

The use of a novel phage-based technology as a practical tool for the diagnosis of tuberculosis in Africa

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Sub-Saharan Africa has experienced a significant increase in tuberculosis cases in recent years, fuelled by high rates of TB-HIV co-infection in the region. The diagnosis of tuberculosis is based largely on clinical assessment, sputum smear microscopy and chest radiography. Although smear microscopy is useful for detecting the most infectious cases, a significant portion of cases are negative on sputum smears, making diagnosis more difficult. New tests are urgently needed. The *FASTPlaqueTB* test, a bacteriophage-based method, has been evaluated in several studies in Africa and elsewhere. Studies in South Africa and Pakistan reported that between half and two-thirds of smear-negative culture-positive TB cases were detected by the *FASTPlaqueTB* test within 2 days. This suggests a beneficial role for this test in the early diagnosis of clinically suspected smear-negative cases. The same technology has been applied to develop a rapid test to indicate multi-drug resistant TB, *FASTPlaqueTB-MDRi*. This test gave equivalent results to conventional drug susceptibility methods, but with more rapid results. The tests are simple to perform and require no specialised equipment, making the technology suitable for widespread implementation.

Key words: Tuberculosis, bacteriophage, diagnostic test, phage amplification technology, multi-drug resistance, drug susceptibility test.

INTRODUCTION

The World Health Organization (WHO) has estimated that tuberculosis (TB) is responsible for the death of approximately two million people each year. TB is the single leading cause of mortality of people living with HIV/AIDS and since 70% of those co-infected live in Sub-Saharan Africa, this region bears the overwhelming burden of the global epidemic of human immunodeficiency virus (HIV)-associated TB (World Health Organisation, 2002).

In low-income countries with a high prevalence of TB, the diagnosis of TB is based largely on clinical assessment, sputum smear microscopy and chest radiography. Techniques utilised in developed countries to obtain pulmonary specimens (such as sputum induction, bronchoscopy and biopsy) and more sensitive or rapid tools for the analysis thereof, are often unavailable in resource-poor settings (Harries et al., 1998a). An array of new diagnostic approaches, including nucleic acid amplification, antibody detection, liquid culture, cellular immune recognition, antigen capture, and

chemical and physical detection, have been developed (Perkins, 2000). However, many of these techniques are expensive and often require sophisticated equipment, making them unsuitable for routine application in low-income countries. In addition, there have been limited evaluations of such methods in field conditions to assess their clinical utility.

The sensitivity of sputum smear microscopy has been reported to be between 22–80% of culture-confirmed TB cases (Colebunders and Bastian, 2000). Although smear microscopy, when properly performed, will detect the most infectious cases, that is those with the largest numbers of TB bacilli, it is recognized that a significant portion of TB cases will be missed when only smear microscopy is used. The quality of results achieved with smear microscopy is heavily dependent on the workload, skill and motivation of the technician reading the slides. Up to 100 fields need to be examined to report a slide as negative (that is no acid-fast bacilli observed), which may be difficult to achieve in high volume, under-resourced laboratories. Culture is a sensitive and specific method, however results take up to 8 weeks using conventional methods, too late to affect the initial diagnosis. It may be used to confirm the diagnosis or for drug susceptibility testing. Culture is unavailable in many low-income settings. Chest X-ray is commonly used to aid in the diagnosis of TB. However, since radiological changes are not specific for TB and do not always reflect active

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Abbreviations: TB, tuberculosis; HIV, human immunodeficiency virus; MDR-TB, multi-drug resistant tuberculosis; WHO, World Health Organisation.

