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ORIGINAL ARTICLE

Development of an antimicrobial formulation for control of specimen-related contamination in phage-based diagnostic testing for tuberculosis

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Abstract

Aims: To develop and evaluate an antimicrobial supplement for use with phage-based tests for rapid detection of drug resistance of tuberculosis (TB).
Methods and Results: An antimicrobial formulation containing nystatin, oxacillin and aztreonam (NOA) (final concentrations of 50 000 IU l^{-1}, 2 mg l^{-1}, and 30 mg l^{-1} respectively) was developed. This formulation was tested for its influence on detection of a number of *Mycobacterium tuberculosis* (MTB) strains using the phage amplification (*FASTPlaque*) assay. Addition of the supplement did not lead to significant reduction in assay sensitivity. Antimicrobial efficacy was assessed with a range of Gram-positive and -negative organisms. The NOA supplement had a broad antimicrobial effect. The supplement was tested for its effect on growth of MTB culture, and on determination of rifampicin resistance using the phage-based methodology (*FASTPlaque–Response*). NOA did not significantly affect the growth of a range of rifampicin susceptible and resistant MTB strains, nor did it have an adverse effect on the number of interpretable results, nor the ability to discriminate between rifampicin susceptibility and resistance.
Conclusion, Significance and Impact of Study: Use of NOA antimicrobial supplement with rapid phage-based tests for TB will increase the proportion of interpretable results obtained, and enable their wider implementation in disease-endemic countries by improved control of specimen-related contamination.

Introduction

Tuberculosis (TB) remains a global health emergency. There is an urgent need for new diagnostic tests that can improve the speed and accuracy of diagnosis of the disease, as well as providing a rapid means of detecting drug resistance. Ideally, these tests should be able to be implemented in district or regional laboratories in high-burden countries, in close proximity to the patients, in order to maximize the benefit of increased speed of diagnosis (Perkins and O'Brien 2006).

However, the widespread implementation of new technologies in high-burden settings may be hampered by laboratory quality issues, such as high levels of contamination, often related to long specimen transport times. Contamination leads to fewer results being reported, and the requirement for further samples to be tested, or may result in TB positive cases being missed. A recent meta-analysis of contamination in mycobacterial cultures from clinical specimens (mostly respiratory specimens) showed the problem to be common, with contamination rates of 12·8% (95% confidence interval, 12·3–13·4%) on solid culture, 8·6% (95% confidence interval, 8·2–9·9%) in BACTEC 960 / MGIT and 4·4% on solid Lowenstein-Jensen medium (95% confidence interval, 4·1–4·7%) (Cruciani *et al.* 2005). Much higher rates of contamination have

been reported (up to 40·4%), particularly in developing country settings (Huang *et al.* 2001; Mbulo *et al.* 2004).

The principal strategy used to address contamination in culture-based TB detection methods is decontamination of the clinical specimen prior to inoculation onto the growth medium. A number of decontamination methods are in common use for processing of respiratory specimens, most commonly using sodium hydroxide with or without a mucolytic agent such as *N*-acetyl-L-cysteine (Kent and Kubica 1985). Decontamination is a balance between the inhibition of growth of bacterial/ fungal contaminants and maintaining the viability of mycobacteria in specimens. Harsher decontamination will effectively inhibit the growth of contaminants, but also reduce the recovery rates of mycobacteria, while milder methods will allow recovery of more mycobacteria, but will result in higher rates of contamination.

Several antibiotic formulations have been developed for use with TB culture media, in conjunction with the above decontamination procedures. Mitchison *et al.* (1972) developed the PACT formulation, containing polymixin B, amphotericin B, carbenicillin and trimethoprim, for selective isolation of mycobacteria in egg and agar-based media. This formulation was later used in conjunction with liquid media, such as Kirchner's liquid media (Allen *et al.* 1983). A variation of this formulation, PANTA, containing polymixin B, amphotericin, nystatin, trimethoprim and azlocillin was developed for use with Middlebrook 7H12 liquid medium (Siddiqi and Hwangbo 1986).

