MYCOBACTERIUM REALTIME PCR KIT

REF RTPCR016-LPD Σ 96

CE 0123 For *in vitro* diagnostic use

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.
The device is intended to be used with general population with suspected infection by the microorganism.
The test is a qualitative and automated assay, intended to be used as an aid to diagnosis.

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 the *16S rRNA* gene for genus *Mycobacterium*.
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. Mycobacteria 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/Cy5.5 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 Mycobacteria (*16S rRNA* gene). Also, as internal control, primers/probe for human *RNAse P* gene. 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/Cy5.5 detection).
-Precision micropipettes.
-Sterile tips with aerosol barrier.
-Microcentrifuge.
-PCR cabinet (recommended).
-Vortexer.

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 *in vitro* diagnostic use only. For professional 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.
22. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

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 (4 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.
   - 1.2. In order to improve safety during the handling of samples in the extraction process, they must be previously inactived by heating them for 30 minutes at 95ºC.
   - 1.3. 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/Cy5.5) 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	Mycobacteria (FAM)	MABSC (HEX/VIC)	MTBC (Texas/ROX)	MAC (Cy5)	IC (Q705/Cy5.5)	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

The result interpretation is described in the tables below:

RESULT	Mycobacteria (FAM)[2]	MABSC (HEX/VIC)	MTBC (Texas/ROX)	MAC (Cy5)	IC (Q705/Cy5.5) [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
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 (Mycobacteria) 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 Mycobacteria.
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.

LIMITATIONS OF USE

1. The performance with other types of specimens different to processed human sputum has not been evaluated.
2. The device is not intended to be used in specimens derived from cerebrospinal fluid or blood.
3. The device is not intended to detect antibodies from lymphocyte secretions immunoassay intended for the detection of active *Mycobacterium tuberculosis* infection.
4. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures.
5. Detection of the pathogens nucleic acids depends on the organism load present in the specimen and may be affected by specimen collection methods, patient factors, stage of infection and/or strain. False negative results may also occur if amplification inhibitors are present in the specimen. The kit was validated with a specific nucleic acid extraction method. Alternative extraction procedures might be also appropriate but require user validation. A 260/280 purity ratio among 1.8-2.0 is acceptable.
6. The test provides qualitative results. No correlation can be drawn between the magnitude of a positive result and the number of microorganisms in the sample.
7. The test only works within the limits of the genomic regions from which the primers and probes have been chosen. The test targets highly conserved regions, however due to the high variability of DNA genomes it is possible that certain subtypes might not be detected. At design time, mutations of the target regions were not detected.
8. A negative test result does not exclude the presence of the target organism at levels below the detection limit of the assay.
9. A positive test does not rule out the possibility that other pathogens may be present.
10. The values obtained in the sensitivity and specificity performance study correspond to the total number of samples tested and may vary depending on the type of sample.
11. This kit is designed for generic detection of MTBC, MAC, MABSC and NTM. It is not possible to differentiate among the different species and subspecies included in the complexes. If distinction of specific species and subspecies is needed, additional testing is required.
12. The performance results showed were generated using the thermocycler CFX96 (Bio-Rad).
13. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.
14. A summary of safety and performance is available on EUDAMED or it can be requested at email address customerservice@vircell.com.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Mycobacterium abscessus complex

Positive processed human sputum samples (n=50) and previously confirmed negative human sputum samples (n=51) were analysed. Samples were tested against a commercial Real-time PCR kit.
Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).
The results were as follows:

Samples No.		101
Sensitivity (%)		100
	95% CI	93-100
Specificity (%)		100
	95% CI	93-100
PPV (%)		100
NPV (%)		100
LR+/LR-		-1.01/-0.99
True Positive		50
True Negative		51
False Positive		0
False Negative		0
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

Mycobacterium avium complex

Positive processed human sputum samples (n=49) and previously confirmed negative human sputum samples (n=51) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		100
Sensitivity (%)		100
	95% CI	93-100
Specificity (%)		100
	95% CI	93-100
PPV (%)		100
NPV (%)		100
LR+/LR-		-1.01/-0.99
True Positive		49
True Negative		51
False Positive		0
False Negative		0
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

