

Logix Smart MTB Kit (MTB-K-006)

Description

The Logix Smart Mycobacteria Tuberculosis (MTB) Test developed by Co-Diagnostics, Inc. detects deoxyribonucleic acid (DNA) of mycobacteria tuberculosis complex members. Real-time polymerase chain reaction (PCR) amplifies specific regions of the pathogen genome, which in this assay is *IS6110*. The amplified product is then detected by fluorescent dyes (FAM, CF560, and CF610). Real-time PCR detection is recommended for detection of MTB during the acute stages of infection. Mycobacteria are commonly present in the lungs; however, Mycobacteria can infect or spread to other parts of the body. Due to the ability of tuberculosis to spread through the air as well as body fluids, early detection is critical to prevent transmission and accelerate treatment. The Logix Smart MTB Test detects the bacteria within 45 cycles from pulmonary and extra-pulmonary samples.

- In each Logix Smart MTB Kit supplied is a complete, ready-to-use master mix. Simply add the extracted DNA sample to the master mix and run!
- A human RNase P gene marker serves as an Internal Positive Control (IPC) to monitor the quality of each reaction and is designed to also detect inadequate samples.

Materials Provided

Kit Contents

Logix Smart Mycobacteria tuberculosis Kit (MTB-K-006)				
Cap Color	Component	Symbol	Catalog Number	Amount
Black	Logix Smart MTB Master Mix	MM	MTB-MM-006	1x500uL (100 reactions)
Red	Logix Smart MTB Positive Control	PC	MTB-PC-006	1x500uL (100 reactions)
Clear	Nuclease Free Water	NTC	GEN-NTC-001	1x500uL (100 reactions)

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 5 µL and tips

- Ice or cooling block
- Vortex
- Centrifuge with rotor for 2mL tubes and minimum 12,000rpm
- 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

Storage:

- **Logix Smart MTB Master Mix and Positive Control 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. Storage at 2-8° should not exceed five days in total.

WARNINGS!**The user should pay attention to the following:**

- **Use sterile pipet tips with filters.**
- **Store and extract positive materials (specimens, controls, and amplicons) separately from other reagents.**
- **Consult appropriate Safety Data Sheets (SDS) for safety.**

To prevent degradation of reagents in Test Kits:

- 1) **Immediately store Logix Smart MTB Master Mix and Positive Control at or below -15°C.**
- 2) **Always work with each Logix Smart MTB Master Mix and Positive Control on ice.**
- 3) **Return components of Logix Smart MTB Master Mix and Positive Control to the freezer immediately after using.**
- 4) **Make aliquots if necessary to avoid more than three freeze/thaw cycles.**

Upon receipt of the **Logix Smart MTB 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, and make aliquots to avoid multiple freeze/thaw cycles.

Intended Use:

The Logix Smart MTB Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

This product is for export only and is not for sale in the United States.

Sample Extraction Recommendations

The Logix Smart MTB Kit has been validated with pulmonary and extra-pulmonary samples using NALC-NaOH decontamination followed by QIAamp DNA Mini Kit Extraction (Cat No. 51304/51306, Qiagen).

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

Real-time PCR Setup

- To prevent contamination, it is recommended to use Good Molecular Laboratory Practices 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.

In the designated negative control setup area:

1. Thaw **Logix Smart MTB Master Mix (MTB-MM-006)** 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 5 uL of **Logix Smart MTB Master Mix** into desired wells.
6. Add 5 uL **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)

7. Thaw extracted/purified DNA on ice (if frozen).
8. Vortex and centrifuge purified DNA for a few seconds.
9. Add 5 uL of purified DNA sample to each well using a new tip between each sample.
10. Thaw **Logix Smart MTB Positive Control (MTB-PC-006)** on ice (for minimum time possible).
11. Vortex and centrifuge **MTB Positive Control** for a few seconds.
12. Add 5 µL of **MTB Positive Control** to appropriate well(s).
13. Place caps on the tubes according to the real-time system being used.
14. Put plate/tubes in real-time PCR machine and start the run.

