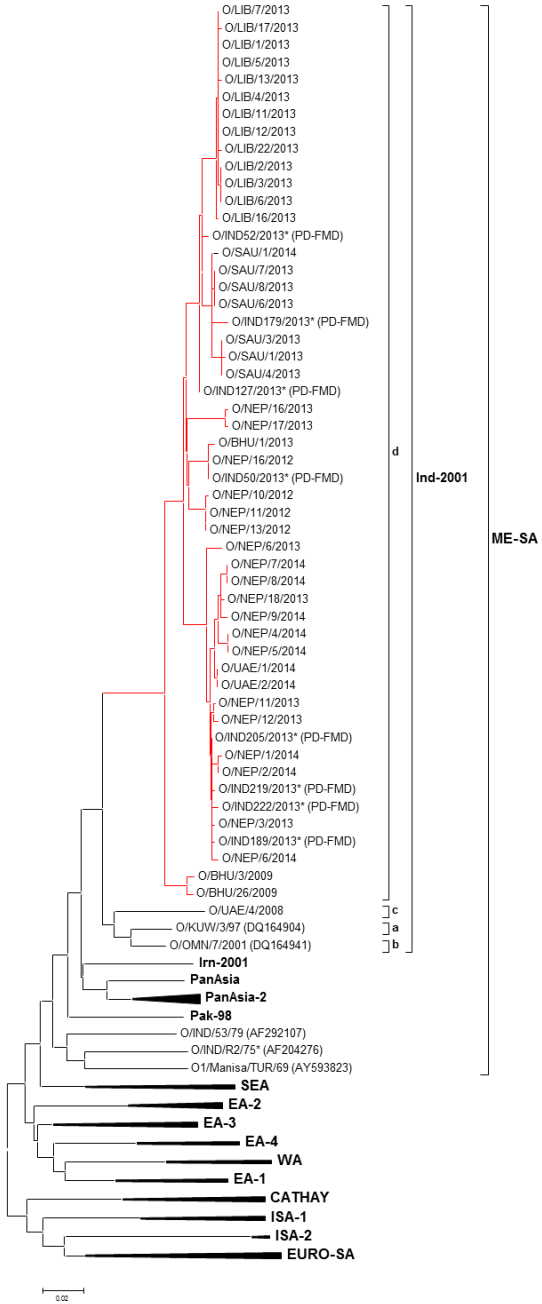
IND-2001 PHYLOGENY:

Phylogeny of representative strains within Ind-2001 lineage (shown in red) in relation to other lineages and topotypes of FMDV-O.



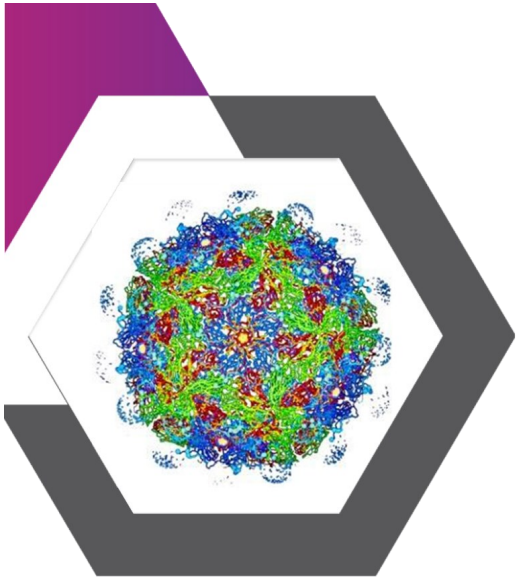
The Pirbright Institute

Preventing and controlling viral diseases

FMDV-O Ind-2001

specific

real-time RT-PCR assay



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A company limited by guarantee, registered in England no. 559784. The Institute is also a registered charity.

Director: Professor John Fazakerley BSc, MBA, PhD, FSB, FRCPath.

The Pirbright Institute receives strategic funding from the Biotechnology and Biological Sciences Research Council.

INTRODUCTION:

This FMDV-O Ind-2001-specific real-time RT-PCR is a molecular tool for detection of foot-and-mouth Disease virus O/ME-SA/Ind2001 lineage (Knowles et al., 2014).

Viruses of this lineage were previously detected in India, Nepal, United Arab Emirates, Saudi Arabia and Libya and caused field outbreaks in North Africa and West EuroAsia during 2013/2014 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	
Ind2001d-FP (working stock 10 µM)	2 µl
Ind2001d-RP (working stock 10 µM)	2 µl
Ind2001d-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')
Ind2001d-FP	CCTCCTTCAAYTACGGTG
Ind2001d-RP	GCCACAATCTTYTGTTTGTG
Ind2001d-P	FAM-CTGCTCGCCATTCACCCG-BHQ-1

THERMAL PROFILE:

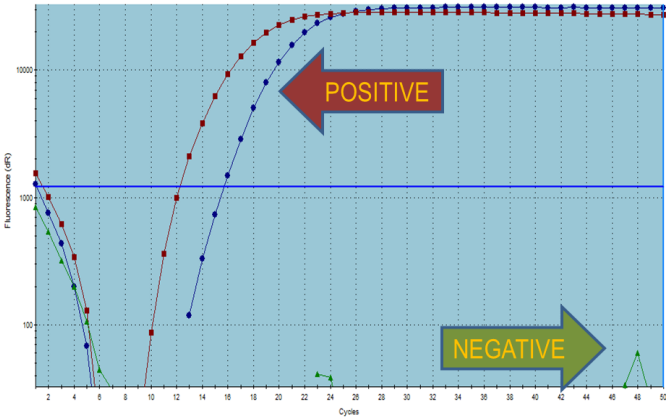
Amplification of reactions is to be carried out using a real-time PCR instrument under following conditions: 60°C for 30 min, 95°C for 10 min followed by 50 cycles of 95°C for 15 sec and 60°C for 1 min. Fluorescence data is collected at the annealing/elongation step.

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