

Instructions for Use

Actiphage[®] TB Blood Test

A rapid bacteriophage assay for the detection of mycobacteria in human blood samples.

For research use only

100 Reactions



Catalogue No. PBD-PC-001 and PBD-PC-004



1 INTENDED USE

PBD Biotech's Actiphage® TB Blood Test is a bacteriophage assay developed for the detection of viable mycobacteria, belonging to the *Mycobacterium tuberculosis* Complex (MTBC), in blood and this procedure describes this application for human blood samples.

2 PRINCIPLE OF ACTIPHAGE® TB BLOOD TEST

M. tuberculosis is a facultative intracellular pathogen that thrives inside the host's macrophages and other cell types and thus, as part of the blood sample preparation protocol, white blood cells (WBCs) need to be isolated and lysed, releasing the mycobacteria and facilitating access for phage infection. The lysed WBCs and contents are then added to sample tubes together with a lysis reagent (Actiphage® Reagent). If viable mycobacterial cells are present, they will be infected by the mycobacteria-specific bacteriophage. The phage replicate intracellularly until new progeny phage are produced which perforate the bacterial wall allowing their release. Following filtration to remove any whole bacteria, genetic material released from the lysed mycobacteria is collected and purified. Finally, mycobacteria are identified using a MTBC specific qPCR.

3 PRODUCT CONTENTS

Each test contains sufficient materials for 100 reactions. The shelf life of each component is indicated on the respective label.

The test must be stored at 2 °C – 8 °C on receipt but is shipped between 10 °C and 24 °C (ambient temperature).

3.1 ITEMS PROVIDED

1 x Actiphage® TB Blood Test & TB PCR Test (PBD-PC-001) which contains:

- 1 x Actiphage TB Blood Test (PBD-PC -004) containing:
 - 1 x Actiphage® Medium sachet (PBD-PC-011)
 - 2 x Actiphage® Growth Supplement bottles (PBD-PC-012)
 - 5 x lyophilised Actiphage® Reagent vials (PBD-PC-010)
 - 1 x lyophilised Actiphage® BCG Control vial (PBD-PC-013)
 - 110 x Rapid tubes (PBD-PN-002)
 - 3 x HetaSep™ solution bottles (PBD-PN-001)

- 1 x Actiphage® TB PCR Test (PBD-PC-009) containing:
 - 4 x TB Lyophilized PCR Beads
 - 4 x PBD Resuspension Buffer x2
 - 4 x Negative Control (NTC - water)
 - 4 x Internal Amplification Control (IAC)

3.2 ITEMS REQUIRED BUT NOT PROVIDED

3.2.1 Reagents

- Molecular biology grade DNase/RNase-free water
- Sterile Phosphate Buffered Saline (PBS) for isolation of WBCs.
- DNA purification Kit
- Genomic DNA from MTBC to use as a PCR Positive Template Control (PTC) (Optional).

3.2.2 Accessories and Equipment

General Equipment:

- Microbiology laboratory consistent with local regulations for working with hazard group 3 pathogens.
- 'Pre-PCR Mix' and 'DNA preparation' dedicated laboratory areas
- Class II biosafety cabinet (or containment facilities compliant with local regulations for potentially infectious agents).
- Fridge (2 °C – 8 °C)
- Freezer (-20 °C – -18° C)
- 37 °C incubator
- 45 °C heat-block or water bath
- Autoclave capable of reaching 121 °C (15 psi).
- Pipettes capable of dispensing 1 mL, 200 µL, 100 µL, 20 µL, 10 µL and 1 µL.
- Electronic pipette for use with 5 mL and 10 mL stripettes.
- Swing out centrifuge rotor suitable for 15 mL or 50 mL conical tubes and swing out rotor capable of generating at least 120 x g.
NOTE: Samples must only be centrifuged in equipment with biocontainment lids.
- Microcentrifuge suitable for 2 mL microcentrifuge tubes and capable of generating at least 16,000 x g.
- Vortex mixer
- Quantitative PCR (qPCR) Instrument
- Racks capable of holding 2 mL, 15 mL, and 50 mL tubes.