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

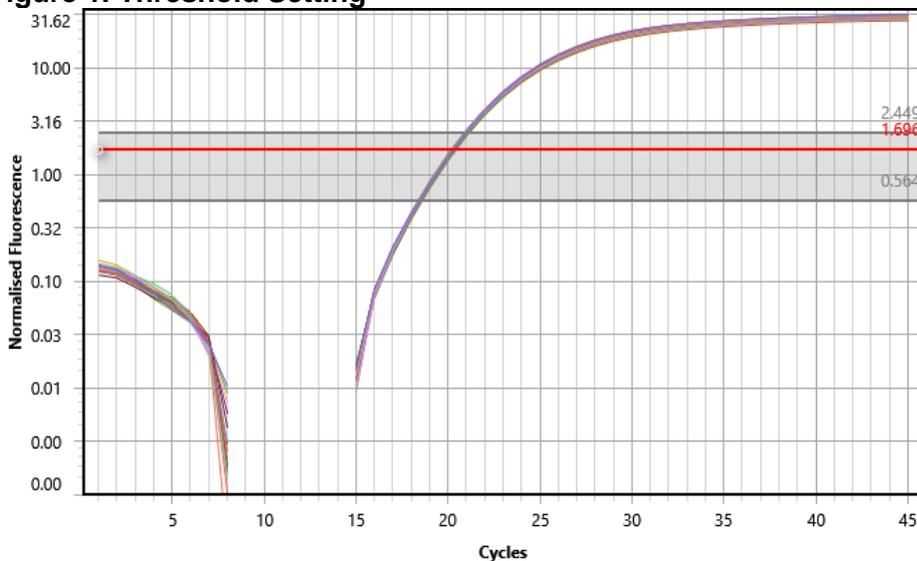
Interpretation of Results

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.

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:

Figure 1. Threshold Setting



If using the magnetic induction cycler 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.

Positive Controls:

Highlight the positive control reaction well. Each positive control should show an amplification curve for the Mycobacteria tuberculosis *IS6110* primary marker in the FAM channel, an amplification curve for Mycobacteria tuberculosis second marker (MTBC) 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 of MTB (FAM)

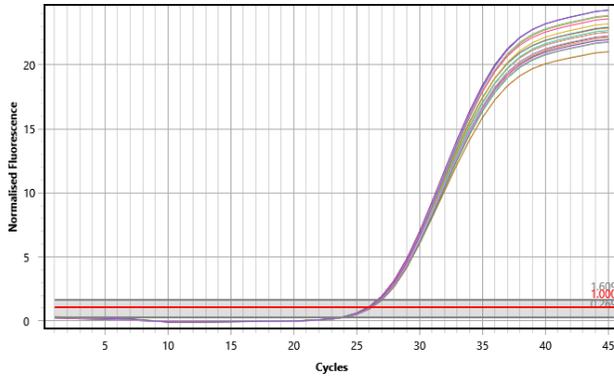


Figure 2b. Positive Control Amplification of MTBC (CF560)

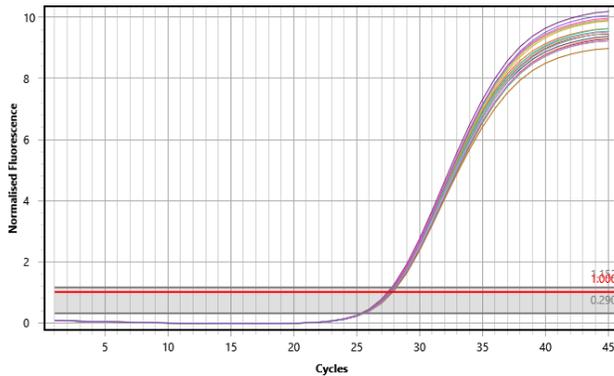
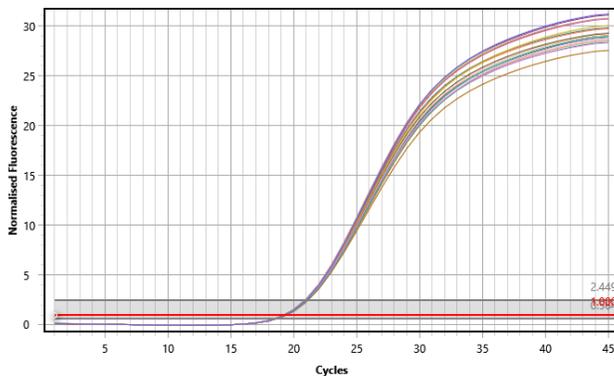


