



Evaluation of a rapid screening test for rifampicin resistance in re-treatment tuberculosis patients in the Eastern Cape

H Albert, A P Trollip, T Seaman, C Abrahams, R J Mole, A Jordaan, T Victor, E Hoosain

Background and objectives. Patients with multidrug-resistant (MDR) tuberculosis (TB) are at high risk of treatment failure. It is anticipated that early identification of MDR-TB and appropriate treatment will improve patient outcome and disease control. We evaluated the rapid detection of rifampicin resistance in previously treated TB patients, directly from acid-fast bacilli (AFB)-positive sputum using a phage-based test, FASTPlaque-Response (Biotec Laboratories Ltd, Ipswich, UK). The ability of rifampicin resistance to predict MDR-TB was also determined.

Design. A prospective study was done comparing performance of the rapid phage test with conventional culture and drug susceptibility testing (DST) in AFB-positive TB patients.

Setting. Five primary health clinics and one TB referral centre in the Port Elizabeth Metropolitan area, Eastern Cape.

Outcome measures. Sensitivity, specificity and overall accuracy of the phage test were determined compared with gold standard culture and DST. Discrepant results were resolved by molecular detection of mutations conferring rifampicin resistance. The proportion of rifampicin-resistant strains that were MDR was also determined.

Results. Previously treated patients were at a high risk of MDR-

TB (35.7%). Sensitivity, specificity and overall accuracy of FASTPlaque-Response for rifampicin resistance determination were 95.4% (95% confidence interval (CI): 91.0 - 99.8%), 97.2% (95% CI: 94.5 - 99.9%) and 96.5% (95% CI: 94.1 - 98.9%) respectively compared with conventional DST (unresolved), calculated for specimens that had both FASTPlaque-Response and conventional DST results available. FASTPlaque-Response results were available in 2 days instead of 28 - 85 days with conventional DST. However, only 70.6% of FASTPlaque-Response results were interpretable compared with 86.3% of conventional DST results. The majority (95.5%) of rifampicin-resistant strains were MDR-TB.

Conclusions. Rapid detection of rifampicin resistance using FASTPlaque-Response could contribute to improved management of patients at risk of MDR-TB, such as previously treated patients. However, improvement in control of specimen-related contamination is needed to ensure that a higher proportion of FASTPlaque-Response results are interpretable. Where indicated, early modification of therapy could improve patient prognosis and reduce disease transmission.

S Afr Med J 2007; 97: 858-863.

South Africa has one of the highest rates of tuberculosis (TB) in the world. It is ranked ninth in the world in terms of the number of cases, and has an estimated incidence of 558 cases per 100 000 population.¹ The TB burden is exacerbated by a high incidence of HIV infection.² Between 28.2% and 71.9% of TB cases in the different provinces are estimated to be HIV-

positive.³ The Eastern Cape suffers a high burden of TB, having a higher-than-national average incidence and the second-highest case load of the 9 provinces.²

Multidrug-resistant TB (MDR-TB), defined as resistance to at least rifampicin and isoniazid, is a threat to successful TB control.⁴ A survey of MDR-TB in South Africa³ reported a mean rate of 1.6% in new cases and 6.6% in previously treated cases. South Africa is reported to have at least 6 000 new cases of MDR-TB per year.⁵

Patients with MDR-TB are at a high risk of treatment failure on short-course chemotherapy.⁶ Conventional drug susceptibility testing for TB has a long turnaround time of several weeks to months, which delays diagnosis of MDR-TB patients. Earlier identification of patients with MDR-TB and appropriate treatment is expected to improve individual patient outcomes and overall disease control.

Phage amplification (FASTPlaque) technology^{7,8} (Biotec Laboratories, Ipswich, UK) is a rapid method for both the diagnosis of TB⁹⁻¹² and rifampicin susceptibility testing.¹³⁻¹⁵ This technology utilises mycobacteriophage (phage; bacterial viruses) to detect viable *Mycobacterium tuberculosis* complex organisms. The FASTPlaque technology has been previously

Biotec Laboratories Ltd, Somerset Hospital, Cape Town

H Albert, PhD (current address: Foundation for Innovative New Diagnostics (FIND), Cape Town)

AP Trollip, DTech

T Seaman, MSc (Med) (current address: Department of Clinical Pharmacology, University of Cape Town)

