

## Performance of a rapid phage-based test, *FASTPlaqueTB*<sup>TM</sup>, to diagnose pulmonary tuberculosis from sputum specimens in South Africa

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### SUMMARY

**SETTING:** Twelve primary health care clinics in the South Peninsula Administration, Cape Town, Western Cape Province, South Africa.

**OBJECTIVE:** To evaluate the performance of *FAST-PlaqueTB*<sup>TM</sup>, a new phage-based test, for the rapid diagnosis of TB in individuals with no previous history of TB treatment presenting at primary health care clinics in Cape Town, South Africa.

**DESIGN:** A comparative study of *FASTPlaqueTB*<sup>TM</sup>, auramine smear microscopy and Löwenstein-Jensen culture of 1692 decontaminated sputum specimens from 853 patients suspected of having TB. Resolution of discrepant results was undertaken by review of clinical information, chest X-ray and follow-up of treatment outcomes.

**RESULTS:** *FASTPlaqueTB*<sup>TM</sup> detected TB in 75.2% of culture-confirmed cases and 70.3% of all cases with a clinical diagnosis of TB, with a specificity of 98.7% and 99.0%, respectively. The performance parameters of *FASTPlaqueTB*<sup>TM</sup> were significantly superior to those of concentrated auramine smear microscopy (63.4% and 61.3% sensitivity, and 97.4% and 97.3% specificity in culture-confirmed and all cases, respectively). Of those patients with two negative sputum smears, *FAST-*

*PlaqueTB*<sup>TM</sup> detected TB in 54.1% of TB cases confirmed by culture and 48.8% of all cases with a clinical diagnosis of TB. A combination of smear microscopy and *FASTPlaqueTB*<sup>TM</sup> enabled 81.2% of culture-confirmed cases and 78.4% of total TB cases to be detected within 2 days of presentation.

**CONCLUSION:** *FASTPlaqueTB*<sup>TM</sup> is a rapid, manual test for the diagnosis of TB. The test has significantly higher sensitivity overall compared with auramine sputum smear microscopy in individuals with no previous history of TB treatment, although smear microscopy did detect the most infectious of the TB cases. The *FAST-PlaqueTB*<sup>TM</sup> test is easy to perform, requires no dedicated equipment, and results are read by eye within 48 hours. This test can be useful for the diagnosis of TB in developing countries with a high burden of TB where other rapid diagnostic tests may not be appropriate. The test shows promising performance, particularly in the diagnosis of smear-negative disease, and could be used in conjunction with smear microscopy to aid in the diagnosis of additional cases of TB.

**KEY WORDS:** mycobacteriophage; tuberculosis; diagnostic test; South Africa

TUBERCULOSIS REMAINS one of the leading causes of mortality in the developing world. Most tuberculosis can be successfully treated if diagnosed in a timely fashion. Currently, most cases of tuberculosis are diagnosed using one or more established approaches. Clinical signs and symptoms of TB, as well as chest X-rays, are commonly used; these criteria are non-specific and their use may lead to both under- and over-diagnosis.<sup>1</sup> Laboratory diagnosis in many parts of the world relies solely on acid-fast bacilli (AFB) microscopy in which stained smears of sputum specimens from TB suspects are examined microscopically. This test is the basis for the diagnostic procedure recommended by the World Health

Organization (WHO). This method will detect the most infectious cases of TB, with the highest numbers of AFB in the sputum, but it suffers from a lack of sensitivity.<sup>2</sup>

Significant morbidity and mortality are associated with TB patients with negative sputum smears. Smear-negative patients are also responsible for transmission of disease,<sup>3</sup> although to a lesser degree than sputum smear-positive patients; they may later become smear-positive if left untreated. In paucibacillary disease, it is often difficult to establish a rapid and definitive diagnosis.<sup>4,5</sup> Culture of digested and decontaminated sputum is a more sensitive test than sputum smear microscopy, but culture results are not

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available for at least several weeks. This causes serious delay in both diagnosis and treatment, with the opportunity for further disease transmission.

The human immunodeficiency virus (HIV) epidemic has led to increases in the incidence of tuberculosis. In HIV-infected individuals, TB is often associated with unusual clinical presentation, with AFB smear-negative disease and atypical chest X-ray findings more common, making its diagnosis more challenging.<sup>4,6-8</sup>

Rapid TB diagnostic tests have been developed and are in routine use in the industrialised world. However, these tests often require sophisticated equipment and may not be appropriate for use in developing countries, which have the highest burden of disease.<sup>9-13</sup>

The *FASTPlaqueTB*<sup>TM</sup> test (Biotec Laboratories Ltd., Ipswich, UK) is based on novel phage amplification technology in which mycobacteriophage (phage specific for mycobacteria) are used as indicators of the presence of viable *Mycobacterium tuberculosis* in a clinical specimen.<sup>14-19</sup> This test utilises mycobacteriophage to reflect the presence of viable *M. tuberculosis*. After phage infection, a virucidal solution is added which destroys all phage that have not infected the tubercle bacilli. The remaining phage in the infected bacilli replicate until new progeny phage are released as the cells lyse. The new phage are amplified by the addition of a non-pathogenic rapid-growing mycobacterial host, *M. smegmatis*, which is also able to support phage replication. Phage can be visualised as clear areas, or plaques, in a lawn of host cells. The number of plaques visualised from a given sample is related to the number of viable tubercle bacilli in the original sample. The test's manual format allows it to be performed in any laboratory that has access to basic microbiological equipment and requires no specialised, dedicated equipment.