Mycobacterium tuberculosis complex

Positive processed human sputum samples (n=58) and previously confirmed negative human sputum samples (n=51) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		109
Sensitivity (%)		100
	95% CI	94-100
Specificity (%)		100
	95% CI	93-100
PPV (%)		100
NPV (%)		100
LR+/LR-		-1.01/-0.99
True Positive		58
True Negative		51
False Positive		0
False Negative		0
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

Non-tuberculous mycobacteria

Positive processed human sputum samples (n=51) and previously confirmed negative human sputum samples (n=51) were analysed. Samples were tested against a commercial Real-time PCR kit.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Samples No.		102
Sensitivity (%)		98
	95% CI	90-100
Specificity (%)		100
	95% CI	93-100
PPV (%)		100
NPV (%)		98
LR+/LR-		-0.99/-0.97
True Positive		50
True Negative		51
False Positive		0
False Negative		1
Borderline		0

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

METHOD COMPARISON

The assay performance for detection of MABSC, MAC, MTBC and NTM was determined relative to liquid culture results.

The results were as follows:

			Culture positive	Culture negative	Total
PCR	MABSC	Positive	25	0	25
		Negative	3	213	216
	MAC	Positive	31	0	31
		Negative	22	213	235
	MTBC	Positive	98	0	98
		Negative	6	213	219
	NTM	Positive	48	0	48
		Negative	48	213	261

Additionally, the assay performance for detection of MTBC and MAC/MABSC/NTM was determined relative to liquid culture results considering the auramine staining.

The results were as follows:

			Culture positive		Culture negative	
			Auramine positive	Auramine negative	Auramine positive	Auramine negative
PCR	MTBC	Positive	63	13	0	0
		Negative	0	6	5	146
	MAC / MABSC / NTM	Positive	24	25	0	0
		Negative	2	29	5	209
		Total	89	73	10	355

PRECISION

6 samples (4 positive and the positive and negative controls) were amplified twice in 2 runs per day in 2 different qRT-PCR thermocyclers on 20 consecutive days. Samples were run in CFX96 (Bio-Rad). Within-run precision, between-run precision, between-day precision and within-laboratory precision were determined.

The results were as follows:

Mycobacterium abscessus complex

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.6	0.8	0.1	1.0

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive sample 1	0.7	1.0	0.6	1.4
Positive sample 2	1.7	0.8	1.0	2.1
Positive sample 3	0.7	1.0	1.0	1.6
Positive sample 4	0.8	0.9	0.3	1.2
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

Mycobacterium avium complex

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.4	1.1	0.7	1.3
Positive sample 1	1.0	1.1	0.7	1.7
Positive sample 2	1.3	1.4	0.7	2.1
Positive sample 3	0.7	0.6	0.6	1.2
Positive sample 4	1.9	0.8	0.3	2.1
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

Mycobacterium tuberculosis complex

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.3	0.6	0.3	0.7
Positive sample 1	0.6	0.5	0.7	1.1
Positive sample 2	0.9	0.2	0.6	1.1
Positive sample 3	0.6	0.3	0.7	1.0
Positive sample 4	0.9	0.7	0.3	1.2
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

Mycobacteria

Sample	Within-run precision %CV	Between-run precision %CV	Between-day precision %CV	Within-laboratory precision %CV
Positive control	0.5	1.0	0.6	1.3
Positive sample 1	0.8	1.2	0.7	1.6
Positive sample 2	0.8	0.8	1.1	1.6
Positive sample 3	0.8	1.5	1.1	2.0
Positive sample 4	1.1	1.5	0.5	2.0
Negative control	No amplification	No amplification	No amplification	No amplification

CV: Coefficient of variation

INTERFERENCES

A study has been performed to evaluate the effect of potentially interfering substances.

Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).