C Abrahams, BSc Hons

RJ Mole, PhD

Medical Research Council Centre for Molecular and Cellular Biology, Department of Medical Biochemistry, University of Stellenbosch, Tygerberg, W Cape

A Jordaan, ND Med Tech

T Victor, PhD

Nelson Mandela Metropolitan Municipality, Port Elizabeth

E Hoosain, MB ChB

Corresponding author: H Albert (alberth@mweb.co.za)



evaluated in South Africa.^{9,12-14} as well as in other high-burden countries.^{10,13,15}

This study sought to evaluate the performance of phage amplification technology in detecting rifampicin resistance directly from acid-fast bacilli (AFB) smear-positive sputum, in a population of patients previously treated for TB (re-treatment cases), who are at increased risk of MDR-TB. The utility of rifampicin resistance as an indicator for MDR-TB in this population was also determined.

Material and methods

Patients

Patients were recruited at 6 clinics (5 primary health care facilities and 1 TB referral centre) in the Port Elizabeth and Uitenhage Metropolitan area in the Eastern Cape between 15 September 2003 and 26 May 2004. Symptomatic patients presenting at the clinics were tested according to TB control programme guidelines.¹⁶ Patients who had been previously treated for TB and whose sputum was found to be AFB smear-positive (re-treatment cases) were selected for inclusion in this study. Patients were categorised as treatment failure (RF), treatment interruption (RI), previously cured (RC), or completed previous treatment (RT). The study was subject to ethical review by the South African Medical Research Council and World Health Organization (WHO) committees. Written informed consent in either English, Xhosa or Afrikaans was obtained from all patients.

Sample size

Published data estimated approximately 8% MDR-TB in previously treated patients in the Eastern Cape.³ Therefore, a sample size of 500 smear-positive patients was estimated to include at least 40 rifampicin-resistant specimens. Forty rifampicin-resistant TB strains would allow estimation of the true sensitivity of the test (ability to detect true rifampicin resistance) to be at least 95% compared with the conventional method, at a significance level of 0.05, and a reliability level of 7%. Based on previous studies,¹³ results of approximately 85% of conventional and phage-based methods were anticipated to be available for analysis (remaining results were expected to be unavailable because of contamination or un-interpretable results). Therefore a sample size of at least 590 smear-positive specimens was set for the study.

Laboratory testing

Before commencing treatment 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 standard re-treatment regimen.¹⁶ Specimens were stored refrigerated for a maximum of 3 days before transport to the laboratory

according to the manufacturer's recommendations.¹⁷ Specimens were transported by courier to the laboratory in Cape Town for testing by smear microscopy, FASTPlaque-Response, conventional indirect drug susceptibility testing (DST) and molecular testing.

On receipt a direct smear was prepared from each specimen, stained using the Ziehl-Neelsen method and examined at $\times 1\,000$ magnification. Smears were graded according to the International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines,¹⁸ as followed by the National Health Laboratory Service (NHLS). Specimens with a smear grading of 1+ or more were included in the study.

Sputum specimens were decontaminated using the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method.¹⁹ Following centrifugation, the pellet was suspended in approximately 1.5 ml of phosphate buffer pH 6.8. Two portions of 0.1 ml were inoculated on Lowenstein-Jensen (LJ) medium and 0.2 ml onto a selective Middlebrook 7H11 plate. A 0.5 ml portion of the processed sediment was removed for DNA sequencing.

Cultures were incubated for up to 8 weeks in a 5 - 10% CO₂ atmosphere. Positive cultures were confirmed as *M. tuberculosis* complex by use of Ziehl-Neelsen staining and *p*-nitrobenzoic acid (PNB) testing. *M. tuberculosis* complex does not grow within 3 days, is non-pigmented and does not grow on PNB-containing medium. Conventional indirect DST was performed using a modified proportion method on Middlebrook 7H11 medium containing 1.0 µg/ml rifampicin and 0.2 µg/ml isoniazid respectively.¹⁹ Isolates were classified as resistant if there was 1% or more growth on the drug-containing medium compared with the control.