A number of new technologies are becoming available for rapid diagnosis and drug susceptibility testing of *Mycobacterium tuberculosis* that may be appropriate for use in high-burden countries (Perkins and O'Brien 2006). One such method, phage amplification (*FASTPlaque*™) technology (Rees *et al.* 1992; Wilson 1997; Wilson *et al.* 1997) has been applied to both rapid diagnosis and drug susceptibility testing. The technique uses bacteriophage to report the presence of viable TB in a specimen within two days. The procedure for *FASTPlaque* testing includes two incubation steps, the first incubation being in liquid medium, during which time any organisms surviving the decontamination process have an opportunity to resuscitate and become susceptible to phage infection. The second incubation step involves visualization of plaques produced in a lawn of rapidly growing helper bacteria (termed Sensor cells). Contamination that overgrows the lawns of Sensor cells may hinder visualization of plaques and interpretation of results. Varying levels of contamination have been reported with this technology, ranging from zero to 18·2% (Albert *et al.* 2002, 2004; Muzaffar *et al.* 2002; Marei *et al.* 2003), usually reflecting the contamination rates for culture experienced in the same setting.

The *FASTPlaque* technique has recently been applied to the detection of rifampicin resistance directly from sputum within 2 days (Albert *et al.* 2004). However, the level of contamination has been highlighted as a potential limiting factor in the widespread implementation of the technology (Mbulo *et al.* 2004; Albert *et al.* 2007).

This paper describes the laboratory evaluation of an antimicrobial formulation containing nystatin, oxacillin and aztreonam (NOA) for control of specimen-related contamination which is compatible with phage-based technology. Aztreonam is bactericidal for a wide spectrum of Gram-negative aerobic organisms, acting by inhibition of bacterial cell wall synthesis, and is highly resistant to beta-lactamases. Oxacillin is active against penicillin-resistant staphylocooci and Group A beta-haemolytic streptococci. Nystatin's main activity is against *Candida* spp. and some other fungi and yeasts (Martindale and Reynolds 1993). The use of this antimicrobial combination is aimed at improving the robustness of rapid phage-based TB diagnostic and rifampicin resistance tests, facilitating their wider implementation, including in high-burden countries.

Materials and methods

Antimicrobials

Individual antibiotics were obtained from MAST Diagnostics (Liverpool, UK). Freeze-dried vials of preformulated NOA antimicrobial supplement (final concentrations in medium of nystatin, oxacillin and aztreonam of 50 000 IU l^{-1}, 2 mg l^{-1}, and 30 mg l^{-1} respectively) were used (Biotec Laboratories Ltd, Ipswich, UK). One vial of NOA antimicrobial supplement was reconstituted in 3 ml FPTB Medium Plus (supplemented Middlebrook 7H9-based medium, supplied by Biotec Laboratories Ltd) and added to 300 ml FPTB Medium Plus. The FPTB Medium Plus was then used throughout the test procedure for the reconstitution of reagents, preparation of controls and neutralization of Virusol, the viricide used to inactivate exogenous phage. All other reagents were obtained from Sigma Chemical Company unless stated otherwise.

FASTPlaque assay procedure

The *FASTPlaque*TB and *FASTPlaque–Response* testing was performed according to manufacturer's instructions (Biotec Laboratories Ltd, 2003, 2005). Positive and negative assay controls were performed with each batch of tests. All work was performed in a Level 2 bio-safety cabinet and according to local safety guidelines for work involving category three micro-organisms.

Cultures

Mycobacterium tuberculosis stock cultures were maintained at −70°C. Following thawing, the strains were inoculated onto Middlebrook 7H11 medium and incubated for 4–6 weeks at 37°C. A suspension of the strains was prepared by addition of a loopful of growth to 5 ml FPTB Medium Plus containing six to eight glass beads. The suspension was homogenized by mixing with a vortex mixer for 30 s then allowed to stand for 15 min. The supernatant was adjusted by comparison with a 0·5 MacFarland standard.

