Protocol for chikungunya (CHIKV) and o'nyong-nyong virus (ONNV) RT-qPCR positive control using CHIKV-ONNV Armored RNA (adapted to TaqMan platform)

For RUO (Research Use Only)

Vial content

CHIKV-ONNV Armored RNA (ArRNA) is a positive control to be used in combination with RT-qPCR assay Lyoph-P&P (lyophilized primers and probe) CHIKV-ONNV duplex for the detection and differentiation of chikungunya virus (CHIKV) and o'nyong-nyong virus (ONNV). The target RNA sequence is encapsidated through an *in vitro* protocol to provide a virus-like particle; therefore, the ArRNA must be extracted (such as virus supernatant or a clinical sample) before RT-qPCR.

Caution

Vials containing ArRNA must be stored at -20°C in the dark after reception. Stable for 4 years under the described conditions.

Instructions

This protocol is adapted to the following kit and extraction/RT-qPCR platforms:

- EZ1 Virus Mini Kit v2.1 (QIAGEN) on EZ1 extraction platform (QIAGEN)
- SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher)
- CFX (BIORAD) RT-qPCR thermal cycler

Use of other kits or extraction/RT-qPCR platforms may require adaptation of the protocol.

I. Rehydration of the ArRNA

- Write the date on the vial before opening.
- Resuspend ArRNA as follows to obtain the stock solution (**these steps are critical to ensure adequate homogenization**):

  ✓ Add 500µL of nuclease-free water

  ✓ Homogenize by pipetting 10 to 20 times the volume up and down in the glass vial

  ✓ Incubate rehydrated ArRNA at room temperature for 10 minutes

  ✓ Perform a second series of 10-times pipetting

II. Preparation of the working solution

- Extract 400µL of the stock solution on EZ1 platform (QIAGEN) using EZ1 Virus Mini Kit v2.1
- Choose 60µL as elution volume
- Dilute 60µL of eluates in 440µL of PBS to obtain working solution

- If the positive control is used several days after extraction, the working solution should be aliquoted to avoid several freezing/thawing cycles, and be stored at -80°C for optimal stability.

III. RT-qPCR

- Prepare PCR mix using RT-qPCR assay Lyoph-P&P (lyophilized primers and probe), previously rehydrated as explained in the SoP 'Protocol for Real-Time RT-PCR assay with Lyoph-P&P (adapted to TaqMan platform)' (Table 1)
- Perform RT-qPCR with the cycling program in Table 2

Table 1. Preparation of the PCR mix.

	Volume/PCR test (µL)
2X Reaction mix	12.5
Rehydrated Lyoph-P&P	7
SSIII/Taq enzyme mix	0.5
Total (1 PCR test)	**20**
Template RNA (H_2O for Ctrl-)	5
Final volume	**25**

Read: FAM (CHIKV), VIC (ONNV)-QSY

Table 2. Cycling program.

50°C	15min	
95°C	2min	
95°C	15sec	45 cycles
60°C	45sec (plate read)	

Using this protocol, a vial contains material for 100 PCR runs.