FASTPlaque-Response test

FASTPlaque-Response tests were provided by Biotec Laboratories Ltd. (Ipswich, UK) and were performed according to the manufacturer's instructions.¹⁷ Briefly, the residual specimen was washed in medium and then split, with one portion incubated overnight with rifampicin, and the other incubated in drug-free medium. The viability of the *M. tuberculosis* strain was assessed by its ability to support phage replication (production of plaques) after incubation with rifampicin. Specimen results were considered valid if 100 plaques or more were observed on the rifampicin-free (RIF-) plate. Strains were determined to be susceptible to rifampicin if less than 50 plaques resulted from the rifampicin-containing sample (RIF+), and rifampicin-resistant if 50 plaques or more were obtained.

Discrepant results

For any specimens in which the FASTPlaque-Response and the conventional drug susceptibility result disagreed, both methods were repeated. FASTPlaque testing was performed



from the positive culture. In addition, molecular testing for rifampicin resistance was performed on all samples.²⁰ These results were used to confirm the presence of mutations related to rifampicin resistance in specimens in which there was a discrepancy between the FASTPlaque-Response and conventional DST result.

Phage-based testing was performed on cultures of all strains in which less than 100 plaques were obtained on the FASTPlaque-Response test but the smear and culture were both positive. This was to determine whether the phage was able to infect these particular strains of TB. The FASTPlaque-Response procedure was followed as described earlier, except that the rifampicin solution used for indirect testing was half the concentration used in the direct test (final concentration, 5 µg/ml). Testing directly from sputum requires a higher concentration of rifampicin because of binding and inactivation of rifampicin in the complex sputum matrix, to allow an equivalent active drug concentration. Interpretation of results was as previously described.¹⁴

Results

A total of 573 specimens were received from 556 patients. Six specimen containers were empty on receipt at the laboratory, and 8 specimens were more than 4 days old and had been stored inappropriately before shipment to the laboratory and were therefore excluded from testing. One hundred and seventy smear-negative specimens and 25 scanty positive specimens were received and were excluded from the study. Of the remaining 364 specimens included in the study, 142, 125 and 97 specimens were 1+, 2+ and 3+ smear-positive respectively. Results of 50 specimens (13.7%) were unavailable using the conventional method because of failure to grow, contamination of the culture, or indirect susceptibility test.

Table I shows the resistance patterns of the strains using the conventional drug susceptibility method, according to patient category. Approximately 10% of re-treatment patients enrolled in the study had previously failed treatment. The highest proportion of MDR-TB was found in patients who had previously failed treatment (73.3%), while lower but substantial levels of MDR-TB were present in patients who had previously been cured (36.9%) or who had interrupted treatment (27.7%). Patients who had previously completed treatment had the lowest MDR rates in this population. A substantially higher level of MDR-TB was obtained compared with the published data which estimated approximately 8% MDR-TB in previously treated cases.³

The FASTPlaque-Response test and conventional DST results are shown in Table II. Overall, 314 specimens (202 rifampicin-susceptible and 112 rifampicin-resistant) were culture-positive for *M. tuberculosis* complex and gave an interpretable conventional susceptibility test result (86.3%). The conventional susceptibility result was unavailable in a total

of 50 specimens (13.7%). Nineteen specimens were culture-negative (5.2%), 17 specimens were contaminated on culture or DST (4.7%), and 14 specimens gave invalid results because of insufficient growth on the DST control plate (3.8%).

Of the rifampicin-resistant strains, 95.5% (107/112) were also resistant to isoniazid (MDR), while 5 strains were isoniazid-susceptible. One hundred and ninety strains were rifampicin and isoniazid-susceptible, while 12 strains were isoniazid-resistant and rifampicin-susceptible. No non-tuberculosis mycobacteria (NTMs) were isolated from any of the specimens.

For the FASTPlaque-Response test, 257 specimens (70.6%) gave interpretable results overall. Assay controls were out of specification for 4 batches of tests, which led to results of 18 specimens not being interpretable (4.9%). In addition, 38 specimens (10.4%) gave less than 100 plaques on the RIF- plate and 51 specimens (14.0%) were contaminated to such an extent that results could not be interpreted.