Effect of NOA antimicrobial supplement on detection of *M. tuberculosis* by phage assay

Mycobacterium tuberculosis H37*Ra* ATCC 25177 and eight clinical isolates (five rifampicin resistant strains and four rifampicin susceptible strains) were cultured on Middlebrook 7H11 medium at 37°C. A 0·5 MacFarland suspension of each strain was prepared in FPTB Medium Plus (without NOA). Dilutions of each suspension were made in Medium Plus and Medium Plus supplemented with NOA (three batches of NOA tested). Ten replicate 1·0 ml aliquots of the 10^{-3} dilution were dispensed into 10 reaction vessels for each dilution series. Samples were incubated overnight at 37°C and tested according to the *FASTPlaque*TB protocol with their corresponding Medium Plus. Plaque numbers on each plate were recorded. Viable counts of the MTB were performed on Middlebrook 7H11 medium by standard methods (Kent and Kubica 1985). The mean number of plaques obtained for each strain (results for three batches combined), with and without NOA, were compared using a two-tailed *t*-test for independent samples using Intercooled STATA 8·0 software (Statacorp LP; College Station, TX, USA).

Effect of NOA antimicrobial supplement on growth of a range of organisms

Supplemented Middlebrook 7H9-based agar mixtures were prepared containing 7H9-agar blend (*FASTPlaque*TB Agar) with 10% oleic acid, albumin, dextrose and catalase (OADC) supplement and 1 mmol l^{-1} calcium chloride. Reconstituted NOA was added to cooled, molten agar and the mixture poured into 90 mm plastic disposable Petri dishes and allowed to set.

A range of Gram-negative and Gram-positive bacteria (*Ps. aeruginosa* (five strains), *Ps. fluorescens, Ps. putida, Kl. pneumoniae, Pr. mirabilis, B. licheniformis* (two strains), *B. cereus, B. megaterium, B. subtilis, B. firmis, Staph. aureus, Salm. typhimurium, E. coli, Ser. liquefaciens, Ser. marcescens, Myco. smegmatis*) were streaked onto *FASTPlaque*TB Agar plates and incubated overnight at 37°C. A loopful of each overnight culture was streaked onto a NOA antimicrobial plate and un-supplemented *FASTPlaque*TB Agar plate and incubated at 37°C for up to 72 h. The growth of each organism on the control plate was compared with growth on the NOA-containing plate.

Effect of NOA and PANTA supplements on growth of *M. tuberculosis* strains in 7H12 liquid medium

Pre-formulated freeze-dried NOA supplement (MAST Diagnostics) was reconstituted by the addition of 3 ml sterile distilled water (SDW) to the vial. The solution was diluted by addition of 0·4 ml of reconstituted supplement to 0·6 ml of SDW. One hundred microlitres (100 μl) of diluted NOA solution was added to a Bactec 12B vial (Middlebrook 7H12 medium), to give usual working concentration of NOA, and 100 μl SDW was added to each control Bactec 12B vial and a drug free control. PANTA supplement was reconstituted according to manufacturer's instructions and 100 μl was added to the appropriate Bactec 12B vials (Becton Dickinson and Co., Sparks, MD, USA). All Bactec 12B vials were inoculated with 100 μl of a 10^{-2} dilution of fresh culture in Medium Plus containing 0·05% Tween 80. Each strain was tested in triplicate with and without each antimicrobial. Vials were incubated at 37°C for two days prior to the first reading, and were read daily thereafter using the BACTEC 460 TB system non-weekend protocol until the growth index (GI) reached the maximum value of 999 (Hawkins 1986).

Effect of NOA on determination of rifampicin resistance of *M. tuberculosis* clinical isolates

A series of clinical isolates of *M. tuberculosis* with known rifampicin susceptibility were used for this experiment. Conventional rifampicin susceptibility testing was performed using the proportion method on Middlebrook 7H11 medium (1 μg ml^{-1} rifampicin; Kent and Kubica 1985). All rifampicin resistant strains in the collection (112 isolates) and an equivalent number of sequentially selected rifampicin susceptible strains from the collection were included. Strains were tested according to the *FASTPlaque–Response* protocol (Biotec Laboratories Ltd, 2003), except that half strength rifampicin solution (final concentration 5 μg ml^{-1}) was used and no decontamination was performed on the isolated cultures. This lower concentration of rifampicin is used in indirect testing, i.e. testing from cultures rather than directly from processed sputum (Albert *et al.* 2001). Testing was performed with and without the addition of NOA antimicrobial to the test medium. Plaque numbers on each plate were recorded and results were interpreted according to *FASTPlaque–Response* product insert. Any strains giving

