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CO-DIAGNOSTICS INC.

Logix Smart Mycobacteria tuberculosis (MTB) Test kit

MARKERS IS6110 AND MPB64
CO-DIAGNOSTICS, INC

REF MTB-K-007

IVD
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Table of Contents

1. Manufacturer and Authorized Representative in the European Union	2
2. Logix Smart Mycobacteria tuberculosis kit description	2
3. Intended Use	2
4. Mycobacteria tuberculosis Complex	3
5. Kit Contents	3
6. Materials Required (not included)	4
7. Sample Information.....	4
8. Important Information and Warnings.....	5
9. Storage and Handling	5
10. Procedure	6
11. Interpretation of Results	7
12. Troubleshooting.....	16
13. Legend of Package Symbols.....	17

1. Manufacturer and Authorized Representative in the European Union

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2. Logix Smart Mycobacteria tuberculosis test kit description

The Logix Smart Mycobacteria Tuberculosis (MTB) Test is designed to detect the presence or absence of deoxyribonucleic acid (DNA) of mycobacteria tuberculosis complex members.

Real-time polymerase chain reaction (PCR) amplifies the *IS6110* and *MPB64* regions of the pathogen genome. The amplified product is then detected by fluorescent dyes (FAM, CF560, and CF610).

- Each Logix Smart MTB Kit contains a complete, ready-to-use master mix.
- A human RNaseP gene marker serves as an Internal Positive Control (IPC) to monitor the quality of each reaction and is designed to also detect inadequate samples.
- Nuclease Free Water is also provided for a negative control.

Each kit provides reagents sufficient for 100 reactions.

3. Intended Use

The Logix Smart MTB Kit is intended for molecular biology applications. This product is released for use as an *in vitro* diagnostic device, for export only and is not for sale in the United States.

The Logix Smart MTB kit was designed and validated for use in respiratory tract fluid specimen for detection of presence or absence of Mycobacteria tuberculosis complex *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti*, *M. microti* and *M. pinnipedii* through amplification of the bacterial DNA markers *IS6110* and *MPB64*.

4. Mycobacteria tuberculosis Complex

The Mycobacteria tuberculosis Complex (MTB-complex) refers to a group of microorganisms genetically related to the Mycobacterium species. These microorganisms are the main cause of tuberculosis in humans and other animals.

According to the WHO - Global Tuberculosis Report of 2017, tuberculosis (TB) is the worldwide leading ninth cause of death, ranking even above HIV/AIDS. In 2016, the estimated number of deaths among HIV-negative population was of 1.3 million (this number was an improvement from the 1.7 million in 2000) with additional HIV-positive population deaths of 374,000.

Due to the ability of tuberculosis to spread through the air as well as body fluids, early detection is critical to prevent transmission, accelerate treatment and continue to fight worldwide to combat the disease.

Real-time PCR detection is recommended for detection of the MTB-complex during the acute stages of infection. The Logix Smart MTB Test detects the bacteria within 40 cycles from respiratory tract fluid samples. Non-tuberculosis mycobacteria are commonly present in the lungs and in the environment, and they can cause a tuberculosis-like disease. Mycobacteria can infect or spread to other parts of the body.

The marker *IS6110* has been used for decades to detect MTB-complex members, although more recent studies showed that the *IS6110* marker was not present in some isolates, and that *MPB64* was found as an additional conserved marker to increase the sensitivity of tests designed for detection of the MTB-complex.

5. Kit Contents

Table 1. Logix Smart Mycobacteria tuberculosis Kit (Cat. No. MTB-K-007)

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Black	Logix Smart MTB Master Mix	MM	MTB-MM-007	Proprietary blend of co-primers and PCR reagents	1x500µL (100 reactions)
Red	Logix Smart MTB Positive Control	PC	MTB-PC-007	Proprietary blend of positive primers	1x500µL (100 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1x500µL (100 reactions)

6. Materials Required (not included)

Important: Ensure that the instruments used in these procedures have been calibrated and maintained according to manufacturer instructions.

- DNA Extraction Kit
- Heating block from 37-95°C
- Pipettes capable of transferring 5µL, with appropriate tips and filters
- Ice or cooling block
- Vortex
- Real-time PCR System with FAM (green), Cal Fluor 560 (yellow), and Cal Fluor 610 (orange) dyes or equivalent and accompanying tubes/plates and caps/films
- Material needed for extraction, for example centrifuge with rotor for 2mL tubes and capable of 12,000 rpm, is also not included

7. Sample Information

7.1 Type

Sputum samples should be collected early morning. It is recommended to use a NALC-NaOH decontamination followed by a DNA extraction method prior to real-time PCR set-up.

7.2 Collection

The sample collection is fundamental to produce a good result for any molecular diagnostic assay. Extreme exposure environments that could not only damaged the microorganism but also its DNA can affect the efficiency of the test and its results.