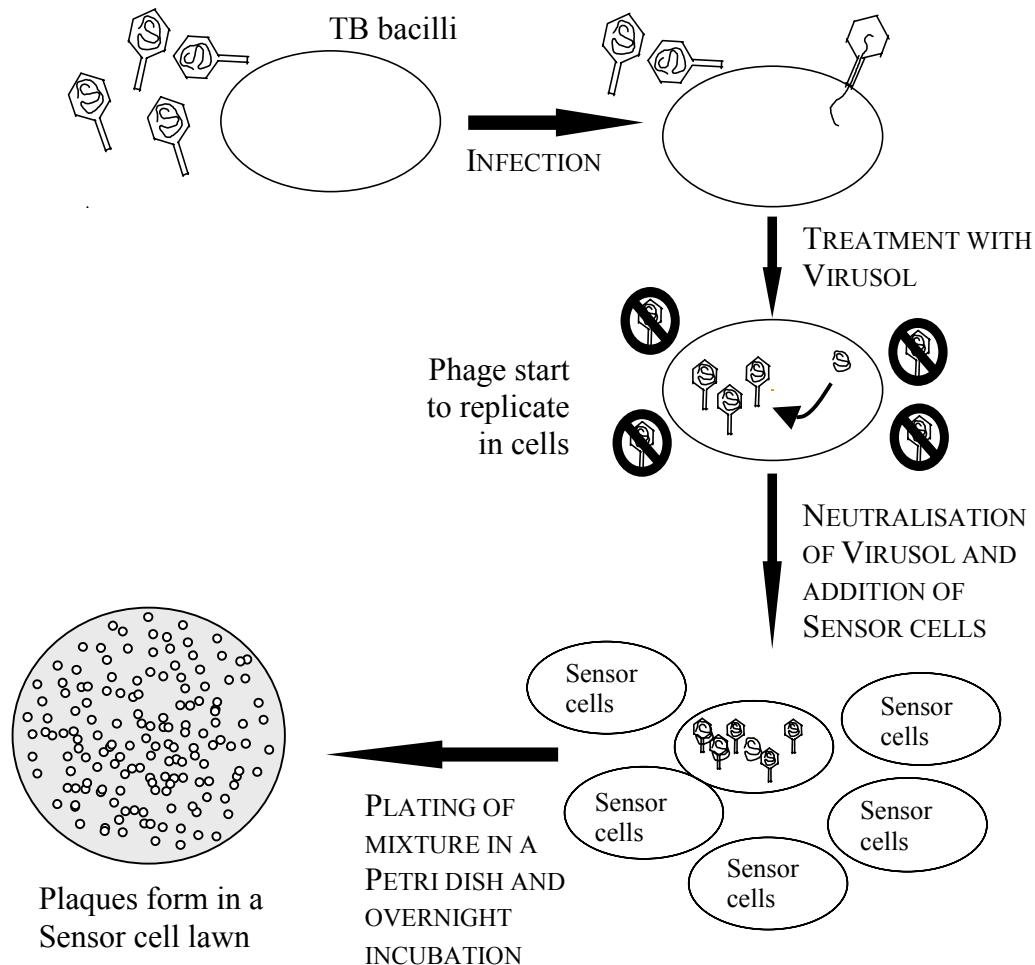


Figure 1. Diagrammatic representation of phage amplification technology.

disease, over-reliance on chest X-ray can lead to misdiagnosis.

The detection and management of infectious smear-positive pulmonary TB cases, responsible for most transmission, is the primary aim of TB Control Programmes. However, it is known that patients whose sputum specimens are negative on sputum smears may also transmit TB (Behr et al., 1999) and are likely to progress to smear positive disease if left untreated (Hong Kong Chest Service, 1981). Smear negative disease is also more common in children and the elderly (Colebunders and Bastian, 2000). HIV-positive individuals often present with smear-negative pulmonary TB (Johnson et al., 1997; Hargreaves et al., 2001) and are more likely to have an atypical presentation (Henn et al., 1999; Johnson et al., 1997) and disseminated disease (Fanning, 1999) than immunocompetent persons. HIV positive patients with smear negative TB are reported to have a higher mortality rate than smear positive cases (Harries et al., 1998b). HIV co-infection also broadens the differential diagnosis of smear-negative pulmonary TB (Colebunders and Bastian, 2000).

There is often a delay in diagnosis in smear negative TB patients as no rapid definitive test is available, which has important consequences in terms of transmission, increased morbidity and mortality for the individual patient, and costs of a prolonged hospital stay while establishing the diagnosis (Hudson et al., 2000).

A delay in TB diagnosis poses a more specific risk to HIV positive patients, as an increase in viral load results from TB-related induction of HIV replication. Such an increase may result in an increased rate of immune suppression and reduced survival. These effects on viral load may be irreversible, despite effective TB treatment. Therefore, a reduction in diagnostic delay for TB is critical in influencing the size of this effect on immune suppression and improving survival (Lawn and Griffin, 2001).

PHAGE AMPLIFICATION TECHNOLOGY

Phage amplification technology forms the basis of the *FASTPlaqueTBTM* test (manufactured by Biotec

Table 1. Performance parameters of the *FASTPlaqueTB* test compared with culture, as reported in several international evaluations (Muzaffar et al., 2002b).

