Quantitative RT-PCR for WNV and house-keeping gene (β-actin)

Primers : WNproC-F 10 (5'-CCTGTGTGAGCTGACAAACTTAGT-3')
WNproC-R 153 (5'-GCGTTTTAGCATATTGACAGCC-3')
ACTBFrw966 : (5'-CAGCACAATGAAGATCAAGATCATC-3')
ACTBrev1096 : (5'-CGGACTCATCGTACTCCTGCTT-3')

Resuspend primers with milliQ water to obtain a 100µM stock solution and store at -20°C (FMDV L3 laboratory).

Probes :
Classical TaqMan probes :
WNproC-probe (5'-FAM-CCTGGTTTCTTAGACATCGAGATCT -TAMRA-3')
ACTB1042-67 probe (5'-VIC-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA-3')

Resuspend probes at 100µM with milliQ water (60µL for each probe). Prepare 5µL aliquotes and store at -20°C (FMDV L3 laboratory). Dilute probes at 10µM before use: add 45µL RNase free water for each 5 µl aliquote.

« Standard » :

- Viral RNA (strain IS98-ST1), stored at -80°C. Initial viral suspension titer: approximately 10^6pfu/µL;

10-fold serial dilutions of viral RNA: 2µL previous dilution + 18µL RNase free water.

- extracted RNA from the horse « Deister »…

5-fold serial dilutions of equine RNA : 4µL previous dilution + 16µL RNase free water.

Kit : AgPath-ID™ One-Step RT-PCR Reagents (Applied Biosystem, 4387424 for 500 reactions), stored in -20°C freezer in Mix Preparation room in FMDV L3 laboratory.

PCR conditions: volumes per tube =

ProC		ProC+β-actine	
H_2O DEPC	5.8µL	H_2O DEPC	5.1µL
Tampon 2X	12,5µL	Tampon 2X	12,5µL
RT-PCR mix 25X	1µL	RT-PCR mix 25X	1µL
Primer ProC F 100µM	0.1µL	Amorce ProC F 100µM	0.1µL
Primer ProC R 100µM	0.1µL	Amorce ProC R 100µM	0.1µL
Probe ProC 10µM	0.5µL	Sonde ProC 10µM	0.5µL
		Amorce b-actin F 100µM	0.1µL
		Amorce b-actin R 100µM	0.1µL
		Sonde b-actin 10µM	0.5µL

Prepare a PCR mix, by calculating the volume required for the PCR reactions + 1 or 2 extra tubes. Distribute 20µL of PCR mix per PCR tube.

Add 5 µl RNA or water per tube. Test dilutions 10^{-2} to 10^{-6} for the viral RNA (from $5x10^4$ $DECP_{50}$ to 5 $DECP_{50}$: one tube per dilution). Test dilutions 1/5 and 1/25 for equine RNA in the β-actin PCR. Add 1 NTC tube (5µL water) and 2 tubes per sample (5µL pure extracted RNA).

Enter the program on the AB7300 thermocycler (be careful to enter the right reaction volume = 25µL). Detector manager = FAM-TAMRA or VIC-TAMRA.

Program :

Reverse transcription	45°C	10 min	
Denaturation	95°C	10 min	
PCR	95°C	15 s	*40 cycles
Analysis mode = quantification	60°C	45s	