

FASTPlaque-Response™

A rapid bacteriophage assay for the determination of rifampicin resistance in sputum specimens containing *Mycobacterium tuberculosis* complex

For *in vitro* diagnostic use only



25 Determinations

FASTPlaque™ Catalogue No. 5/200

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1 INTENDED USE

BIOTEC's *FASTPlaque-Response*TM test is a rapid bacteriophage assay for determination of rifampicin resistance of *Mycobacterium tuberculosis* (MTB) complex organisms in smear positive sputum specimens.

2 SUMMARY

Rifampicin susceptibility is critical to the successful outcome of short-course chemotherapy (SCC) for tuberculosis. SCC has been shown to be effective even if the patient is initially resistant to isoniazid, streptomycin or other drugs. However, if rifampicin resistance occurs, the likelihood of successful treatment outcome is severely reduced¹.

Rifampicin resistance rarely occurs in isolation, and is usually found in combination with isoniazid resistance². Resistance to at least these two key drugs is termed multi-drug resistant tuberculosis (MDR-TB). MDR-TB is an increasing health risk and a threat to TB control programmes in many countries. Treatment of MDR-TB requires use of less effective, more toxic and expensive drugs for longer periods². Delay in diagnosing patients with MDR-TB leads to poorer prognosis for the individual patient and ongoing transmission of the disease.

The *FASTPlaque-Response*TM test will determine the rifampicin susceptibility of MTB directly from the patient's sputum specimen. The test has a manual format and results are read by eye. Results are available within 2 days. This test utilises basic microbiological equipment and skills available in most laboratories.

3 PRINCIPLE OF *FASTPlaque-Response*TM

*FASTPlaque-Response*TM is based on the *FASTPlaque*TM principle (Phage Amplification, Figure 1) which utilises mycobacteriophage (viruses that specifically infect [or target] mycobacteria) to reflect the presence of viable MTB within a sputum specimen³.

The sputum specimen is first decontaminated. This kills most bacteria present in the specimen other than the target mycobacteria. A portion of the decontaminated and washed specimen is incubated in the presence (RIF+) or absence (RIF-) of rifampicin. Following incubation, the viability of MTB in each sample is assessed using the *FASTPlaque*TM principle (Figure 1). The target mycobacterial cells are rapidly infected by the target-specific bacteriophage (Actiphage) added to the decontaminated sample. The resulting mixture is then treated with a virucidal solution (Virusol) that destroys all bacteriophage which have not infected the target cells. After treatment with the virucidal solution the only bacteriophage that remain are those that are protected within viable target mycobacteria. These bacteriophage continue to replicate until new progeny phage are released as the cells break open (lyse).

These progeny bacteriophage are then amplified by the introduction of a non-pathogenic rapid growing mycobacterial cell host (Sensor cells). Progeny bacteriophage undergo rapid cycles of infection, replication and lysis, which are seen as clear areas (plaques) in a lawn of confluent growth of Sensor cells. The number of plaques generated from a given sample reflects the number of viable MTB cells containing mycobacteriophage. If there are no viable target bacilli in the original sample, there will be no phage amplification and therefore no bacteriophage to detect at the end of the assay.

The number of plaques in the rifampicin-free sample (RIF -) is compared with the number of plaques produced from the sample incubated in the presence of rifampicin (RIF +). The absence of plaques in the rifampicin-containing sample indicates that those MTB are susceptible to rifampicin (*ie.* they are no longer viable and can not support phage replication). The presence of plaques in the rifampicin-containing sample indicates that viable MTB have survived and the strain is resistant to rifampicin (Figure 2).

Figure 1: FASTPlaque™ test principle.

