

***FASTPlaque*TB™**

A rapid bacteriophage assay for the detection of *Mycobacterium tuberculosis* complex in clinical samples

For *in vitro* diagnostic use only

Specimen copy

50 Determinations

FASTPlaque Catalogue No. 5/100

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1 INTENDED USE

BIOTEC's *FASTPlaqueTB* test is a rapid bacteriophage assay for the determination of *Mycobacterium tuberculosis* (MTB) complex in decontaminated sputum samples.

2 INTRODUCTION

Tuberculosis is a bacterial disease spread by infectious airborne droplets containing *Mycobacterium tuberculosis* (occasionally *Mycobacterium bovis* or *Mycobacterium africanum*). It is estimated by the World Health Organisation (WHO) that approximately one third of the world's population is infected with *M. tuberculosis*, resulting in 9 million new cases of TB each year, leading to 3 million deaths annually¹.

Today, in locations where most tuberculosis is found, the diagnosis of tuberculosis is often based upon both laboratory findings and clinical signs and symptoms. The laboratory test most often used to diagnose TB is AFB (acid-fast bacilli) microscopy [also called sputum smear microscopy] for the examination of sputum specimens obtained from symptomatic patients who take the initiative to visit a health care facility. Sputum smear microscopy will identify the most infectious cases of TB. However, it is accepted that this approach will also miss a substantial number of cases of TB. Some of these missed cases will be infectious². Other cases, if left undiagnosed, may later become sputum smear microscopy positive and infectious. Culture of digested and decontaminated sputum is a more sensitive test for the diagnosis of TB. However, a culture of *M. tuberculosis* takes several weeks to grow. This will result in delays in diagnosis of TB that may lead to further spread of disease.