	Cape Town, South Africa (n=1618)§	Barcelona, Spain (n=857)ψ	Karachi, Pakistan (n=514)*	Small scale evaluations (n=441)**
Sensitivity (%)	72.5	64.6	81.6	83.4
Specificity (%)	99.0	98.5	97.7	97.4
PPV	0.91	0.78	0.97	0.95
NPV	0.96	0.97	0.85	0.90

§ (Albert et al., 2002a)

ψ (Orús et al., 2001)

* (Muzaffar et al., 2002a)

** Seven separate studies (mean n=63, range 19-141) conducted in India (3 studies), Pakistan, Egypt, Kenya and Zimbabwe.

Sensitivity is the percentage of TB positive specimens correctly identified as positive by the test method.

Specificity is the percentage of TB negative specimens correctly identified as negative by the test method.

PPV, positive predictive value (the proportion of test positive results that are true TB positive).

NPV, negative predictive value (the proportion of test negative results that are true TB negative).

Laboratories Ltd., Ipswich, UK), a rapid, manual test for the diagnosis of TB within two days. Phage amplification technology relies on the ability of mycobacteriophage (phage specific for mycobacteria) to specifically infect and replicate in viable TB bacilli (Wilson et al., 1997; Mole and Maskell 2001; Trollip et al., 2001).

Bacteriophage infect TB bacilli in the sample. Following infection, the sample is treated with a potent virucide (Virusol™) to destroy mycobacteriophage that have not infected host cells. Following this step the virucide is neutralised so that subsequently released phage are not affected. The only remaining phage are those protected within the host organisms. These phage undergo a replication cycle resulting in the release of phage from host cells. Released phage are allowed to infect a lawn of rapid-growing, non-pathogenic mycobacteria (Sensor™ cells) within an agar plate, which also support the replication of the bacteriophage. Zones of clearing (plaques) appear in the presence of TB cells that were originally infected, causing successive rounds of replication in the Sensor cells leading to localised zones of cell death and clearing of the lawn (Figure 1). The appearance of plaques is indicative of a sample containing viable TB (Mole and Maskell, 2001). Results are read by eye and are simple to interpret. The test's manual format allows it to be performed in any laboratory that has access to basic microbiological equipment, since no specialised, dedicated equipment is required.

RAPID DIAGNOSIS OF TB

For application of the technology to the rapid diagnosis of pulmonary TB, sputum specimens are first decontaminated using a standard N-acetyl-cysteine–sodium hydroxide method. Following a wash step in the *FASTPlaqueTB* medium, samples are incubated

overnight prior to testing by the *FASTPlaqueTB* method, as described above. Results are available within 48 hours of receipt of the patient's specimen.

The *FASTPlaqueTB* test has been evaluated for the diagnosis of pulmonary TB at several locations in Africa, as well as in Asia and Europe. In addition to several small-scale in-country evaluations, three large-scale studies have been performed in South Africa (Albert et al., 2002a), Pakistan (Muzaffar et al., 2002a) and Spain (Orús et al., 2001). The performance of *FASTPlaqueTB* was compared with smear microscopy and conventional culture, using Löwenstein-Jensen medium, or a combination of solid and liquid culture methods. A summary of the results from these evaluations, and several small-scale evaluations, is shown in Table 1.

In each study, specificity was very high (>97%), giving a high degree of confidence that a positive *FASTPlaqueTB* result was representative of active disease. High specificity was reported even in the presence of a high number of non-tuberculous mycobacterial isolates (32% of total isolates) in one of the populations studied (Orús et al., 2001). Overall, the test detected 65-83% of confirmed TB cases within two days, compared to culture, which took up to eight weeks. Furthermore, *FASTPlaqueTB* sensitivity of 48% to 67% and specificity of 98% was reported for the detection of smear-negative TB in the studies carried out in Pakistan and South Africa (Muzaffar et al., 2002a, Albert et al., 2002a). When the use of smear microscopy and *FASTPlaqueTB* were combined, an overall sensitivity of 90% was reported in Pakistan (Muzaffar et al., 2002a).

Revathi et al. (2001) reported encouraging results from a preliminary study of the use of *FASTPlaqueTB* in the diagnosis of TB in 141 sputum specimens in Nairobi, Kenya. All TB culture positive specimens were positive by *FASTPlaqueTB* (n=35) while only 1 out of 10 specimens growing non-tuberculous mycobacteria was

