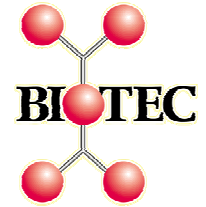


# Rifampicin resistance screening directly from sputum in 2 days using **FAST**Plaque-Response™ test



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## SUMMARY

Resistance to rifampicin is of critical importance to the success of short-course chemotherapy, and is a good predictor for multi drug resistant TB in many settings. Where resistance to other drugs is present, e.g. isoniazid, treatment with standard first-line regimens is still effective. However, when rifampicin resistance occurs the chance of treatment success is greatly reduced.

The **FAST**Plaque-Response test is based on phage amplification technology which uses bacteriophage to detect viable TB bacilli in the specimen. The test is a simple, manual method that provides results within 2 days from receipt of the patient's specimen.

This study is evaluating the performance of **FAST**Plaque-Response in determining rifampicin resistance from smear-positive sputum specimens from re-treatment patients in the Port Elizabeth metropolitan area, South Africa. The performance of **FAST**Plaque-Response was compared with conventional LJ culture and 7H11 susceptibility testing. Analysis of *rpdB* mutations was used to resolve discrepant results.

Excellent agreement was reported between **FAST**Plaque-Response and the conventional method (n=229). All rifampicin resistant patients were multidrug resistant. **FAST**Plaque-Response may provide a rapid means for screening patients not susceptible to standard rifampicin-based regimens, and allow alternative treatment strategies to be adopted, thus improving outcome for individual patients and reducing spread of multidrug resistant disease.

## METHODS

Patients were recruited at six clinics in the Port Elizabeth and Uitenhage Metropolitan area. An additional sputum specimen was collected from re-treatment patients who had a recent positive smear result (usually within the previous week) and had returned to the clinic to commence the re-treatment regimen. Specimens were transported by courier to the Cape Town laboratory.

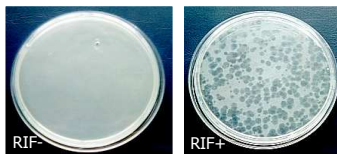
A direct ZN smear was performed and graded according to the IUATLD guidelines. Smears with a grading of 1+ or greater were included in the study. Primary isolation of *M. tuberculosis* complex from sputum specimens was performed using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method. Following centrifugation, the pellet was suspended in approximately 1.5ml of phosphate buffer pH 6.8. Two portions of 0.1ml were inoculated on Lowenstein-Jensen medium and 0.1ml onto a selective Middlebrook 7H11 plate. A concentrated auramine smear was also performed. A sample was submitted for *rpdB* mutation analysis using dot-blot method.

The remaining pellet was tested using the **FAST**Plaque-Response test according to manufacturer's instructions.

Cultures were incubated for up to 8 weeks in 5-10% CO<sub>2</sub> atmosphere. Positive cultures were confirmed as *M. tuberculosis* complex by ZN staining and *p*-nitrobenzoic acid (PNB) testing. Conventional susceptibility testing was performed using the modified proportion method on Middlebrook 7H11 medium containing 1.0µg/ml rifampicin and 0.2µg/ml isoniazid respectively



## FASTPlaque-Response



Incubate sputum sample with and without rifampicin at 37°C for 24 hours. Rifampicin resistant strains survive drug treatment and produce plaques, whilst susceptible strains do not.

## RESULTS

**Table 1. Overall comparison of **FAST**Plaque-Response test with indirect 7H11 proportion method susceptibility test, unresolved results per specimen (n=345)**

	7H11 Resistant	7H11 Susceptible	Culture negative	Contam <sup>3</sup>	Invalid <sup>4</sup>	Total
<b>FAST</b> Plaque-Response Resistant	83	4 <sup>2</sup>	4	3	1	95
<b>FAST</b> Plaque-Response Susceptible	4	138	9	6	5	162
RIF- <100 plaques <sup>1</sup>	3	27	1	3	3	37
Contam	15	25	1	5	5	51
Total	105	194	15	17	14	345

<sup>1</sup> less than 100 plaques obtained on the RIF- plate

<sup>2</sup> three of these specimens had mutations in the *rpdB* gene associated with rifampicin resistance

<sup>3</sup> contaminated on either culture or 7H11 susceptibility test

<sup>4</sup> invalid result on 7H11 method due to insufficient growth on 7H11 control plate

Unexpected high level of contamination on **FAST**Plaque-Response was mostly due to storage of plates at room temperature after incubation and prior to reading results, leading to overgrowth of contaminants.

**Table 2. Comparison of **FAST**Plaque-Response with resolved susceptibility test results (n=229)**

	7H11 Resistant	7H11 Susceptible	Total
<b>FAST</b> Plaque-Response Resistant	86	1	87
<b>FAST</b> Plaque-Response Susceptible	4	138	142
Total	90	139	229

Sensitivity = 86/90 = 95.6%  
Specificity = 138/139 = 99.3%  
Overall accuracy = 224/229 = 97.8%

## CONCLUSIONS

**FAST**Plaque-Response performance was comparable with indirect susceptibility testing, with results in 2 days from receipt of specimen compared with up to 11 weeks by the conventional method.

Use of a simple, rapid test, such as **FAST**Plaque-Response, could play an important role in DOTS-Plus programmes enabling the rapid detection of MDR-TB cases, improving the patient prognosis and reducing the period of infectiousness of these patients.

Rifampicin resistant cases could be rapidly treated with an appropriate standardised second-line regimen. Further susceptibility testing to second-line drugs may then be carried out to individualise therapy, if available. This could contribute to reducing the ongoing transmission of MDR-TB strains in the community and ultimately decreasing the overall burden of disease.

## ACKNOWLEDGEMENTS

We are grateful to the Port Elizabeth Department of Health, clinic staff and patients for their support, and to Mrs. C. Dolley, clinical nurse coordinator.

This investigation received financial support from the UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Diseases (TDR).



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