Table III shows the FASTPlaque-Response results compared with the conventional DST, subdivided according to smear grading (1+, 2+ or 3+). Of the specimens that were culture-positive and had a conventional DST result, 72.9% (229/314) specimens gave interpretable results using the FASTPlaque-Response test. Seventy-three per cent (65/89) of 3+ smear-positive specimens, 78.3% (83/106) of 2+ smear-positives and 68.1% (65/89) of 1+ smear-positive specimens gave interpretable results. A further 28 specimens gave interpretable results on the FASTPlaque-Response test but were culture-negative, contaminated or gave uninterpretable results using conventional DST.

LJ cultures took 29.5 ± 8.4 days (mean \pm standard deviation (SD)) to become positive, with times ranging from 7 to 64 days, and cultures on selective 7H11 medium took 17.9 ± 5.6 days (mean \pm standard deviation (SD)), ranging from 10 to 39 days. Conventional DST took 21 days. Therefore the total turnaround time for the conventional testing ranged from 28 to 85 days, with a mean \pm SD of 50.5 ± 8.4 days on LJ medium and 38.9 ± 5.6 days on selective 7H11 medium. FASTPlaque-Response results were available in 2 days from receipt of the specimen in the laboratory.

Of the 31 specimens that had less than 100 plaques on the RIF- plate but had a positive culture and DST result, 96.8% (30/31) of the strains could be infected by the phage when tested from the positive culture.

Contamination of 14.0% (51/364) on the FASTPlaque-Response test was much higher than anticipated from previous studies,^{9,13} and appeared to be related to growth of contaminants which occurred during storage of plates at room temperature for up to several days after the final overnight incubation but before reading plates. Contamination was also found in at least 1 of the 3 cultures inoculated in 42 specimens (11.5%), although contamination precluded a result being obtained by conventional susceptibility testing in only 19



Table I. Drug resistance patterns of the *Mycobacterium tuberculosis* strains using the conventional proportion method according to patient category (N)

Patient category	H ^S R ^S	H ^R R ^S	H ^S R ^R	H ^R R ^R	Total (N (%))
Previously cured (RC)	71	6	0	45	122 (38.9)
Treatment failure (RF)	6	1	1	22	30 (9.6)
Treatment interrupted (RI)	94	4	4	39	141 (44.9)
Previously completed treatment (RT)	19	1	0	1	21 (6.7)
Total (%)	190 (60.5)	12 (3.8)	5 (1.6)	107 (34.1)	314 (100)

H^S = isoniazid-susceptible; H^R = isoniazid-resistant; R^S = rifampicin-susceptible; R^R = rifampicin-resistant.

Table II. Overall comparison of the FASTPlaque-Response test with the indirect 7H11 proportion method susceptibility test, unresolved results per specimen (N = 364)

FASTPlaque-Response	Indirect 7H11 proportion method					Total (%)
	Resistant	Susceptible	Culture-negative	Contaminated [†]	Invalid [‡]	
Resistant	83	4 [§]	5	3	0	95 (26.1)
Susceptible	4	138	12	6	2	162 (44.5)
RIF- < 100 plaques*	4	27	4	3	0	38 (10.4)
Contaminated	15	25	4	4	3	51 (14.0)
Assay control out of specification	6	8	4	0	0	18 (4.9)
Total (%)	112 (30.8)	202 (55.5)	19 (5.2)	17 (4.7)	14 (3.8)	364 (100)

* Less than 100 plaques obtained on the RIF- plate.

[†] Contaminated on either culture or 7H11 susceptibility test.

[‡] Invalid result on 7H11 method due to insufficient growth on 7H11 control plate.

[§] Three of these specimens had mutations in the *rpoB* gene associated with rifampicin resistance.

RIF = rifampicin.

Table III. Comparison of FASTPlaque-Response with indirect 7H11 proportion method susceptibility test results related to smear grading (1+ to 3+ smear-positive), unresolved data

FASTPlaque-Response	Indirect 7H11 proportion method				
	Resistant	Susceptible	Culture-negative	Contaminated [†]	Invalid
3+ smear-positive (N = 97)					
Resistant	29	2 [‡]	0	0	0
Susceptible	0	34	2	2	0
RIF- < 100 plaques*	1	0	0	0	0
Contaminated	9	7	1	3	0
Assay control out of specification	2	5	0	0	0
2+ smear-positive (N = 125)					
Resistant	25	1 [‡]	1	1	1
Susceptible	4	53	2	1	4
RIF- < 100 plaques*	1	11	0	3	0
Contaminated	4	6	1	0	3
Assay control out of specification	0	1	2	0	0
1+ smear-positive (N = 142)					
Resistant	29	1 [‡]	3	2	0
Susceptible	0	51	5	3	1
RIF- < 100 plaques*	2	16	1	3	0
Contaminated	2	12	0	1	2
Assay control out of specification	4	2	2	0	0

*Less than 100 plaques obtained on the RIF- plates.