This study assesses the performance of *FAST-PlaqueTB*<sup>TM</sup> in the diagnosis of TB among individuals suspected of having pulmonary tuberculosis who have not previously been treated for the disease (or who have not been on TB treatment for the previous 3 years) in a high incidence setting. Performance is compared with detection of *M. tuberculosis* using concentrated auramine smear microscopy and culture on Löwenstein-Jensen (LJ) medium. Discrepant results were resolved using clinical information, including signs and symptoms, chest X-ray findings and response to treatment.

## MATERIALS AND METHODS

### *Clinical specimens*

Sputum specimens ( $n = 1692$ ) were obtained from 853 suspects for pulmonary tuberculosis presenting at 12 primary health care clinics in the City of Cape Town South Peninsula Administration area of Cape Town, South Africa. Specimens were collected between March and December 2000.

Patients were enrolled in the study if they met the following inclusion criteria: 1) pulmonary TB suspects or patients with confirmed pulmonary TB who were not yet on anti-tuberculosis therapy, 2) persons over 18 years of age, 3) persons who had not been treated for TB within at least the last 3 years. Two expectorated sputum specimens were normally collected from each patient.

### *Decontamination of sputum specimens for microscopy, culture and FASTPlaqueTB*<sup>TM</sup>

Specimens were kept at room temperature and processed on the day of collection, or stored overnight at 2–8°C and processed on the following day. Sputum specimens were processed by a standard N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method.<sup>20</sup> Following neutralisation of the pellet in 67 mM phosphate buffer pH 6.8 and subsequent centrifugation, the pellet was suspended in 1 ml phosphate buffer. A smear was prepared from several drops of the suspension and 0.1 ml was used to inoculate LJ medium slants.

The remaining sediment was washed by adding *FASTPlaqueTB*<sup>TM</sup> (FPTB) Medium Plus (supplemented Middlebrook 7H9-based medium) up to the 15 ml graduation mark on the centrifuge tube. After shaking, the mixture was centrifuged at 2200 × *g* for 20 minutes. The supernatant fraction was carefully decanted and discarded, and the resulting pellet was suspended in 1 ml fresh FPTB Medium Plus. A 1 ml sample was removed for testing by *FASTPlaqueTB*<sup>TM</sup>.

### *Microscopy*

Smears were stained using auramine-O stain,<sup>21</sup> and the entire slide was observed by an experienced technologist using a fluorescent microscope with an oil immersion lens at ×450 magnification. All stained smears were stored for the duration of the study and were available for review. All positive smears and smears from specimens giving discrepant results were reread by a second technologist. Smears were considered positive if any AFB were observed.

### *Culture*

Inoculated LJ slants were incubated at 37°C and growth was recorded at weekly intervals for up to 8 weeks.

### *Identification of mycobacteria*

Smears of positive LJ cultures were prepared and stained by the Ziehl-Neelsen method. Confirmation of the presence of *M. tuberculosis* complex was made by p-nitrobenzoic acid (PNB) testing.<sup>22</sup> Lack of growth on the PNB-containing media confirmed the presence of *M. tuberculosis* complex organisms.

### *FASTPlaqueTB*<sup>TM</sup> test

*FASTPlaqueTB*<sup>TM</sup> was performed daily, immediately following receipt and processing of specimens. One millilitre of the sample was transferred to a reaction

tube and incubated overnight (approximately 15–18 hours). *FASTPlaqueTB*<sup>TM</sup> testing was performed according to the pack insert.<sup>23</sup>

Following overnight incubation, 100 µl of Acti-phage<sup>TM</sup> bacteriophage reagent was added. Samples were incubated at 37°C for 1 hour, after which 100 µl of Virusol<sup>TM</sup> (virucidal solution) was added. The contents of the reaction tubes were thoroughly mixed by rolling and inverting the vessels, to ensure that the Virusol<sup>TM</sup> came into contact with the entire inner surface of the vessels to aid efficient exogenous phage inactivation. Samples were incubated at room temperature for 5 minutes. Five millilitres of FPTB Medium Plus was added to neutralise the Virusol<sup>TM</sup> activity, then 1 ml of Sensor<sup>TM</sup> cells (*M. smegmatis*) was added. The entire contents of the reaction tube were then incorporated into 5 ml molten *FAST-PlaqueTB*<sup>TM</sup> Agar (Middlebrook 7H9-based agar, kept at 50–60°C in a water bath) in a sterile Petri dish. Once set, the Petri dishes were inverted and incubated at 37°C for 18–24 hours. The number of plaques (zones of clearing) in the lawn of Sensor<sup>TM</sup> cells was recorded.

Positive and negative assay controls, supplied as part of the kit, were prepared and tested with each batch according to the manufacturer's instructions. Negative assay controls should have 10 plaques or less and positive assay controls should have 20–300 plaques (inclusive) for the batch of tests to be considered valid. The cut-off value of 20 plaques or greater being positive for sputum specimens was defined by the manufacturer.<sup>23</sup> *FASTPlaqueTB*<sup>TM</sup> results were not reported to the medical officers at the time of testing, and therefore did not influence patient management.

#### *Clinical data and chest X-ray*

Clinical data forms were completed for all persons enrolled in the study and reviewed by a medical officer (BH, ES or RH). In the case of patients for whom their specimens gave discrepant results, clinical data were re-evaluated by a medical officer. Clinical assessment included patient history, signs and symptoms, miniature chest X-ray (90 mm × 90 mm), follow-up information, and results of testing of subsequent specimens from the same patient. The outcome of subsequent testing and treatment of patients was also determined by examination of patient files.

All patients with at least one culture positive for *M. tuberculosis* were considered to have TB disease. Also, patients who were culture-negative but were ill and with clinical evidence of TB were initiated on anti-tuberculosis treatment. A response to treatment in culture-negative TB patients was defined as improvement in the clinical picture and amelioration of TB radiographic abnormalities.