- 500 mL autoclavable clear glass screw top bottle.
- Howie style side fastening laboratory coat.
- Safety glasses

General Disposables:

- Autoclavable containers for discarding of waste (various sizes).
- Disposable gloves
- Sterile disposable 1.5 mL microcentrifuge tubes capable of withstanding at least 16,000 x g.
- Sterile disposable 2.0 mL screw cap microcentrifuge tubes capable of withstanding at least 16,000 x g.
- Sterile filtered pipette tips for all listed pipette volumes.
- 10 mL graduated pipettes stripettes
- Sterile 15 mL and 50 mL conical polypropylene screw cap centrifuge tubes.
- BD Vacutainer plastic sodium heparin tubes or equivalent.
- qPCR plates and adhesive seals.
- Suitable mycobactericidal disinfectant

3.3 STORAGE, RECONSTITUTION, SHELF-LIFE, AND RE-USE OF TEST COMPONENTS

Prior to use, the test components should be stored at 2 °C – 8 °C. Ensure the components to be used are removed from the fridge and brought to room temperature (RT; 18 °C – 25 °C) prior to starting the assay. The components should not be used beyond the expiry date shown on the respective label. To ensure optimal performance, it is important that all test components are reconstituted as directed below and that any unused test components are stored according to the following instructions.

3.3.1 Actiphage® Medium base

To prepare Actiphage® Medium base, in a suitable autoclavable 500 mL clear glass screw top bottle, mix the contents of one Actiphage® Medium base sachet (2.35 g) with 450 mL molecular biology grade water. Gently mix to dissolve and autoclave at 121 °C for 10 minutes.

Once reconstituted and autoclaved, the Actiphage® Medium base can be stored for up to four weeks at RT before use. Actiphage® Medium base should not be used if there are any visual signs of contamination (turbidity).

3.3.2 Actiphage® Media Plus

Working aseptically, Actiphage® Media Plus is made by adding both bottles of Actiphage® Growth Supplement to 450 mL of Actiphage® Medium base. Ensure the Actiphage® Medium base is at RT before addition of Actiphage® Growth Supplement. Aliquot the Actiphage® Media Plus into sterile 50 mL conical tubes.

Actiphage® Media Plus is used to reconstitute the lyophilised Actiphage® Reagent (see section 3.3.3) and Actiphage® BCG Positive Control vials (see section 3.3.4).

Actiphage® Media Plus can be stored for up to four weeks at 2 °C – 8 °C before use, but no longer than the expiry date. Use a fresh aliquot of Actiphage® Media Plus each time the Actiphage® TB Blood test is carried out and discard any leftover media. Actiphage® Media Plus should not be used if there are any visual signs of contamination (turbidity).

3.3.3 Actiphage® Reagent

Working aseptically, Actiphage® Reagent is prepared by adding 2.3 mL of Actiphage® Media Plus to the lyophilised Actiphage® Reagent vial and mixing gently to re-hydrate the freeze-dried bacteriophage.

One vial of Actiphage® Reagent is sufficient for 20 reactions.

Once reconstituted, Actiphage® Reagent can be stored for up to one month at 2 °C – 8 °C before use, but no longer than the expiry date.

3.3.4 Actiphage® BCG Positive Control

Actiphage® BCG Positive Control contains attenuated *M. bovis* BCG. To reconstitute the Actiphage® BCG Positive Control, add 1 mL of Actiphage® Media Plus to the lyophilised Actiphage® BCG Positive Control vial and mix gently to re-hydrate the freeze-dried mycobacteria. Incubate the reconstituted Actiphage® BCG Positive Control without shaking at 37°C overnight (approx. 18 hours) to allow BCG cells to recover prior to starting the assay.

One vial of Actiphage® BCG Positive Control is sufficient for running all reactions included in the Actiphage® TB Blood Test.