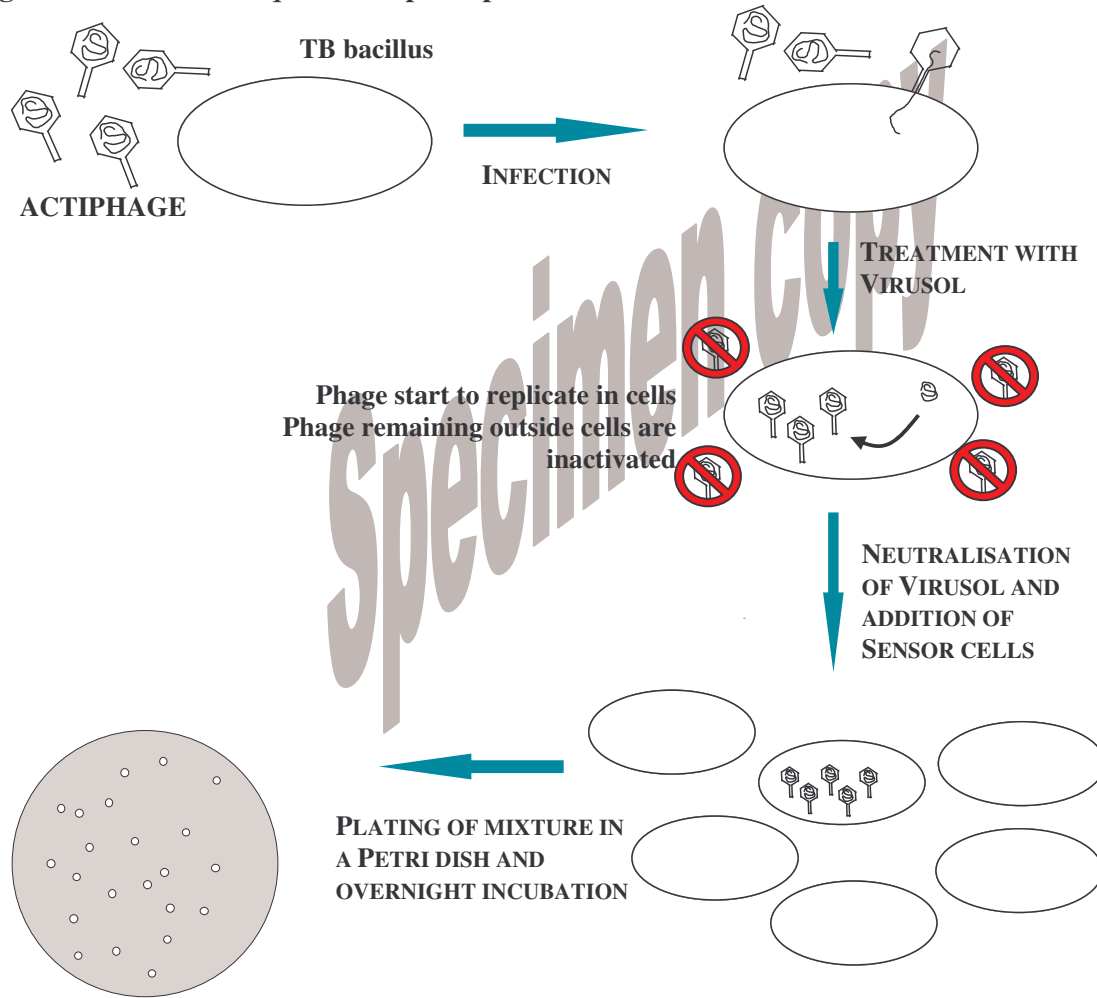
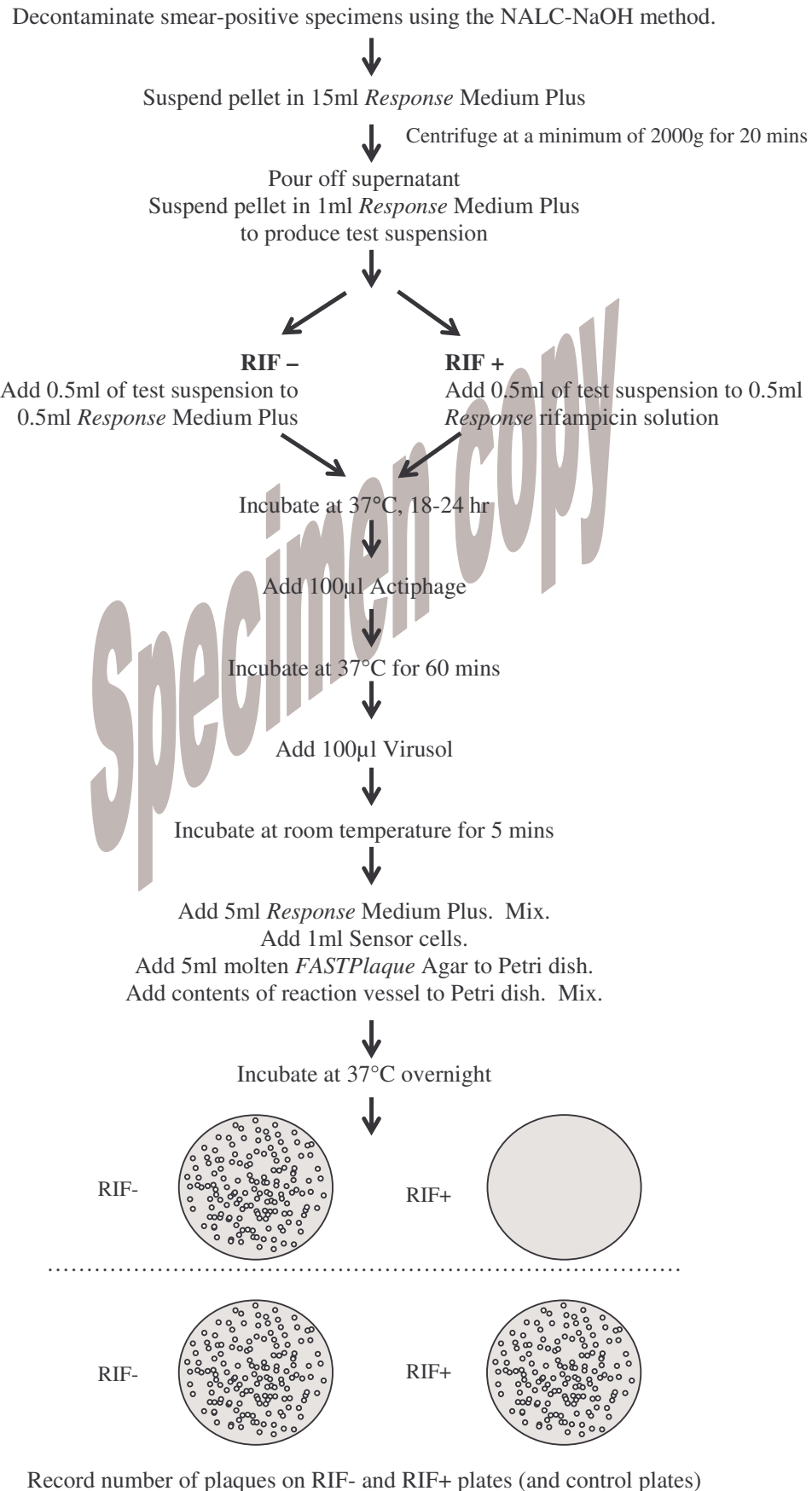