The results were as follows:

Interfering substances	Samples No.	Maximum added concentration without interference
Mucin	2	2.5 mg/mL
Paracetamol	2	1324 µmol/L
Rifampicin	2	25 µg/mL
Ibuprofen	2	2425 µmol/L
Nicotine	2	6,2 µmol/L
Acetylcystein	2	10,2 mmol/L
Ebastine	2	0,78 µmol/L
Human whole blood	2	2% (v/v)
Saline nasal spray	2	10% (v/v)

CROSS REACTIVITY

A study has been performed to evaluate the effect of potentially cross-reactive microorganisms. Samples were run in CFX96 (Bio-Rad).

The results were as follows:

Microorganism	Samples No.	Positives No.
Acinetobacter baumannii	1	0
Adenovirus 31	1	0
Adenovirus 7	1	0
Adenovirus 8	1	0
Aspergillus fumigatus	1	0
Bordetella bronchiseptica	1	0
Bordetella holmesii	1	0
Bordetella parapertussis	1	0
Bordetella pertussis	1	0
Candida albicans	1	0
Chlamydophila pneumoniae	1	0
Chlamydophila psittaci	1	0
Citrobacter freundii	1	0
Corynebacterium diphteriae	1	0
Cytomegalovirus	1	0
Enterobacter cloacae	1	0
Epstein-barr virus	1	0
Escherichia coli (EIEC)	1	0
Fusobacterium nucleatum	1	0
Haemophilus influenzae	1	0
HCoV-NL63	1	0
Human metapneumovirus	1	0
Influenza A virus H1N1	1	0
Influenza B virus	1	0
Klebsiella pneumoniae	1	0
Legionella bozemanii (Fluoribacter bozemanae)	1	0
Legionella dumoffii (Fluoribacter dumoffii)	1	0
Legionella longbeachae	1	0
Legionella micdadei	1	0
Legionella pneumophila	1	0
Measles virus	1	0
MERS-CoV	1	0
Moraxella catarrhalis	1	0
Mycoplasma pneumoniae	1	0
Neisseria meningitidis serogroup A	1	0
Nocardia asteroides	1	0
Parainfluenza 1 virus	1	0
Parainfluenza 2 virus	1	0
Parainfluenza 3 virus	1	0
Parainfluenza 4 virus	1	0
Prevotella melaninogenica	1	0
Propionibacterium acnes	1	0
Pseudomonas aeruginosa	1	0
Respiratory syncytial virus subtype A	1	0
Respiratory syncytial virus subtype B	1	0
Rhinovirus	1	0
Rhodococcus equi	1	0
Staphylococcus aureus (mecA-)	1	0

Microorganism	Samples No.	Positives No.
Streptococcus agalactiae	1	0
Streptococcus constellatus	1	0
Streptococcus pneumoniae	2	0
Streptococcus pyogenes	1	0
TOTAL	53	0

In addition, an in-silico analysis of the primers/probes sequences comparing to other microorganisms that could be found in clinical samples was performed.
The results were as follows:

	Homology >80%			
Microorganism	MABSC	MAC	MTBC	Mycobacteria
Actinomyces meyeri	No	No	Yes	No
Actinomyces naeslundii	No	Yes	Yes	Yes
Actinomyces pyogenes (Trueperella pyogenes)	No	Yes	No	No
Bocavirus	No	No	No	No
Chlamydia caviae	No	No	No	Yes
Corynebacterium pseudodiphtheriticum	No	No	Yes	Yes
Corynebacterium xerosis	No	Yes	Yes	Yes
Eikenella corrodens	No	No	Yes	Yes
Pasteurella multocida	Yes	No	No	Yes
Porphyromonas gingivalis	Yes	Yes	Yes	No
Stenotrophomonas maltophilia	Yes	Yes	Yes	Yes
Streptococcus mitis	No	Yes	No	No
Streptococcus mutans	No	No	No	Yes

"YES" indicates microorganisms that showed > 80% homology with respect to one of the primers but not with any other primers included in the assay. Cross-reaction and/or interference with the assay due to the presence of these organisms could not be tested, but it is unlikely to occur.