Once reconstituted, Actiphage® BCG Positive Control can be stored for up to two weeks at 2 °C – 8 °C before use, but no longer than the expiry date.

3.3.5 Rapid tubes

Rapid tubes supplied with the test are for single use only. The test contains sufficient Rapid tubes for 100 reactions, plus 10 extra tubes for preparation of five Actiphage® BCG Positive Control samples. Only tubes supplied with the test should be used for the assay procedure.

3.3.6 Actiphage® TB PCR Test

Reconstitute the TB lyophilized PCR bead by adding 280 µL of PBD Resuspension Buffer x2.

One TB lyophilized PCR bead vial is sufficient for 25 reactions.

Resuspended PCR reagents are stable at 2 °C – 8 °C for three weeks, but no longer than the expiry date.

3.3.7 HetaSep™ solution

The Actiphage® TB Blood Test includes HetaSep™ solution for depletion of red blood cells (RBCs) from fresh blood samples and isolation of nucleated cells – WBCs.

The test includes enough HetaSep™ solution to process up to 100 samples of 2 mL of whole blood collected in sodium heparin anticoagulant.

HetaSep™ solution can be stored at RT or 2 °C – 8 °C and is stable until the expiry date. Ensure the solution warms up to RT and then invert the bottle to mix the contents prior to use.

4 ACTIPHAGE® TB BLOOD TEST PROCEDURE

4.1 BLOOD SAMPLE PREPARATION

Blood samples (3 – 10 mL) **MUST** be collected using sodium heparin as the anticoagulant (BD Vacutainer or equivalent) following the manufacturer's instructions for use.

If blood samples are to be stored prior to testing, they **MUST be kept between 10 °C and 24 °C for no longer than 48 hours from collection. Whole blood samples should not be refrigerated below 10 °C.** Longer storage, or refrigeration of samples is likely to result in failure to achieve effective recovery of mycobacteria.

PBD Biotech recommend that blood samples are handled in a class II biosafety cabinet or in compliance with local regulations for handling potentially infectious agents.

PBD Biotech recommend using HetaSep™ solution (Stemcell Technologies) to aggregate erythrocytes and quickly separate nucleated cells from RBCs in whole blood samples. Detailed

directions for use of HetaSep™ solution can be found on the Product Information Sheet, available at <https://www.stemcell.com>

4.1.1 WBC isolation using HetaSep™

1. Ensure the whole blood sample is homogenous by inverting the sample tube several times.
2. Gently mix 400 µL of HetaSep™ with 2 mL of whole blood (1-part HetaSep™ solution to 5-parts of blood) by pipetting in a 15 mL centrifuge tube.
3. Centrifuge the sample at 90 x g for 1 minute (24-hour-old blood) or 2 minutes (48-hour-old blood) at RT with the brake off.

NOTE: Centrifugation time is based on 2 mL of 24-hour-old or 48-hour-old blood sample. If processing fresh blood or different volume of blood, centrifugation time needs to be adjusted according to manufacturer.

4. Let the sample stand for 10 minutes at RT to allow further sedimentation of the RBCs and improve recovery of the nucleated cells.
5. Harvest the plasma layer **carefully** with a 1 mL pipette.
6. Transfer the harvested plasma into 5 mL (at least 4-fold volume) of sterile PBS in a clean 15 mL centrifuge tube and mix well by pipetting.
7. Centrifuge the sample at 120 x g for 10 minutes at RT with the brake off.
8. Carefully remove the supernatant with a pipette and discard taking care not to disrupt the pellet.

If processing the sample on the same day, proceed to WBC lysis (section 4.1.2).

SAFE STOPPING POINT (Optional) - If sample is to be stored overnight:

- Resuspend the pellet in 1 mL of Actiphage® Media Plus by pipetting gently until a uniform suspension is formed.
- Transfer the cell suspension into a clean 2.0 mL screw cap microcentrifuge tube and leave overnight at RT.
- After overnight incubation, centrifuge sample at 13,000 x g for 3 minutes at RT.
- Carefully remove the supernatant with a pipette and discard taking care not to disrupt the pellet.

