

rtRT-PCR for the detection of O/ME-SA/SA-2018 FMDV

This real-time RT-PCR is a molecular tool for detection of Foot-and-mouth disease virus lineage O/ME-SA/SA-2018, as it is an emerging lineage in South Asia since 2018. The assay has been tested on 34 samples with a specificity of 91,7% (11/12 SA-2018 samples detected). The primers and probes are indicated hereafter:

Oligo name (final concentration)	Sequence (5'-3')	Use
SA2018_F3 (0.4 μ M)	ACAACACCACCAATCCAAC	Forward Primer
SA2018_P3 (0.3 μ M)	FAM-ACTCACCCGACTTGCACTGCCGT-TAMRA	Probe
SA2018_Rev2 (0.4 μ M)	CGTTGTAAACAGTAGCCATGA	Reverse Primer

The SA-2018 has been validated using Ag-Path kit in a duplex system with β -actin, and following the volumes and concentrations as follow (5 μ l of RNA):

	Volume (μ l)	Concentration		
	For one tube	Initial	Final	
Ultrapure water (DNase RNase Free)	1,15	/		
Buffer 2X (kit AgPath-ID™)	12,5	2	1	X
Primer F	1	10	0,4	μ M
Primer R	1	10	0,4	μ M
Probe FAM-TAMRA	0,75	10	0,3	μ M
Primer F β -actine	1	10	0,4	μ M
Primer R β -actine	1	10	0,4	μ M
Probe VIC-TAMRA β -actine	0,6	5	0,12	μ M
RT-PCR mix 25X (Enzyme)	1	25	1	X

Real-time PCR program:

Cycles of RTq-PCR		
T°	Time	nb cycles
45°C	10min	1
95°C	10min	1
95°C	15s	45
62°C	1min	

NB: This system has been validated on a small number of samples and should therefore be tested against other samples from this lineage.