Figure 2c. Positive Control Amplification of IPC (CF610)



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

Table 1. Positive Control Cycle Threshold Values

Range for Positive Control Ct Values*	
MTB (FAM)	21.00-29.99
MTBC (CF560)	23.00-31.99
IPC (CF610)	15.00-23.99

MTB (FAM): Mycobacteria tuberculosis Primary Marker for *IS6110*

MTBC (CF560): Mycobacteria tuberculosis Secondary Marker

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 into a reaction well.

Negative Controls:

Next highlight the negative control. The results of the negative control should show no amplification that passes the threshold within 45 cycles similar to those seen below:

Figure 3a. Negative Control Amplification

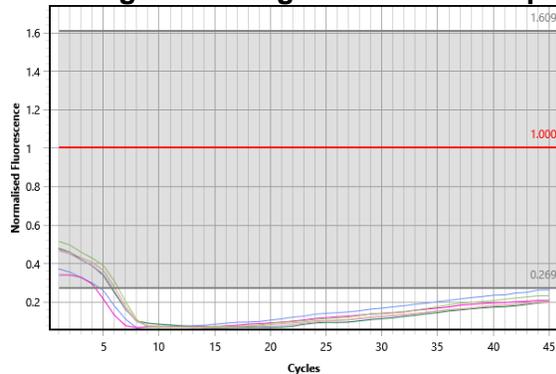
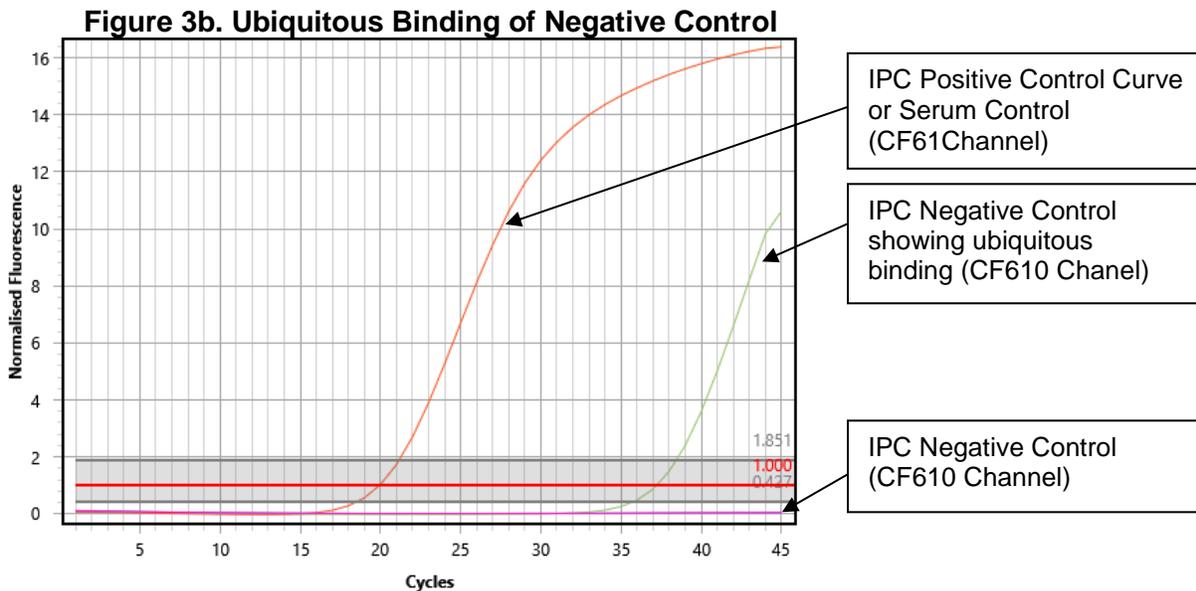