#### *Statistical analysis*

The rationale for determining the number of specimens to be tested assumed a 12% prevalence of TB

among previously untreated individuals suspected of having TB. On this basis a sample size of 1600–1700 would be expected to yield approximately 200 culture-positive specimens. This would be sufficient to identify a 5% differential in performance between *FAST-PlaqueTB*<sup>TM</sup> and smear microscopy at a one-sided probability level of 0.05, using the sign test for equiprobability of results.<sup>24</sup>

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of *FAST-PlaqueTB*<sup>TM</sup> were calculated by comparison with the culture results on both a per specimen basis and a per patient basis. A resolved analysis was made in comparison with culture results combined with the patients' clinical data. Significance calculations were performed using the sign test.

## RESULTS

A total of 1692 specimens from 853 patients were included in the study. Specimens whose culture and/or *FASTPlaqueTB*<sup>TM</sup> results could not be interpreted due to contamination were excluded from the analysis (52, 20 and two specimens were contaminated on culture, *FASTPlaqueTB*<sup>TM</sup> and both tests, respectively). Results from 1618 specimens were therefore analysed: 781 patients provided two specimens each, and a further 56 patients produced a single specimen.

Of the 1618 specimens analysed, 10.6% (171) were smear-positive and 89.4% (1447) were smear-negative by concentrated auramine smear microscopy. Two hundred and seven specimens were found to be positive on LJ culture for *M. tuberculosis*. Of these, 129 (62%) were from smear-positive specimens and 78 (38%) from smear-negative specimens.

#### *Comparison of FASTPlaqueTB<sup>TM</sup> with auramine smear and LJ culture*

Table 1 shows a comparison of the performance of the *FASTPlaqueTB*<sup>TM</sup> test with LJ culture for all specimens ( $n = 1618$ ), smear-positive specimens ( $n = 171$ ) and smear-negative specimens ( $n = 1447$ ). Overall sensitivity, specificity, PPV and NPV of *FAST-PlaqueTB*<sup>TM</sup> were 72.5%, 99.0%, 0.91 and 0.96, respectively, when calculated on a per specimen basis (unresolved data).

*FASTPlaqueTB*<sup>TM</sup> was a significantly better predictor of culture result than smear microscopy overall and in both smear-positive and smear-negative sub-groups ( $P \leq 0.005$  overall and for both sub-groups). *FASTPlaqueTB*<sup>TM</sup> was also a better predictor of both positive and negative culture results when analysed for each sub-group ( $P \leq 0.005$ ).

*FASTPlaqueTB*<sup>TM</sup> detected 86.8% of smear-positive, culture-positive specimens and 48.7% of smear-negative, culture-positive specimens (Table 1). Whilst the specimens took  $3.4 \pm 1.2$  weeks and  $4.5 \pm 1.3$  weeks to become positive by LJ culture (mean  $\pm$  stan-

**Table 1** FASTPlaqueTB™ compared with Löwenstein-Jensen culture (with p-nitrobenzoic acid [PNB] test confirmation); unresolved data per specimen

	No. of specimens positive for TB on LJ culture		Sensitivity %	Specificity %	PPV	NPV
	Positive	Negative				
All specimens (n = 1618)			72.5	99.0	0.91	0.96
FASTPlaqueTB positive	150	14				
FASTPlaqueTB negative	57	1397				
Smear-positive specimens (n = 171)			86.8	83.3	0.94	0.67
FASTPlaqueTB positive	112	7				
FASTPlaqueTB negative	17	35				
Smear-negative specimens (n = 1447)			48.7	99.5	0.84	0.97
FASTPlaqueTB positive	38	7				
FASTPlaqueTB negative	40	1362				

PPV = positive predictive value; NPV = negative predictive value.

dard deviation for the smear-positive and smear-negative groups, respectively), the FASTPlaqueTB™ results were obtained within 2 days. Forty-one per cent (32/78) of the smear-negative, culture-positive specimens took 5 weeks or longer to become positive on LJ culture. Of the specimens taking 5-8 weeks to become positive by conventional culture, FASTPlaqueTB™ detected 37.5% (12/32) in 2 days.

#### Discrepant results

A total of 71 specimens gave discrepant results between FASTPlaqueTB™ and LJ culture, 24 from smear-positive specimens and 47 from smear-negative specimens: 57 results were FASTPlaqueTB™-negative and culture-positive, and 14 results were FASTPlaqueTB™-positive and culture-negative.

#### False-negative FASTPlaqueTB™ results compared with LJ culture

Fifty-seven specimens (17 smear-positive and 40 smear-negative) were negative by FASTPlaqueTB™ but positive by culture (Table 1). Of the 17 smear-positive specimens (from 12 patients) that were FASTPlaqueTB™ false-negative, eight contained less than 40 bacilli per slide, seven specimens contained 40–399 bacilli, one specimen had 4–40 per field and one specimen had more than 40 bacilli per field. For the smear-negative specimens that were FASTPlaqueTB™ false negatives (40 specimens from 32 patients), nine patients had only one out of two positive cultures, indicating low numbers of AFB or poor mycobacterial viability. This was also suggested by the longer than average time for the cultures to become positive (six out of nine cultures took 6 weeks or longer to become positive). This may suggest that the FASTPlaqueTB™ false-negative results arise at least partly from sampling bias, related to uneven distribution of low numbers of organisms in the sputum. Very low numbers of viable mycobacteria may be present in some specimens and may be below the detection limit of the FASTPlaqueTB™ test.