unexpected results [discordant results, controls out of specification, contaminated plates, or <100 plaques obtained on the rifampicin-free (RIF-) plates] were retested with and without the antimicrobial supplement. Sensitivity (ability to correctly detect rifampicin resistance), specificity (ability to correctly detect rifampicin susceptibility) and overall accuracy of the *FASTPlaque–Response* test were calculated by standard methods compared with the proportion method as the reference standard, in specimens in which results of both index test and reference standard were available. Statistical analysis was performed using Intercooled STATA 8·0 software (Statacorp LP, College Station, TX, USA).

Results

Effect of NOA antimicrobial supplement on detection of *M. tuberculosis* by phage assay

The mean number of plaques (and standard deviation) for different MTB strains tested with and without the NOA supplement are shown in Table 1. The mean of ten replicates was recorded for samples without NOA, and the mean of 30 replicates (all NOA batches) was recorded with NOA. *P*-values <0·05 were considered statistically significant.

The results show that there was some variation in the numbers of plaques obtained in different strains of MTB. The viable counts of the MTB test suspensions varied between $1{\cdot}7 \times 10^6$ and $6{\cdot}9 \times 10^7$ CFU ml^{-1}. This is consistent with other findings (unpublished data) and reflects variability in the preparation of suspensions, age of cultures, and dispersion of cells in suspension. There was a significant difference in the number of plaques obtained with and without NOA supplement in four of the MTB strains (R391, R213, S1730 and S56), with three strains giving higher values without NOA, and one strain giving lower plaque numbers. There was no significant difference in the other strains tested. When results of all the strains were considered together, there was no significant difference in the numbers of plaques obtained with and without the antimicrobial supplement.

Effect of NOA on growth of a range of organisms

Satisfactory growth was seen on all the control plates within 24 h, except for *Pr. mirabilis* and *Staph. aureus* which took 72 h incubation. The NOA supplement inhibited growth of all organisms except *B. cereus* and *Myco. smegmatis*, which were not affected by the presence of NOA. Incubation of organisms in the presence of NOA resulted in either complete inhibition of growth or in substantial reduction in the number of colonies.

Effect of NOA on growth of *M. tuberculosis* strains in 7H12 liquid medium

There was no significant difference in the mean time to reach the maximum growth index reading (GI 999) between the NOA supplement, PANTA and the un-supplemented 12B medium, when results of all MTB strains were considered together (Table 2). There was, however, some variation in the growth rate of individual strains of MTB, and the effect of PANTA and NOA formulations thereon (Fig. 1). The NOA appeared to have little effect on the growth of 5 strains when compared with unsupplemented medium, whilst the growth of three strains was somewhat delayed. PANTA supplement delayed the

Table 1 Comparison of plaque numbers for phage assay performed with and without NOA supplement

MTB strain	Rifampicin resistance (mutation)	Viable count (CFU ml^{-1})	With NOA		Without NOA		
			Mean plaque count*	Standard deviation	Mean plaque count †	Standard deviation	*t*-test *P*-value‡
H37ra	RIF^S	$6{\cdot}9 \times 10^7$	280·8	26·35	283·6	29·12	0·7782
S1	RIF^S	$2{\cdot}6 \times 10^7$	520·41	72·07	516·8	91·15	0·8990
S56	RIF^S	NA	231·03	49·57	293·8	39·45	0·0008
S6	RIF^S	$5{\cdot}2 \times 10^6$	29·37	7·29	28·6	7·62	0·7772
BT10	RIF^R (NA)	$5{\cdot}0 \times 10^7$	301·57	74·30	316·9	34·18	0·5346
R453	RIF^R (*rpo*B 526)	$1{\cdot}1 \times 10^7$	303·33	58·21	335·5	57·51	0·1725
S1730	RIF^R (*rpo*B 533)	$1{\cdot}7 \times 10^6$	124·67	25·86	91	13·27	0·0004
R213	RIF^R (*rpo*B 516)	$1{\cdot}2 \times 10^7$	338	63·90	435·2	50·31	0·0001
R391	RIF^R (*rpo*B 516)	$3{\cdot}6 \times 10^6$	195·1	58·45	368·33	99·94	<0·0001
Overall		–	243·97	139·42	273·90	161·10	0·0782

NA result not available.
*Mean of 10 replicates.
†Mean of 30 replicates over three batches of NOA reagent.
‡Comparison between with and without NOA.

