Version 1.1
March 2017

MTB assay

For the detection of *Mycobacterium tuberculosis* complex using the BD MAX™ system

Instructions for use

Distributed by

Biolegio

Contents

Introduction 3
Contact information 3

1. Protocol 4
   - 1.1 Materials required 4
   - 1.2 Run settings 4
   - 1.3 Setting up the experiment 5

2. Results interpretation 5

Introduction

This protocol describes the system settings and run setup protocol for the detection of *Mycobacterium tuberculosis* complex (MTB) using the BD MAX system. The assay targets the IS6110 gene.

The MTB assay was optimized for the BD MAX system by PAMM laboratories, Veldhoven. Validation was performed on BAL and sputum samples.

Contact information

For information regarding ordering dried snap-in tubes for the MTB assay:
info@biolegio.com

For information regarding to the protocol:
secretariaatMM@pamm.nl

1. Protocol

This protocol describes the assay settings that are required for a to run the MTB assay on the BD MAX system. The MTB snap-ins contain primers and probes for the detection of MTB and require no further preparation. For monitoring failure in extraction procedure an internal Sample Process Control (SPC) is present in the BD MAX Sample Buffer tube which is detected using the BD MMK (SPC) mastermix.

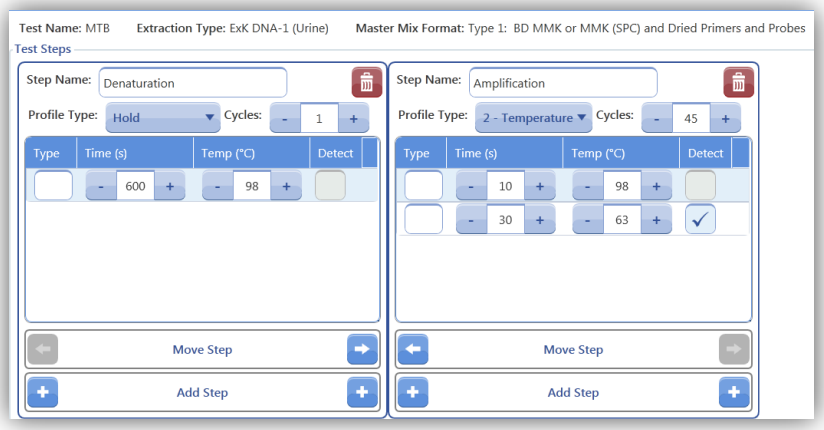
1.1 Materials needed

- BD MAX instrument
- BD ExK DNA-1 Extraction kit (BD cat no: 442818)
- BD MMK (SPC) mastermix (BD cat no: 442829)
- BD MAX PCR Cartridges (BD cat no: 437519)
- Dried snap-ins MTB (Biolegio cat no: BDT-14004)
- Vortex Mixer
- Micropipettes
- Pipette tips with filters
- Disposable gloves
- Lab. coat