Figure 2: FASTPlaque-Response™ assay procedure flow diagram



4 PRODUCT CONTENTS

Each kit contains sufficient materials for 25 determinations, and comprises two boxes, one for the reagents, the other for the Reaction vessels.

The shelf life of the kit is indicated on the outer box labels. The box of reagents must be stored at 2-8°C, while the box containing Reaction vessels may be stored between 2-30°C.

4.1 KIT CONTENTS

- 5 x *Response* Medium sachets ^A
- 5 x *Response* Growth Supplement vials
- 5 x lyophilised Actiphage (mycobacteriophage) vials
- 5 x lyophilised Sensor cells (rapid growing, non-pathogenic *Mycobacterium* species) vials
- 5 x Virusol tablets (virucide) in vials ^B
- 5 x FPTB Agar sachets ^A
- 5 x *Response* Rifampicin vials
- 50 x Reaction vessels (sterile)
- 1 x Instructions for use

^A Hazard information: **Xi: R 36/37/38** irritating to eyes, respiratory system and skin. **S 24/25 36 22 7** Avoid contact with skin and eyes. Wear suitable protective clothing. Do not breathe dust. Keep container tightly closed.

^B Hazard information: **Xi: R 36/38**. Irritating to eyes and skin. **S 26 36** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

4.2 ITEMS REQUIRED BUT NOT PROVIDED

4.2.1 Reagents

- Purified water (distilled or reverse osmosis water)
- NOA Antimicrobial Supplement[#]
- Sodium hydroxide (NaOH)
- Sodium citrate dihydrate
- N-acetyl-L-cysteine (NALC)
- Potassium dihydrogen phosphate, anhydrous (KH₂PO₄)
- Disodium hydrogen phosphate, anhydrous (Na₂HPO₄)

4.2.2 Accessories and Equipment

- Microbiology laboratory consistent with local regulations for use with *M.tuberculosis*
- Biosafety cabinet
- Personal protective equipment (disposable gloves, masks, gowns)
- Discard container and suitable mycobactericidal disinfectant
- Vortex mixer
- Centrifuge with sealed centrifuge cups suitable for 50ml conical tubes and capable of spinning to at least 2000g
- Autoclavable containers (various sizes)
- Means of melting agar (*e.g.* boiling waterbath)
- Waterbath (55°C ± 2°C)
- Autoclave capable of reaching 121°C (15psi) *
- 37°C incubator *
- Refrigerator (2-8°C) *
- Sterile 90mm Petri dishes
- Sterile 50ml conical polypropylene screw cap tubes (aerosol free and graduated)
- Pipettes to dispense 1.1ml, 1ml and 0.1ml plus sterile pipette tips
- 10ml graduated pipettes
- Sterile tubes (for preparation of assay controls)

[#] Used to control contamination – Biotec Laboratories Product code 5/250. * To include means of confirming correct operating temperature

4.3 STORAGE, RECONSTITUTION, SHELF-LIFE AND RE-USE OF KIT COMPONENTS

Prior to use, the reagents should be stored between 2-8°C. The components should not be used beyond the date shown on the outside of the kit box.

In order to ensure optimal kit performance, it is important that all kit components are reconstituted as directed below and that any unused kit components are stored according to the following instructions.

4.3.1 *Response Medium*

Response Medium should be reconstituted by adding the contents of the sachet to 180ml purified water, in a suitable autoclavable container. Allow the powder to hydrate for 10 minutes. Mix gently, before sterilising by autoclaving at 121°C for 10 minutes. Once reconstituted and autoclaved the medium can be stored for up to 4 weeks at room temperature (less than 25°C) prior to addition of *Response Growth Supplement*. Aseptic technique should be used when handling autoclaved medium. *Response Medium* should not be used if there are any visual signs of contamination (turbidity). One sachet of *Response Medium* is sufficient for 5 determinations.

4.3.2 *Response Growth Supplement*

This is added to cooled sterile *Response Medium*. One vial of *Response Growth Supplement* is sufficient for 5 determinations.

4.3.3 *Response Medium Plus*

To prepare *Response Medium Plus* aseptically add one bottle of *Response Growth Supplement* and 2ml of reconstituted NOA Antimicrobial supplement (Product code 5/250, see NOA Antimicrobial Supplement Instructions for further information) to reconstituted, autoclaved and cooled *Response Medium*. Mix gently before use. *Response Medium Plus* can be stored for up to 7 days at 2-8°C (but no longer than the expiry date of the *Response Medium*), provided aseptic techniques are employed throughout usage. *Response Medium Plus* should not be used if there are any visual signs of contamination (turbidity). This volume of *Response Medium Plus* is sufficient for 5 determinations.