#### False-positive FASTPlaqueTB™ results compared with LJ culture

Fourteen specimens from 12 patients (seven smear-positive and seven smear-negative specimens) were positive by FASTPlaqueTB™ but negative by culture. Table 2 shows clinical data and results of additional laboratory tests for these patients. The review of these data has allowed four smear-positive and two smear-negative specimens (from five patients) to be reclassified as true TB positive results. Results of specimens from the remaining patients have been considered as FASTPlaqueTB™ false-positive results for the purposes of the resolved data analysis (Table 3). In two specimens (from one patient), the false-positive result was due to cross-reactivity with *M. intracellulare* in a patient with smear-positive disease. One patient was smear-positive on one specimen but culture-negative with no further confirmation of active TB. In the remaining specimens, fairly low numbers of plaques were achieved on the FASTPlaqueTB™ test (70 plaques or less), and since all these patients were both smear and culture-negative, these patients were difficult to follow up. There was no further evidence of TB in these patients.

#### False-positive smear microscopy results compared with LJ culture

In 35 specimens, the smear result was positive and the FASTPlaqueTB™ and culture results were negative. Low numbers of AFB were observed on the smears of these specimens. Twenty specimens contained four bacilli or less per slide, six specimens contained 5–8 bacilli per slide, four specimens had between nine and 12 bacilli per slide and three specimens had 13–16 bacilli per slide. Four patients (eight specimens tested) were started on TB treatment based on smear result and clinical suspicion and were considered to have TB, although this was not confirmed by culture of *M. tuberculosis* from these specimens. However, these patients were considered to be positive for TB for the purposes of the analysis of total cases. The remaining 27 specimens

**Table 2** Analysis of discrepant results between FASTPlaqueTB™ (FPTB) positive and Löwenstein-Jensen (LJ) culture-negative specimens

Patient number	Clinical information	Laboratory results	History of previous TB	Final interpretation of FPTB
1	Signs and symptoms and CXR suggestive of TB. TB treatment initiated. Patient remained smear-positive after 3 months treatment.	One specimen was 2+ smear-positive, FPTB-positive and LJ-negative. Another specimen from this patient was 1+ smear-positive, FPTB-positive, LJ-negative. <i>M. intracellulare</i> was isolated from a subsequent specimen	10 years previously	False positive
2	Signs and symptoms suggestive of TB. Mediastinal nodes. <i>M. tuberculosis</i> was cultured from aspirated lymph glands. TB treatment initiated.	One specimen was scanty 3 smear-positive, FPTB-positive, LJ-negative. Another specimen from same patient was smear-negative, FPTB-positive, LJ-negative	No	True positive
3	Signs and symptoms and CXR suggestive of TB. TB treatment initiated.	Specimen was 3+ smear-positive, FPTB-positive and LJ-negative. Another specimen from this patient was 3+ smear-positive, FPTB-positive and LJ-positive	No	True positive
4	Signs and symptoms and CXR suggestive of TB. TB treatment initiated.	Specimen was 2+ smear-positive, FPTB-positive and LJ-negative. Another specimen from this patient was 2+ smear-positive, FPTB-positive and gave sparse growth on LJ after 8 weeks' incubation.	No	True positive
5	Signs and symptoms and CXR suggestive of TB. TB treatment initiated.	Specimen was scanty 1 smear-positive, FPTB-positive, LJ-negative. Another specimen from this patient was scanty 5 smear-positive, FPTB contaminated and LJ-negative. Repeat specimens 4 months after initial presentation were 2+ and 1+ smear-positive and both FPTB-positive.	6 and 9 years previously	True positive
6	CXR normal. Patient was not started on TB treatment. No further follow-up was possible.	One specimen was scanty 3 smear-positive, FPTB-positive, LJ-negative. Another specimen from this patient was smear-negative, FPTB-negative, LJ contaminated.	No	Inconclusive
7	CXR suggestive of TB (atypical). HIV positive. Subsequent CXR normal. Disseminated TB with pleural effusion. Biopsy was AFB+. Prostate biopsy was also AFB+. TB treatment was initiated.	Specimen was smear-negative, FPTB-positive, LJ-negative. Another specimen from this patient was smear-negative, FPTB-negative, LJ-negative.	No	True positive
8	CXR abnormal but not suggestive of TB (deformed chest—distorted lungs).	Specimen was smear-negative, FPTB-positive, LJ-negative. Other specimen from this patient was smear-negative, FPTB-negative, LJ-negative.	No	False positive
9	CXR normal. Follow-up CXR 6 months after initial presentation was normal. No evidence of TB.	Specimen was smear-negative, FPTB-positive, LJ-negative. Other specimen from this patient was smear-negative, FPTB-negative, LJ-negative.	No	False positive
10	CXR normal. History of contact with TB.	Specimen was smear-negative, FPTB-positive, LJ-negative. Other specimen from this patient was smear-negative, FPTB-negative, LJ-negative. Repeat specimens 5 months later were smear negative, FPTB-negative.	No	False positive
11	CXR normal. Subsequent CXR also normal. Symptoms improved. No TB treatment given.	Specimen was smear negative, FPTB-positive, LJ-negative. Other specimen from this patient was smear-negative, FPTB-negative, LJ-negative.	No	False positive
12	CXR normal	Specimen was smear negative, FPTB-positive, LJ-negative. Other specimen from this patient was smear-negative, FPTB-negative, LJ-negative. Repeat specimens 6 months later were smear-negative.	No	False positive

CXR = chest X-ray; HIV = human immunodeficiency virus; AFB = acid-fast bacilli.

were considered to be false-positive smear microscopy results, as there was no further evidence of TB in these patients. Due to the low numbers of AFB obtained on many of these slides, it is possible that false-positive smear results are due to laboratory cross-contamination, e.g., in microscope immersion

oil, since the slides were read in a very high throughput laboratory with a high TB positivity rate among routinely tested specimens. In routine practice, repeat specimens would be examined to confirm the diagnosis of TB in patients with low numbers of bacilli on their first smear.

