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Bacteriophage-based technologies for the rapid diagnosis and drug susceptibility testing of tuberculosis

BY ANDRÉ TROLLIP, HEIDI ALBERT, AND TIM MASKELL

It is estimated that about one-third of the world's population is infected with *Mycobacterium tuberculosis*, the organism that causes tuberculosis. There are about eight million new cases and two million deaths from tuberculosis each year.[1] Most active disease results from infections that occur in the developing world.[2]

Tuberculosis and human immunodeficiency virus (HIV) infection form a lethal combination, each speeding up the other's progress. Tuberculosis in HIV-infected persons can present atypically, and is often negative on sputum smears, the most commonly used diagnostic strategy. There is a rapid progression of tuberculosis in HIV-infected patients, making the early diagnosis of these persons critical.[3]

Any test that will be broadly accepted by the global tuberculosis diagnostic community needs to be cost effective, accurate, simple to perform, and easy to implement within the current infrastructure.

Multidrug-resistant tuberculosis (MDR-TB) is tuberculosis resistant to at least two of the most effective drugs, isoniazid and rifampicin. In a study conducted by the World Health Organization (WHO, Geneva, Switzerland) and the International Union Against Tuberculosis and Lung Disease (Paris, France), MDR-TB was found in all 35 countries surveyed.[4] Resistance to rifampicin has been identified as a good predictor of MDR-TB in many parts of the world.[5] Testing the rifampicin susceptibility of clinical isolates of *M. tuberculosis* can have important benefits for both the patient and the community at large: Rifampicin susceptibility testing will identify those patients most likely to fail standard treatment regimens. Delay of appropriate treatment can lead to more serious disease and additional opportunities for dissemination.

Conventional tuberculosis diagnosis

Classical methods for the diagnosis of pulmonary tuberculosis include acid-fast bacilli (AFB) smear microscopy and culture. Smear microscopy involves the visualization of AFB in stained smears prepared from sputum specimens obtained from tuberculosis suspects. This test is the diagnostic recommended as part of the WHO directly observed therapy (short course), or DOTS strategy. Although AFB microscopy is both rapid and simple, it is relatively insensitive.[6] Culture techniques are sensitive and specific, but are slow. Cultures performed in liquid on semiautomated systems are more rapid, but the systems are expensive, making them impractical for the developing world.[7]

Currently available techniques for drug susceptibility testing are culture based, and include the proportion and absolute concentration methods. Conventional methods may require several months before results can be reported from receipt of the initial specimen. Delays in reporting results lead to delays in delivery of appropriate treatment. Rapid methods to determine drug susceptibility have recently been developed. These include a radiometric method and a variety of molecular techniques. The procedures are costly and are not appropriate for use in developing countries, where they are most needed.

Phage amplification technology

Any test that will be broadly accepted by the global tuberculosis diagnostic community needs to be cost effective, accurate, simple to perform, and easy to implement within the current infrastructure. Phage amplification technology offers the potential for such testing, and would be well suited for use in those countries

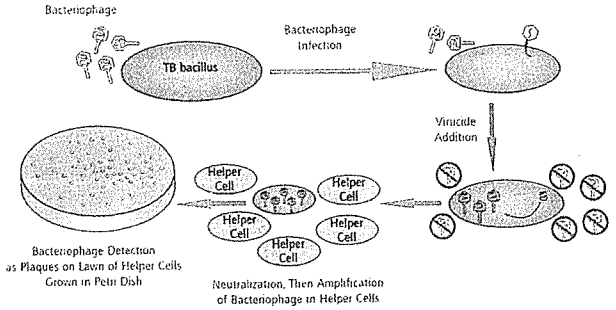

Figure 1 *Diagrammatic representation of the* FASTPlaque*TB assay.*

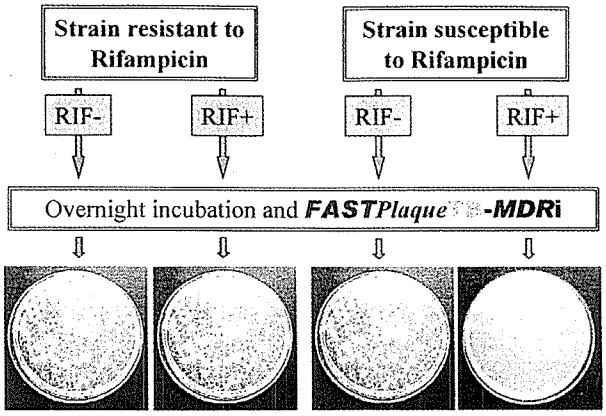

Figure 2 *Diagrammatic representation of the* FASTPlaque *TB-MDRi assay.*

with a high prevalence of tuberculosis. Use of the technology as a diagnostic tool for the detection of bacterial pathogens has been well documented.[8,9]