4.3.4 *Actiphage*

Actiphage is prepared by adding 1.1ml of sterile *Response Medium Plus* into the vial and mixing gently to dissolve. One bottle of *Actiphage* is sufficient for 5 determinations. Reconstituted *Actiphage* may be stored for up to 7 days if kept at 2-8°C (but no longer than the expiry date of *Response Medium*), provided aseptic techniques are used throughout usage. Mix well prior to use.

4.3.5 *Sensor cells*

Sensor cells are prepared by adding 11 ml of *Response Medium Plus* into the vial and mixing gently to suspend. One bottle of *Sensor cells* is sufficient for 5 determinations. Reconstituted *Sensor cells* may be stored for up to 7 days if kept at 2-8°C (but no longer than the expiry date of *Response Medium*), provided aseptic techniques are employed throughout usage. Mix well prior to use. *Sensor cells* will appear as a turbid suspension once mixed.

4.3.6 *Virusol tablet*

Add 5ml sterile purified water to the *Virusol* vial and dissolve. Tablets that are broken can still be used. Once reconstituted, *Virusol* can be stored for up to 7 days at 2-8°C. *Virusol* solution must be mixed well prior to use to ensure all the material is dissolved. Discoloration and precipitation may occur on storage. This does not affect the performance of the reagent. Shake gently to re-dissolve before use.

4.3.7 *FASTPlaqueTB™ Agar*

FASTPlaqueTB™ Agar should be reconstituted by adding the contents of the sachet to 60ml purified water. Allow the powder to hydrate for 10 minutes. Mix gently before sterilising by autoclaving at 121°C for 10 minutes. Aseptic techniques should be used when handling autoclaved agar. If using immediately, allow to cool to 55°C ($\pm 2^\circ\text{C}$) in a waterbath before use. Once autoclaved the agar can be stored for up to 4 weeks at room temperature (up to 25°C). Melt solidified agar completely and allow to cool to 55°C ($\pm 2^\circ\text{C}$) before use. The agar may be reheated up to 3 times, enabling subsequent re-uses of the kit if necessary. This volume of *FASTPlaqueTB™ Agar* is sufficient for 5 determinations.

4.3.8 Response Rifampicin

Reconstitute the *Response* Rifampicin in 3ml *Response* Medium Plus and ensure it is fully dissolved before use. Reconstituted rifampicin solution can be stored at 2-8°C for up to 7 days (but no longer than the expiry date of *Response* Medium). Mix prior to use. One vial of *Response* Rifampicin is sufficient for 5 determinations.

4.3.9 Reaction Vessels

The reaction vessels supplied with the kit are sterile and for single use only. The kit contains sufficient reaction vessels for the number of determinations. Only the reaction vessels supplied with the kit should be used for the assay procedure. Do not use for preparation of assay controls. Reaction vessels should arrive closed. Open vessels should not be used.

5 PRECAUTIONS

5.1 SAFETY PRECAUTIONS

Acid-fast bacilli (AFB) smear-positive sputum specimens may contain *Mycobacterium tuberculosis*, some of which may be drug resistant strains, or other mycobacteria. In addition, clinical samples may contain other infectious agents such as Hepatitis B virus and Human Immunodeficiency Virus (HIV). Suitable safety precautions⁷ must be used at all times when handling such specimens, including working in an appropriate bio-safety cabinet and using personal protective equipment such as gloves, gowns and masks. Local guidelines must be followed when working with pathogenic material and for disposal of pathogenic biological waste including in the event of laboratory accidents.

Several components of the test kit contain products of animal origin and may cause irritation to some individuals. These constituents have been obtained from accredited TSE (Transmissible Spongiform Encephalopathy) free sources. NOA Antimicrobial Supplement contains a penicillin antibiotic which may be allergenic to certain individuals.

5.2 TECHNICAL PRECAUTIONS

- For professional *in vitro* diagnostic use only, by personnel competent in aseptic technique and experienced in working with *M. tuberculosis*.
- The operator should seek assistance from their country distributor, or directly from Biotec, if they do not understand anything in these instructions or have problems running the test (see Troubleshooting, Section 10). Additional training material is available upon request.
- If the kits or reagents appear damaged upon arrival, do not use and contact your local supplier.
- Components must not be used beyond the expiry date printed on the outer kit. Do not freeze any kit component or reconstituted reagent.
- The reagents are provided at defined working concentrations. Assay performance may be lost if reagents are modified or not stored under recommended conditions as detailed in Section 4.3.
- Do not mix different batch or lot numbers of reagents.
- Employ aseptic techniques throughout the reagent preparation and assay procedure.
- All pipettes, pipette tips, glassware and plastic-ware used must be sterile.
- Ensure that all equipment used is correctly maintained, calibrated and monitored as appropriate.
- All reagents must be brought to room temperature before use
- Reconstitute sufficient reagents required to perform the test. Do not return excess reagents to bottles after use.
- Care needs to be taken to treat all specimens and control reactions in the same manner, according to Section 7.2 of these instructions for use. Omission of, or deviation from, any step could lead to inaccurate results.
- A rifampicin-free control (RIF-) and rifampicin-containing sample (RIF+) must be performed for each specimen to enable results to be interpreted.
- Disposal of Actiphage and Sensor cells, and any equipment that has come into contact with these reagents, should be in accordance with local regulations for the disposal of microbiological waste.