Table 2 Time to reach GI 999 (days) for *M. tuberculosis* strains grown in Bactec 12B medium with and without antimicrobial (NOA and PANTA)

	Mean time to GI 999 (days)		
Strain	No antimicrobial	NOA	PANTA
H37*ra* (ATCC 25177)	10	6·3	7
R213	17	17	14·3
S6	10	10	10
BT10	10	11	17
R453	17	17	14·7
R391	10	10	10
S1	7	5·7	6·3
S56	10	10	6
Overall	11·4	10·9	10·7

Mean time to reach GI 999 is average of three replicate vials.

time to achieve a growth index of 999 compared with un-supplemented medium for BT10 strain (rifampicin resistant strain), whilst growth in the NOA-containing medium was unaffected. PANTA supplemented medium supported more rapid growth of R213, R453 and H37*ra* strains compared with NOA supplemented medium. When the mean of all strains was considered, there did not appear to be any substantial trend between either antimicrobial and without antimicrobial.

Effect of NOA on determination of rifampicin resistance of *M. tuberculosis* clinical isolates

A comparison was made of the indirect *FASTPlaque–Response* results performed with and without antimicrobial. In addition, results were compared with conventional rifampicin susceptibility test results, performed by a modified proportion method on Middlebrook 7H11 medium (Kent and Kubica 1985). Table 3 shows data comparing the performance of the *FASTPlaque-Response* testing with and without the antimicrobial. The results obtained from the first round of testing are shown. In addition, results from the repeat tests of one batch whose assay controls were initially out of specification (which all agreed with the original results) are also included.

Of 214, 204 isolates gave interpretable results when tested both with and without the antimicrobial supplement. Of the remaining 10 isolates, seven isolates gave <100 plaques when tested both with and without antimicrobial (too few for interpretation of results). Upon repeat testing two additional samples gave interpretable results (concurring with each other), while the remainder remained un-interpretable, assumed to be due to lost viability and hence inability to support infection with the Actiphage. The remaining three isolates were all contaminated without antimicrobial, but were either interpretable (resistant), <100 plaques or also contaminated in the presence of antimicrobial.

The results of the test with antimicrobial supplement agreed with the un-supplemented test results in 97·5% (199/204) isolates. 97·0% of results agreed when the strain was rifampicin resistant and 98·1% of results when the strain was rifampicin susceptible. Three isolates were resistant without antimicrobial but susceptible with antimicrobial, and two isolates were susceptible without antimicrobial but resistant when tested with antimicrobial.

Sensitivity, specificity and overall accuracy (with 95% confidence interval) of the *FASTPlaque–Response* test without NOA antimicrobial were 94·0% (89·4–98·7), 94·2% (89·7–98·7) and 94·1% (90·9–97·3). Sensitivity, specificity and overall accuracy of the *FASTPlaque–Response* test with NOA were 94·1% (89·5–98·7), 95·2% (91·1–99·3) and 94·6% (91·8–97·4). There was no significant difference in the performance parameters of the *FASTPlaque–Response* test compared with the 7H11 proportion method when performed with and without the antimicrobial supplement (Table 4).

Discussion

These results suggest that the NOA supplement may be an effective addition to the *FASTPlaque* test medium, for use in detecting MTB from clinical specimens as well as for rapid determination of rifampicin resistance using the *FASTPlaque–Response* test. The results presented herein demonstrate that the NOA formulation does not have a detrimental effect on the sensitivity of the phage assay to detect TB, nor does it significantly inhibit growth of a range of drug-susceptible and drug-resistant *M. tuberculosis* strains in liquid culture medium. The supplement appears to have good antimicrobial efficacy against a range of Gram-positive and -negative organisms, which are likely to be encountered as specimen contaminants. Data on the effect of addition of NOA on the determination of rifampicin resistance from TB cultures suggests that the antimicrobial formulation does not interfere with the discrimination of rifampicin resistance and susceptibility by the phage assay.

