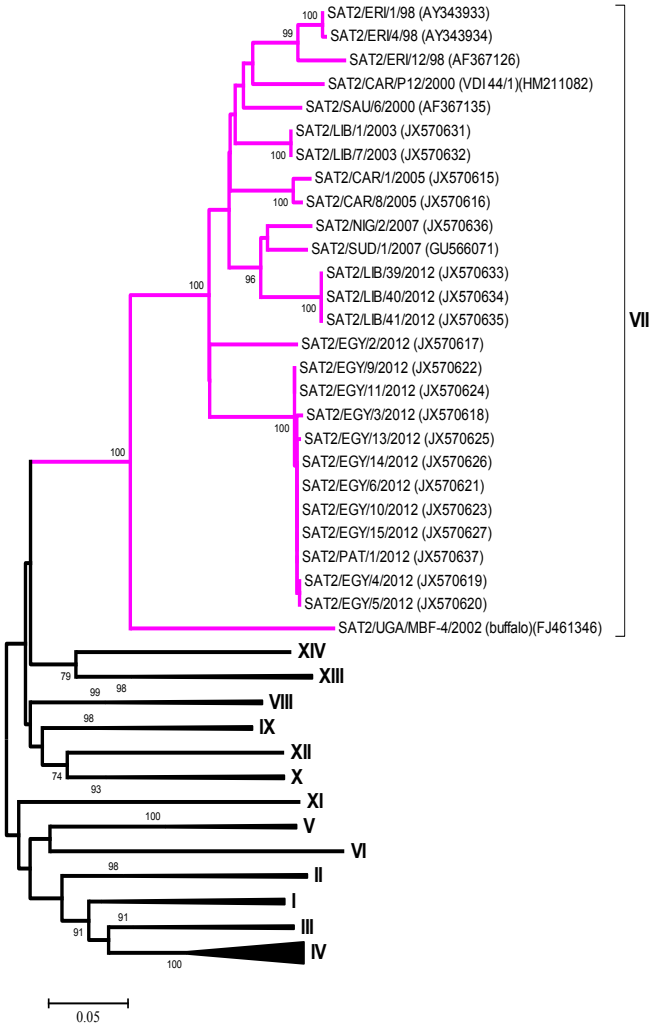
PHYLOGENY:

Phylogeny of representative strains within FMDV/ SAT2/VII, shown in purple, in relation to other top-otypes of FMDV-SAT 2.



Preventing and controlling viral diseases

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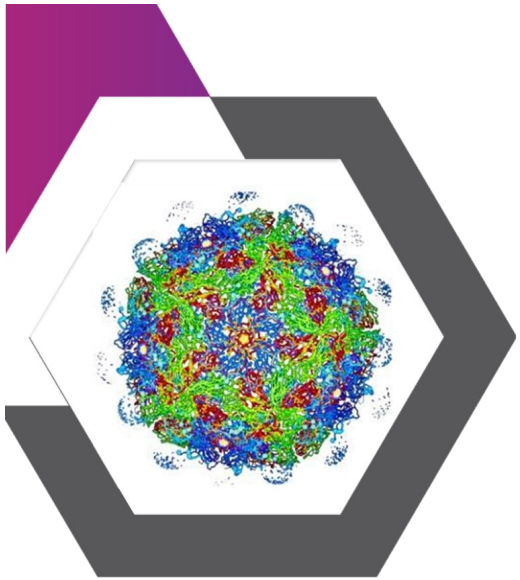
Preventing and controlling viral diseases

FMDV/SAT2/VII

specific

real-time RT-PCR assay



INTRODUCTION:

This FMDV/SAT2/VII specific real-time RT-PCR is a molecular tool for detection of foot-and-mouth Disease virus SAT2/VII topotype and was developed in response to the SAT2 outbreak in Egypt in 2012 (Ahmed et al., 2012).

Viruses of this lineage were previously detected in Libya, Egypt, Palestinian Autonomous Territories, Eritrea and Cameroon as indicated on the map below.

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	
SAT2/VII-FP (working stock 10 µM)	2 µl
SAT2/VII-RP (working stock 10 µM)	2 µl
SAT2/VII-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 of the oligonucleotides are custom synthetized and their sequences are listed below:

OLIGO NAME	NUCLOTIDE SEQUENCE (5'→3')
SAT2/VII-FP	TGAAGAGGGCTGAGCTGTACTG
SAT2/VII-RP	CTCAACGTCTCCTGCCAGTTT
SAT2/VII-P	FAM-ACAGATTCGACGCGCCCATCG-TAMRA

THERMAL PROFILE:

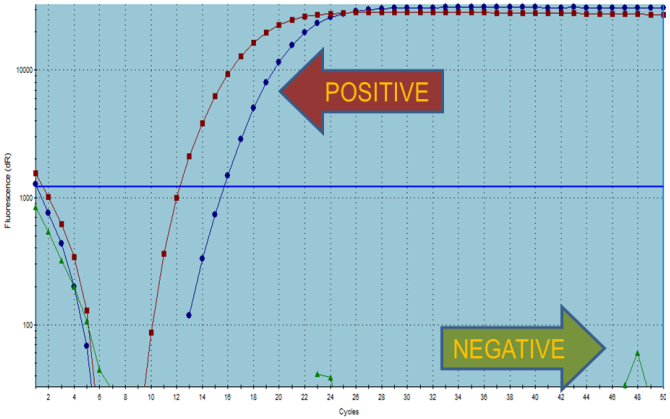
Amplification of reactions is to be carried out using a real-time PCR instrument under following conditions: 60°C for 30 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. Fluorescence data is collected at the annealing/elongation step.

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