6 SPECIMENS

The *FASTPlaque-Response*TM test has been evaluated for use with the following specimen types:

Smear-positive expectorated sputum specimens.

A sputum smear should be prepared from all specimens prior to processing using the *FASTPlaque-Response*TM test to confirm the AFB smear-positive status of the specimen. This may be a direct smear (prior to decontamination)^{4, 5}, or concentrated smear (following decontamination)⁴. Sputum smears may be stained using either Ziehl-Neelsen stain, or by a fluorochrome stain (*e.g.* auramine-O)⁵. Smear-positive sputum specimens (at least 1+ smear positive, greater than 10 bacilli per 100 fields) may be tested directly using the *FASTPlaque-Response*TM test⁴.

Sputum specimens should be processed as soon as possible after collection. Specimens may be stored for a maximum of 3 days at 2-8°C prior to decontamination and testing. Testing specimens stored for longer than three days is likely to result in a greater number of un-interpretable results.

6.1 SAMPLE PREPARATION

Sputum digestion and decontamination should be carried out using the N-acetyl-L-cysteine-sodium hydroxide method as described in the Clinical Microbiology Procedures Handbook⁶ (referred to hereafter as the NALC-NaOH method).

The following reagents are required:

4% sodium hydroxide (NaOH) solution

2.9% sodium citrate solution

0.067M phosphate buffer pH 6.8

N-acetyl-L-cysteine (NALC) powder

The above solutions should be prepared using purified water, and sterilised by autoclaving at 121°C for 15 minutes. They should be stored at less than 30°C. To prepare the NALC-NaOH-sodium citrate decontamination solution, a 1:1 mixture of 4% NaOH and 2.9% sodium citrate solution is then prepared, and 0.50g NALC is added per 100ml solution (0.5% w/v). Once the NALC is added, the solution is stable for up to 24 hours if stored at 2-8°C.

1. Add an equal volume of the NALC-NaOH-sodium citrate decontamination solution to the sputum specimen in a sterile 50ml conical centrifuge tube. If the volume of specimen is greater than 10ml, transfer 10ml of the most purulent or mucoid portion of the specimen to the centrifuge tube using a sterile pipette. Tightly close the lid.
2. Mix the contents of the tube using a vortex mixer, for not more than 30 seconds.
3. Incubate for 15 minutes at room temperature.
4. Dilute the tube contents by adding the phosphate buffer up to the 45ml mark on the centrifuge tube. Tightly close the lid.
5. Centrifuge for 20 minutes at a minimum of 2000g.
6. Gently pour off the supernatant into a discard container containing a suitable mycobactericidal disinfectant. Take care not to pour off the pellet. If additional tests (*i.e.* culture) are required, a portion of the supernatant should be removed at this point.
7. Suspend the resultant pellet in 15ml *Response Medium Plus*.
8. Centrifuge for 20 minutes at a minimum of 2000g.
9. Gently pour off the supernatant into a discard container containing a suitable mycobactericidal disinfectant. Take care not to pour off the pellet.
10. Suspend the resultant pellet in 1ml *Response Medium Plus* to produce the test suspension.

7 PROCEDURE

7.1 INCUBATION OF TEST SUSPENSION

1. Aseptically add 0.5ml of *Response* Rifampicin solution to “RIF +” reaction vessel. Using a separate sterile pipette aseptically add 0.5ml *Response* Medium Plus to “RIF -” reaction vessel.
2. Aseptically add 0.5ml of the test suspension (Section 6.1.10) to each of the “RIF +” and “RIF -” reaction vessels labeled with specimen identifier. Use a separate sterile pipette for each dispensing action. Mix gently and then incubate for 18-24 hours at 37°C.

7.2 PROCESS CONTROL

A Negative and Positive process control must be performed on every occasion the assay procedure is run. The controls will be prepared at the point of assay and require no pre-incubation step.

7.2.1 Negative control

Dispense 1ml *Response* Medium Plus into a reaction vessel labelled “Negative Control”. The Negative Control should result in 0-10 plaques.

7.2.2 Positive control

Aseptically prepare a dilution series of Sensor cells by adding 0.1ml of reconstituted Sensor cells to 10ml of *Response* Medium Plus. Mix thoroughly. This should be further diluted by taking 0.1ml and adding this to 10ml of *Response* Medium Plus. Mix thoroughly. A further dilution is made by taking 0.1ml of this dilution and adding to 10ml of *Response* Medium Plus. 1ml of this final dilution should then be added to a reaction vessel labelled “Positive Control”. The Positive Control should result in 20 plaques or greater.

