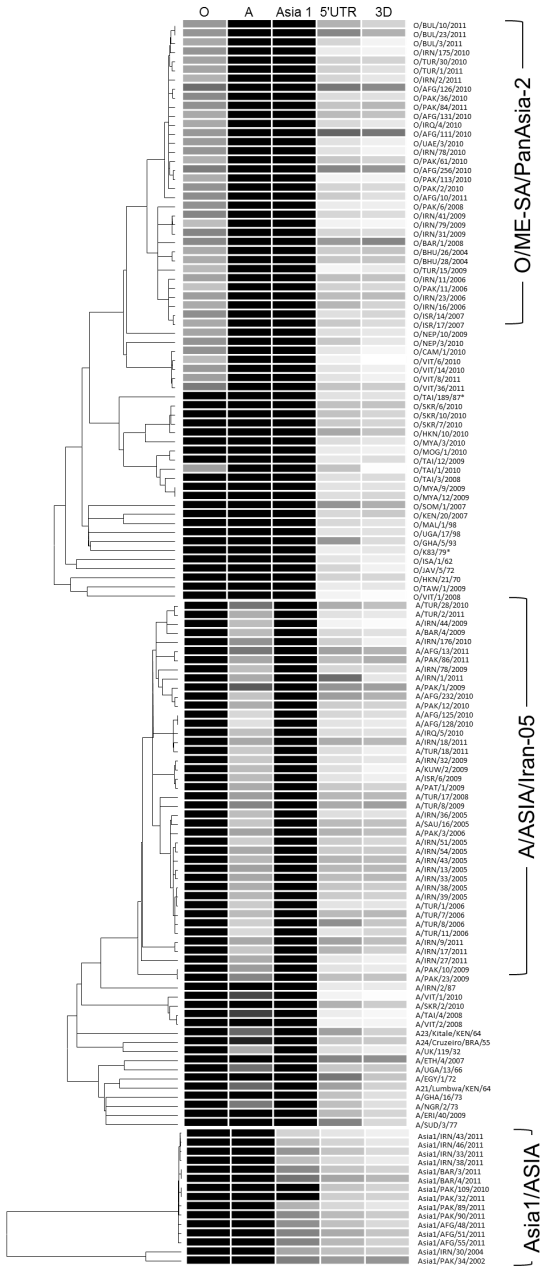
PHYLOGENY:

Illustration of phylogenetic relationship of viruses used for validation of the assays in relation to numeric results is shown below.



Preventing and controlling viral diseases

Real-time RT-PCR assays for detection of FMDV serotype O, A and ASIA1 specific to West Euroasia

Preventing and controlling viral diseases

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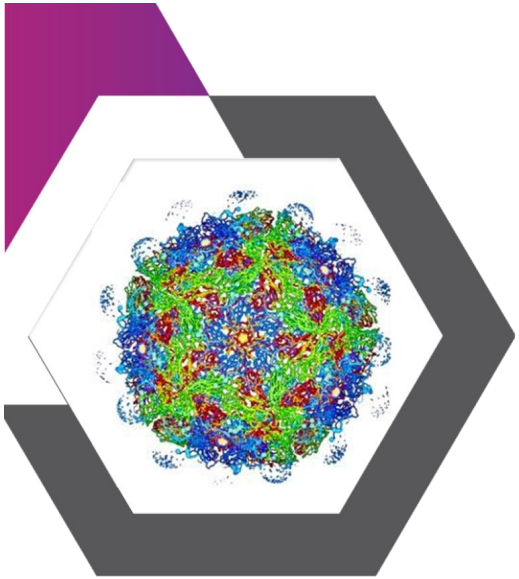


INTRODUCTION:

The set of real-time RT-PCR type-specific assays was designed for the detection of FMDV strains currently circulating in West Euroasia as illustrated in the figure below (Reid et al., 2014).

The set of real-time RT-PCR assays aims to detect FMDV O/ME-SA/PanAsia-2, A/ASIA/Iran-05, Asia1/ASIA/Group 1, 2 and 6.

ASSAY COMPOSITION:

The composition of the assay is presented in the table below.

Reagents indicated with an asterisk (*) are part of SuperScript III/ Platinum Taq One-Step qRT-PCR Kit (Invitrogen).

Due to high sensitivity of the test, care needs to be taken when handling samples and reagents to avoid possibility of contamination.

REAGENT	
FP (working stock 10 µM)	2 µl
RP (working stock 10 µM)	2 µl
P (working stock 5 µM)	1 µl
SuperScript III RT/Platinum *Taq* Mix*	0.5 µl
2x Reaction Mix*	12.5 µl
Nuclease free water	2 µl
RNA	5 µl
total volume	25 µl

All oligonucleotides were custom synthetized and their sequences are listed below:

OLIGO NAME	NUCLOTIDE SEQUENCE (5'→3')
O/ME-FP	CCGAGACAGCGTTGGATAACA
O/ME-RP	CCATACTTGCAGTTCCCGTTGT
O/ME-P	FAM-CCGACTTGCACTGCCTTACACGGC-TAMRA
A/ME-FP	ACGACCATCCACGAGCTYC
A/ME-RP	RCAGAGGCCTGGGACAGTAG
A/ME-P	FAM-CGTGCGCATGAAACGTGCCG-TAMRA
Asia1/ME-FP	GCAGTWAAGGCYGAGASCATYAC
Asia1/ME-RP	GCARAGGCCTAGGGCAGTATG
Asia1/ME-P	FAM-AGCTGTTGATCCGCATGAAACGYGCG-TAMRA

THERMAL PROFILE:

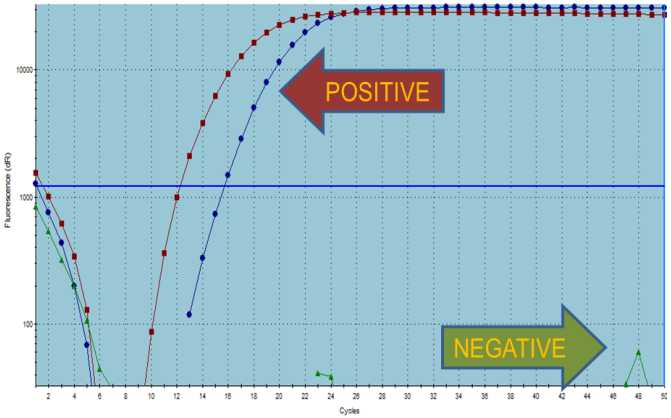
Amplification of reactions is to be carried out using a real-time PCR instrument under following conditions: 60°C for 30 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. Fluorescence data is collected at the annealing/elongation step.

RESULTS INTERPRETATION:

In **positive** samples, fluorescence signal accumulated during amplification, crosses the threshold value. A Ct value is calculated at the end of the assay.

Negative results (for assays that did not reach the threshold) are reported as "No Ct".

Examples of typical amplification curves are presented below.



TROUBLE SHOOTING:

Should you encounter difficulties with these assays or with interpretation of data, please contact the Vesicular Disease Laboratory WRLFMD at the Pirbright Institute, UK