[†] Contaminated on either culture or 7H11 susceptibility test.

[‡] One 1+ smear-positive, one 2+ smear-positive and one 3+ smear-positive specimen had a mutation in the *rpoB* gene associated with rifampicin resistance.

RIF = rifampicin.



specimens (5.2%), owing to contamination on all 3 cultures (data not shown).

Results of both the FASTPlaque-Response and conventional DST were available for 229 specimens (Table IV). Sensitivity, specificity and overall accuracy of the test were 95.4% (95% CI: 91.0 - 99.8%), 97.2% (95% CI: 94.5 - 99.9%) and 96.5% (95% CI: 94.1 - 98.9%) respectively compared with conventional culture and DST (unresolved) in specimens for which both results were available. Calculation of the kappa statistic²¹ showed 'almost perfect agreement' ($\kappa = 0.93$) of the FASTPlaque-Response and conventional method in specimens for which interpretable results were available for both methods.

Table IV. Comparison of FASTPlaque-Response test with indirect 7H11 proportion method susceptibility test, unresolved data per specimen (N = 229)

FASTPlaque-Response	Indirect 7H11 proportion method		
	Resistant	Susceptible	Total
Resistant	83	4 *	87
Susceptible	4	138	142
Total	87	142	229

* Three of these specimens had mutations in the *rpoB* gene associated with rifampicin resistance.

Four discrepant results were obtained in which the FASTPlaque-Response test identified a strain as being resistant, whereas the indirect DST determined the strain to be susceptible. Repeat testing confirmed the original results of both methods. However, molecular testing reported that 3 of the 4 strains had mutations that conferred rifampicin resistance. These 3 strains were therefore considered to be rifampicin-resistant, while the fourth strain was considered susceptible. In addition, 4 discrepant results were obtained in which the FASTPlaque-Response test gave a susceptible result whereas the indirect susceptibility test result was resistant. Molecular testing found mutations conferring rifampicin resistance in 3 of the strains. Repeat indirect testing results agreed with the initial results in all cases. These 4 strains were assumed to be true rifampicin-resistant cases.

Discussion

MDR-TB is already impacting on TB control in South Africa. Treatment of MDR-TB uses more toxic and expensive drugs, for longer periods, and is less effective than treatment of drug-susceptible disease. The cost of treating 1 case of MDR-TB is up to 25 times the cost of treating 1 drug-susceptible case.⁵ Drug susceptibility testing is critical in the management of patients with MDR-TB. However, conventional drug susceptibility methods have a slow turnaround time of weeks to months which hinders rapid decision making by clinicians. MDR-TB patients who remain undetected will be

infectious for longer, leading to further opportunity for disease transmission.²²

In this study FASTPlaque-Response, a rapid test for rifampicin resistance was compared with conventional DST in re-treatment TB patients, who are at increased risk of MDR-TB. The FASTPlaque-Response test showed good correlation with the conventional DST results in those specimens for which interpretable results were available. The FASTPlaque-Response test also identified 3 strains as being resistant that were susceptible by conventional DST but were found to be rifampicin-resistant by molecular testing.²⁰ In 97% of cases (539/556 patients) a single specimen was tested per patient, but for 3% of patients (17/556) 2 specimens were included in the study. This may affect the independence of the results, but since such a small percentage of patients submitted multiple specimens it is unlikely that substantial bias was introduced.

A limitation of the study was that all testing was performed by the same technologists and blinding of specimens did not occur. However, the possibility of introducing bias is not expected to be substantial since the FASTPlaque-Response results were reported first and there was a substantial delay before results were reported for the conventional culture and DST.

Results of the FASTPlaque-Response test were available in 2 days from receipt of the specimen, a reduction of between 26 and 83 days compared with conventional testing. No specialised equipment is required to perform the test, which is an important consideration in high-burden countries where procurement and maintenance of equipment can be a hurdle to implementation of new technologies. The rate of contamination experienced in this study was significantly higher than in previous studies in South Africa^{9,13} although higher rates of contamination have been reported elsewhere.¹¹ This was a limitation of the test in this study since only 70.6% of specimens yielded an interpretable result compared with 86.3% of conventional DST results.