If needed consult the CDC (Center for Disease Control) TB education page. On the CDC's manual "Chapter 4: Diagnosis of TB Disease", page 88, see link <https://www.cdc.gov/tb/education/corecurr/pdf/chapter4.pdf> for instructions on standard protocols for appropriate collection of sputum samples.

7.3 Storage

Once collected, patients should be instructed to keep samples refrigerated (insulated boxes and ice packs could be provided) until samples are delivered to the laboratory, or samples could be collected at clinic and kept refrigerated until delivery to the laboratory.

8. Important Information and Warnings

WARNINGS!



Users should pay attention to the following:

- Use sterile pipette tips with filters.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents.
- Consult appropriate Safety Data Sheets (SDS) for safety. The SDS is provided with the shipment, if absent, please, contact Co-Diagnostics, Inc on the contacts at the end of this document for an electronic version of the SDS.

9. Storage and Handling

9.1 Stability

Logix Smart MTB Master Mix (MTB-MM-007) and Positive Control (MTB-PC-007) must be stored at or below -15°C and are stable until the expiry date on the label. Reduce freezing and thawing to three times. Expired reagents should be disposed of according to the laboratory's waste disposal protocols for non-hazardous goods. Storage at $2-8^{\circ}\text{C}$ should not exceed five days in total.

To prevent degradation of reagents in Test Kits:

- 1) Immediately store Logix Smart MTB Master Mix (MTB-MM-007) and Positive Control (MTB-PC-007) at or below -15°C
- 2) Always work with each Logix Smart MTB Master Mix (MTB-MM-007) and Positive Control (MTB-PC-007) on ice.
- 3) Return components of Logix Smart MTB Master Mix (MTB-MM-007) and Positive Control (MTB-PC-007) to the -15°C freezer immediately after using
- 4) Make aliquots if necessary to avoid multiple freeze/thaw cycles
- 5) Handle all biological sample material using standard precautions.

9.2 Handling

Upon receipt of the **Logix Smart MTB Test Kit**, check to make sure there is excess dry ice in the shipment and that the reagents are still completely frozen. If there are any problems with the shipment, please contact your distributor immediately. **Immediately store the Logix Smart MTB Kit at or below -15°C .**

If you work in an area prone to power outages it is extremely important that you have a back-up generator for your freezer as well as a temperature data log to **ensure that the Logix Smart MTB Kit remains frozen at or below -15°C**. Reagents must remain frozen at all times (or on ice when in use) to prevent degradation.

While working with thawed components of **Logix Smart MTB Kit** it is extremely important that the **reagents are kept on ice at all times**. Return the reagents to the freezer immediately after use.

10. Procedure

10.1 Recommendations

10.1.1 Extraction of sample's DNA

The Logix Smart MTB Kit has been validated for use in respiratory tract fluid samples using NALC-NaOH decontamination protocol followed by QIAamp DNA Mini Kit Extraction (Cat No. 51304/ 51306, Qiagen).

Any other commercial extraction kit that are capable of producing a trustworthy and good quality DNA yield can also be used with Logix Smart MTB kit.

10.1.2 Real-Time PCR Setup

Logix Smart MTB Test kit was designed to successfully amplify DNA extracted using any Real-Time PCR thermo cycler, although validation data has been acquired with the following instruments: Mic qPCR 4-channel Cycler (Bio Molecular Systems), Eco 48 (Cole-Parmer), and StepOne™ Real-Time PCR System (Applied BioSystem, ThermoFisher Scientific).

- To prevent contamination, it is recommended to use Good Laboratory Practices for Molecular Genetic testing that follow a uni-flow process and separation of negative and positive materials.
- All real-time PCR master mix, positive controls, negative controls, and samples should be briefly spun down to remove residue from the lids after storage or mixing.
- Thaw all reagents and samples on ice before starting setup.
- All reagents must remain on ice for the duration of real-time PCR setup.

10.2 Procedure on Designated Areas for Real Time PCR Assay

In the designated negative control setup area:

1. Thaw **Logix Smart MTB Master Mix** (MTB-MM-007) on ice (for minimum time possible).
2. Vortex and centrifuge **Logix Smart MTB Master Mix** for a maximum 3 seconds.
3. Put the **Logix Smart MTB Master Mix** on ice.

***All steps involving Logix Smart MTB Master Mix should be performed on ice or on a frozen tray**

4. Put PCR tubes on ice.
5. Aliquot 5µL of **Logix Smart MTB Master Mix** into desired wells.
6. Add 5µL **Nuclease Free Water (GEN-NTC-001)** to the appropriate well(s).