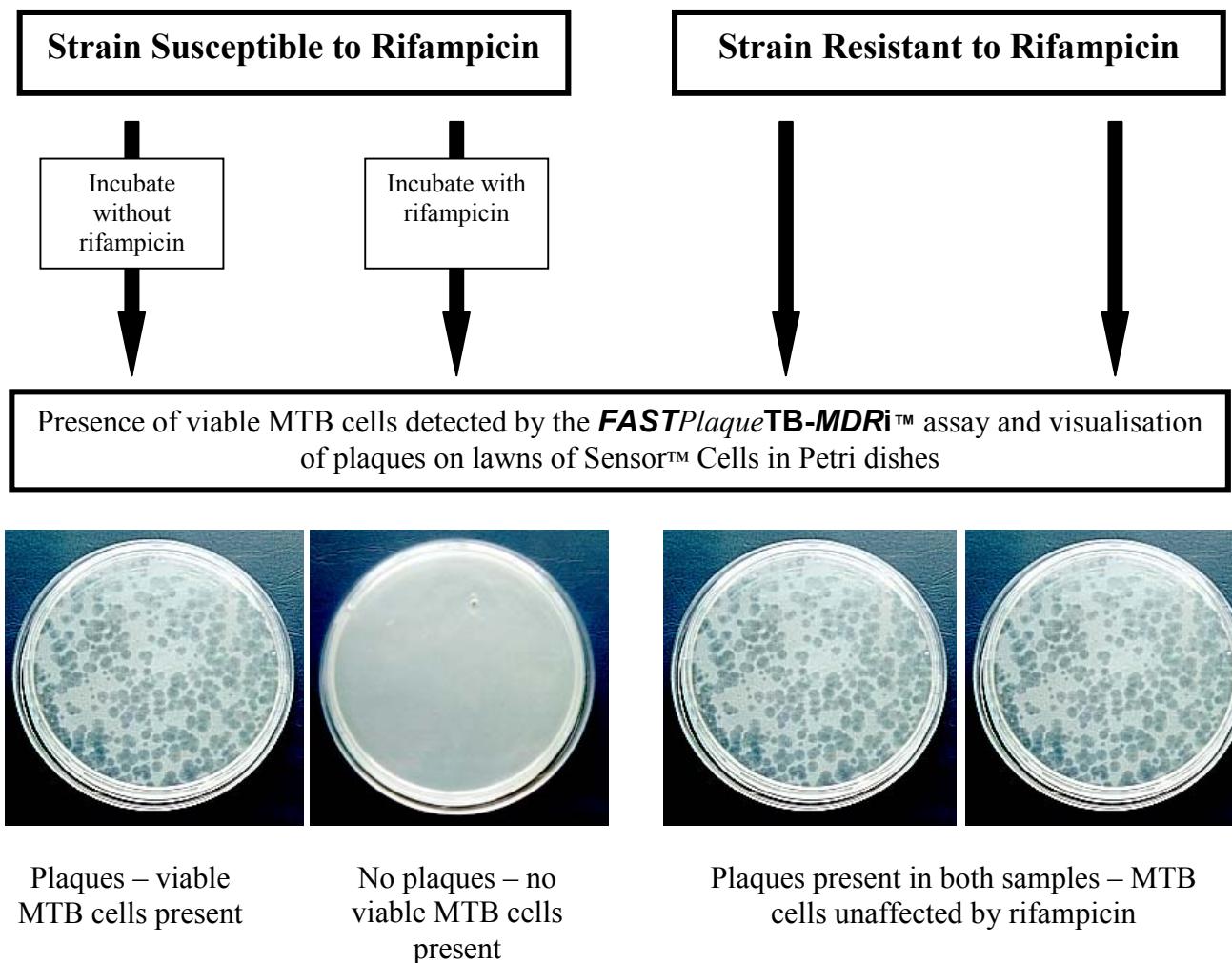


Figure 2. Principle of the *FASTPlaqueTB-MDRi* test, a rapid determination of rifampicin resistance as a marker for multi-drug resistance.

positive by the *FASTPlaqueTB* test. Furthermore, they also reported good performance of the *FASTPlaqueTB* in detecting *M. tuberculosis* in body fluids and aspirates with a sensitivity and specificity of 93.5% and 98.4% respectively (Revathi, 2002). The authors concluded that the test has potential for the rapid diagnosis of TB, and was cheaper than polymerase chain reaction (PCR) testing for the rapid diagnosis of TB in their setting.

MULTI-DRUG RESISTANT TB

The occurrence of drug resistant TB is generally low in many parts of Africa, including Kenya (Githui et al., 1998). However, even in these countries drug resistance may be a problem in certain high-risk populations, such as refugees (Githui et al., 2000). A number of African countries, including the Ivory Coast, Sierra Leone, Zimbabwe and Swaziland, have been highlighted as

multi-drug resistant TB (MDR-TB) transmission “hot spots” (Becerra et al., 2000). In addition, Mozambique has also been reported to have a significant number of MDR-TB cases (Espinal et al., 2001).