A phage amplification assay for rapid tuberculosis diagnosis, the *FASTPlaque*TB™ test (**Biotec Laboratories Ltd.**, Ipswich, Suffolk, U.K.), utilizes specific mycobacteriophage or phage (Actiphage™) to reflect the presence of viable *M. tuberculosis* in sputum samples (*Figure 1*). After phage infection, a virucidal solution (Virusol™) destroys all phage that have not infected the tubercle bacilli. The phage replicate in the infected bacilli until new progeny phage is released as the cells lyse. The new phage are amplified by the addition of a nonpathogenic rapid-growing mycobacterial host (Sensor™ cells) that is also able to support phage replication. The resulting phage can be visualized as clear areas (plaques) in a lawn of Sensor cells. The number of plaques visualized from a given sample is related to the number of viable tubercle bacilli in the original sample.

The *FASTPlaque*TB-*MDRi*™ assay is a bacteriophage-based test for the rapid indication of MDR-TB within 48 hr, based on the susceptibility of strains to rifampicin (*Figure 2*).[10–12] The test can be applied to solid or liquid cultures of *M. tuberculosis*,[13,14] and work aimed at developing a direct test from sputum is under way.[15]

Clinical performance

The *FASTPlaque*TB assay relies on the pretreatment of sputum with *n*-acetyl-L-cysteine-sodium hydroxide. After treatment, the sample is washed, incubated overnight in a recovery medium to allow a period of recovery for the tubercle bacilli, and then assayed.

A trial was conducted in collaboration with the South Peninsula Municipality Health Services (Cape Town, South Africa).[16] It evaluated the performance of the assay, AFB smear microscopy, and Löwenstein-Jensen culture in 1692 decontaminated sputum specimens from 853 new tuberculosis suspects. The assay detected *M. tuberculosis* in 73.1% of all cases with a clinical diagnosis of tuberculosis with a specificity of 99.0%. Performance parameters of the test were significantly superior to that of concentrated AFB smear microscopy (64.2% sensitivity and 97.3% specificity). In specimens with very low numbers of AFB, false-positive smear results were obtained in a number of cases in which the diagnosis of tuberculosis may be difficult to confirm. *FASTPlaque*TB may play a role in the rapid confirmation of tuberculosis in such cases, since a very low incidence of false-positive results occurs and the positive predictive value of the test is high. In the study, 35% of the tuberculosis cases had two negative sputum smears. The assay was positive in either one or both of the specimens tested in 55.3% of these patients. It can therefore play an important role in the rapid diagnosis of patients with smear negative disease.

A comparative study of the *FASTPlaque*TB-*MDRi* assay and conventional drug susceptibility methods was performed in two laboratories of the South African Institute for Medical Research (Johannesburg, South Africa).[17] Performance of the assay was comparable to the gold standard proportion methods for rifampicin susceptibility testing (see *Table 1*).

Conclusion

The *FASTPlaque*TB and *FASTPlaque*TB-*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 specialized equipment, and rely on basic microbiological techniques. Since the tests detect viable *M. tuberculosis*, their performance is not affected by HIV coinfection. The as-

Table 1

Sensitivity, specificity, and overall accuracy of *FASTPlaque*TB-*MDRi* test compared with indirect proportion method

	Sensitivity[a]	Specificity[b]	Overall accuracy[c]
Laboratory 1 (n = 94)	1.00 (32/32)	0.97 (60/62)	98% (92/94)
Laboratory 2 (n = 97)	1.00 (49/49)	0.94 (45/48)	97% (94/97)

[a]Sensitivity is the ability to detect true resistance.
[b]Specificity is the ability to detect true susceptibility.
[c]Overall accuracy is the number of correct results over the total number of results expressed as a percentage.

says are appropriate for widespread application in the fight against tuberculosis and MDR-TB in both the developing and the industrialized world.

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