7.3 ASSAY PROCEDURE

1. Prepare Positive and Negative Controls as per Section 7.2.
2. Remove pre-incubated specimens (Section 7.1) from the 37°C incubator and process immediately.
3. To each sample and prepared control add 0.1ml of Actiphage, taking care not to touch the sides of the reaction vessel with the pipette tip. Shake gently ensuring that the contents remain in the bottom of the vessel. Incubate at 37°C for 60 minutes.
4. After incubation add 0.1ml of Virusol solution to each reaction vessel.
5. Ensure that the lid is firmly secured. Mix the contents of the reaction vessel well by inverting and rolling the reaction vessel to ensure that Virusol comes into contact with all interior surfaces of the vessel.
6. Leave to stand at room temperature (20-25°C) for 5 minutes.
7. Add 5 ml of *Response* Medium Plus to the vessel. Mix by inverting the reaction vessel once.
8. To each vessel add 1ml of Sensor cells.
9. Remove the molten *FASTPlaqueTB*TM Agar from the 55°C water bath and add 5ml to an empty sterile pre-labelled Petri dish. A single plate should be poured at a time.
10. Immediately pour the entire contents of the reaction vessel into the Petri dish. Replace the lid of the Petri dish and mix the contents well, by swirling in both directions, ensuring that the entire bottom surface of the plate is covered. Care should be taken to prevent agar touching the lid. Repeat steps 9 and 10 for each sample.
11. Leave at room temperature to allow the agar to set.
12. Once the agar has set (approximately 30 minutes at 20-25°C), invert each Petri dish and place in a 37°C incubator overnight (18-24 hours).
13. Remove the plates from the incubator and read results by counting the number of plaques formed. Refer to section 8 for interpretation. Record the results.

8 RESULTS

Results of the *FASTPlaque-Response*TM test are read as plaques (zones of clearing) on a lawn of Sensor cell growth. Sensor cell lawns should appear evenly opaque and creamy white in colour. Plaques will appear in the lawns if infected viable cells are present in the sample. Plaques will be clear and circular of approximately 1-4mm diameter. Individual plaques may be present, or if sufficient plaques are present, they may fuse to give either confluent or complete lysis of the lawn. Confluent or completely lysed plates usually contain more than 300 plaques.

Care needs to be taken when interpreting results to differentiate growth of Sensor cell lawns from lawns of contaminating bacteria that may sometimes result from heavily contamination specimens. Contamination may be present as discrete colonies on a lawn, or as a generalised growth. If plaques can be seen in the presence of contamination, then results may be read and interpreted.

If there is any doubt whether lawns are completely lysed, contain no plaques or are contaminated, plates should be re-incubated for up to 24 hours along with control plates and lawn appearance and intensity re-evaluated.

8.1 INTERPRETATION

For interpretation of results of the *FASTPlaque-Response*TM test, the Positive and Negative controls should be within the following specified limits. If control results are outside these limits, interpretation of clinical specimens should not be made (refer to Section 10, Troubleshooting).

Negative control 10 plaques or less
Positive control 20 plaques or greater

Interpretation of results for sputum specimens is as follows:

RIF- plate 100 plaques or greater to allow interpretation of results

If RIF+ plate has 50 plaques or greater **RIFAMPICIN RESISTANT**
If RIF+ plate has less than 50 plaques **RIFAMPICIN SUSCEPTIBLE**

For specimens giving less than 100 plaques on the RIF - plate, refer to Section 10, Troubleshooting. If contamination appears on either the RIF- or RIF+ plates to an extent that the result can not be read, then no interpretation of the specimen is possible.

Examples of possible *FASTPlaque-Response*TM results and their interpretation:

Specimen	RIF- plate (no. plaques)	RIF+ plate (no. plaques)	Interpretation
1	>300	>300	RESISTANT
2	150	90	RESISTANT
3	>300	1	SUSCEPTIBLE
4	79	0	Not interpretable - Invalid RIF-
5	23	22	Not interpretable - Invalid RIF-

9 CLINICAL PERFORMANCE

***FASTPlaque-Response*TM clinical performance data in smear-positive specimens, Cape Town, South Africa⁷**

*FASTPlaque-Response*TM correctly determined rifampicin susceptibility in all smear-positive specimens ($n=145$; resolved data). 82.6% of specimens tested gave interpretable results by the *FASTPlaque-Response*TM test.

	7H11 resistant	7H11 susceptible	Total
<i>FASTPlaque-Response</i> TM resistant	11	0	11
<i>FASTPlaque-Response</i> TM susceptible	0	134	134
Total	11	134	145

***FASTPlaque-Response*TM clinical performance data in smear-positive previously treated TB patients in Port Elizabeth, South Africa⁸**

*FASTPlaque-Response*TM performance was comparable with indirect 7H11 proportion method, with results in 2 days compared with up to 11 weeks. Overall agreement (resolved data) was 97.8%. Resolved sensitivity and specificity were 95.6% and 99.3% respectively. 72.9% of smear-positive culture-positive specimens gave interpretable results.

	7H11 resistant	7H11 susceptible	Total
<i>FASTPlaque-Response</i> TM resistant	86	1	87
<i>FASTPlaque-Response</i> TM susceptible	4	138	142
Total	90	139	229

Studies were carried out in a high TB burden setting, with a low incidence of NTMs. All smear-positive specimens tested were confirmed to contain *M.tuberculosis* complex organisms.