Figure 2a shows no amplification of a negative control. The threshold of 1.000 is represented by the red line and the lines at the bottom show that no amplification curve formed; therefore, the amplification never crossed the threshold to produce a cycle threshold value.

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.



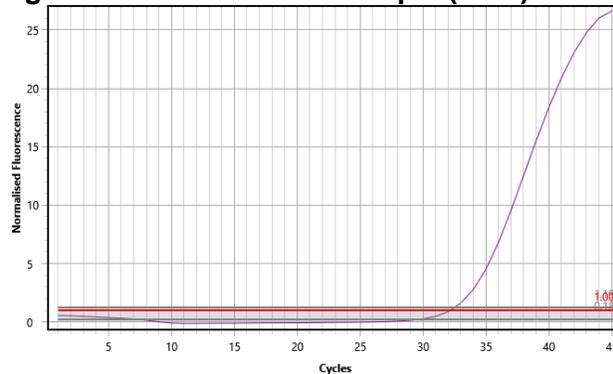
If the negative control shows any amplification of MTB <45 cycles the results are invalid and the entire experiment must be repeated. Amplification of MTB in a negative control indicates contamination in one or more of the reagents or pipetting error. Amplification of MTB >45 cycles is outside the detectable range and is considered negative.

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):

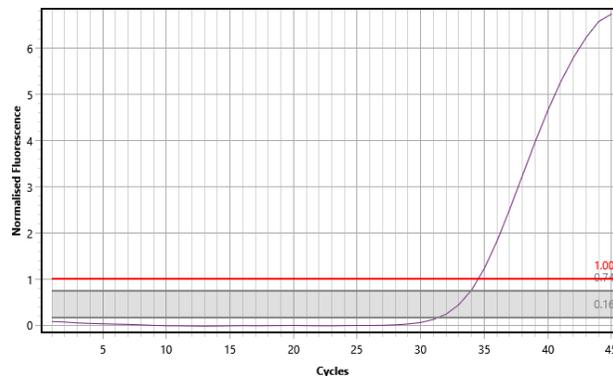
1. Positive (figures 4 and 5)
2. Negative (figure 6)
3. Negative Due To Inadequate Nuclear Material (figure 7)

A **Positive** result will show an amplification curve or cycle threshold value for MTB below 45 cycles. Amplification curves greater than 45 cycles for MTB are outside of the detection limits for the assay. The following results are examples of positive MTB samples:

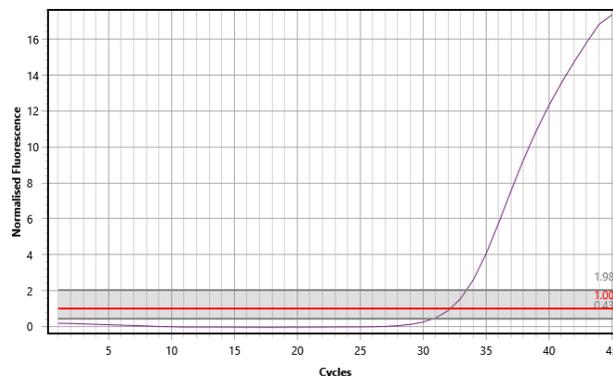
Figure 4a-c. MTB Positive Sample Results for Each Channel
Figure 4a. MTB Positive Sample (FAM)


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 Mycobacteria complex members.