4.1.2 WBC lysis

1. Resuspend the pellet in 1 mL of sterile distilled water and mix well by pipetting.
2. Place into to a clean 2.0 mL screw cap microcentrifuge tube.
3. Incubate sample for 20 minutes at RT to lyse the WBCs.
4. Centrifuge sample at 13,000 x g for 3 minutes at RT to collect released mycobacteria.
5. Remove the supernatant and discard taking care not to disrupt the pellet.
6. Resuspend in 100 µL of Actiphage® Reagent by pipetting gently until a uniform suspension is formed.

Sample processing will carry on in section 4.4.

4.2 ACTIPHAGE® BCG POSITIVE CONTROL PREPARATION

Extracellular DNA (eDNA) is an important component of the bacterial extracellular matrix and plays a fundamental structural role by promoting attachment to surfaces and bacterial aggregation. Preparation of Actiphage® BCG Positive Control is required to remove the background eDNA that could mask the mycobacterial DNA released due to the action of the bacteriophage lytic cycle.

1. Pre-heat a heating block or water bath to 45 °C.
2. After overnight incubation at 37°C, collect the reconstituted Actiphage® BCG Positive Control vial and mix well by gently pipetting up and down five times.
3. Transfer 100 µL of Actiphage® BCG Positive Control into a 1.5 mL microcentrifuge tube and incubate at 45 °C for 30 minutes.
4. Prepare a 1/10 dilution by adding 900 µL of Actiphage® Media Plus into the heated Actiphage® BCG Positive Control and mix well by gently pipetting up and down five times.
5. Add 100 µL of diluted Actiphage® BCG Positive Control directly onto the filter column contained within a pre-labelled Rapid tube. Prepare Actiphage® BCG Positive Control **in duplicate**.

NOTE: These **two** control samples will be used in section 4.3 to prepare the Actiphage® Reagent Positive Control (+ phage) and Actiphage® Media Plus positive control (- phage) to evaluate the phage activity.

6. Centrifuge at 400 x g in a microcentrifuge for 5 minutes at RT. If there is residual liquid left on the filter column, repeat this step.

7. Dispose of the filtrate containing the background eDNA of the Actiphage[®] BCG Positive Control.
8. Wash BCG cells by adding 500 µL of Actiphage[®] Media Plus to the Rapid tube column.
9. Centrifuge at 400 x g for 5 minutes at RT. If there is residual liquid left on the filter column, repeat this step.
10. Discard the filtrate.
11. Repeat wash steps 8 -10 twice.
12. Collect two new Rapid tubes and remove the unused filter columns (discard). Place the filter columns containing BCG cells into the clean Rapid tubes.

4.3 PREPARATION OF ACTIPHAGE[®] TEST CONTROLS

Negative and positive phage activity controls must be included on every occasion the Actiphage[®] TB Blood test is carried out. The positive phage activity controls evaluate the performance of the phage lytic ability against the Actiphage[®] BCG Positive Control.

Prepare each control as described below and label accordingly.

- **Actiphage[®] Reagent Positive Control:** add 100 µL of reconstituted Actiphage[®] Reagent onto the filter column containing the Actiphage[®] BCG Positive Control previously prepared (see section 4.2). Resuspend BCG cells by pipetting up and down five times.
- **Actiphage[®] Media Plus Positive Control:** add 100 µL of Actiphage[®] Media Plus onto the filter column containing the Actiphage[®] BCG Positive Control previously prepared (see section 4.2). Resuspend BCG cells by pipetting up and down five times.
- **Actiphage[®] Reagent Negative Control:** add 100 µL of reconstituted Actiphage[®] Reagent onto the filter column contained within a new pre-labelled Rapid tube.
- **Optional: Actiphage[®] Media Plus Negative Control:** add 100 µL of Actiphage[®] Media Plus onto the filter column contained within a new pre-labelled Rapid tube.