Phage amplification technology has also been applied to produce a test for drug resistance, which provides results within two days from a TB culture (Albert et al., 2001; Albert et al., 2002b; Wilson et al., 1997). The *FASTPlaqueTB-MDRi™* test (formerly known as *FASTPlaqueTB-RIF™*) uses rifampicin resistance as an indication of multi-drug resistance in strains of *Mycobacterium tuberculosis*. The viability of TB following treatment with rifampicin is determined by comparison of the presence of plaques in samples incubated with and without rifampicin (Figure 2). Rifampicin resistance is a good predictor of MDR-TB (World Health Organisation, 1997) and is a useful tool to identify those patients unlikely to be successfully treated on standard regimens. Rapid determination of drug resistance can improve the

outcome of the individual patient, as well as being important from a public health perspective, reducing further spread to health care workers and the community.

The evaluation of *FASTPlaqueTB-MDRi* in two South African studies yielded very high sensitivities (100%) and specificities (>94%) compared with conventional methods (Albert et al., 2001; Albert et al., 2002b). The test also showed excellent correlation with the well-established Bactec 460 method in determination of rifampicin susceptibility. Results of the *FASTPlaqueTB-MDRi*TM test were available within 2 days compared with approximately 1 week and 3-4 weeks for the Bactec 460 and solid culture proportion methods, respectively.

A simple, accurate test, such as *FASTPlaqueTB-MDRi*TM, may have a role in the rapid determination of multi-drug resistance both for individual patient management as well as a tool for ongoing surveillance.

DISCUSSION

The high sensitivity and specificity of the *FASTPlaqueTB*TM assay, coupled with the rapid availability of the results, suggests a beneficial role for this test in the early diagnosis of clinically suspected smear-negative cases, thus improving case finding (Albert et al., 2002a; Muzaffar et al., 2002b). Although smear positive patients have higher numbers of TB bacilli in their sputum, and are therefore more likely to infect close contacts, the delay in diagnosis of smear negative patients may lead to extended opportunities for transmission from these patients (Behr et al., 1999). In addition, certain patient groups such as children, the elderly and the immunocompromised, suffer from a disproportionately high rate of smear negative disease. Although smear microscopy has lowered sensitivity in these groups of patients it remains a useful tool for the diagnosis of TB (Di Lonardo et al., 1995). However, the incorporation of additional tests, such as *FASTPlaqueTB*, into the diagnostic algorithm could improve case finding, particularly in smear negative cases.

The rapid indication of multi-drug resistant TB, and appropriate treatment, is critical to reducing the spread of this often-fatal disease. The *FASTPlaqueTB-MDRi* offers more rapid results than conventional methods. However, even more significant benefit could be derived from performing the test directly from the patient's sputum sample, rather than requiring a culture to grow prior to performing the test. Ongoing developments of the *FASTPlaqueTB-MDRi* test are focused on producing a test directly from sputum that will indicate drug resistance within 2 days from receipt of the specimen. Such a simple and rapid test could make a substantial contribution towards the control of multi-drug resistant TB.

The *FASTPlaqueTB* and *FASTPlaqueTB-MDRi* tests provide rapid and reliable results for the diagnosis and drug susceptibility testing of *M. tuberculosis*. The tests

are easy to perform, require no specialised equipment, and rely on basic microbiological techniques. Furthermore, these tests overcome the problems of diagnostic delay and insensitivity encountered by traditional diagnostic methods. Since the tests directly detect viable *M. tuberculosis* present in clinical specimens, rather than relying on antigen or antibody detection, their performance is anticipated to be unaffected by HIV co-infection. The results presented here demonstrate that the *FASTPlaqueTB* test performs consistently well across a range of settings.

All these factors make the *FASTPlaqueTB* and *FASTPlaqueTB-MDRi* tests appropriate for widespread application in the fight against tuberculosis and multi-drug resistant tuberculosis in Africa. The acceptance and use of such new technology in routine diagnostic algorithms will follow the demonstration of its positive impact on the overall cost of effective TB diagnosis. Such cost effectiveness analysis should include not only the benefits of reduced transmission of TB, but also the potential for cost savings by reducing the number of clinic visits, reducing hospital stay while a diagnosis is established, and unnecessary testing and treatment of incorrectly diagnosed patients. Rapid diagnosis of TB and initiation of appropriate treatment will reduce further transmission of the disease in the hospital and community and may lead to prolonged survival in HIV-infected TB patients.

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