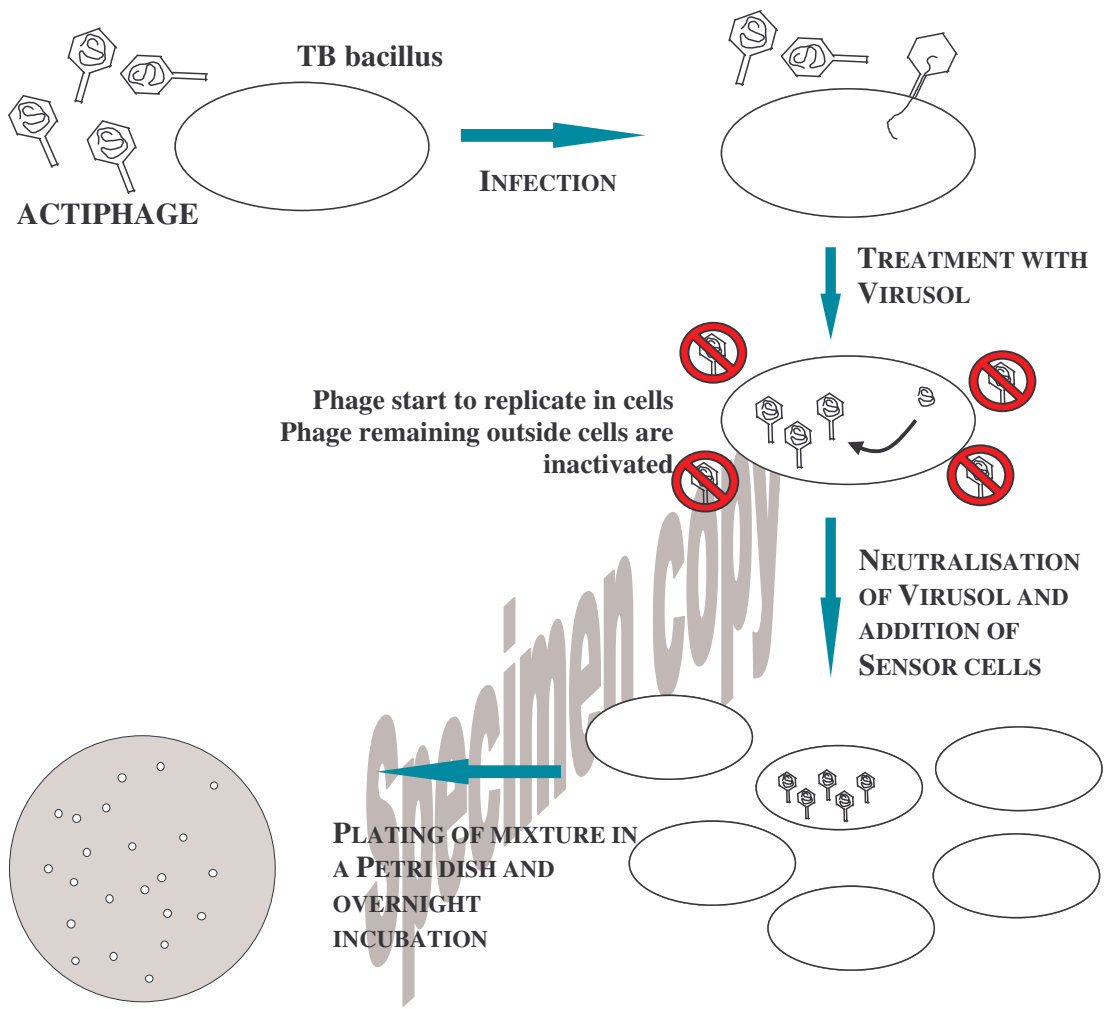
3 PRINCIPLE OF *FASTPlaqueTB*

FASTPlaqueTB is based on the *FASTPlaque*TM principle (Phage Amplification, Figure 1) and utilises mycobacteriophage (viruses that specifically infect [or target] mycobacteria) to reflect the presence of viable MTB within a sputum specimen. The sputum specimen is first decontaminated. This kills most bacteria present in the specimen other than the target mycobacteria. Next, the target mycobacterial cells are rapidly infected by the target-specific bacteriophage (Actiphage) added to the decontaminated specimen. The resulting mixture is then treated with a virucidal solution (Virusol) that causes the destruction of 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 is related to the number of viable MTB cells containing mycobacteriophage. If there are no target bacteria (*i.e.* no viable MTB cells) in the original sample, there will be no phage amplification and therefore no bacteriophage to detect as plaques at the end of the assay.

Figure 1: FASTPlaque™ test principle.



4 PRODUCT CONTENTS

Each kit contains sufficient materials for 50 determinations.

The shelf life of the kit is indicated on the outer box label. The kit must be stored at 2-8°C.

4.1 REAGENTS

- 5 x FPTB Medium sachets ^A
- 5 x FPTB 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
- 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)
- 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
- Disposable gloves
- Discard container
- Vortex mixer
- Centrifuge with sealed centrifuge cups suitable for 50 ml 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 centrifuge tubes (aerosol free and graduated)
- Pipettes to dispense 1.1ml, 1ml and 0.1ml plus sterile pipette tips
- 10ml graduated pipettes
- Suitable mycobactericidal disinfectant
- Sterile tubes (for preparation of assay controls)

*To include means of confirming correct operating temperature.

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

Prior to use, the test kit and components 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 FPTB Medium

FPTB Medium should be reconstituted by adding the contents of the sachet to 270ml 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. Allow to cool to room temperature before addition of FPTB Growth Supplement (see 4.3.3). 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 FPTB Growth Supplement. Aseptic techniques should be used when handling autoclaved medium. FPTB Medium should not be used if there are any visual signs of contamination (turbidity). One sachet of FPTB Medium is sufficient for 10 tests.

4.3.2 FPTB Growth Supplement

This is added to cooled sterile FPTB Medium (see 4.2.3 for storage requirements of FPTB Medium Plus). One vial of FPTB Growth Supplement is sufficient for 10 tests.

4.3.3 FPTB Medium Plus

FPTB Medium Plus is used to reconstitute Actiphage and Sensor cells. To prepare FPTB Medium Plus, aseptically add one bottle of FPTB Growth Supplement to reconstituted, autoclaved and cooled FPTB Medium. Mix gently before use. FPTB Medium Plus can be stored for up to 4 weeks at 2-8°C, (but no longer than the expiry date of FPTB Medium), provided aseptic techniques are employed throughout usage. FPTB Medium Plus should not be used if there are any visual signs of contamination (turbidity). This volume of FPTB Medium Plus is sufficient for 10 tests.

4.3.4 Actiphage

Actiphage is prepared by adding 1.1ml of FPTB Medium Plus into the vial and mixing gently to dissolve. One bottle of Actiphage is sufficient for 10 tests. Reconstituted Actiphage may be stored for up to 7 days if kept at 2-8°C, (but no longer than the expiry date of FPTB Medium), provided aseptic techniques are employed throughout usage. Mix well prior to use.