Table 1. Preparation of Actiphage® test controls

Control	Actiphage BCG Positive Control	Actiphage Reagent
Actiphage® Reagent Positive Control	✓	✓
Actiphage® Media Plus Positive Control	✓	X
Actiphage® Reagent Negative Control	X	✓
Actiphage® Media Plus Negative Control (Optional)	X	X

4.4 ACTIPHAGE® TEST

1. Transfer the sample resuspended in Actiphage® Reagent (section 4.1.1) onto the filter column contained within a pre-labelled Rapid tube.
2. Ensure cap is firmly closed and incubate the sample for 3 hours 30 minutes at 37 °C. **Treat the positive and negative controls prepared in section 4.3 in the same way as the test samples.**
3. Centrifuge the sample at 13,000 x g in a microcentrifuge for 3 minutes at RT. If all the liquid from the upper chamber does not pass through the Rapid tube filter column, this is usually due to cell debris blocking the filter.
NOTE: In this case, the filter column can be rotated through 180° and the centrifugation step repeated.
4. After centrifugation, remove the filter column from the Rapid tube using tweezers and immediately dispose to the appropriate biocontainer of disinfectant.
5. Collect the flow-through in the collection tube which contains the released mycobacterial genomic DNA.

SAFE STOPPING POINT (Optional)

The DNA (before purification) can either be used directly for molecular analysis or can be stored frozen at -20 °C until required.

The output from the Actiphage® test is mycobacterial genomic DNA, which can be used as a target for molecular diagnostic analyses. Each DNA sample should be sufficient for at least two nucleic acid amplification tests (5 µl each test).

4.5 DNA PURIFICATION

The flow-through must be purified and concentrated to a final volume of 12 µL eluted in nuclease free water, using an appropriate **DNA purification system**.

We recommend using the Monarch® PCR & DNA Cleanup Kit (New England BioLabs) or the DNA Clean-up and Concentrator (Zymo Research) following the manufacturer's instructions.

4.6 MOLECULAR ANALYSIS

If using PBD-PC-001 (which includes PBD-PC-009 Actiphage® TB PCR Test), the Actiphage® TB PCR Test contains a lyophilized PCR bead allowing homogeneous detection of:

- Mycobacteria belonging to MTBC by targeting the insertion sequence IS6110 with hydrolysis probe FAM labelling, with a limit of detection of one genomic copy.
- An Exogenous internal amplification control IAC DNA, with hydrolysis probe Cy5 labelling, to confirm absence of PCR inhibitors.

This PCR test has been validated on a Cobas® z 480 Analyser 96-well thermal block cycler unit (Roche Diagnostics) with halogen lamps using FAM (465-510) and Cy5 (610-670) detection channels. Software version: LCS480 1.5.1.62 SP3 - UDF 2.1.0.26.

Our PCR lyophilized bead contains Uracil N-glycosylase (UNG) to eliminate potential post-PCR carryover contamination associated with routine molecular testing.

Alternatively, if using PBD-PC-004 (which does not contain PBD-PC-009 Actiphage® TB PCR Test) then you should use an alternative DNA qPCR or sequencing assay to interrogate the DNA released by the Actiphage® TB Blood Test. Interpretation of results in this case would need to be as per the manufacturer's instructions or validated and defined by the user. The acceptance criteria (specified in section 5) should not be used.

4.6.1 PCR set-up

1. Establish qPCR plate setup defining each sample position including Actiphage® positive and negative controls and NTC.
2. In a 'Pre-PCR Mix' dedicated area, prepare a Master Mix by mixing 10 µL of resuspended PCR bead solution (prepared as per section 3.3.6) and 5 µL of IAC per reaction.
3. Transfer 15 µL of Master Mix into each well of interest.

4. In the 'DNA Preparation' dedicated area, add 5 µL of each DNA sample in the corresponding well.
5. Add 5 µL of nuclease-free water in the NTC well.
6. Seal the plate and spin down before placing it in the thermocycler.
7. Run the following qPCR program settings.

