OROPOUCHE-MAYARO VIRUS REALTIME PCR KIT

REF RTPCR032-LP-R Σ 24

RUO For research use only

INTENDED PURPOSE

Real Time RT-PCR kit to detect nucleic acid from Oropouche and Mayaro viruses in human serum and plasma samples.

INTRODUCTION

Oropouche virus (OROV) is a negative-sense single-stranded RNA spherical enveloped arbovirus belonging to the species Orthobunyavirus, family Peribunyaviridae. An increase in the detection of cases of Oropouche virus disease was observed from 2024 in some areas of the Americas including Bolivia, Peru, Cuba, Colombia and Brazil, with imported cases in other areas. Oropouche virus disease has an incubation period of 4 to 8 days (range: 3-12 days). The onset is sudden, usually with fever, headache, myalgia, rash, chills, and sometimes persistent nausea and vomiting for up to 5 to 7 days. Most cases recover within 7 days; however, convalescence could take weeks. Current recommendations include specials precautions for pregnant women.

Mayaro virus (MAYV) is a positive-sense single-stranded RNA icosahedral enveloped arbovirus belonging to the Alphavirus genus, Togaviridae family.
Mayaro fever has an incubation period of 1 to 12 days. The course of the disease is self-limiting, lasting 3 to 5 days. In the first few days, the patient presents a non-specific clinical picture: fever, headache, myalgia, chills, dizziness, nausea, photophobia, anorexia, joint edema, leukopenia, and thrombocytopenia. Hemorrhagic manifestations and encephalopathy and have been described in some cases. Imported and local cases were reported in Bolivia, Brazil, Colombia, Ecuador, French Guiana, Haiti, Mexico, Panama, Peru, and Venezuela among other.

OROV and MAYV could produce similar symptoms to other relevant arboviruses such as dengue, Zika and chikungunya highlighting the importance of a rapid and precise identification.

TEST PRINCIPLE

It is based on the reverse transcription (RT) and amplification in the same reaction well of specific fragments of Oropouche virus (OROV) and Mayaro virus (MAYV) by real time PCR.
One lyophilized master mix (RT-PCR MIX) is provided for screening and confirmation using one independent target for each virus.
PCR mix targets a specific fragment of the NP gene (S fragment) for OROV and a specific fragment of NSP1 gene for MAYV.
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 a bacteriophage MS2 RNA and a specific oligo pair/probe for its amplification.
The technique is divided into 2 main steps: 1) RNA extraction and 2) reverse transcription and amplification/detection with specific oligo pairs and probes. OROV RNA is detected in FAM channel, and MAYV RNA is detected in HEX/VIC channel. The internal control (MS2 RNA) is detected in Cy5 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 reverse transcription, amplification and detection using real time PCR.

MATERIALS PROVIDED

[1] VIRCELL OMV RT-PCR MIX LP: 3 strips with 8 tubes containing reverse transcriptase, Taq polymerase, buffer and specific primers/probe for Oropouche virus (NP gene) and Mayaro virus (NSP1 gene). Also, as internal control, primers/probe for bacteriophage MS2 RNA. 1 reaction per tube. Lyophilized.
[3] VIRCELL OMV 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: 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 6: 6 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 and Cy5 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 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 RNA purification kits.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen and avoid microbial contamination.
The kit can be used with specimens such as serum and plasma.
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 48h after collection at -20ºC or lower; avoid freezing at higher temperatures and freeze-thaw cycles.
Recommended guidance: Separated Serum or Plasma. p. 5.5.1.1.1. GP44-A4_Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests, 4th 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.
2. Amplification using RT-PCR (performed in the Amplification area):
   2.1. Preparation of the VIRCELL RT-PCR MIX tubes: 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	51 ºC	20 minutes
1 cycle	95 ºC	2 minutes
45 cycles	95 ºC	15 seconds
	58 ºC	45 seconds*

* Fluorescence data (FAM, HEX/VIC and Cy5) 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	OROV (FAM)	MAYV (HEX/VIC)	IC (Cy5)[1]	Interpretation
VIRCELL OMV POSITIVE CONTROL	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	Invalid
VIRCELL NEGATIVE CONTROL	No amplification or Ct >40	No amplification or Ct >40	Amplification (Ct < 40)	Correct
	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40)	Invalid

INTERPRETATION OF RESULTS

The result interpretation is described in the tables below:

RESULT	OROV (FAM)	MAYV (HEX/VIC)	IC (Cy5)[1]	Interpretation
1	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 >40	No amplification or Ct >40	Amplification (Ct < 40)	Negative
3	Amplification (Ct < 40)	No amplification or Ct >40	Amplification (Ct < 40) or no amplification	OROV
4	No amplification or Ct >40	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	MAYV
5	Amplification (Ct < 40)	Amplification (Ct < 40)	Amplification (Ct < 40) or no amplification	OROV + MAYV

[1] In case of a high copy number of the target nucleic acid, the amplification of the internal control (IC) in results 3 to 5 may be affected. The late amplification or absence of IC amplification does not change the interpretation of the result.

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

RUO	For research use only
	Use-by (expiry date)
	Store at x-y °C
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
RCNS Xµl	Reconstitute in <X> µl
	Manufacturer

Current version Nr.: L-RTPCR032-LP-R-EN-01
Date: 2024/09/26
Updates: New reference