Although there was no significant difference overall in the numbers of plaques obtained with and without the antimicrobial supplement added to the medium when MTB cultures were tested, in some strains there was a small absolute reduction. However, this small reduction is unlikely to lead to poorer performance of the *FASTPlaque–Response* test since the test is designed for use only with smear-positive specimens, which would be expected to contain large numbers of bacilli, and the *FASTPlaque–Response* result plates usually have 300

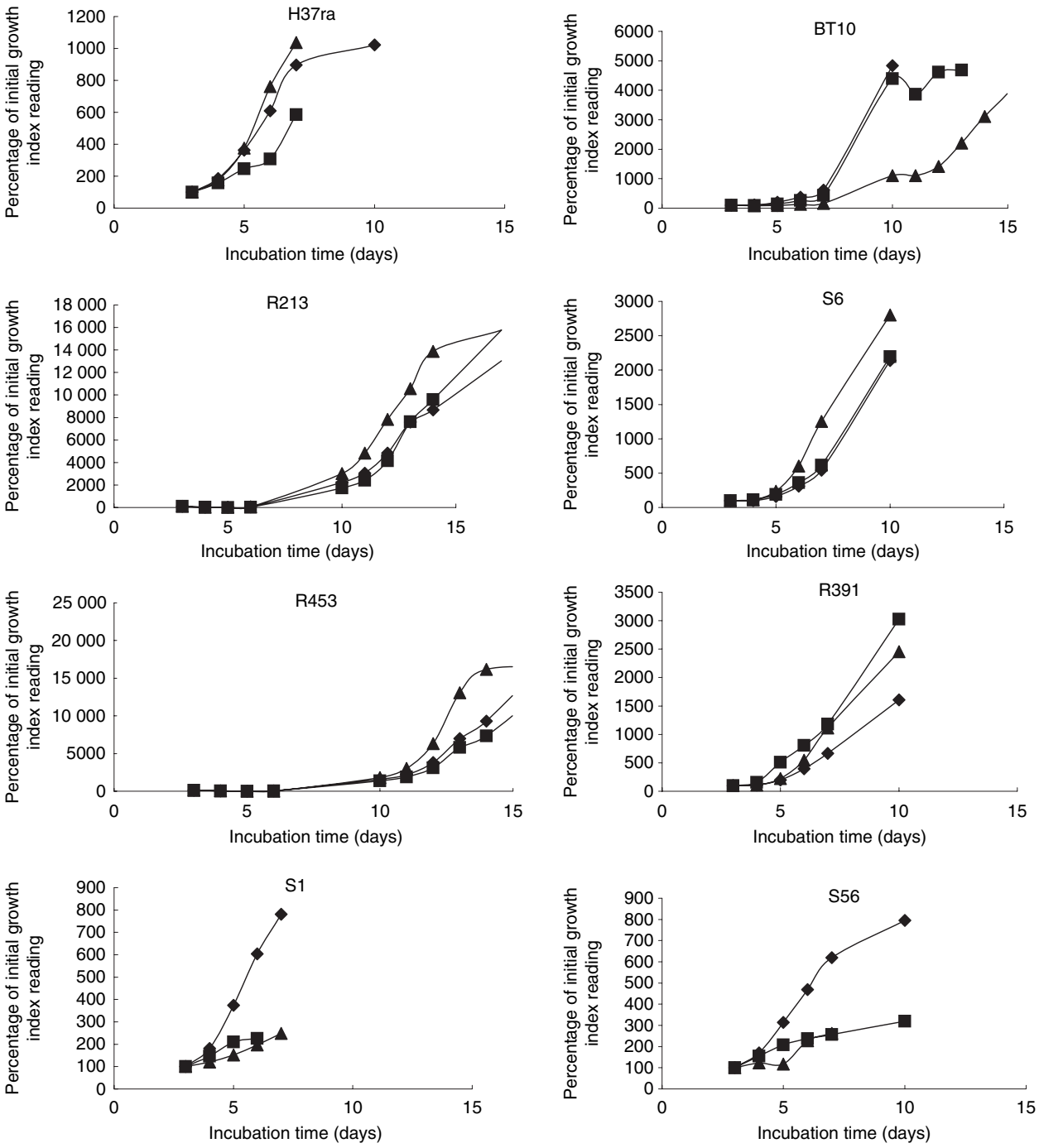

Figure 1 Growth of *M. tuberculosis* strains in Bactec 12B medium with and without antimicrobials (NOA and PANTA); rate of increase of growth index readings over time (days). Each point represents the mean of three readings. ◆, No AM; ■, NOA; ▲, PANTA.