**Table 3** FASTPlaqueTB™ performance in culture-confirmed TB cases and all TB cases; resolved data per patient

	No. of patients with culture-confirmed TB* (resolved data for all TB cases)		Sensitivity† (%)	Specificity† (%)	PPV†	NPV†
	Positive	Negative				
All patients (n = 781)			75.2 (70.3)	98.7 (99.0)	0.89 (0.92)	0.96 (0.95)
FASTPlaqueTB positive (1 or both results positive)	76 (78)	9 (7)				
FASTPlaqueTB negative	25 (33)	671 (663)				
Patients with 2 negative sputum smears (n = 694)			54.1 (48.8)	99.2 (99.2)	0.80 (0.81)	0.97 (0.93)
FASTPlaqueTB positive (1 or both results positive)	20 (21)	5 (5)				
FASTPlaqueTB negative	17 (22)	652 (646)				

\* Values in this table show patients in whom the diagnosis of TB was confirmed by culture of *M. tuberculosis* from either one or both sputum specimens collected in this study. Values in parentheses are based on data following resolution of discrepant results. Patients with a diagnosis of TB included those patients with culture-confirmed TB from additional specimens tested as well as patients determined as having TB based on chest X-ray, smear results and decision to initiate TB treatment.

† Sensitivity, specificity, positive and negative predictive values calculated using culture-confirmed TB; all TB diagnoses in parentheses. PPV = positive predictive value; NPV = negative predictive value.

#### Analysis of FASTPlaqueTB™ compared with clinical diagnosis of TB

The results of 781 patients were analysed in which complete data (FASTPlaqueTB™, smear and LJ culture) were available for two sputum specimens. Table 3 shows the results on a per patient basis of patients diagnosed with TB, both culture-confirmed TB and cases, in whom TB treatment was initiated due to clinical and chest X-ray data. Of the 101 patients with at least one positive culture, FASTPlaqueTB™ detected 75.2% (76/101) of all patients and 54.1% (20/37) of smear-negative cases.

An additional 10 patients whose specimens processed as part of this study were culture-negative, but who were initiated on TB treatment, were confirmed to have TB either by positive smears and/or cultures on subsequent specimens tested, and/or were initiated on TB treatment due to signs and symptoms and chest X-ray abnormalities consistent with TB and showed good response to TB treatment. A total of 111 patients were diagnosed as having TB on this basis. Four of these patients had positive sputum smears but were negative on culture. Six patients were both smear- and culture-negative but were identified as having TB for the following reasons: four patients had positive cultures from other specimens tested subsequently; one patient had a pleural effusion and subsequently a smear-positive prostate biopsy and responded well to treatment; one patient was a true 'culture-negative' case and had typical signs and symptoms which resolved with TB treatment. Five of these six patients were negative by FASTPlaqueTB™. In at least one case, the false-negative laboratory test results appear to be due to poor specimen quality. One of these patients was documented as having difficulty producing sputum.

FASTPlaqueTB™ was positive in either one or both of the specimens tested in 70.3% (78/111) of all

TB cases. In patients with two negative sputum smears, FASTPlaqueTB™ was positive in either one or both of the specimens in 48.8% (21/43) of total cases.

Auramine smear microscopy performance parameters were 63.4% and 61.3% sensitivity, 97.4% and 97.3% specificity, 0.78 and 0.79 positive predictive value and 0.95 and 0.94 negative predictive value for culture-confirmed TB cases and all TB cases, respectively. A combination of smear microscopy and FASTPlaqueTB™ enabled 81.2% (82/101) of culture-confirmed cases and 78.4% (87/111) of all TB cases to be detected within 2 days of presentation.

#### DISCUSSION

FASTPlaqueTB™ had good sensitivity (70.3%) and high specificity (99.0%) in the diagnosis of TB among previously untreated patients suspected of having TB. The overall performance of FASTPlaqueTB™ was significantly superior to that of the auramine smear microscopy.

In South Africa, diagnosis of TB in new suspects is based largely on sputum smear microscopy. The smear positivity rate of 10.6% among specimens from patients suspected of having TB in this study is similar to that reported in Malawi (11.9%).<sup>25</sup> This equates to 9.5 sputum specimens tested for every one smear-positive specimen, or approximately 19 specimens requested for the diagnosis of one patient (with two positive sputum smears). In this study, 38.7% (43/111) of the TB cases (including culture-confirmed TB and clinically diagnosed disease) had two negative sputum smears. In this sub-group, FASTPlaqueTB™ was positive in either one or both of the specimens tested in 54.1% of culture-confirmed cases and 48.8% of all cases. FASTPlaqueTB™ may therefore play a role in the rapid diagnosis of patients with smear-

negative disease. The sensitivity of *FASTPlaqueTB*<sup>TM</sup> in detection of smear-negative TB is within the range of the sensitivity of 46–70% reported for rapid molecular-based diagnostic tests.<sup>26–33</sup>

Smear microscopy sensitivity has been reported to vary from 30% to more than 70%.<sup>2</sup> The smear microscopy used in this study is based on fluorescence microscopy of concentrated sputum sediments following centrifugation, and is commonly considered to have superior sensitivity compared with direct smear microscopy performed in many countries. Therefore, the proportion of TB cases missed when direct smear microscopy is solely used is likely to be higher than the 36% of cases (38% of specimens) found in this study.

