

Protocol for the use of a vial of inactivated virus as T+ arbovirus

Contents

An inactivated virus vial contains non-infectious, lyophilised material: inactivated virus spiked in plasma. This vial can be extracted and tested using the usual molecular diagnostic protocol.

Storage

Vials of inactivated virus must be stored at -20°C away from light after receipt. Stable for at least 6 months under the conditions described.

Materials

This protocol has been validated with the following kit and extraction and RT-qPCR machines :

- EZ1/EZ2 Virus Mini Kit v2.1 (QIAGEN) on EZ1/EZ2 (QIAGEN)
- SuperScript™ III Platinum™ One-Step qRT-PCR Kit (ThermoFisher)
- Thermocycler CFX (BIORAD)

The use of other kits or other extraction or RT-qPCR machines may require adaptation of the protocol.

I. Reconstitution du flacon,

- Write the date on the bottle before opening it.
- Resuspend the lyophilisate as follows: **(these steps are essential to ensure adequate homogenisation)**
 - ✓ Add 400µL of water to the vial.
 - ✓ Pipette the volume up and down in the vial 10 to 20 times to ensure adequate homogenisation
 - ✓ Leave the rehydrated lyophilisate at room temperature for 10 minutes
 - ✓ Perform a second pipetting step after incubation

II. Preparation of the working solution

- Extract 400 µL of the stock solution using the EZ1/EZ2 automated system (QIAGEN) with EZ1/EZ2 Virus Mini Kit v2.1
- Select 60 µL as the elution volume
- If the positive control is used several days after extraction, the working solution must be aliquoted to avoid multiple freeze/thaw cycles and stored at -80°C for optimal stability.

III. RT-qPCR

- Prepare the PCR mix using the Lyoph-P&P (Lyophilized primers and probe) RT-qPCR system, previously reconstituted as described in the SoP 'Protocol for real-time RT-qPCR with Lyoph-P&P (adapted to a TaqMan platform)'. (Table 1)
- Perform RT-qPCR with the thermal profile described in Table 2

Table 1. Preparation of the RT-qPCR mix (using SuperScript™ III Platinum™ One-Step qRT-PCR Kit - ThermoFisher)

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

Reading : FAM-QSY

Table 2. Thermal profil

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

Using this protocol, one vial of lyophilised inactivated virus can be used to perform 100 RT-qPCR runs.

NB: This inactivated virus cannot be used as a specificity control. It is not guaranteed to be free of other viral RNAs.