In the designated positive control area (preferably separated from the master mix setup area to avoid contamination)

1. Thaw extracted/purified DNA on ice (if frozen).
2. Vortex and centrifuge purified DNA for a few seconds.
3. Add 5µL of purified DNA sample to each well using a new tip between each sample.
4. Thaw **Logix Smart MTB Positive Control (MTB-PC-007)** on ice (for minimum time possible).
5. Vortex and centrifuge **Logix Smart MTB Positive Control** for a few seconds.
6. Add 5µL of **Logix Smart MTB Positive Control** to appropriate well(s).
7. Place caps on the tubes according to the real-time system being used.
8. Put plate/tubes in real-time PCR machine and start the run.

10.3 Thermal Cycling Setup

Program the thermal cycler to the following conditions for a total reaction volume of 10µL:

Temperature	Time	Cycles	Capture
95°C	20 seconds	45	N/A
95°C	3 seconds		N/A
55°C	32 seconds		FAM (Green), Cal Fluor 560 (yellow), and Cal Fluor 610 (Orange)

1. When the run is finished, ensure that the run file is saved.
2. Check to see that the controls passed.
3. If controls pass, then interpret the sample results. If controls fail, then the run is invalid, and all should be repeated

11. Interpretation of Results

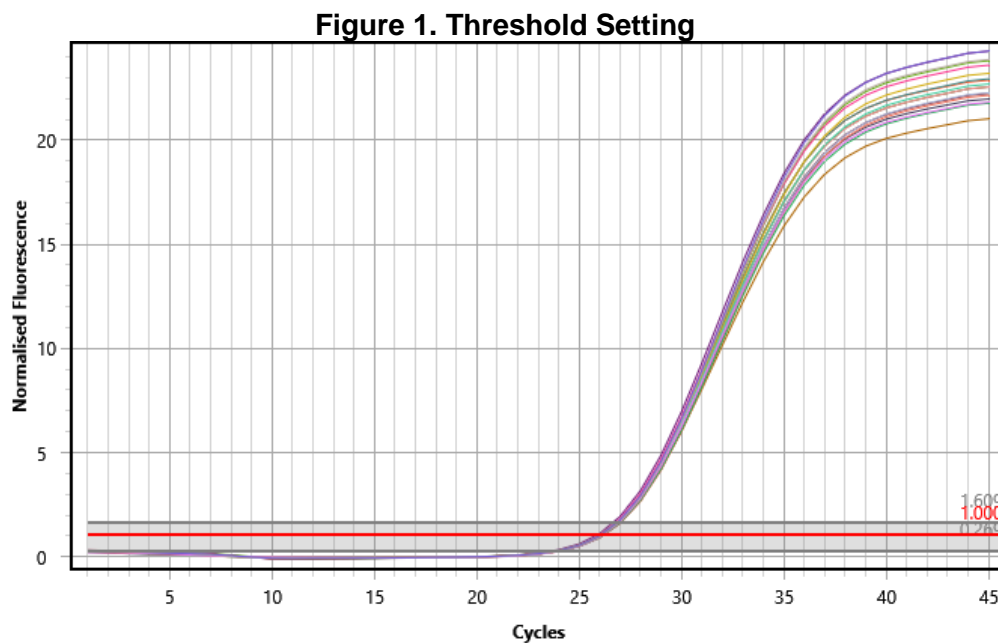
11.1 Limit of detection

The limit of detection of a molecular diagnostic test is the lowest concentration of analyte that was consistently detected (95% or higher) during performance evaluation studies. Because some isolates may not have the DNA markers for *IS6110* AND *MPB64*, the non-amplification of one or the other does not preclude a positive result. Having a sample showing positive for *IS6110* and negative for *MPB64* indicates that the strain may have only one of the markers, and the result in this case is still positive for tuberculosis.

The limit of detection validated with Logix Smart MTB kit is 50 copies per reaction in a 5 μ L sample. This value takes in account the lowest concentration for the detection of the two markers, *IS6110* and *MPB64*, for Mycobacteria tuberculosis complex.

11.2 Analyzing Controls

The analysis parameters should be set so that the threshold is above the noise background and placed at a point during the exponential phase of amplification that best reflects all orders of magnitude across the run. The following is an example of a proper threshold setting:



If using the magnetic induction cyler (Mic) from Co-Diagnostics or BioMolecular Systems, set the method to dynamic, threshold to 1.000, ignore cycles before 5, and ensure all the other parameters are set to default settings. Select the icon to display chart with a linear y-axis.