However, smear microscopy does detect those patients with the highest number of TB bacilli in the sputum. Diagnosis using smear microscopy is the mainstay of the WHO strategy to control tuberculosis. It detects the cases thought to be responsible for the majority of disease transmission. In this study, the *FASTPlaqueTB*<sup>TM</sup> test gave false-negative results in 13.6% (9/66) patients who had at least one positive sputum smear. This result may appear surprising, as many smear-negative sputum specimens, containing fewer bacilli, gave positive results by the *FASTPlaqueTB*<sup>TM</sup> test. There may be several reasons for the occurrence of these false-negative results. *FASTPlaqueTB*<sup>TM</sup> requires viable bacilli and intact phage receptors in the cell surface to allow phage attachment and replication. Expression of phage receptors and efficiency of phage replication may vary depending on the strain of TB or the physiological state of the bacilli. However, unpublished data show that the phage has close to 100% coverage of *M. tuberculosis* strains from diverse geographical locations. In addition, phage inhibitory substances are known to be present in unprocessed sputum (unpublished data). The level of these substances appears to vary between specimens, and if present in high concentrations may inhibit the phage-TB interaction.

False-positive results were obtained by both smear microscopy and *FASTPlaqueTB*<sup>TM</sup>. In the case of smear microscopy false positives, low numbers of AFB were observed in some specimens but a diagnosis of TB could not be confirmed by culture or other means. In routine practice, where the interpretation of such results may be in doubt, additional sputum specimens would be tested to confirm the diagnosis. Either an additional smear or a chest X-ray suggestive of TB would be used to confirm a single positive sputum smear result. Such false-positive results may occur due to laboratory cross-contamination, particularly in laboratories with high numbers of positive specimens.

One patient gave false-positive results on both smear and *FASTPlaqueTB*<sup>TM</sup>. This patient had disease due to *M. intracellulare*, with high numbers of organisms present in the sputum. Other false-positive *FAST-*

*PlaqueTB*<sup>TM</sup> results were based on low numbers of plaques (20–30) close to the cut-off of 20 plaques determined by the manufacturer for this test. In some cases, particularly when low plaque numbers were obtained, these results may be caused by incomplete destruction of exogenous phage by the virucidal solution, possibly due to a protective effect on the phage by sputum components.

Several patients provided specimens that were positive with the *FASTPlaqueTB*<sup>TM</sup> test but were both smear- and culture-negative. Only some of these patients received TB treatment. Patients with positive *FASTPlaqueTB*<sup>TM</sup> results but negative on other laboratory tests, in whom treatment was not initiated, proved difficult to follow up as there was no specific evidence of TB other than suggestive signs and symptoms. There was a lower index of suspicion of TB in these patients, and they were lost from the TB control programme follow-up procedures. Other patients who were culture-negative but where there was a decision to treat for TB by the medical officers could be followed up, and confirmation of TB in these cases was possible either by subsequent cultures or response to treatment.

This study supports the clinical utility of *FASTPlaqueTB*<sup>TM</sup> in the rapid diagnosis of pulmonary tuberculosis. The test may be used in conjunction with sputum smear microscopy to detect additional cases that would be missed by smear alone. The test offers the potential for widespread application in high burden countries due to its reliance on basic microbiological techniques and its lack of requirement for specialised equipment. The acceptance of such new technology into routine diagnostic algorithms will follow the demonstration of its positive impact on the overall cost of effective TB diagnosis, including minimising clinic visits, unnecessary treatment and testing of misdiagnosed patients, as well as reducing the opportunity for further spread of the disease.

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#### References

- 1 Lale A P. Role of bronchoscopy and allied procedures to evaluate overdiagnosis of tuberculosis. *Indian J Tuberc* 1999; 46: 193–196.
- 2 Foulds J, O'Brien R. New tools for the diagnosis of tuberculosis: the perspective of developing countries. *Int J Tuberc Lung Dis* 1998; 2: 778–783.
- 3 Behr M A, Warren S A, Salamon H, et al. Transmission of