Table 2. qPCR program settings.

Cycles	Time	Temperature
UNG*	2 minutes	25 °C
1 cycle start	5 minutes	95 °C
40 cycles	10 seconds	95 °C
	1 minute	60 °C

*Activation step for Uracil N-glycosylate. Alternatively, once PCR samples and reagents are combined in the plate allow to stand for at least 2 minutes at RT to allow activation of UNG.

NOTE: An optional PCR Positive Template Control (PTC) can be included by adding 15 µL of Master Mix and 5 µL of quantitative genomic DNA from MTBC. An optional PCR Negative Control (NC) can be included by mixing 15 µL of resuspended PCR bead and 5 µL of NTC (water).

5 ACCEPTANCE CRITERIA

5.1 PCR Controls

The threshold cycle (Ct or Cp) is the intersection between the amplification curve and the threshold line. It allows the relative measurement of the concentration of the target in the PCR reaction when a reference sample is analysed in the same run. To analyse and interpret the signals obtained by qPCR, the threshold must be set up. On the Cobas z 480 Analyser the FAM and Cy5 channels are individually analysed using "AbsQuant/Fit points" method using the default settings (Background 2-6, Auto baseline and Auto threshold calling).

The qPCR results are validated if the controls (NTC, IAC, NC, PTC) present valid results (see table 3).

To validate the assay, the PCR negative control sample (NTC and NC) must not exhibit a qPCR signal in the relevant target channel (FAM).

IAC is a reaction-independent control designed to co-amplify a genomic region other than our target, using the same reagents (except probe), under the same annealing temperature and reaction conditions. It controls for machine or PCR reagent failure.

To validate the assay from an IAC perspective, any samples with IAC (Cy5) Ct values ≥ 35 should be considered invalid and repeated.

Table 3. Acceptance criteria for PCR controls.

Control	Ct (FAM)	Ct (Cy5)	Interpretation
NTC - Negative Template control	≥ 40		Plate Pass
	<40		Plate Fail
IAC - Internal Amplification Control		< 35	Sample Pass
		≥ 35	Sample Fail
NC - Negative Control (Optional)	≥ 40		Plate Pass
	<40		Plate Fail
PTC - Positive Amplification Control (Optional)	≥ 40		Plate Fail
	<40		Plate Pass

5.2 Actiphage® Controls

To confirm phage-mediated lysis of target cells by the Actiphage® Reagent, a positive PCR result should be generated with the Actiphage® positive control samples, and no target DNA should be detected in the negative control samples.

To validate the assay, a shift of at least +2 Δ Ct (FAM channel) must be observed between the Actiphage® Reagent Positive Control and the Actiphage® Media Plus Positive Control.

Full acceptance criteria for the Actiphage® assay controls are summarized in the table below.

Table 4. Acceptance criteria for Actiphage® controls table.

Control	Ct (FAM)	Δ Ct (FAM)	Interpretation
Actiphage® Reagent Positive Control (A)		B - A ≥ 2 B - A ≤ 2	Plate Pass
Actiphage® Media Plus Positive Control (B)			Plate Fail
Actiphage® Reagent Negative Control	≥ 40		Plate Pass
	< 40		Plate Fail
Actiphage® Media Plus Negative Control	≥ 40		Plate Pass
	< 40		Plate Fail

5.3 Test samples

Once the PCR and Actiphage controls are validated as described in section 5.1 and 5.2, sample results can be interpreted according to the table below.

Table 5. Interpretation of test samples results.

Targets		Interpretation
MTBC (FAM)	IAC (Cy5)	
Negative	Positive	Negative
Positive	Positive	Positive
Positive	Negative	Invalid - Repeat analysis Presence of inhibitors, competition with the main target or omission of IAC addition.
Negative	Negative	Invalid - Repeat analysis DNA omission, degradation or not in contact with Master Mix. Presence of inhibitors or omission of IAC addition.

Negative: FAM Ct \geq 40 or Cy5 Ct \geq 35.