4.3.5 Sensor cells

Sensor cells are prepared by adding 11 ml of FPTB Medium Plus into the vial and mixing gently to suspend. One bottle of Sensor cells is sufficient for 10 tests. 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 FPTB Medium), provided aseptic techniques are employed throughout usage. Mix well prior to use.

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 FPTB Agar

The 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 (±2°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 (±2°C) before use. The agar may be reheated no more than 3 times, enabling subsequent re-uses of the kit if necessary. This volume of FPTB Agar is sufficient for 10 tests.

4.3.8 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

Sputum specimens may contain *Mycobacterium tuberculosis*, some of which may be drug resistant strains. In addition, clinical samples may contain other infectious agents such as Hepatitis B virus and Human Immunodeficiency Virus (HIV). Suitable safety precautions³ 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 and 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.

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 Trouble Shooting, Section 11). 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 and 10 test box labels. 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.
- 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*TB assay has been evaluated for use with the following specimen types:

Sputum, expectorated.

If specimens are to be stored prior to testing, they should be kept at between 2-8°C for no longer than 3 days. Storage of specimens is likely to increase the rate of contaminant growth on results plates and the number of false negative results.

6.1 SPECIMEN 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 Handbook ⁴ (referred to hereafter as the NALC-NaOH method).

The following reagents are required:

4% sodium hydroxide 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. A 1:1 mixture of 4% NaOH and 2.9% sodium citrate solution is then prepared. To prepare the decontamination solution, 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 decontamination solution to the 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.
7. Suspend the resultant pellet in 15ml FPTB 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 FPTB Medium Plus.
11. Aseptically transfer 1ml of the suspension to a reaction vessel labelled with specimen identifier.
12. Incubate samples overnight (18-24 hours) at 37°C.

7 PROCEDURE

7.1 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.

8.1.1 Negative control

Dispense 1ml of FPTB Medium Plus into a reaction vessel labelled “Negative control”. The Negative control should result in 0-10 plaques.

7.1.2 Positive control

Aseptically prepare a dilution series of Sensor cells by adding 0.1ml of reconstituted Sensor cells to 10ml of FPTB Medium Plus. Mix thoroughly. This should be further diluted by taking 0.1ml and adding this to 10ml of FPTB Medium Plus. Mix thoroughly. A further dilution is made by taking 0.1ml of this dilution and adding to 10ml of FPTB 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.

7.2 ASSAY PROCEDURE

1. Prepare Positive and Negative controls as per Section 7.1.
2. Remove pre-incubated specimens (as per section 6.1) from 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 vessel 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. Allow the reaction vessel to stand at room temperature (20-25°C) for 5 minutes.
7. Add 5 ml of FPTB 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 FPTB Agar from the 55°C water bath and add 5 ml to an empty pre-labelled sterile 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 plate has set (approximately 30 minutes at 20-25°C), invert each Petri dish and place in a 37°C incubator overnight.
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 *FASTPlaqueTB* 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 1-4 mm 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 constitute a positive result and 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 be present in a specimen. 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 *FASTPlaqueTB* test results, 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, and the Troubleshooting section should be referred to.

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

Interpretation of *FASTPlaqueTB* results for decontaminated sputum specimens are as follows:

0-19 plaques	Negative <i>FASTPlaqueTB</i> result
20 plaques or greater	Positive <i>FASTPlaqueTB</i> result

9 CLINICAL PERFORMANCE

***FASTPlaque*TB clinical performance data in adult pulmonary TB suspects in Cape Town, South Africa**

*FASTPlaque*TB results were compared to concentrated auramine smear microscopy and LJ culture (confirmed with PNB testing). Performance of *FASTPlaque*TB was compared to culture results for all, smear positive and smear negative specimens.⁵

	All specimens <i>n</i> =1618	Smear positive <i>n</i> =171	Smear negative <i>n</i> =1447
Sensitivity	72.5%	86.8%	48.7%
Specificity	99.0%	83.3%	99.5%
PPV *	0.91	0.94	0.84
NPV #	0.96	0.67	0.97

* PPV – positive predictive value; # NPV – negative predictive value

*FASTPlaque*TB was shown to be a significantly better predictor of culture result than concentrated auramine smear microscopy.