11.3 Positive Controls

Highlight the positive control reaction well. Each positive control should show an amplification curve for the *IS6110* primary marker in the FAM channel, an amplification curve for *MPB64* second marker in the CF560 channel, and amplification of the internal positive control for *RNaseP* (IPC) in the CF610 channel similar to that seen below:

Figure 2a. Positive Control Amplification with marker *IS6110* (FAM)

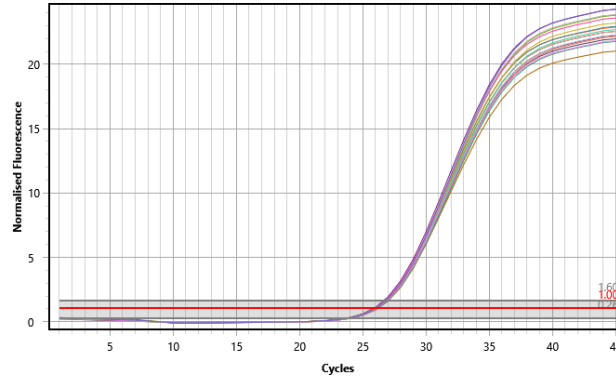


Figure 2b. Positive Control Amplification of with marker *MPB64* (CF560)

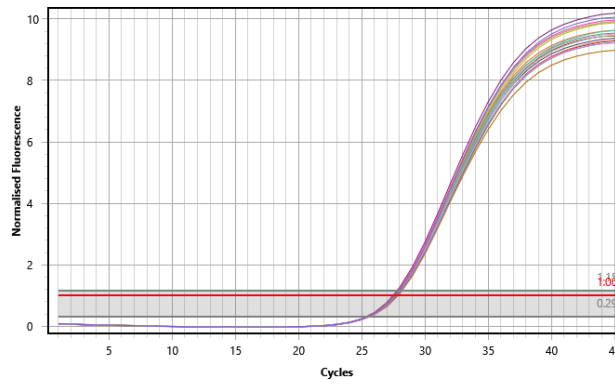
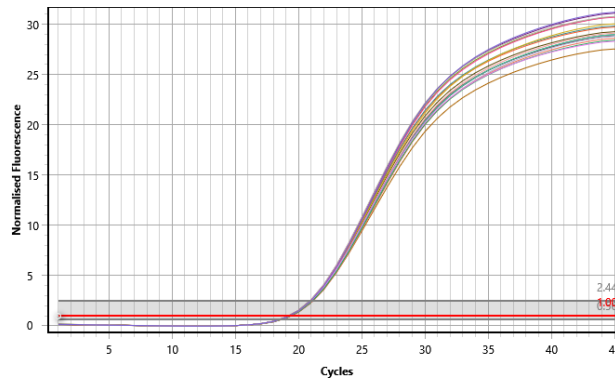


Figure 2c. Positive Control Amplification of IPC (CF610)



Using the established analysis parameters, the positive control cycle thresholds shall be within the following ranges:

Table 2. Positive Control Cycle Threshold Values

Range for Positive Control Ct Values*	
MTB for <i>IS6110</i> (FAM)	21.00-29.99
MTB for <i>MPB64</i> (CF560)	21.50-25.20
IPC (CF610)	15.00-23.99

IS6110 (FAM): Mycobacteria tuberculosis Primary Marker for *IS6110*
MPB64 (CF560): Mycobacteria tuberculosis Secondary Marker for *MPB64*
IPC (CF610): RNaseP Internal Positive Control Marker

*Ct values may vary by ± 2 cycles based on instrument differences.

If the Ct values for the controls do not match the graph, then the threshold or the baseline must be manually changed until the values represent the growth curves. The threshold must be set as close to the start of the growth curve and above the background noise. The baseline must be set before the first amplification curve. Check the instrument user manual for instructions for adjusting the threshold or baseline.

If the positive control does not show amplification, then the tests are invalid. Loss of amplification for a positive control is indicative of primer degradation which may result from reagents being at temperatures above -15°C for more than one hour or being used past the expiration date. Pipetting error may also account for lack of positive control amplification by pipetting control into the wrong well, missing a well, or pipetting an inadequate amount of reagent or sample into a reaction well.

11.4 Negative Control

Next highlight the negative control. The results of the negative control should show no amplification that passes the threshold within 40 cycles similar to those seen below:

Figure 3a. Negative Control Amplification

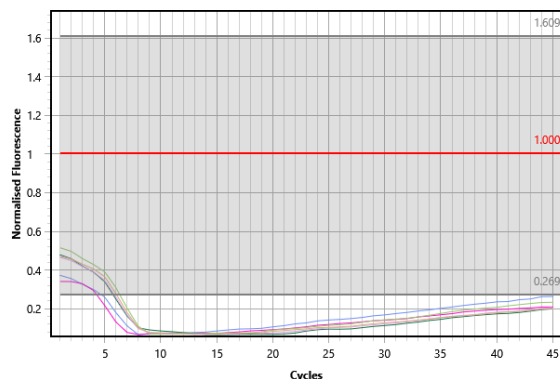
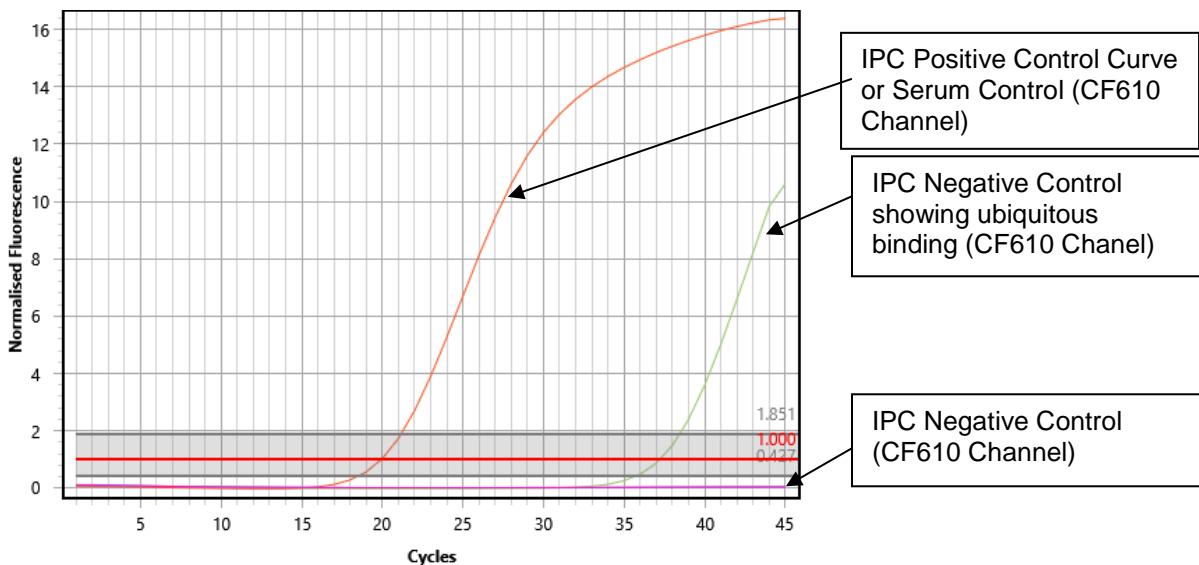


Figure 3a shows no amplification of a negative control. The threshold of 1.000 is represented by the red line and the lines at the bottom show that no amplification curve formed; therefore, the amplification never crossed the threshold to produce a cycle threshold value.

Occasionally, ubiquitous binding will cause amplification of RNaseP in a negative control of water, but the amplification is limited only to the IPC channel and crosses the threshold at cycle 35 or higher as shown below. Note, if using a sample matrix of serum or plasma as a negative control the IPC will show a positive amplification curve if extraction was successful.

Figure 3b. Ubiquitous Binding of Negative Control



If the negative control shows any amplification of *IS6110* or *MPB64* <40 cycles the results are invalid. Troubleshooting process may be initiated and the entire experiment must be repeated. Amplification of *IS6110* or *MPB64* in a negative control indicates contamination in one or more of the reagents or pipetting error, and the results cannot be trusted. Amplification of *IS6110* or *MPB64* >40 cycles is outside the detectable range and is considered negative.

11.5 Analyzing Samples

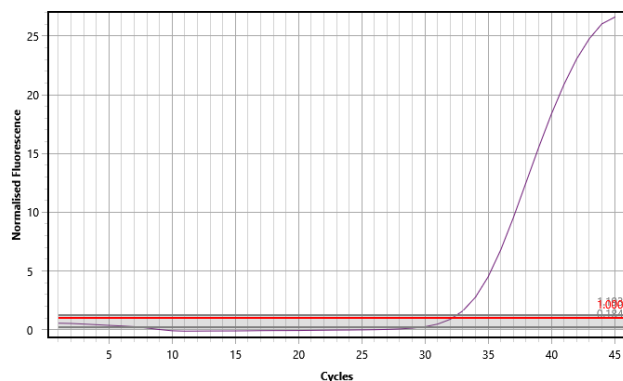
Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes (figures may vary based on machine used and quantity of MM and sample):

1. Positive (figures 4 and 5)
2. Negative (figure 6)
3. Negative Due to Inadequate Nuclear Material (figure 7)

A **Positive** result will show an amplification curve or cycle threshold value for *IS6110* and *MPB64* below 40 cycles. Amplification curves greater than 40 cycles for *IS6110* and *MPB64* are outside of the detection limits for the assay and is considered negative. The following results are examples of positive MTB samples:

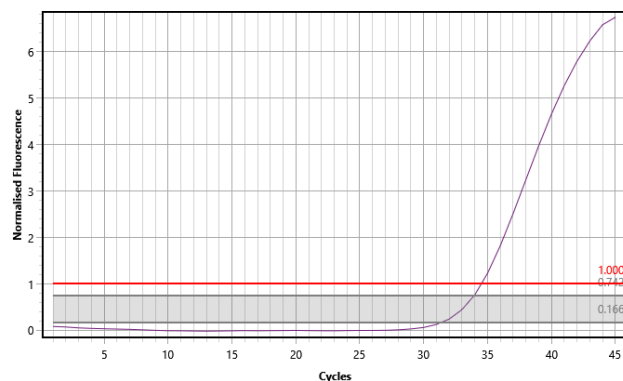
Figure 4a-c. MTB Positive Sample Results for Each Channel