Positive: FAM Ct $<$ 40 or Cy5 Ct $<$ 35.

6 LIMITATIONS

Detection of mycobacteria by the Actiphage[®] TB Blood Test is dependent on the quantity and quality of the sample collected, its storage prior to processing, and the number of live organisms present.

The Actiphage[®] TB Blood Test is dependent on the level of mycobacteria infected WBCs present in the circulating blood and therefore, in the early stages of infection where very low-level bacteraemia may occur it is possible to achieve a false negative result.

A false positive result will only occur if there is cross-contamination of the sample with mycobacterial DNA from the target organism.

7 PRECAUTIONS

7.1 SAFETY PRECAUTIONS

- Suitable safety precautions must be always used when handling clinical samples, including working in an appropriate biosafety cabinet, and using personal protective equipment. Local guidelines must be followed when working with pathogenic material, and for disposal of pathogenic biological waste, and in the event of laboratory accidents.

- The procedure involves working with *M. tuberculosis* and this work must be carried out in a facility suitable for handling this organism.
- All chemicals and biological materials are potentially hazardous. Samples are potentially infectious and must be treated as biohazardous materials.
- Any remaining sample material that generates a positive result using the Actiphage® TB Blood Test should be treated as containing viable cells of the organism detected and decontaminated using an appropriated disinfectant such as 70% ethanol for 30 minutes.

7.2 TECHNICAL PRECAUTIONS

- For use only by personnel competent in aseptic technique and experienced in working with pathogenic *Mycobacterium spp.*
- If the Actiphage® TB Blood Test or any included component appear damaged upon arrival, do not use and contact PBD Biotech.
- Test reagents must not be used beyond the expiry date printed on the label.
- Do not substitute the reagents in the test or mix between batches or lot numbers, as this may affect performance.
- Do not freeze any test component or reconstituted reagent.
- The reagents are provided at defined working concentrations. Assay performance may be compromised if reagents are modified or not used or stored under recommended conditions, as detailed in section 3.3.
- Reconstitute sufficient reagents required to perform the test. Do not return excess reagents to bottles after use.
- All reagents must be brought to RT before use.
- Use aseptic and PCR contamination control techniques, as appropriate, throughout the reagent preparation and assay procedure. All pipettes, pipette tips, glassware and plastic-ware used must be sterile and or DNA/ RNAse free.
- Care must be taken to treat all samples and control reactions in the same manner, according to sections 4.4 and 4.5 detailed in these instructions for use. Omission of, or deviation from, any step could lead to inaccurate results.
- 'Pre-PCR Mix' and 'DNA preparation' need to be carried out in two physically separated areas to reduce the chance of contamination.

- Use individual, dedicated pipets for setting up reaction mixes and adding positive control reagents.
- Use extreme caution to prevent contamination of the controls and reaction mix reagents with the synthetic materials that are contained in the IAC reagent.
- Do not use qPCR reaction volumes (reaction mix plus sample) of less than 20 μ L.
- Do not open the PCR plate after the qPCR run has finished.
- Refer to the Cobas® z 480 Analyser user manual (available at www.diagnostics.roche.com) for additional qPCR instrument warnings, precautions, and procedures.
- Caution must be observed to ensure correct sample testing with emphasis on wrong sample entry, loading error, and pipetting.
- To prevent cross-contamination between samples leading to false positive results, it is mandatory to use disposable materials for single use and to avoid direct contact between samples.
- Disposal of test components and any consumables that have been in contact with these reagents, should be carried out in accordance with local regulations for the disposal of microbiological waste.
- Any instrumentation, or connecting parts, including (but not limited to) qPCR instruments, centrifuges and pipettes must be calibrated, maintained, and cleaned as directed by the manufacturer.
- The operator should seek assistance directly from PBD Biotech if they do not understand anything in these instructions or have problems running the test.

8 MATERIAL SAFETY DATA SHEETS

A material safety data sheet (MSDS) for this test is available from PBD Biotech, upon request.