MYCOBACTERIUM REALTIME PCR KIT

REF RTPCR016-LPD-R Σ 96

RUO For research use only

INTENDED PURPOSE

Real Time RT-PCR kit to detect nucleic acid from *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* complex (MABSC) species and other non-tuberculous mycobacteria (NTM) in human sputum samples.

INTRODUCTION

Mycobacteria are among the groups of microorganisms with the greatest clinical importance, being the causal agents of several human infections with high mortality and morbidity. Currently they represent one of the most serious health problems worldwide. They are found in various sources, such as soil, water, and animals, and can cause infections in humans and animals.

Mycobacteria are classified as tuberculous and non-tuberculous, depending on their ability to cause tuberculosis:

Tuberculous mycobacteria mainly include the *Mycobacterium tuberculosis* complex (MTBC), which is the cause of tuberculosis in humans, a chronic, granulomatous disease that mainly affects the lungs, although it can have other localized and even disseminated presentations. This complex is composed of several species (*M. tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. africanum*, *M. microti*, *M. caprae*, *M. canettii*, *M. pinnipedii*, *M. mungi* and *M. suricattae*). *M. tuberculosis* is the most common in humans.

On the other hand, nontuberculous mycobacteria (NTM) encompass a wide variety of species that can cause disease in humans. Among the most frequently isolated are those belonging to the *Mycobacterium avium* complex (MAC), which includes species such as *Mycobacterium avium* (*M. avium* subsp. *avium*, *M. avium* subsp. *hominissuis*, *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum*), *Mycobacterium intracellulare* and *Mycobacterium chimaera*. These are common in the environment and can cause infections in people with weakened immune systems, such as HIV/AIDS patients.

Another relevant complex is the *Mycobacterium abscessus* complex (MABSC), which includes the subspecies *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii*. These behave as opportunistic pathogens and can cause infections in different areas of the body, such as the lungs, skin and soft tissues. They are especially problematic due to their resistance to multiple antibiotics, which makes their treatment difficult.

Differentiation between MTBC, MAC, and MABSC complexes is important due to differences in treatment approaches and transmission.

TEST PRINCIPLE

It is based on the amplification, in the same reaction well, of specific fragments of nucleic acids from mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium avium* complex (MAC), *Mycobacterium abscessus* complex (MABSC), as well as other non-tuberculous mycobacteria (NTM) by real time PCR.

One lyophilized master mix (RT-PCR MIX) is provided for screening and confirmation using one independent target for each pathogen.

The PCR mix targets a specific fragment of the *IS1081* insertion sequence for MTBC, of the *ITS* region for MAC and MABSC, and of the *16S rRNA* gene for NTM.

An amplification control is included to check the absence of carry-over of amplification inhibitors and the correct amplification set-up. This control consists of human *RNAse P* gene and a specific oligo pair/probe for its amplification.

The technique is divided into 2 main steps: DNA extraction and amplification/detection with specific oligo pairs and probes. NTM is detected in FAM channel, MTBC is detected in Texas/ROX channel, MABSC is detected in HEX/VIC channel and MAC is detected in Cy5 channel. The internal control (*RNAse P*) is detected in Q705 channel.

KIT FEATURES

VIRCELL RT-PCR MIX and VIRCELL POSITIVE CONTROL are lyophilized. It is necessary to reconstitute them before use (see "Preparatory treatment of the device" section). The rest of the reagents are ready to use.

This kit is based on amplification and detection using real time PCR.

MATERIALS PROVIDED

[1] VIRCELL MTBAVAB RT-PCR MIX LPD: 1 plate with 96 tubes divisible into 12 strips with 8 tubes containing Taq polymerase, buffer and specific primers/probe for MTBC (*IS1081*), MAC (*ITS*), MABSC (*ITS*) and NTM (*16S rRNA gene*). Also, as internal control, primers/probe for *RNAse P*. 1 reaction per tube. Lyophilized.

[3] VIRCELL MTBAVAB POSITIVE CONTROL: 1 vial containing a mixture of lyophilized non-infectious nucleic acids to be used as positive control. Red cap.

[4] VIRCELL NEGATIVE CONTROL: 200 µl of deionized water to be used as negative control. Green cap.

[5] VIRCELL PCR MIX RECONSTITUTION SOLUTION: 2 x 1 ml of aqueous solution to reconstitute the PCR mix. Yellow cap.