- Mycobacterium tuberculosis* from patients smear negative for acid fast bacilli. *Lancet* 1999; 53: 444–449.
- 4 Harries A D, Banda H T, Boeree M J, Welby S, Wirima J J, Subramanyam V R. Management of pulmonary tuberculosis suspects with negative sputum smears and normal or minimally abnormal chest radiographs in resource-poor settings. *Int J Tuberc Lung Dis* 1998; 2: 999–1004.
  - 5 Lucas S B, De Cock K M, Hounnou A, et al. Contribution of tuberculosis to slim disease in Africa. *BMJ* 1994; 308: 1531–1533.
  - 6 Van de Broek, J, Mfinanga, S, Moshiri, C, et al. Impact of human immunodeficiency virus infection on the outcome of treatment and survival of tuberculosis patients in Mwanza, Tanzania. *Int J Tuberc Lung Dis* 1998; 2: 547–552.
  - 7 Sepkowitz K A, Raffalli, J, Riley L, et al. Tuberculosis in the AIDS era. *Clin Microbiol Rev* 1995; 8: 180–199.
  - 8 Hargreaves N J, Kadzakanja O, Phiri S, et al. What causes smear-negative pulmonary tuberculosis in Malawi, an area of high HIV seroprevalence? *Int J Tuberc Lung Dis* 2001; 5: 113–122.
  - 9 Kambashi B, Mbulo G, McNerney R, et al. Utility of nucleic acid amplification techniques for the diagnosis of pulmonary tuberculosis in sub-Saharan Africa. *Int J Tuberc Lung Dis* 2001; 5: 364–369.
  - 10 Garrino M G, Glupczynski Y, Degraux J, Nizet H, Delmee M. Evaluation of the Abbott LCx *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in human samples. *J Clin Microbiol* 1999; 37: 229–232.
  - 11 Pfyffer G E, Kissling P, Jahn E M, Welscher H M, Salfinger M, Weber R. Diagnostic performance of amplified *Mycobacterium tuberculosis* direct test with cerebrospinal fluid, other non respiratory, and respiratory specimens. *J Clin Microbiol* 1996; 34: 834–841.
  - 12 Benjamin W H Jr, Waites K B, Beverly A, et al. Comparison of the MB/BacT system with a revised antibiotic supplement kit to the BACTEC 460 system for detection of mycobacteria in clinical specimens. *J Clin Microbiol* 1998; 36: 3234–3238.
  - 13 Hanna B A, Ebrahimzadeh A, Bruce Elliott L. Multicenter evaluation of the BACTEC MGIT 960 system for the recovery of mycobacteria. *J Clin Microbiol* 1999; 37: 748–752.
  - 14 Wilson S M, Al-Suwaidi Z, McNerney R, Porter J, Drobniowski F. Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of *Mycobacterium tuberculosis*. *Nature Med* 1997; 3: 465–468.
  - 15 Watterson S A, Wilson S M, Yates M D, Drobniowski F A. Comparison of three molecular assays for rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*. *J Clin Microbiol* 1998; 36: 1969–1973.
  - 16 Eltringham I J, Drobniowski F A, Mangan J A, Butcher P D, Wilson S M. Evaluation of reverse transcription-PCR and a bacteriophage-based assay for the rapid phenotypic detection of rifampicin resistance in clinical isolates of *Mycobacterium tuberculosis*. *J Clin Microbiol* 1999; 37: 3524–3527.
  - 17 Eltringham I J, Wilson S M, Drobniowski F A. Evaluation of a bacteriophage-based assay (phage amplified biologically 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.
  - 18 McNerney R, Kiepiela P, Bishop K S, Nye P M, Stoker N G. Rapid screening of *Mycobacterium tuberculosis* for susceptibility to rifampicin and streptomycin. *Int J Tuberc Lung Dis* 2000; 4: 69–75.
  - 19 Albert H, Heydenrych A, Mole R, Trollip A P, Blumberg L. Evaluation of FASTPlaqueTB-RIF™, a rapid, manual test for the determination of rifampicin resistance from *M. tuberculosis* cultures. *Int J Tuberc Lung Dis* 2001; 5: 906–911.
  - 20 Master R N. Mycobacteriology. In: Isenberg H D, ed. *Clinical Microbiology Procedures Handbook*. Section 3. Vol. 1. Washington DC: American Society for Microbiology, 1992.
  - 21 Strong B E, Kubica G P. Isolation and identification of *Mycobacterium tuberculosis*. A Guide for the Level II Laboratory. US Department of Health and Human Services. Public Health Service. Atlanta, GA: Centers for Disease Control, 1981.
  - 22 Allen B W. Tuberculosis bacteriology in developing countries. *Med Lab Sci* 1984; 41: 400–409.
  - 23 FASTPlaqueTB—a rapid bacteriophage assay for the detection of *Mycobacterium tuberculosis* complex in clinical samples. Product Insert. Ipswich, UK: Biotec Laboratories Ltd., 2000.
  - 24 Neave H R. *Elementary Statistics Tables*. p. 29. London, UK: George Allen and Unwin, 1981.
  - 25 Harries A D, Nyirenda T E, Banerjee A, et al. District sputum smear microscopy services in Malawi. *Int J Tuberc Lung Dis* 1998; 2: 914–918.
  - 26 Abe S, Hirano K, Wada M, et al. Detection of *Mycobacterium tuberculosis* in clinical specimens by polymerase chain reaction and Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test. *J Clin Microbiol* 1993; 31: 3270–3274.
  - 27 D'Amato R F, Wallman A A, Hochstein L H, et al. Rapid diagnosis of pulmonary tuberculosis by using Roche AMPLICOR *Mycobacterium tuberculosis* PCR test. *J Clin Microbiol* 1995; 33: 1832–1834.
  - 28 Bradley S P, Reed S L, Catanzaro A. Clinical efficacy of the amplified *Mycobacterium tuberculosis* direct test for the diagnosis of pulmonary tuberculosis. *Am J Respir Crit Care Med* 1996; 153: 1606–1610.
  - 29 Chinn D P, Yajko D M, Hadley W K, et al. Clinical utility of a commercial test base on the polymerase chain reaction for detecting *Mycobacterium tuberculosis* in respiratory specimens. *Am J Respir Crit Care Med* 1995; 151: 1872–1877.
  - 30 Nolte F S, Metchock B, McGowan J E, et al. Direct detection of *Mycobacterium tuberculosis* in sputum by polymerase chain reaction and DNA hybridisation. *J Clin Microbiol* 1993; 31: 1777–1782.
  - 31 Shawar R M, Zaatari F A K, Nataraj A, Clarridge J E. Detection of *Mycobacterium tuberculosis* in clinical samples by two-step polymerase chain reaction and nonisotopic hybridisation methods. *J Clin Microbiol* 1993; 31: 61–65.
  - 32 Kwiatkowska A, Marczak J, Zieba M, Nowak D. Clinical utility of a commercial ligase chain reaction kit for the diagnosis of smear negative pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1999; 3: 421–425.
  - 33 Noordhoek G T, Kolk A H J, Bjune G, et al. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. *J Clin Microbiol* 1994; 32: 277–284.

## R É S U M É

**CONTEXTE :** Douze dispensaires de soins de santé primaire des services administratifs de la Péninsule du Sud, Cape Town, Province du Cap Ouest, Afrique du Sud.

**OBJECTIF :** Evaluer les performances du FASTPlaqueTB™, un nouveau test basé sur les phages, pour le diagnostic

rapide de la tuberculose chez les individus sans antécédents de traitement de la tuberculose, se présentant à des dispensaires de soins de santé primaire à Cape Town, Afrique du Sud.