10 LIMITATIONS

- Detection and susceptibility testing of *M. tuberculosis* by the *FASTPlaque-Response*[™] test is dependent on the quantity and quality of the specimen collected its storage prior to processing, and the number of live organisms present. Inappropriate storage of specimens may lead to un-interpretable results, due to either overgrowth of contaminants or too few plaques on the RIF- plates.
- The *FASTPlaque-Response*[™] test has only been evaluated with smear-positive sputum specimens. Other specimen types and smear-negative sputum specimens have not been fully evaluated with the *FASTPlaque-Response*[™] test. An AFB smear should be performed prior to performing the *FASTPlaque-Response*[™] test.
- Decontamination must be performed using the recommended NALC-NaOH method. The use of other decontamination methods has not been fully evaluated. Some decontamination agents have been shown to have detrimental effects on assay efficacy. Use of lower centrifuge speeds will reduce the number of TB bacilli sedimented, which may adversely affect the performance of the test. Omission of the overnight incubation step (Section 6.1) will have a negative effect on assay performance. Omission of the NOA Antimicrobial Supplement will lead to a higher level of contamination and consequently fewer interpretable results.
- The *FASTPlaque-Response*[™] test requires viable TB bacilli to be present in order to obtain >100 plaques on the rifampicin-free (RIF-) sample and be able to interpret the test result. Patients on TB treatment who are responding to therapy may give a positive smear result after the TB are dead. These specimens would not give an interpretable result with *FASTPlaque-Response*[™], which requires live TB. Approximately 10-15% of smear-positive culture-positive specimens may not be detected by the test, due to the presence of inhibitory factors in the sputum (see Section 9). These specimens should be submitted for culture and indirect susceptibility testing.
- The *FASTPlaque-Response*[™] test determines rifampicin resistance of MTB. Whilst rifampicin resistance is a good marker for multidrug-resistance in many settings ², and usually occurs in combination with isoniazid resistance, rifampicin mono-resistance, or resistance in combination with other drugs, can occur. Local guidelines for management of drug resistant TB should be consulted.
- A specimen that produces >100 plaques on the RIF- plate may contain *M.tuberculosis*, *M.bovis*, *M.africanum* or *M.microti*. Laboratory studies (testing of decontaminated sputum model) have sometimes given >100 plaques with certain species of Non-tuberculous Mycobacteria (NTMs). NTMs may be rifampicin susceptible or resistant. The presence of *M.tuberculosis* complex organisms in the specimen should be confirmed.
- The presence of high concentrations of blood in sputum specimens may adversely affect the performance of the test.
- *FASTPlaque-Response*[™] results should be interpreted in relation to other laboratory and clinical information. False resistant and false susceptible results can occasionally occur. Results may be confirmed by a conventional susceptibility test method, such as the proportion method. Please refer to the expected performance and limitations of the test, as outlined in these instructions.

11 TROUBLESHOOTING

For interpretation of results of the *FASTPlaque-Response*[™] test, the positive and negative controls must fall within the specified limits and the RIF- plate should have 100 plaques or greater. The following table lists several possible reasons for results being obtained outside the recommended specifications. For further information contact your local distributor.

FAULT	CAUSE	ACTION
Greater than 10 plaques obtained on the negative control	Insufficient Virusol was added, or it was not adequately mixed to inactivate all excess Actiphage.	<i>See Assay Procedure (Section 7.3).</i>
	Media or equipment may have been contaminated with <i>M. tuberculosis</i> or Sensor cells.	<i>Check for contamination. Discard or re-sterilise all suspected contaminated reagents and equipment.</i>
Less than 20 plaques obtained on the positive control	Incorrect dilution of Sensor cells during preparation of positive control.	<i>See Process Control (Section 7.2).</i>
	Sensor cells or Actiphage exceeding recommended expiry date	<i>See Storage, Reconstitution, Shelf-life and Re-use of Kit Components (Section 4.3)</i>
	Insufficient Actiphage was added.	<i>See Assay Procedure (Section 7.3).</i>
Less than 100 plaques obtained on the RIF- plate	Specimen stored for longer than recommended prior to testing.	<i>See Sample Preparation (Section 6.1).</i>
	There is a low number of viable MTB, below the limit of detection of the test	<i>Perform a sputum smear prior to testing to ensure specimen is smear-positive. There may be a low number (or no) viable MTB in smear-positive specimens of patients on TB treatment. Attempt to culture specimen and perform indirect susceptibility test if possible.</i>
	The strain tested is not MTB, but may be a non-tuberculous mycobacterium [NTM] (also known as mycobacterium other than tuberculosis, or atypical mycobacterium).	<i>Confirm the identity of the mycobacterial isolate being tested.</i>
Growth of contaminating bacteria on plate obscures lawn of Sensor cells and does not allow interpretation of plaques	Decontamination procedure may not be sufficient.	<i>Follow instructions for sample preparation carefully (Section 6.1).</i>
	NOA Antimicrobial Supplement may have been omitted	<i>Ensure that NOA Antimicrobial Supplement is added to the Response Medium Plus.</i>
	There may be a long delay between collection of specimen and processing, allowing overgrowth of organisms.	<i>Specimens may be stored for up to 72 hours between collection and processing, if stored at 2-8°C.</i>
	Reagent contamination	<i>Discard all reagents and prepare fresh. Review aseptic technique.</i>
Lower rate of valid results than expected.	Sample decontamination procedure may be too harsh, e.g. higher NaOH concentration or longer incubation time used.	<i>Follow instructions for sample preparation carefully (Section 6.1). Use of higher NaOH concentration or longer incubation time, or use of other methods may adversely affect test performance.</i>

12 REFERENCES

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13 MATERIAL SAFETY DATA SHEETS

A material safety data sheet (MSDS) for the kit is available from the manufacturer or their distributor, upon request.

