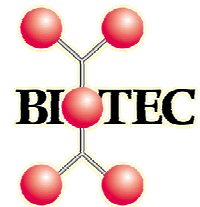


Improved contamination control using a new antimicrobial supplement developed for rapid phage-based rifampicin susceptibility testing



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SUMMARY

The *FASTPlaque-Response* test is a phage-based test to determine rifampicin resistance of *M. tuberculosis* (MTB) strains directly from sputum within 2 days. The method uses smear-positive sputum samples that have been decontaminated using the NALC-NaOH method and then incubated in nutrient rich medium. The decontamination process may in some cases be ineffective in inhibiting growth of certain contaminating organisms found in sputum. This may lead to un-interpretable results in culture-based methods, as well as in the phage-based *FASTPlaque-Response* test.

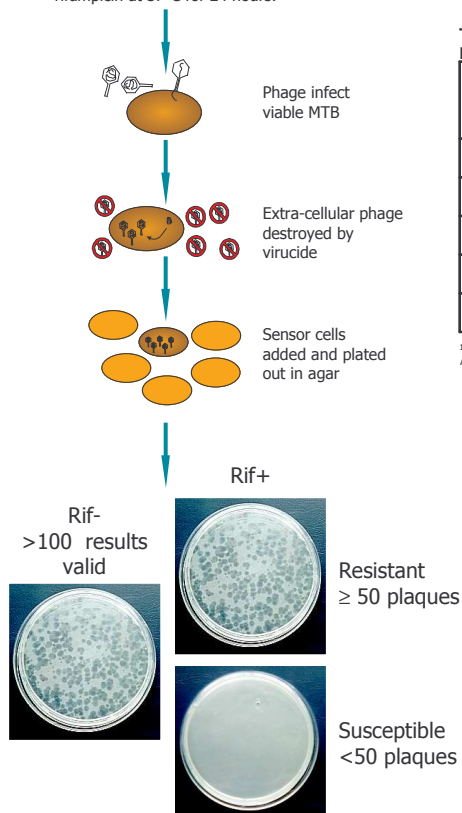
An antimicrobial formulation (NOA Antimicrobial Supplement) has been specifically developed for use with the phage-based test. In experimental studies, the formulation of aztreonam, oxacillin and nystatin was found to have no detrimental effect on the test's ability to detect rifampicin resistance in MTB strains, but was effective in controlling the growth of the bacterial and fungal organisms commonly causing contamination in sputum specimens. The performance of the *FASTPlaque-Response* test with and without NOA Antimicrobial Supplement was compared in 632 smear-positive sputum. Antibiotic supplementation resulted in comparable sensitivity and specificity but substantially increased the number of interpretable results.

METHOD

Sputum specimens were stored at 2-8°C prior to testing. After NALC-NaOH processing, smear positive (1+ to 3+) specimens were split after re-suspension in phosphate buffer:

- 0.5ml processed with the *FASTPlaque-Response* test with NOA Antimicrobial Supplement
- 0.5ml processed with the *FASTPlaque-Response* test
- 0.5ml used to inoculate MGIT cultures, followed by determination of rifampicin susceptibility by the indirect LJ Middlebrook method.

Incubate sputum sample with and without rifampicin at 37°C for 24 hours.



CONCLUSIONS

- NOA Antimicrobial supplement improves contamination control in the *FASTPlaque-Response* test. In specimens of ≤3 days old, 88% of specimens are interpretable.
 - When specimens >3 days (up to 14 days) are included in the analysis, 79.1% of results are interpretable.
- Presence of NOA Antimicrobial Supplement does not affect discrimination of resistant and susceptible results.
- Results were available in 2 days with the *FASTPlaque-Response* test, compared to 26 to 77 days (mean 35.2 days) for the conventional DST test.

RESULTS

Table 1. Comparison of interpretability of results for the *FASTPlaque-Response* test, with and without NOA, and conventional rifampicin DST – specimens 3 days or less, as specified for the *FASTPlaque-Response* test

| n=238 | <i>FASTPlaque-Response</i> with NOA | | <i>FASTPlaque-Response</i> WITHOUT NOA | | Conventional DST | |
|-----------------------------------|-------------------------------------|------|--|------|------------------|------|
| | number | % | number | % | number | % |
| Total interpretable | 209 | 87.8 | 165 | 69.3 | 167 | 70.1 |
| Contamination | 2 | 0.8 | 34 | 14.3 | 61 | 25.6 |
| Results not reported ¹ | 0 | 0 | 0 | 0 | 10 | 4.2 |
| Un-interpretable FPR ² | 27 | 10.3 | 39 | 16.4 | 0 | 0 |

¹ Culture results not reported due to culture being culture negative, presence of NTM, or results lost ² Un-interpretable by the *FASTPlaque-Response* method (Controls or Rif-out of specification).

Table 2. Comparison of interpretability of results for the *FASTPlaque-Response* test, with and without NOA, and conventional rifampicin DST – all specimens (Specimen age: 0-14 days, mean 3.9 days)

| n=631 | <i>FASTPlaque-Response</i> with NOA | | <i>FASTPlaque-Response</i> WITHOUT NOA | | Conventional DST | |
|-----------------------------------|-------------------------------------|------|--|------|------------------|------|
| | number | % | number | % | number | % |
| Total interpretable | 499 | 79.1 | 436 | 69.1 | 465 | 73.7 |
| Contamination | 26 | 4.1 | 90 | 14.3 | 128 ¹ | 20.3 |
| Results not reported ² | 0 | 0 | 0 | 0 | 38 | 6.0 |
| Un-interpretable FPR ³ | 106 | 16.8 | 105 | 16.7 | 0 | 0 |

¹ 94.5% were contaminated on initial culture ² Culture results not reported due to culture being culture negative, presence of NTM, or results lost ³ Un-interpretable by the *FASTPlaque-Response* method (Controls or Rif-out of specification).

Table 3. Performance of the *FASTPlaque-Response* test, in the presence and absence of NOA Antimicrobial Supplement, compared to un-resolved conventional rifampicin DST (LJ proportion method)

| | <i>FASTPlaque-Response</i> with NOA | | | <i>FASTPlaque-Response</i> WITHOUT NOA | | |
|-------------------|-------------------------------------|------|------------|--|------|------------|
| | Number | % | 95% CI | Number | % | 95% CI |
| Overall agreement | 369/384 | 96.1 | 94.2-98.0 | 326/342 | 95.3 | 93.1-97.5 |
| Sensitivity | 51/52 | 98.1 | 94.4-100.0 | 39/42 | 92.9 | 85.1-100.0 |
| Specificity | 318/332 | 95.8 | 93.6-99.0 | 287/300 | 95.7 | 93.4-98.0 |

OUTCOME

- Use of a simple, rapid test, such as *FASTPlaque-Response*, could play an important role in TB control programmes enabling the rapid detection of MDR-TB cases, improving the patient prognosis and reducing the period of infectiousness of these patients.
- Applications may include:
 - Patients failed or defaulted treatment
 - Patients not responding to therapy
 - New cases at high risk of MDR-TB
 - Suspects at risk of MDR e.g. contacts

ACKNOWLEDGEMENTS

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