This contamination appeared to be owing to storage of some plates at room temperature in the laboratory for several days before reading results, during which time growth of contaminants obscured the results. The recommendation that results should be read immediately after overnight incubation, or if necessary stored at 2 - 8°C, should reduce contamination to acceptable levels. Furthermore, the inclusion of antimicrobials in the test medium has since been implemented and this has resulted in reduction in contamination and increase in the level of interpretable results to 87.8% in specimens up to 3 days old or 79.1% in specimens up to 14 days old.²³ The phage was able to detect the majority of different TB strains. It is likely that the lack of interpretable results, because of low numbers of plaques in the drug-free sample, may be due to inhibitory substances present in the sputum and/or the metabolic state of the cells *ex vivo*.^{11,14}



The FASTPlaque-Response test only determines the rifampicin resistance of TB, although MDR-TB is defined as resistance to at least rifampicin and isoniazid. However, in this setting, rifampicin resistance was a good indicator of MDR-TB, with 95.5% of rifampicin-resistant strains also being isoniazid-resistant. This is in agreement with data from other countries³ showing that rifampicin-resistance is often a good marker for multidrug resistance. This high level of correlation may be related to the exclusive use of fixed-dose combination tablets for first-line TB treatment in South Africa and therefore limited opportunity for effective monotherapy owing to non-compliance with treatment. The rate of MDR-TB in this group of patients was substantially higher than the overall rate estimated for the Eastern Cape. This was likely because of inclusion of a TB referral centre as one of the study sites, from which the majority of the MDR-TB patients in the study were enrolled.

The FASTPlaque-Response test was evaluated in AFB-positive sputum specimens only, as currently recommended by the manufacturer. AFB-positive patients are the most infectious cases and therefore are high priority in terms of early detection, allowing suitable infection control measures and initiation of appropriate treatment. Smear-negative specimens could first be cultured and then tested using the FASTPlaque technology as previously described,¹⁴ or using conventional culture-based susceptibility testing.

The appropriate timing of DST has been highlighted by the Stop TB Working Group for MDR-TB²⁴ as an important research focus for DOTS-Plus initiatives. A high proportion of patients who go on to fail standard treatment have a positive sputum smear at 2 - 3 months (74%), while only a minority of patients who go on to be cured have a positive smear at that time.²⁴ Delay in detection and appropriate treatment of MDR-TB patients may also lead to acquisition of further resistance.⁴

Use of a rapid screening test for rifampicin resistance such as FASTPlaque-Response could play an important part in the TB control programme. Earlier detection of MDR-TB cases would allow appropriate management and therapy and could improve patient outcomes as well as reduce disease transmission.

We would like to thank the Port Elizabeth Department of Health for supporting this project. We are grateful to the clinic staff and patients who made this study possible and to Mrs C Dolley, the clinical nurse co-ordinator (employed by Biotec Laboratories Ltd.) who supervised collection and transport of specimens. FASTPlaque-Response tests were supplied free of charge by Biotec Laboratories Ltd. This investigation received financial support from the United Nations Development Programme (UNDP)/World Bank/World Health Organization Special Programme for Research and Training in Tropical

Diseases (WHO/TDR). The Foundation for Innovative New Diagnostics (FIND), a Geneva-based non-profit foundation focused on new TB diagnostic technologies for use in high-burden countries, has a co-development agreement with Biotec Laboratories Ltd., which supports the development, evaluation and demonstration of the FASTPlaque-Response test. FIND is currently evaluating the test for public-sector use. The authors are grateful to Drs Mark Perkins and Rick O'Brien for reviewing an earlier version of the manuscript.