***FASTPlaque*TB clinical performance data in pulmonary sputum specimens from TB suspects in Karachi, Pakistan**

*FASTPlaque*TB results were compared to concentrated ZN smear microscopy and LJ culture (confirmed with PNB testing and PCR confirmation). Performance of *FASTPlaque*TB was compared to culture results for all, smear positive and smear negative specimens.⁶

	All specimens <i>n</i> =514	Smear positive <i>n</i> =192	Smear negative <i>n</i> =322
Sensitivity	81.6%	87.4%	67.1%
Specificity	97.7%	88.2%	98.4%
PPV *	0.97	0.99	0.92
NPV #	0.85	0.40	0.91

*PPV – positive predictive value; # NPV – negative predictive value

A combination of *FASTPlaque*TB and smear identified 80.7 to 90.6% of culture positive specimens in each study^{5,6}.

Further information on the performance of the *FASTPlaque*TB test, including other recent publications, can be accessed from www.biotec.com/fptb.

10 LIMITATIONS

1. Detection of *M. tuberculosis* by the *FASTPlaque*TB test is dependent on the quantity and quality of the specimen collected, its storage prior to processing, and the number of live organisms present.
2. In specimens with few TB bacilli, clumping may affect the sensitivity of the test.
3. The *FASTPlaque*TB assay has only been evaluated with sputum specimens. Other specimen types have not been fully evaluated using *FASTPlaque*TB.
4. Decontamination must be performed using the recommended NALC-NaOH method. The use of other decontamination methods in association with the *FASTPlaque*TB test has not been fully evaluated. Some decontamination agents are believed to have detrimental effects on assay efficacy. Omission of the overnight resuscitation step (section 6.1, no. 12) will significantly reduce the sensitivity of the test.
5. Use of lower centrifugation speeds will reduce the number of TB bacilli sedimented, which may reduce the sensitivity of the *FASTPlaque*TB assay.
6. The *FASTPlaque*TB test requires viable TB bacilli to be present. Anti-tuberculosis chemotherapy may damage and destroy TB bacilli so that they are not detected by the *FASTPlaque*TB test. The effect of broad spectrum antibiotics has not been fully evaluated with the *FASTPlaque*TB test.
7. The presence of high concentrations of blood (>10%) in sputum specimens may adversely affect the performance of the test.
8. A specimen that is positive by *FASTPlaque*TB may contain *M. tuberculosis*, *M. bovis*, *M. africanum* or *M. microti*. Laboratory studies (testing of pure cultures and without decontamination) have occasionally given positive results with certain strains of Mycobacteria other than Tuberculosis (MOTT's).
9. *FASTPlaque*TB results should be interpreted in relation to other laboratory and clinical information. False positive and false negative results can occur. Please refer to the expected performance and limitations of the test, as outlined in these instructions.

11 TROUBLESHOOTING

For interpretation of results of *FASTPlaque*TB, the Positive and Negative controls must fall within the specified limits. The following table shows the most common 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).</i>
	Media or equipment may have been contaminated with <i>M. tuberculosis</i> , Sensor cells or Actiphage.	<i>Check for contamination. Discard all contaminated reagents and re-sterilise 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 7.1).</i>
	Sensor cells exceeding recommended expiry date have been used to prepare Positive control.	<i>See Reconstitution, Storage and Re-use of Kit Components (Section 4.3)</i>
	Actiphage exceeding recommended expiry date was used.	<i>See Reconstitution, Storage and Re-use of Kit Components (Section 4.3)</i>
	Insufficient Actiphage was added.	<i>See Assay Procedure (Section 7).</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>
	There may be a long delay between collection of specimen and processing, allowing overgrowth of organisms.	<i>Review local guidelines for specimen collection and transport. (Section 6)</i>
	Reagents contaminated.	<i>Prepare fresh reagents and review sterilisation and aseptic procedures.</i>
Lower rate of positive 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>
Plaques uneven in appearance	Agar not completely melted allowing solid agar fragment to be added to plates	<i>Ensure agar is heated sufficiently to allow complete melting</i>
Agar not completely set	Agar insufficiently mixed or not completely molten before use	<i>Ensure agar fully melted before use. Mix thoroughly with sample.</i>