plaques or greater. Therefore a small reduction in plaque numbers would be unlikely to cause a substantial difference in the proportion of interpretable results, which requires 100 plaques or greater on the drug-free control plates.

A large scale clinical evaluation of the effect of the NOA supplement on performance of the *FASTPlaque–Response* test directly from smear-positive sputum specimens is required to determine whether the results of the indirect testing can be replicated. Furthermore, the anti-

Table 3 Comparison of indirect *FASTPlaque–Response* results performed with and without NOA antimicrobial

FASTPlaque–Response with NOA	*FASTPlaque–Response* without NOA				
	Resistant	Susceptible	<100 pl.	Contam	Total
Resistant	97	2	0	1	100
Susceptible	3	102	0	0	105
<100 pl.	0	0	7	1	8
Contam	0	0	0	1	1
Total	100	104	7	3	214

<100 pl. less than 100 plaques obtained on RIF- plate; Contam, contaminated plate

Table 4 Comparison of indirect *FASTPlaque–Response* results performed with and without NOA antimicrobial with 7H11 proportion method (reference standard)

	7H11 proportion method	
	Resistant	Susceptible
FASTPlaque–Response without NOA		
Resistant	94	6*
Susceptible	6	98
<100 pl on RIF-	4	3
Contaminated	3	0
FASTPlaque–Response with NOA		
Resistant	95	5†
Susceptible	6	99
<100 pl on RIF-	5	3
Contaminated	1	0

*Two of these isolates had *rpo*B mutations linked to rifampicin resistance (*rpo*B 516 and 531)

†One strain had *rpo*B mutation at *rpo*B 531. The strain with *rpo*B 516 mutation was susceptible when *FASTPlaque–Response* performed with antimicrobial.

microbial efficacy of the formulation must be determined when the testing is performed directly from the clinical specimens, as well as an assessment of the level of interpretable results. Such a study has been performed and will be reported separately.

Further work is also required to assess the use of the NOA supplement in detecting MTB in paucibacillary specimens, although data presented here suggest little effect on the detection limit where low numbers of MTB were present in cultures. The effect of the NOA supplement on detection of non-tuberculous mycobacteria (NTM) should be studied in settings in which NTMs are commonly isolated and clinically relevant, although laboratory testing of NTM cultures (data not shown) did not reveal a significant effect of the NOA supplement. Use of this antimicrobial supplement with rapid phage-based diagnostic tests for TB will increase the robustness of such tests and facilitate their wider implementation in settings in which contamination may present a challenge to growth-based laboratory testing.

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References

Albert, H., Heydenrych, A., Mole, R., Trollip, A.P. and Blumberg, L. (2001) Evaluation of *FASTPlaque*TB-*RIF*, a rapid, manual test for the determination of rifampicin resistance from *M. tuberculosis* cultures. *Int J Tuberc Lung Dis* **5**, 906–911.

Albert, H., Heydenrych, A., Brookes, R., Mole, R.J., Harley, B., Subotsky, E., Henry, R. and Azevedo, V. (2002) Performance of a rapid phage-based test, *FASTPlaque*TB, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. *Int J Tuberc Lung Dis* **6**, 529–537.

Albert, H., Trollip, A., Seaman, T. and Mole, R.J. (2004) Simple, phage-based (*FASTPlaque*) technology to determine rifampicin resistance of *Mycobacterium tuberculosis* directly from sputum. *Int J Tuberc Lung Dis* **8**, 1114–1119.

Albert, H., Trollip, A.P., Seaman, T., Abrahams, C., Mole, R.J., Jordaan, A., Victor, T. and Hoosain, E. (2007) Evaluation of a rapid screening test for rifampicin resistance in re-treatment tuberculosis patients in the Eastern Cape. *SA Med J* (in press).