14 OTHER INFORMATION

A large text version of these instructions is available from the manufacturer or the local Biotec distributor, upon request.

The following symbols have been used on this product:



Store at 2-8°C



Irritant



Expiry



Consult IFU (Instructions for use)

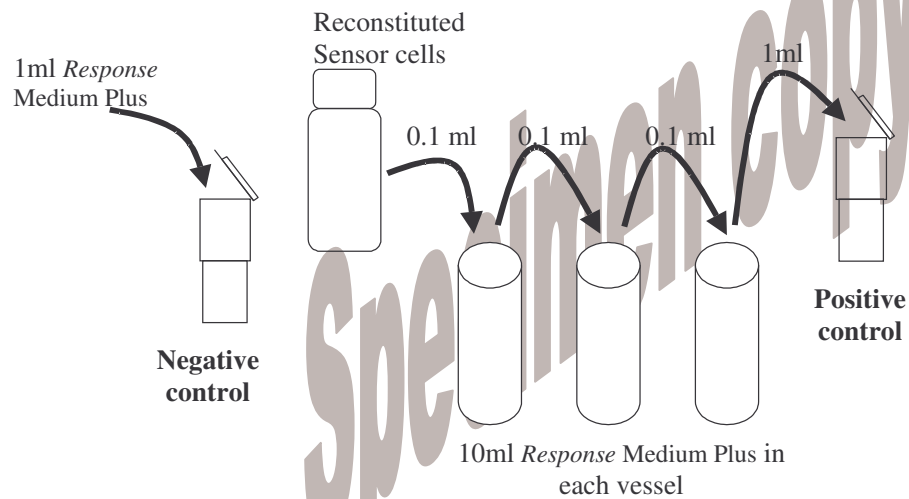


Manufacturer

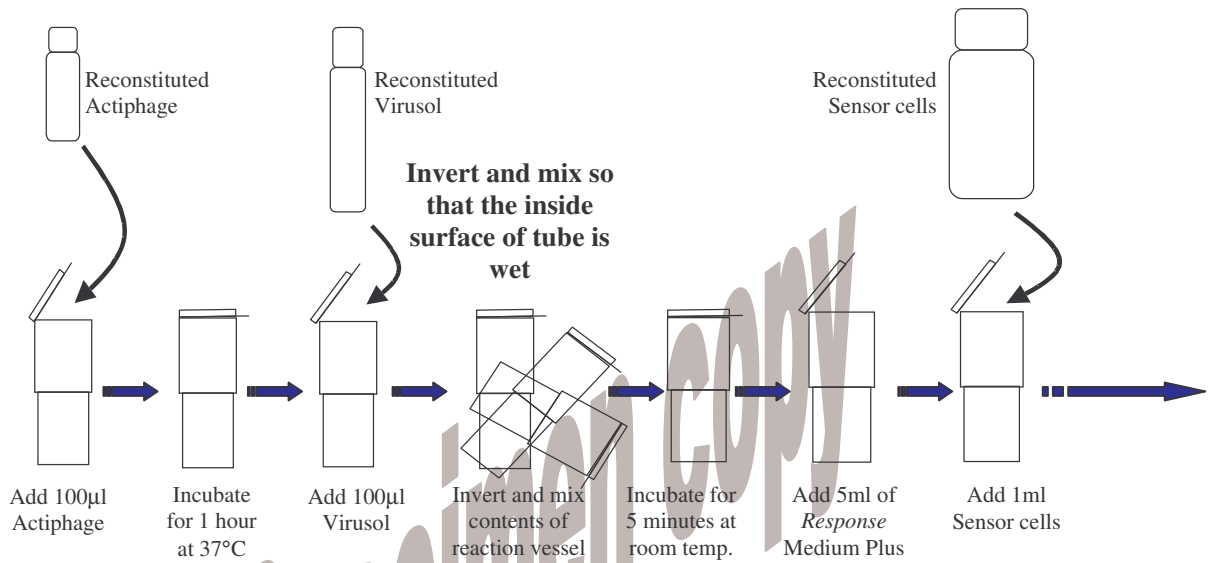
WORK FLOW

Day 0	Arrival of specimen Decontaminate with NALC-NaOH Wash with <i>Response</i> Medium Plus Incubate samples overnight with and without rifampicin
Day 1	Prepare process controls Assay
Day 2	Read results

PREPARATION OF CONTROLS



SCHEMATIC REPRESENTATION OF ASSAY PROCEDURE



Molten agar should not be too hot - touch the top of your hand with bottle

Mix agar and sample quickly and thoroughly. Avoid splashing lid or creating air bubbles