12 REFERENCES

1. Harries, A and Maher, D. **1997**. TB. A Clinical Manual for South-East Asia. World Health Organisation, Geneva.
2. Behr, MA. **1999**. Transmission of *Mycobacterium tuberculosis* from patients smear negative for acid-fast bacilli. *Lancet*. **353**: 444-449.
3. Bozzi CJ *et al.* **1994**. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in Health Care Facilities. *MMWR* **43** (RR13): 1-132.
4. Master, R.N. **1992**. Mycobacteriology. In Isenburg, H.D. (ed.). *Clinical Microbiology Procedures Handbook*. Section 3. Vol. 1. American Society for Microbiology, Washington DC.
5. Albert H, Heydenrych A, Brookes R, Mole R, Harley B, Subotzki E, Henry R, Azevedo V **2002** Performance of a rapid phage-based test, *FASTPlaqueTB*TM, to diagnose pulmonary tuberculosis from sputum specimens in South Africa. *Int. J. Tuberc. Lung Dis.* **6** (6): 529-537
6. Muzaffar, R., Batool, S., Aziz, F., Naqvi, A., Rizvi, A. **2002**. Evaluation of the *FASTPlaqueTB*TM assay for direct detection of *Mycobacterium tuberculosis* in sputum specimens. *Int. J. Tuberc. Lung Dis.* **6** (7): 635-640.

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:

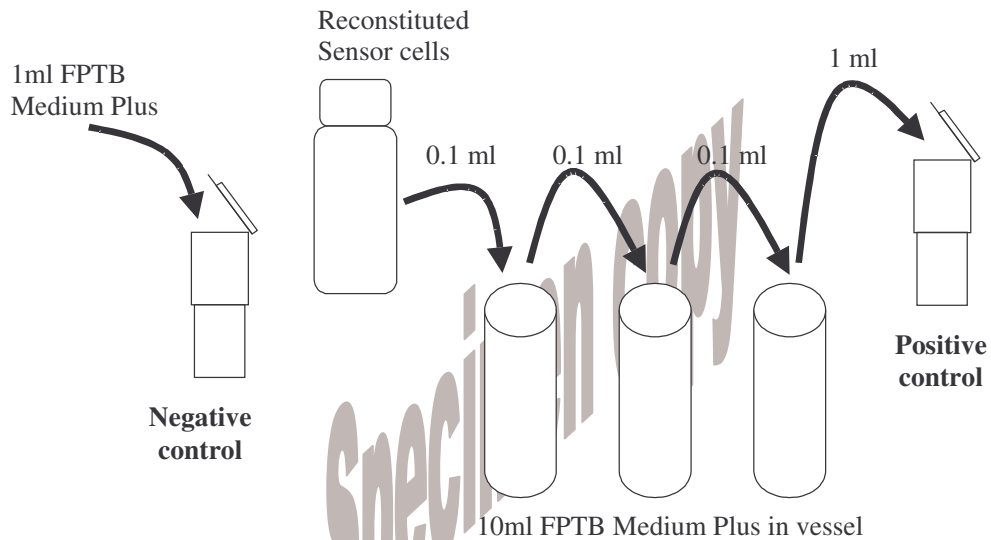


WORK FLOW

Day 0	Arrival of specimen Decontaminate with NALC-NaOH Wash with FPTB Medium Plus Incubate overnight
Day 1	Assay
Day 2	Read results

