Protocol for Pan Phlebovirus RT-qPCR positive control using Pan Phlebo Armored RNA (adapted to TaqMan platform)

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Vial content

Pan Phlebo Armored RNA (ArRNA) is a positive control to be used in combination with RT-qPCR assay Lyoph-P&P (lyophilized primers and probe) Pan Phlebo matsuno for the detection of Pan Phlebovirus**.** 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)
- QuantiNova SYBR Green PCR Kit (2500) (QIAGEN)
- 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) ; NB: resuspend the vial in 4.8µL*number of tests
- Perform RT-qPCR with the cycling program in Table 2

Table 1. Preparation of the PCR mix.

	Volume/PCR test (µL)
2X Reaction mix	10
Rehydrated Lyoph-P&P	4.8
SSIII/Taq enzyme mix	0.2
Total (1 PCR test)	**15**
Template RNA (H_2O for Ctrl-)	5
Final volume	**20**

Read: FAM-MGB NFQ

Table 2. Cycling program.

50°C	10min	
95°C	2min	
95°C	10sec	45 cycles
50°C	20sec	
72°C	40sec (lecture)	
95°C	1min	

melt curve 60°C to 94°C, increment 0,5°C for 5 sec

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