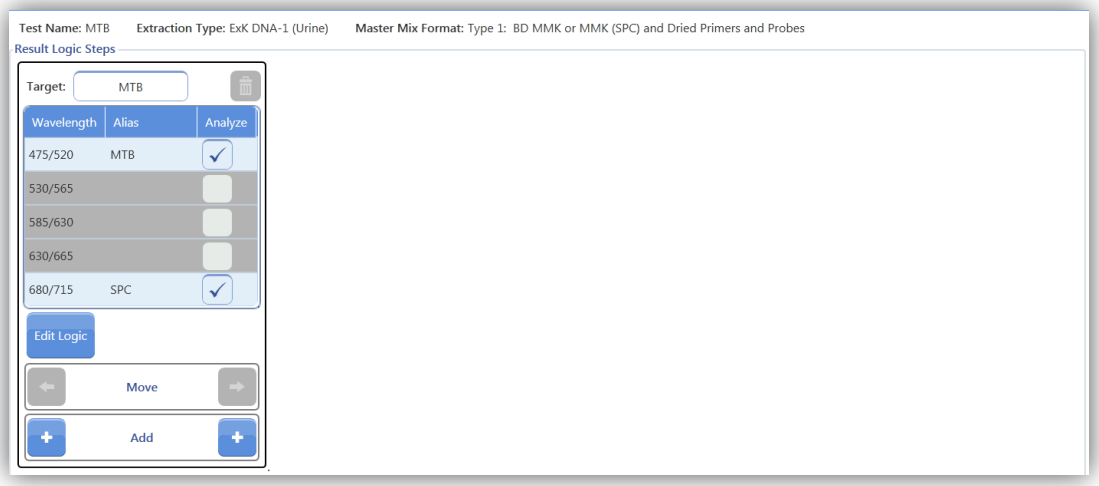
1.2 Run settings

The assay is performed on the BD MAX with use of the BD MMK(SPC) in combination with the ExK DNA-1 kit for extraction.
Create a full process assay in the test editor named "MTB assay" and use the following parameters:

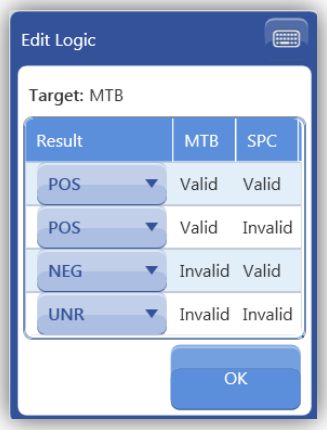
Edit the test steps using the following settings:



If desired apply the result logic as follows;



And edit the logic for each target as follows;



1.1 Setting up the experiment

a. Create a Work List on the BD MAX instrument using the MTB assay (created in step 1.2) and label the lanes with appropriate sample names.

b. Load the prepared Sample Buffer Tubes into their corresponding position in the BD MAX racks.

c. Load the BD MAX racks with the corresponding Unitized Reagent Strips. Note: Shake the strip to ensure liquid is at the bottom of tubes.

d. Snap-in the BD Extraction tubes (position 1), MMK(SPC) tubes (position 2) and MTB tubes (position 3) into the Reagent Strip.

e. Load the racks and cartridges into the BD MAX and Start Run.

2. Results interpretation

2.1 For a run to be valid:
- No BD MAX System failures.
- Negative control (optional) has a Cq value of -1 for all channels except 680/715 (SPC).
- Positive control (optional) has a Cq values for both the 475/520 as the 680/715 channel.

2.2 Interpretation if run is valid:
- A Cq value of -1 indicates a negative result.
- A Cq value of 0 indicates that no Cq value could be obtained. The curve needs to be investigated visually.
- A Cq value for either of the targets indicates a positive result for the corresponding target.
- The SPC (channel 680/715) should always give a Cq value. A negative value for the SPC indicates inhibition and therefore this sample should be repeated.
- All curves need to be visually checked for right interpretation.

3. Results interpretation

3.1 For a run to be valid:
- No BD MAX System failures.
- Negative control (optional) has a Cq value of -1 for all channels except 680/715 (SPC).
- Positive control (optional) has a Cq value for all channels.

3.2 Interpretation if run is valid:
- A Cq value of -1 indicates a negative result.
- A Cq value of 0 indicates that no Cq value could be obtained. The curve needs to be investigated visually.
- A Cq value for either of the targets indicates a positive result for the corresponding target.
- The SPC (channel 680/715) should always give a Cq value. A negative value for the SPC indicates inhibition and therefore this sample should be repeated.
- All curves need to be visually checked for right interpretation.

Disclaimer:
PAMM Laboratories is not responsible for the results of the MTB assay on the BD MAX system. Using the "open protocol" (i.e. MTB dried snap in tubes with primer and probes, together with the BD mastermix) the laboratory itself is responsible for the validation of the assay.