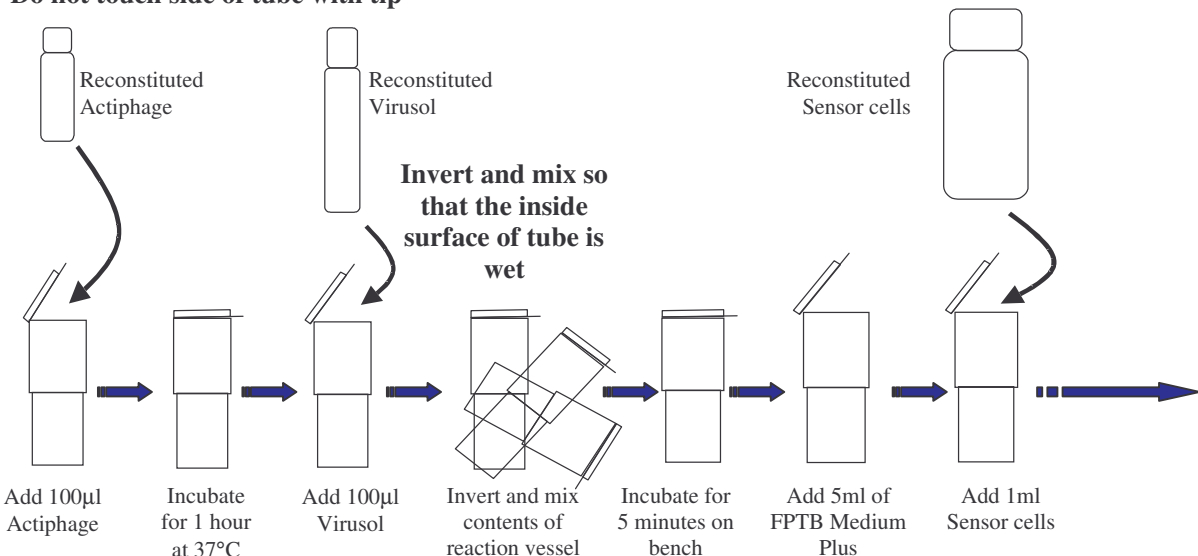
PREPARATION OF CONTROLS

Change pipette tip between each dilution step

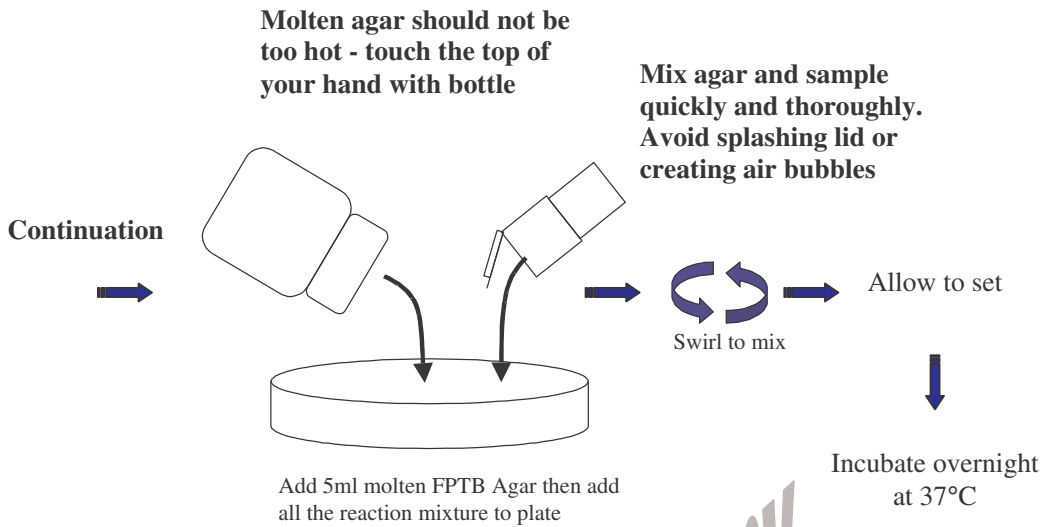


SCHEMATIC REPRESENTATION OF ASSAY PROCEDURE

Do not touch side of tube with tip

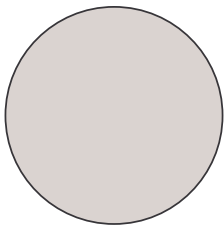


CONTINUATION OF ASSAY PROCEDURE

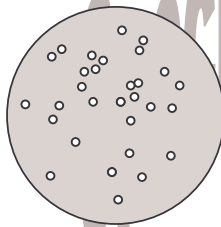


INTERPRETATION OF RESULTS

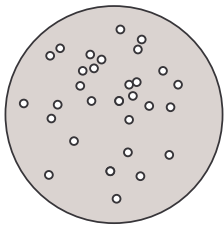
Negative Control - less than 10 plaques



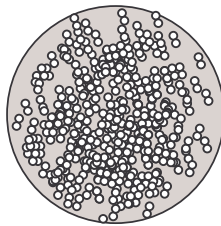
Positive Control - 20 plaques or greater



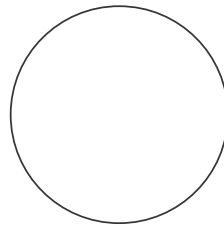
Positive Specimen - 20 plaques or greater



Positive Specimen - confluent lysis - 20 plaques or greater



Positive Specimen - complete lysis - 20 plaques or greater



Negative Specimen - less than 20 plaques