ANALYTICAL SENSITIVITY

A preliminary LoD (limit of detection) was determined by testing serial dilutions of quantified MTBC, MAC, MABSC and NTM samples. Samples were extracted using OptiPure Viral kit on Maelstrom 4800 instrument (TANBead) and run in CFX96 (Bio-Rad).
Once an approximated LoD is determined, the final concentration was confirmed by testing 3 serial dilutions. A minimum of 20 replicates is tested for each dilution. The LoD is determined as the lowest concentration where ≥ 95% of the replicates are positive.

	MABSC	MAC	MTBC	NTM
LoD (copies/µl)	3	3	0.2	6
LoD (copies/ml)	400	400	23.1	800
LoD (copies/reaction)	15	15	0.9	30

INCLUSIVITY

An in-silico analysis for the primered genes included in the assay was performed to determine the inclusivity for the different MTBC, MAC, MABSC and NTM species/subspecies sequences available.
The criteria selected for including the different sequences in the analysis was geographic and the date when the sequence was deposited. Different lineages, types or subtypes were included in the analysis of each microorganism.
GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) was used for accessing sequences.
The results of the in-silico analysis show that the kit is predicted to detect all genome variants included in the analysis.

ANALYTICAL INCLUSIVITY

The analytical inclusivity of the kit for MTBC, MAC, MABSC and NTM detection was analysed by testing representative samples of the different species and subspecies. Samples were run in CFX96 (Bio-Rad).
The results were as follows:

Microorganism	Samples No.	Positives No.	Results Interpretation
Mycobacterium tuberculosis	1	1	MTBC
Mycobacterium microti	1	1	MTBC
Mycobacterium bovis BCG	1	1	MTBC
Mycobacterium caprae	1	1	MTBC
Mycobacterium bovis	1	1	MTBC
Mycobacterium africanum	1	1	MTBC
Mycobacterium avium	1	1	MAC
Mycobacterium intracellulare	1	1	MAC
Mycobacterium colombiense	1	1	MAC
Mycobacterium avium subsp. *silvaticum*	1	1	MAC
Mycobacterium abscessus	1	1	MABSC
Mycobacterium abscessus subsp. *bolletii*	1	1	MABSC
Mycobacterium abscessus subsp. *massiliense*	1	1	MABSC
Mycobacterium kansasii	1	1	NTM
Mycobacterium ulcerans	1	1	NTM
Mycobacterium smegmatis	1	1	NTM
Mycobacterium malmoense	1	1	NTM
Mycobacterium marinum	1	1	NTM
Mycobacterium gastri	1	1	NTM
Mycobacterium peregrinum	1	1	NTM
Mycobacterium scrofulaceum	1	1	NTM
Mycobacterium terrae	1	1	NTM
Mycobacterium xenopi	1	1	NTM
Mycobacterium celatum	1	1	NTM
Mycobacterium genavense	1	1	NTM
Mycobacterium chelonae	1	1	NTM
Mycobacterium lentiflavum	1	1	NTM
Mycobacterium mageritense	1	1	NTM
Mycobacterium chimaera	1	1	MAC
Mycobacterium fortuitum	1	1	NTM
Mycobacterium phlei	1	1	NTM
Mycobacterium mucogenicum	1	1	NTM
Mycobacterium elephantis	1	1	NTM
TOTAL	33	33	

All species and subspecies tested resulted in positive amplification.

EXTERNAL CONTROL

Controls that are required but not provided with the kit will be the following:
- as positive extraction control, AMPLIRUN® TOTAL MTB CONTROL (SPUTUM) Cat. MBTC013-R (Vircell), AMPLIRUN® TOTAL MTB RIF RESISTANT CONTROL (SPUTUM) Cat. MBTC014-R (Vircell) and AMPLIRUN® TOTAL MTB INH RESISTANT (SPUTUM) Cat. MBTC015-R (Vircell)

External controls help monitoring any cross-contamination that occurs during the extraction process, additionally serve as validation tools for extraction reagents.

SYMBOLS USED IN LABELS

In vitro diagnostic medical device


Use-by (expiry date)

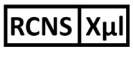
Store at x-y °C

Contains sufficient for <n> test

Batch code

Catalogue number


Consult instructions for use



Reconstitute in <X> µl


Manufacturer

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