3+ positive.[7] The combined culture contamination rate was 7% (13/188), 57.5% (108/188) of the samples were culture-negative, and 35.6% (67/188) were culture-positive.

Twenty-eight *FASTPlaque*TB results were invalid due to the failure of the controls at day two (most likely due to procedural errors). Irrespective of the culture result, there were significantly more positive results using the modified protocol (P = 0.0016), particularly in smear-negative samples (11 [9%] positives on *FASTPlaque*TB as compared to 36 [28.8%] using the modified protocol [$P < 0.001$]).

The contamination rates of the *FASTPlaque*TB tests were low (respectively 2.1% and 2.7% for the *FASTPlaque*TB and the modified protocol). The performance of the *FASTPlaque*TB tests as compared to culture is shown in Table. The overall sensitivity of the *FASTPlaque*TB test was 44.3% (27/61) as compared to 56.7% (38/66) for the modified protocol. Specificity was respectively 95.8% (91/95) and 81.1% (77/95). There was no statistically significant difference in the overall sensitivity between the *FASTPlaque*-TB and modified protocol tests as compared to culture (P = 0.1283). However, when the performance was compared by smear result, a significant difference in sensitivity was found in the smear-negative group (*FASTPlaque*TB = 28.6%; modified protocol = 57.1%; P = 0.0312). There was a statistically significant difference in the overall specificity between the *FASTPlaque*TB and modified protocol tests as compared to culture (P = 0.0015). When performance was compared by smear result, there was a significant difference in specificity in the smear-negative group (*FASTPlaque*TB = 96.4%; modified protocol = 78.8%; P = 0.0005). The likelihood ratios (LR) suggest that the *FASTPlaque*TB is a better overall predictor of TB if the test is positive (*FASTPlaque*TB LR = 10.5; modified protocol LR = 3).

DISCUSSION

In this study, the performance of the *FASTPlaque*TB test and a modified protocol prototype was compared

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to culture (gold standard) in a setting of high TB-HIV co-infection. In all, 72.9% of samples were found to be smear-negative, which is higher than previously reported,[1] but not surprising given the high rate of TB-HIV co-infection in the study population. The overall sensitivity of *FASTPlaque*TB was approximately 20% lower than previously reported,[2,3,8] and the sensitivity using the modified protocol was not, as in vitro results suggested, significantly better. Possible explanations for the low sensitivities include the detrimental effect of the modified decontamination method on the mycobacteria (including prolonged exposure to NALC), splitting of the sample resulting in insufficient quantity of mycobacteria in the sample, or an HIV-associated phenomenon.

In the smear-negative, culture-positive group, there was a 28.5% increase in sensitivity using the modified prototype. However, this increase was associated with a significant decrease in the specificity of the modified prototype test. The specificity of *FASTPlaque*-TB was similar to that observed in other studies,[2,3,8] suggesting that the low specificity of the modified prototype is not associated with sample decontamination (common to both protocols), but is more likely to be due to non-specific phage replication during the post-virucide amplification or inadequate neutralisation of phage particles.

It is concluded that further work is required to improve the specificity of the test using the modified protocol. Furthermore, the poor performance of the *FAST-Plaque*TB test in this study needs to be resolved before investigation of a modified protocol can be made.

Acknowledgements

The authors thank the staff at Site B and Site C, Médecins Sans Frontières, Khayelitsha, Cape Town, for their help with the collection of the samples; the staff of the National Health Laboratory Service, Greenpoint, Cape Town, for smear microscopy; and Biotec Laboratories (Pty) Ltd. for providing the *FASTPlaque* reagents used in the study.

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RÉSUMÉ

Les modifications du protocole du test *FASTPlaque*TB™ ont entraîné une élévation de la limite analytique de détection. Cette étude cherche dans quelle mesure la performance d'un prototype modifié est apte à augmenter la détection d'échantillons à bacilloscopie négative et à culture positive par comparaison avec le test *FASTPlaque*-TB de première génération. Les modifications du *FAST-Plaque*TB ont entraîné une augmentation de la détection des échantillons à bacilloscopie négative, mais sont en association avec une diminution de la spécificité du test. Avant de considérer le *FASTPlaque*TB comme un remplacement viable de l'examen microscopique direct et de la culture pour l'identification de la tuberculose, des travaux complémentaires s'imposent pour résoudre les problèmes de performance identifiés dans cette étude.

RESUMEN

Las modificaciones introducidas al protocolo de la prueba *FASTPlaque*TB™ tuvieron como consecuencia un mayor límite de detección del método. En el presente estudio se investigó si, comparado con el protocolo original, un prototipo modificado de la prueba mejoraba la detección de bacilos acidorresistentes en muestras con examen microscópico y cultivo negativos. Las modificaciones condujeron en efecto a una mayor detección de muestras con frotis negativo, pero este efecto se asoció con una disminución de la especificidad de la prueba. Antes de considerar la *FASTPlaque*TB como sucedáneo viable de la microscopia y el cultivo en la detección de *Mycobacterium tuberculosis*, se precisan nuevos trabajos que resuelvan los problemas del desempeño de la prueba encontrados en el presente estudio.