**SCHÉMA :** Etude comparative du test FASTPlaqueTB™,



de l'examen microscopique des frottis colorés à l'auramine et de la culture sur Löwenstein-Jensen pour 1.692 échantillons d'expectoration décontaminés provenant de 853 patients suspects de tuberculose. On a entrepris de résoudre les résultats discordants en revoyant les informations cliniques, les clichés thoraciques et le suivi des résultats du traitement.

**RÉSULTATS :** Le test *FASTPlaqueTB*<sup>TM</sup> a détecté la tuberculose dans 75,2% des cas confirmés par la culture et dans 70,3% de l'ensemble des cas où le diagnostic clinique de tuberculose a été porté avec une spécificité respectivement de 98,7% et de 99,0%. Les paramètres de performance du test *FASTPlaqueTB*<sup>TM</sup> ont été significativement supérieurs à ceux de l'examen microscopique des frottis des expectorations concentrées colorés à l'auramine (sensibilité de 63,4% et de 61,3% et spécificité de 97,4% et de 97,3% respectivement dans les cas confirmés par la culture et dans l'ensemble des cas). Parmi les patients ayant deux frottis d'expectation négatifs, le test *FASTPlaqueTB*<sup>TM</sup> a détecté la tuberculose dans 54,1% des cas de tuberculose confirmée par la culture et dans 48,8% de tous les cas où un diagnostic clinique de tuberculose a été porté. Une combinaison

de l'examen microscopique des frottis et du test *FASTPlaqueTB*<sup>TM</sup> a permis de détecter dans les 2 jours après leur consultation 81,2% des cas confirmés par la culture et 78,4% de l'ensemble des cas de tuberculose.

**CONCLUSION :** Le *FASTPlaqueTB*<sup>TM</sup> est un test rapide, manuel pour le diagnostic de la tuberculose. Le test a une sensibilité globale significativement plus élevée par comparaison avec la microscopie des frottis d'expectation colorés à l'auramine chez les individus sans antécédents de traitement antituberculeux antérieur ; l'examen microscopique des frottis détecte pourtant les plus contagieux des cas de tuberculose. Le test *FASTPlaqueTB*<sup>TM</sup> est aisé à pratiquer, ne demande pas un équipement spécifique et ses résultats sont lus à l'œil nu dans les 48 heures. Ce test peut être utile pour le diagnostic de la tuberculose dans les pays en développement où la charge de la tuberculose est importante, là où d'autres tests rapides de diagnostic peuvent ne pas être appropriés. Le test a des performances prometteuses, particulièrement pour le diagnostic des maladies à bacilloscopie négative, et pourrait être utilisé en conjonction avec la microscopie des frottis pour aider au diagnostic de cas supplémentaires de tuberculose.

## RESUMEN

**MARCO DE REFERENCIA :** Doce dispensarios de atención primaria de salud en el Distrito de la Península del Sur, Ciudad del Cabo, Sudáfrica.

**OBJETIVO :** Evaluar el rendimiento de *FASTPlaqueTB*<sup>TM</sup>, un nuevo test basado en fagos, para el diagnóstico rápido de la tuberculosis (TB), en sujetos sin antecedentes de tratamiento previo de la TB, que acuden a los dispensarios de atención primaria de salud en Ciudad del Cabo, Sudáfrica.

**MÉTODO :** Estudio comparativo del test *FASTPlaqueTB*<sup>TM</sup>, de la baciloscopia con auramina y del cultivo en Löwenstein-Jensen, en 1.692 muestras descontaminadas de esputo, provenientes de 853 pacientes sospechosos de TB. El problema de los resultados discrepantes se resolvió revisando la información clínica, las radiografías de tórax y el seguimiento de los resultados del tratamiento.

**RESULTADOS :** El test *FASTPlaqueTB*<sup>TM</sup> detectó una TB en el 75,2% de los casos confirmados por cultivo y en el 70,3% de todos los casos con un diagnóstico clínico de TB, con una especificidad de 98,7% y de 99,0%, respectivamente. Los parámetros de rendimiento de *FASTPlaqueTB*<sup>TM</sup> fueron significativamente superiores a los de la baciloscopia con auramina concentrada (63,4% y 61,3% de sensibilidad y 97,4% y 97,3% de especificidad, para los casos confirmados por cultivo y

en el conjunto de los casos, respectivamente). Entre los pacientes con dos baciloscopias negativas, el test *FASTPlaqueTB*<sup>TM</sup> detectó una TB en el 54,1% de los casos confirmados por cultivo y en el 48,8% de todos los casos con diagnóstico clínico de TB. La combinación de la baciloscopia y del test *FASTPlaqueTB*<sup>TM</sup> detectó, dentro de los 2 días que siguieron a la consulta, el 81,2% de los casos confirmados por cultivo y el 78,4% del total de casos de TB.

**CONCLUSIÓN :** El *FASTPlaqueTB*<sup>TM</sup> es un test manual rápido para el diagnóstico de la TB. Tiene una sensibilidad global significativamente más elevada, en comparación con la baciloscopia con auramina, en sujetos sin tratamiento previo de la TB, aunque la baciloscopia detecta los casos más contagiosos de TB. El *FASTPlaqueTB*<sup>TM</sup> es un test de realización fácil, que no requiere un equipamiento específico y los resultados se leen al ojo desnudo, dentro de las 48 horas. Este test puede ser útil para el diagnóstico de la TB en los países en desarrollo con alta prevalencia, donde otros tests rápidos pueden ser inapropiados. El test muestra un rendimiento prometedor, en particular para el diagnóstico de la enfermedad con baciloscopia negativa y puede ser utilizado en conjunto con la baciloscopia para ayudar al diagnóstico de los casos adicionales de TB.