[6] VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION: 500 µl of aqueous solution to reconstitute the positive control. Brown cap.

[7] VIRCELL RT-PCR MIX CAPS: 12 strips of 8 caps RT-PCR compatible.

Special materials required but not provided:

-Microbiological safety cabinet.
-DNA/RNA extraction kit (see recommendations in "Assay procedure").
-Real Time PCR thermocycler (compatible with low profile white tubes with FAM, HEX/VIC, Texas/ROX, Cy5 and Q705 detection).
-Precision micropipettes.
-Sterile tips with aerosol barrier.
-Microcentrifuge.
-PCR cabinet (recommended).
-Vortex.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCELL POSITIVE CONTROL reconstituted: store it between -25°C and -15°C and use until expiration date. Avoid more than 10 freeze-thaw cycles during this time period.

VIRCELL RT-PCR MIX reconstituted: store it between 2ºC and 8ºC and use before 60 minutes.

Rest of reagents: Refer to package label for expiration date (at 2-8°C).

VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For research use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
11. Keep containers for samples and reagents closed while they are not being handled.
12. Avoid using samples subjected to repeated freeze-thaw cycles.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Reagents in this kit could include nucleic acids. Observe the local regulations for waste disposal.
15. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only VIRCELL NEGATIVE CONTROL, VIRCELL PCR MIX RECONSTITUTION SOLUTION and VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION are compatible with the equivalents in other RTPCR VIRCELL references and lots.

17. Specimens should be handled as in the case of infectious samples using safety laboratory procedures. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in deionized or distilled water.
18. Testing of all the samples at the earliest interval following collection will help ensure the most accurate test results. Variation in storage times during specimen shipment has not been assessed.
19. It is recommended to have two different areas to perform the test: Pre-Amplification and Amplification areas.
20. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored to purity.
21. It is recommended to use conventional DNA/RNA purification kits.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

The kit has been validated for human sputum samples. The use of fresh samples is recommended.
It is recommended to avoid delay on transport and laboratory investigations. If immediate delivery to the laboratory is not possible, store specimens in a refrigerator (2 to 8ºC). Store specimens for which testing will be delayed beyond 14 days after collection at -70ºC or lower; avoid freezing at higher temperatures and freeze-thaw cycles.
Recommended guidance: Sputum. p.6.4.13. MM13-A_Collection, Transport, Preparation and Storage of Specimens for Molecular Methods, 1st ed. CLSI.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use, except for the VIRCELL RT-PCR MIX [1] and the VIRCELL POSITIVE CONTROL [3].
[1] VIRCELL RT-PCR MIX. For reconstitution add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube.

⚠The reconstituted VIRCELL RT-PCR MIX must be used within 60 minutes of adding the reconstitution solution stored at 2-8ºC if the start of the test is delayed. In this case, a freeze rack is recommended.
[3] VIRCELL POSITIVE CONTROL. Follow the next steps to reconstitute it:
- Centrifuge the corresponding tube for 5 seconds at 5000 g.
- Add 100 µl of VIRCELL POSITIVE CONTROL RECONSTITUTION SOLUTION [6]
- Mix with vortex for 1-2 seconds.
- Centrifuge the tube for 5 seconds at 5000 g.

After reconstitution, the VIRCELL POSITIVE CONTROL [3] can be frozen at temperature between -25°C and -15°C to be used in subsequent reactions.

ASSAY PROCEDURE

1. DNA/RNA extraction (performed in the Pre-Amplification area):
   1.1. It is recommended to use a commercial extraction kit for DNA/RNA extraction. In order to use commercial extraction kits, follow the manufacturer instructions. Consult with Customer Service.
   In order to improve safety during the handling of samples in the extraction process, it is recommended to previously inactivate them by heating them for 30 minutes at 95ºC.
   The use of a minimum volume of 600 µl of sample in the extraction process improves the DNA extraction yield obtained in the process.
2. Amplification using RT-PCR (performed in the Amplification area):
   2.1. Preparation of the VIRCELL RT-PCR MIX tubes: the plate with 96 tubes provided could be easily torn off into one or more individual 8-tube strips depending of the samples to be tested. Add 15 µl of VIRCELL PCR MIX RECONSTITUTION SOLUTION [5] per tube. Maintain cold after reconstitution.
   2.2. Addition of the sample: Add 5 µl of each extracted DNA/RNA sample to each tube. Add 5 µl of VIRCELL POSITIVE CONTROL [3] and VIRCELL NEGATIVE CONTROL [4] to the corresponding tubes. The negative control is water.
   2.3. Secure caps VIRCELL RT-PCR MIX CAPS [7] on the tubes.
   2.4. It is recommended to briefly centrifuge the plate/strips of tubes with the purpose of ensuring vial content is at the bottom of the tube.
   2.5. RT-PCR program: Insert the PCR tubes in the real time thermocycler and run the following program*:

1 cycle	95 ºC	3 minutes
45 cycles	95 ºC	15 seconds
	60 ºC	45 seconds*

* Fluorescence data (FAM, HEX/VIC, Texas/ROX, Cy5 and Q705) should be collected.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control testing before releasing, complying with strict specifications.

VALIDATION PROTOCOL FOR USERS

It is recommended to include one negative control in each run performed. The negative control will monitor reagent or environmental contamination.
The positive control is recommended to be included on each run. The positive control monitors for reagent failures and for correct operation of essential procedure.
The thermocycler software is likely to automatically calculate the baseline fluorescence value (threshold) based on the amplification curve for each target (fluorescence detection). Nevertheless, it is recommended to set the thresholds for the different detection channels individually. In order to set a threshold for each target, it is recommended to use as a reference the amplification curves of the positive and negative controls. The threshold should be fixed at the beginning of the exponential reading of fluorescence and above the background signal.
The controls result interpretation is as follows:

CONTROL	NTM (FAM)	MABSC (HEX/VIC)	MTBC (Texas/ROX)	MAC (Cy5)	IC (Q705)	Interpretation
VIRCELL MTBAVAB POSITIVE CONTROL	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Correct
	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	Invalid
VIRCELL NEGATIVE CONTROL	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	No Amplification or Ct >40	Invalid

INTERPRETATION OF RESULTS

RESULT	NTM (FAM) [2]	MABSC (HEX/VIC)	MTBC (Texas/ROX)	MAC (Cy5)	IC (Q705) [1]	Interpretation
1	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	Invalid (sample/kit/setup related)
2	No Amplification or Ct >37	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	Amplification (Ct < 40)	Negative
3	Amplification (Ct < 37)	No Amplification or Ct >40	No Amplification or Ct >40	No Amplification or Ct >40	Amplification (Ct < 40) or No amplification	NTM

RESULT	NTM (FAM) [2]	MABSC (HEX/VIC)	MTBC (Texas/ROX)	MAC (Cy5)	IC (Q705) [1]	Interpretation
4	Amplification (Ct < 40)	Amplification (Ct < 40)	No Amplification or Ct >40	No Amplification or Ct >40	Amplification (Ct < 40) or No amplification	MABSC
5	Amplification (Ct < 40)	No Amplification or Ct >40	No Amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	MAC
6	Amplification (Ct < 40)	No Amplification or Ct >40	Amplification (Ct < 40)	No Amplification or Ct >40	Amplification (Ct < 40) or No amplification	MTBC
7	Amplification (Ct < 40)	Amplification (Ct < 40)	No Amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	MABSC + MAC
8	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	No Amplification or Ct >40	Amplification (Ct < 40) or No amplification	MABSC + MTBC
9	Amplification (Ct < 40)	No Amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	MAC + MTBC
10	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or No amplification	MABS + MAC + MTBC

[1] In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 10 may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.

[2] In borderline positive samples, there may be amplification in the HEX/VIC (MABSC), Cy5 (MAC) and/or Texas/ROX (MTBC) channels and no amplification in the FAM (NTM) channel or amplification with Ct >37. The result will be considered valid.

Amplification curves with Ct >37 could be observed in the FAM channel due to environmental NTM.

In case of invalid or inconclusive result, it is recommended to re-extract DNA/RNA from original specimen and re-test it. In the case of failure of amplification of internal control, improper extraction of nucleic acids or inhibition of amplification could be assumed. Testing a new sample is recommended.

SYMBOLS USED IN LABELS

For research use only

Use-by (expiry date)

Store at x-y °C

Contains sufficient for <n> test

Batch code

Catalogue number

Consult instructions for use

Reconstitute in <X> µl

Manufacturer

Current version Nr.: L-RTPCR016-LPD-R-EN-01
Date: 2023/12/29
Updates: New reference