Figure 4a. IS6110 Positive Sample (FAM)



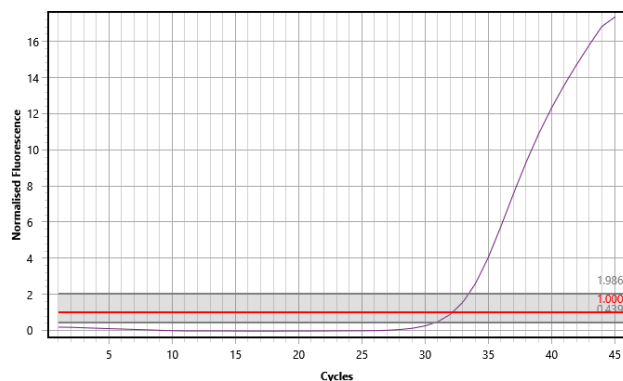
The cycle threshold generated for this sample was 32.16 for FAM. Positive samples do not all have the same cycle threshold values or amplification curves. When running more than one sample, the results will vary. To prevent misinterpretation, it is recommended to view one sample at a time. The results from this channel indicate MTB DNA is present in the sample from one of the *Mycobacterium* complex members.

Figure 4b. MPB64 Positive Sample (CF560)



Viewing the same sample in the CF560 channel, the cycle threshold is calculated as 34.50 indicating it is positive for *Mycobacterium tuberculosis* complex. This marker may be more conserved in some populations than others.

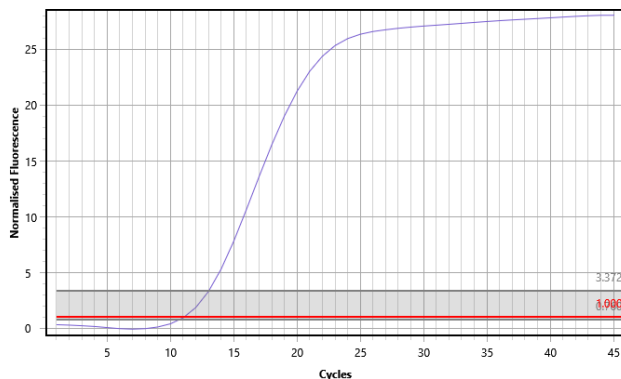
Figure 4c. IPC Positive Sample (CF610)



The CF610 results for each sample determines if the extraction was successful. When amplification occurs, it suggests that genomic DNA is present in the reaction, therefore it is indicative that the DNA extraction was successful enough to allow amplification of DNA. This marker is used as the internal control for each reaction. For the selected sample, the IPC (CF610) cycle threshold is 32.16.

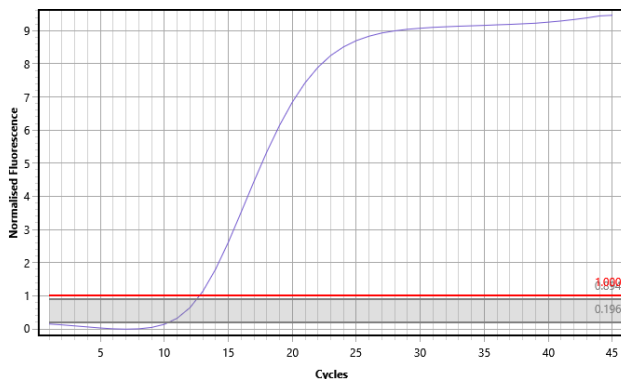
Figure 5. High Concentration MTB Positive Sample Results for Each Channel

Figure 5a. IS6110 Positive Sample in High Concentration (FAM)



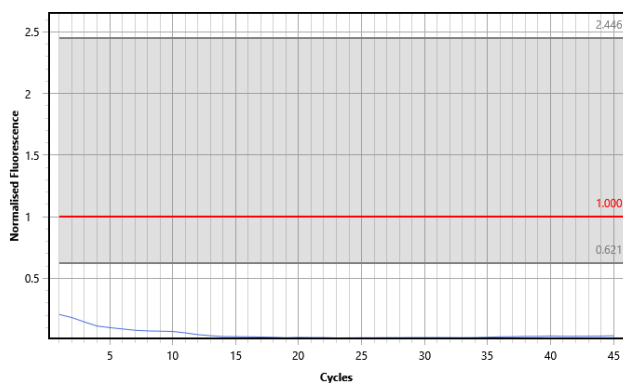
The cycle threshold generated for this sample was 11.02 for FAM. This sample had a known high concentration of marker *IS6110*. When samples are highly concentrated it is important to check for crossover contamination and to prevent misinterpretation of low concentration samples that are in the same run. It is recommended to view the samples one at a time. The results from this channel indicate MTB DNA is present in the sample from one of the Mycobacteria complex members but the results do not indicate a quantitative value.