Figure 4b. MTB Positive Sample (CF560)


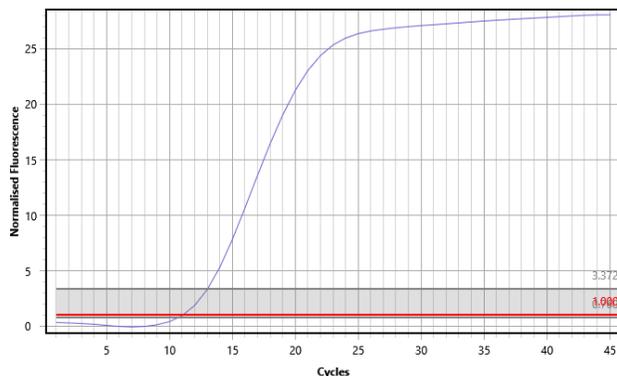
Viewing the same sample in the CF560 channel, the cycle threshold is calculated as 34.50 indicating it is positive for MTB using a second marker. Amplification of this marker is weaker than the primary and its amplification cannot solely determine presence of MTB in the sample.

Figure 4c. MTB Positive Sample (CF610)


The CF610 results for each sample determines if the extraction was successful. When amplification occurs it is representative of DNA being present in the reaction whether or not that DNA is infectious. 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

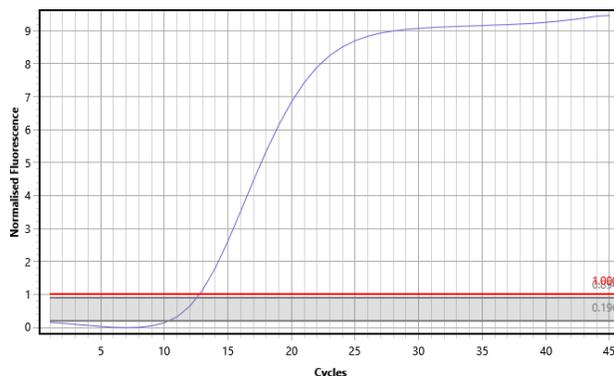
Figure 5a. MTB Positive Sample in High Concentration (FAM)



The cycle threshold generated for this sample was 11.02 for FAM. This sample had a known high concentration of MTB. When samples are highly concentrated it is important to check for cross over contamination and to prevent misinterpretation of low concentration samples that are in the same run. It is recommended to view the

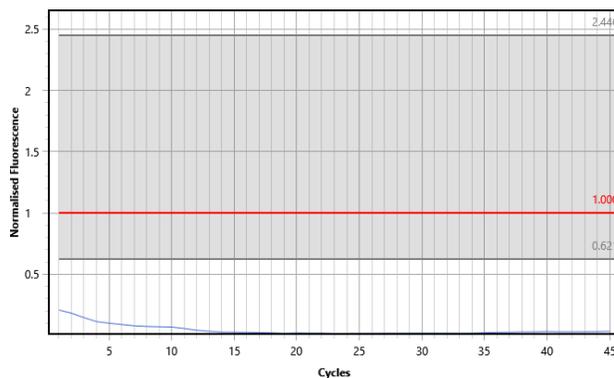
samples one at a time. The results from this channel indicate MTB DNA is present in the sample from one of the Mycobacteria complex members but the results do not indicate a quantitative value.

Figure 5b. MTB 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. Amplification of this marker is weaker than the primary and its amplification cannot solely determine presence of MTB in the sample.