Allen, B.W., Mitchison, D.A., Darbyshire, J., Chew, W.W.K. and Gabriel, M. (1983) Examination of operation specimens from patients with spinal tuberculosis for tubercle bacilli. *J Clin Pathol* **36**, 662–666.

Biotec Laboratories Ltd (2003) *FASTPlaque–Response product insert. For performance evaluation only*. Version 180903. Biotec Laboratories Ltd, Ipswich, UK.

Biotec Laboratories Ltd (2005) *FASTPlaqueTB Product Insert*. Version 4. Biotec Laboratories Ltd, Ipswich, UK.

Cruciani, M., Scarparo, C., Malena, M., Bosco, O., Serpelloni, G. and Mengoli, C. (2005) Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *J Clin Microbiol* **42**, 2321–2325.

Hawkins, J.E. (1986) Non-weekend schedule for BACTEC drug susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* **23**, 934–937.

Huang, T.S., Chen, C.S., Lee, S.S., Huang, W.K. and Liu, Y.C. (2001) Comparison of the BACTEC MGIT 960 and BACTEC 460 TB systems for detection of mycobacteria in clinical specimens. *Ann Clin Lab Sci* **31**, 279–283.

Kent, P.T. and Kubica, G.P. (1985) *Public Health Mycobacteriology. A Guide for the Level III Laboratory*. Atlanta, GA: Centers for Disease Control.

Marei, A.M., El-Behedy, E.M., Mohtady, H.A. and Afify, A.F. (2003) Evaluation of a rapid bacteriophage-based method for the detection of *Mycobacterium tuberculosis* in clinical samples. *J Med Microbiol* **52**, 331–335.

Martindale, W. and Reynolds, J.E.F. (eds.). (1993) *Martindale: The Extra Pharmacopoiea*. 30th edn. London: The Pharmaceutical Press.

Mbulo, G.M., Kambashi, B.S., Kinkese, J., Tembwe, R., Shumba, B., Godfrey-Faussett, P. and McNerney, R. (2004) Comparison of two bacteriophage tests and nucleic acid amplification for the diagnosis of pulmonary tuberculosis in sub-Saharan Africa. *Int J Tuberc Lung Dis* **8**, 1342–1347.

Mitchison, D.A., Allen, B.W., Carrol, L., Dickinson, J.M. and Aber, V.R. (1972) A selective oleic acid albumin agar medium for tubercle bacilli. *J Med Microbiol* **5**, 165–175.

Muzaffar, R., Batool, S., Aziz, F., Naqvi, A. and Rizvi, A. (2002) Evaluation of the *FASTPlaque*TB assay for direct detection of *Mycobacterium tuberculosis* in sputum specimens. *Int J Tuberc Lung Dis* **6**, 635–640.

Perkins, M.D. and O'Brien, R.J. (2006) New diagnostics for tuberculosis: an essential element for global control and elimination. In *Reichman and Hershfield's Tuberculosis: A Comprehensive, International Approach*, 3rd edn. ed. Raviglione, M.C. pp. 1115–1133. New York: Informa Healthcare.

Rees, C.E.D., Rostas-Mulligan, K., Park, S.F., Denyer, S.P., Stewart, G.S.A.B. and Jassim, S.A.A. (1992). *Methods for rapid microbial detection*. PCT Patent WO92/02633.

Siddiqi, S.H. and Hwangbo, C.C. (1986) A new antimicrobial mixture (PANTA) for effective suppression of non-mycobacterial contamination during primary isolation of mycobacteria. Abstr. U-35, p125. In *Abstracts of the 86th Annual Meeting of the American Society for Microbiology*. American Society for Microbiology, Washington DC.

Wilson, S.M. (1997) *Method to detect bacteria*. PCT Patent WO97/022713.

Wilson, S.M., Al-Suwaidi, Z., McNerney, R., Porter, J. and Drobniewski, F. (1997) Evaluation of a new rapid bacteriophage-based method for the drug susceptibility testing of *Mycobacterium tuberculosis*. *Nat Med* **3**, 465–468.