Figure 5b. MPB64 Positive Sample (CF560)



Viewing the same sample in the CF560 channel, the cycle threshold is calculated as 12.74 indicating it is positive for MTB using a second marker.

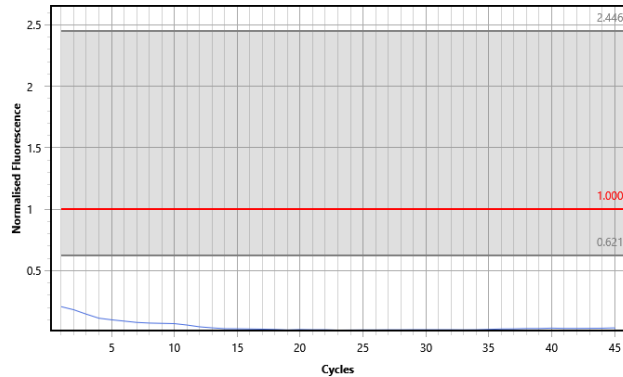
Figure 5c. IPC Positive Sample (CF610)



The CF610 results for this sample are negative due to no human DNA being present, or a very high concentration of MTB DNA. Most likely the MTB DNA flooded the extraction and reduced the amount of normal DNA to undetectable levels. This can also occur in sterile samples (i.e. spinal fluid or pure culture).

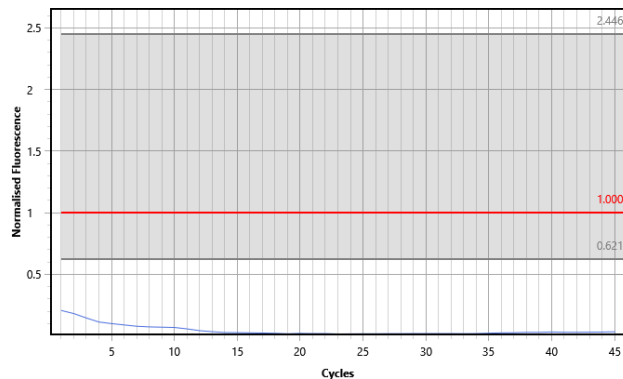
A **Negative Sample** result will show no amplification for MTB. Any amplification curves greater than 40 cycles for markers *IS6110* and *MPB64* are outside of the detection limits for the assay. A negative sample result will have the following curves:

Figure 6. MTB Negative Sample Results for Each Channel
Figure 6a. *IS6110* Negative Sample (FAM)



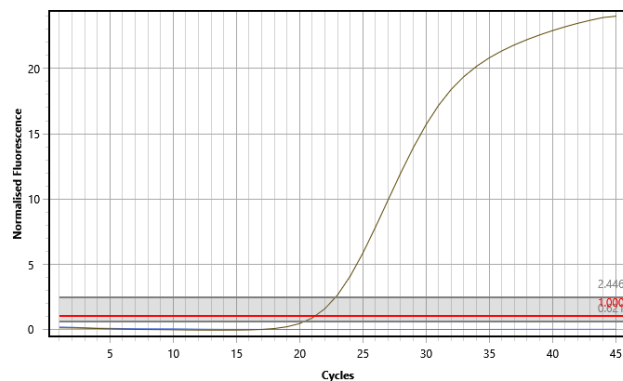
For this sample, no cycle threshold or amplification curve was generated because no MTB DNA was present.

Figure 6b. *MPB64* Negative Sample (CF560)



Viewing the same sample in the CF560 channel, the cycle threshold and amplification curve are also absent because no MTB DNA is present.

Figure 6c. MTB Negative Sample (CF610)

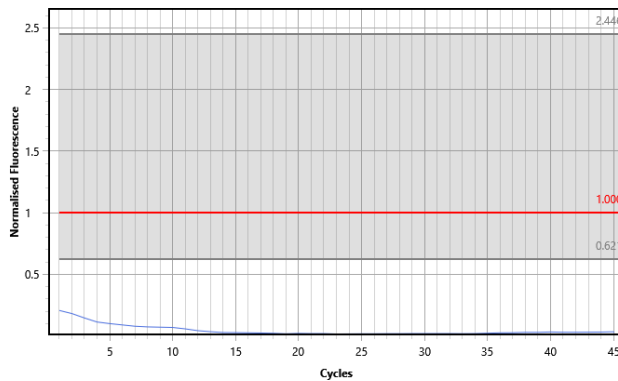


The CF610 results for this sample is a cycle threshold of 21.15. This amplification curve indicates that DNA was successfully extracted from this sample, but no MTB complex DNA was present in the sample.