Figure 5c. MTB Positive Sample (CF610)

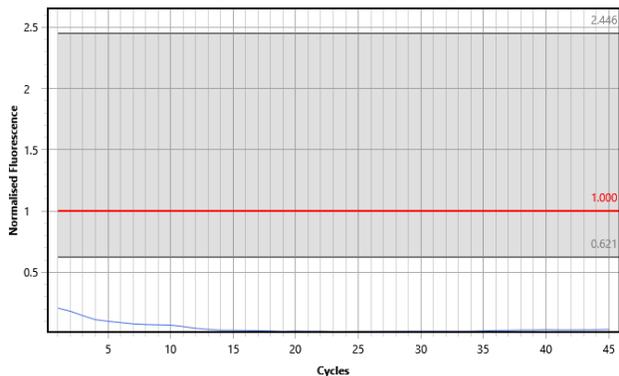


The CF610 results for this sample are negative due to a very high concentration of MTB DNA. Most likely the MTB DNA flooded the extraction and reduced the amount of normal DNA to undetectable levels. This can also occur in sterile samples (i.e. spinal fluid or pure culture).

A **Negative Sample** result will show no amplification for MTB. Any amplification curves greater than 45 cycles for MTB are outside of the detection limits for the assay. A negative sample result will have the following curves:

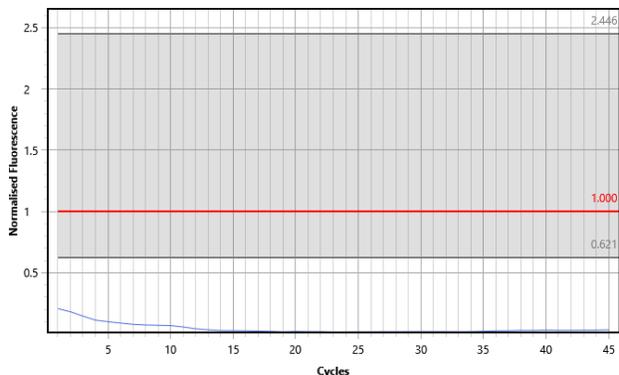
Figure 6. MTB Negative Sample Results for Each Channel

Figure 6a. MTB Negative Sample (FAM)



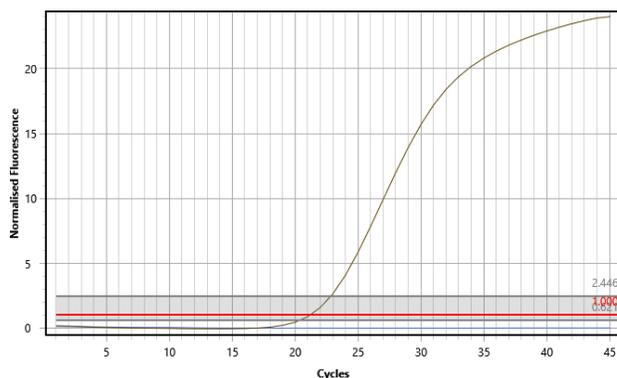
For this sample, no cycle threshold or amplification curve was generated because no MTB DNA was present.

Figure 6b. MTB 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)



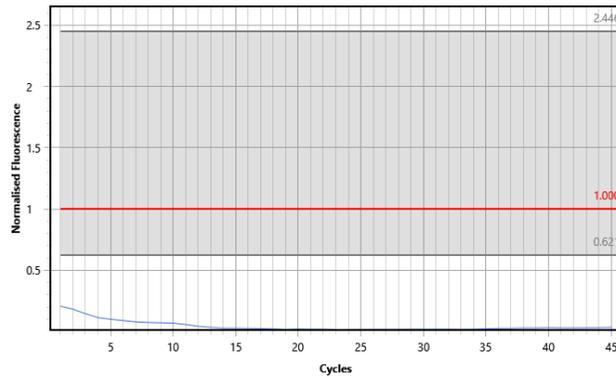
The CF610 results for this sample is a cycle threshold of 21.15. This amplification curve indicates that DNA was successfully extracted from this sample but no MTB DNA was present in the sample.

A Negative Sample Result Due To Inadequate Nuclear Material

If the IPC (CF610), MTB (FAM), and MTBC (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.

For Information or Questions:

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