References

1. World Health Organization. Global Tuberculosis Control. Surveillance, Planning, Financing. 2004. Geneva: WHO, 2004.
2. Bamford L, Loveday M, Verkuil S. Tuberculosis. In: Jumba P, Day C, Ntuli A, eds. *South African Health Review 2003 / 04*. Durban: Health Systems Trust, 2004.
3. World Health Organization. Anti-tuberculosis Drug Resistance in the World. Report No. 3. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance 1999 - 2002. Geneva: WHO, 2004.
4. Farmer P, Bayona J, Becerra M, et al. The dilemma of MDR-TB in the global era. *Int J Tuberc Lung Dis* 1998; 2: 869-876.
5. Department of Health. *DOTS-Plus for Standardised Management of Multidrug-Resistant Tuberculosis in South Africa*. Policy Guidelines. Pretoria: DOH, 2004.
6. Espinal MA, Kim SJ, Suarez PG, et al. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. *JAMA* 2000; 283: 2537-2545.
7. Wilson SM. Method to detect bacteria. PCT Patent WO97/022713. 1997.
8. Wilson SM, Al-Suwaidi Z, McNeerney R, Porter J, Drobniowski F. Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of *Mycobacterium tuberculosis*. *Nature Medicine* 1997; 3: 465-468.
9. Albert H, Heydenrych A, Brookes R, et al. Performance of a rapid phage-based test, FASTPlaqueTB, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. *Int J Tuberc Lung Dis* 2002; 6: 529-537.
10. Marei AM, El-Behedy EM, Mohtady HA, Afify AF. Evaluation of a rapid bacteriophage-based method for the detection of *Mycobacterium tuberculosis* in clinical samples. *J Med Microbiol* 2003; 52: 331-335.
11. Muzaffar R, Batool S, Aziz F, Naqvi A, Rizvi A. Evaluation of the FASTPlaque TB assay for direct detection of *Mycobacterium tuberculosis* in sputum specimens. *Int J Tuberc Lung Dis* 2002; 6: 635-640.
12. Wilson, D, Maartens G. Identifying sputum smear-negative tuberculosis in HIV infected adults using a bacteriophage assay. First South African AIDS Conference, Durban, South Africa, August 2003 (Abstract).
13. Albert H, Trollip A, Seaman T, Mole RJ. Simple, phage-based (FASTPlaque) technology to determine rifampicin resistance of *Mycobacterium tuberculosis* directly from sputum. *Int J Tuberc Lung Dis* 2004; 8: 1114-1119.
14. Albert H, Heydenrych A, Mole R, Trollip AP, 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.
15. Kisa P, Albay A, Bedir O, Baylan O, Doganci L. Evaluation of FASTPlaqueTB-RIF for determination of rifampicin resistance in *Mycobacterium tuberculosis* complex isolates. *Int J Tuberc Lung Dis* 2003; 7: 284-288.
16. Department of Health. *The South African Tuberculosis Control Programme Practical Guidelines*. Pretoria: DOH, 1996.
17. Biotec Laboratories Ltd. FASTPlaque-Response product insert. For performance evaluation only. Version 180903. Biotec Laboratories Ltd., Ipswich, UK, 2003.
18. Enarson DA, Rieder HL, Arnadottir T, Trebusq A. *Management of Tuberculosis. A Guide for Low Income Countries*. Paris: International Union Against Tuberculosis and Lung Disease, 2000.
19. Kent PT, Kubica GP. *Public Health Mycobacteriology. A Guide for the Level III Laboratory*. Atlanta, GA: Centers for Disease Control, 1985.
20. Victor TC, Jordaan AM, van Rie A, et al. Detection of mutations in drug resistance genes of *Mycobacterium tuberculosis* by a dot-blot hybridisation strategy. *Tuberc Lung Dis* 1999; 79: 343-348.
21. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159-174.
22. Teixeira L, Perkins MD, Johnson JL, et al. Infection and disease among household contacts of patients with multidrug-resistant tuberculosis. *Int J Tuberc Lung Dis* 2001; 5: 321-328.
23. Mole RJ, Trollip AP, Seaman T, Abrahams C, Albert H. Improved contamination control using a new antimicrobial supplement developed for rapid phage-based rifampicin susceptibility testing. *Int J Tuberc Lung Dis* 2005; 9: Suppl 1, S271.
24. Chavez Pachas AM, Blank R, Smith Fawzi MC, Bayona J, Becerra MC, Mitnick CD. Identifying early treatment failure on category I therapy for pulmonary tuberculosis in Lima Ciudad, Peru. *Int J Tuberc Lung Dis* 2004; 8(1): 52-58.

Accepted 13 December 2006