11.6 Negative Sample Result Due to Inadequate Nuclear Material

If the IPC (CF610), *IS6110* (FAM), and *MPB64* (CF560) are all negative with no amplification curve and no cycle threshold value, then the result is negative due to inadequate nuclear material. The graphs for each marker would look like the following no matter what channel was selected:

Figure 7. Negative Sample Result Due to Inadequate Nuclear Material (FAM, CF560, and CF610)



Inadequate nuclear material can be the result of error in sample prep, sample degradation, or an inadequate sample. The test may be repeated with a new sample or called negative due to inadequate nuclear material. It may also be the result of expired reagents or degraded reagents from improper storage or handling. It is recommended to investigate all possibilities to determine the cause.

Note: Samples obtained from culture or sterile/pure sites (e.g. CSF, urine, cell lysates, etc.) may not contain RNaseP. In such case, the two negative markers indicate a true negative result for MTB.

11.7 Results interpretation table

The interpretation of results with Ct values can be translated to the following table:

Table 3. Results translation for easy interpretation

IS6110	MPB64	Positive Control (IPC)	Negative Control (NTC)	Final Result
Zero or no Amplification	Zero or no Amplification	Positive amplification*	Zero or no Amplification	Negative (-)
Zero or no Amplification	Positive amplification*	Positive amplification*	Zero or no Amplification	Positive (+)
Positive amplification*	Zero or no Amplification	Positive amplification*	Zero or no Amplification	Positive (+)
Positive amplification*	Positive amplification*	Positive amplification*	Zero or no Amplification	Positive (+)
Any result	Any result	Zero or no Amplification	Any result	Invalid**
Any result	Any result	Any result	Positive amplification*	Invalid**

* Amplification inside normality ranging from 1 to 40 cycles.

** Test is invalid. See section 12, Troubleshooting.

12. Troubleshooting

12.1 Stability

Real-time and Accelerated Shelf-life, and In-use Stability Studies are currently under testing and results will be communicated as soon as they are released. A new version of this Instructions for Use will be issued to replace this one. Please discard this document when a new version is issued.

Without the stability studies results it is imperative that the storage conditions are followed as best possible to assure the product's performance is maintained.

The expiration date of this product has been established as 12 months and it does not exceed the expiration date of any of its reagents used in manufacturing.

12.2 User Errors

Polymerase Chain Reaction (PCR) Assays is a technique that uses Molecular Biology technology to amplify a single copy or a few copies of a segment of a particular DNA sequence.

In order to ensure the Good laboratory Practices for Molecular Biology Assays it is essential that the user has good skills on pipetting to prevent errors as splashes, cross-over contamination, and errors on volume selection. Pipette tips must be replaced for every pipetting. Gloves must be replaced often. Equipment must have calibration up to date for the pipettes and thermo cyclers.

Verification and validation studies performed for MTB-K-007 have been conducted following the current Good laboratory Practices for Molecular Biology Assays. If these conditions are not met the performance will show higher variability due to User Errors while conducting the experiment.

12.3 Invalid Results

Positive Control and Negative Control are validated, manufactured and tested along with the Master Mix. The purpose of these controls is to attest about the performance of the Master Mix, as well as validates the user technique used during the experiment. If the user has a poor use of the techniques required to perform a Molecular Biology Assay, it is more likely that it will be shown by the Positive Control not amplifying or the Negative Control showing amplification.

12.3.1 Positive Control not amplifying











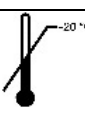

It means that for some reason (e.g. bad conservation, break of stability, expired reagent, volumes errors, pipetting error, it is not the right reagent) an error occurred while conducting the experiment, because it is more likely that the same error happened with the sample the results cannot be trusted, and the test must be invalidated. An investigation should be conducted to identify possible causes for





error and the test has to be reprocessed from extraction or not, depending on the investigation results and risks identified in the process.

12.3.2 Negative Control amplifying

It means that for some reason (e.g. contamination from pipetting, splashes on the PCR plate, user contamination) an error caused the negative control to be contaminated with the positive control or with the sample, because it is more likely that the same error could have happened to the sample the results cannot be trusted, and the test must be invalidated. An investigation should be conducted to identify possible causes for error and the test has to be reprocessed from extraction or not, depending on the investigation results and risks identified in the process.

13. Legend of Package Symbols

	<i>In vitro</i> diagnostic medical device
	Catalogue number
	Batch Code
	Cap color
	Component
	Content/Volume
	Number
	Use-by-date
	Contains sufficient for 100 tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use

	Non-Sterile product – Do not sterilize
	Manufacturer
	Authorized representative in the European Community
	CE-Marking for IVD in compliance to EU Directive 98/79/EC