Lineage-specific real-time RT-PCR assays

The table below describes primers and probes that can be used in these individual assays

1. **A/ASIA/G-VII** Saduakassova et al., 2017 *In print*.

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
A	ASIA	G-VII	G-VII/FP	TGCTCAACTCCCTGCCTC
			G-VII/RP	GAGTTCGGCACGCTTCAT
			G-VII/P	FAM-CCACYACCATCCACGAGCTG-BHQ1

2. **FMDV-SAT 2 (originally developed to detect 2012 outbreak in Egypt).** Ahmed et al., 2012.

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
SAT 2	VII	n/a	SAT2/VII/FP	TGAAGAGGGCTGAGCTGTACTG
			SAT2/VII/RP	CTCAACGTCTCCTGCCAGTTT
			SAT2/VII/P	FAM-ACAGATTCGACGCGC CCATCG-TAMRA

3. **FMDV-A, O and Asia 1 in the Middle East.** Reid et al. 2015.

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
O	ME-SA	PanAsia-2	O/PAsia2/FP	CCGAGACAGCGTTGGATAACA
			O/PAsia2/RP	CCATACTTGCAGTTCCCGTTGT
			O/PAsia2/P	FAM-CCGACTTGCACTGCCTTACACGGC-TAMRA
A	ASIA	Iran-05	A/Iran/FP	ACGACCATCCACGAGCTYC
			A/Iran/RP	RCAGAGGCCTGGGACAGTAG
			A/Iran/P	FAM-CGTGCGCATGAAACGTGCCG-TAMRA
Asia 1	ASIA	Group 1, 2 and 6	Asia1/FP	GCAGTWAAGGCYGAGASCATYAC
			Asia1/RP	GCARAGGCCTAGGGCAGTATG
			Asia1/P	FAM-AGCTGTTGATCCGCATGAAACGYGCG-TAMRA

4. **FMDV-A, O and Asia 1 in the Middle East.** Jamal and Belsham (2015)

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
O	ME-SA	PanAsia-2	O-JB-F	GAGACAGCGTTGGAYAACACC
			O-JB-R	TGWGGTGCCGTGTAAGGCAG
			O-JB-F-P	Fam-AATCCAACGGCTTACCACAAGGCACC-Tamra
A	ASIA	Iran-05	A-JB-F	GCCACGACCATCCACGAGCT
			A-JB-R	GTCCTGYGACRACACTTCCAC
			A-JB-F-P	Fam-CTCGTGCGYATGAAACGTGCYGAGCT-Tamra
Asia 1	ASIA	Group 2 and 7	As-JB-F	TGCCYACYTCXTTYAAYTACGG
			As-JB-R	CARAGGYCTRGGGCAGTATGT
			As-JB-F-P	Fam-CGTTTCATGCGRATYAAMAGCTCAGTGAT-Tamra

5. O/ME-SA/Ind-2001.

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
O	ME-SA	Ind-2001	O/Ind2001/FP	CCTCCTTCAAYTACGGTG
			O/Ind2001/RP	GCCACAATCTTYTGTTTGTG
			O/Ind2001/P	FAM-CTGCTCGCCATTCACCCG-BHQ-1

6. FMDV-A, O, SAT 1 and SAT 2 in East Africa.

type	topotype	lineage	oligo name	nucleotide sequence (5'→3')
A	AFRICA	G-I	A/G-I/FP	GCCACRACCATCCACGA
			A/G-I/RP	GAAGGGCCCAGGGTTGGACTC
			A/G-I/P	FAM-CTCGTGCGMATGAARCGGGC-BHQ1
O	EA-2 and EA-4	unnamed	O/EA2+4/FP	CCTCCTTCAAYTACGGTG
			O/EA2+4/RP	GCCACAATCTTYTGTTTGTG
			O/EA2+4/P	FAM-CCCTCTTCATGCGGTARAGCAG-BHQ1
SAT1	I	unnamed	SAT1 I/FP	CTYGACCGGTTCACYCTG
			SAT1/I/RP	CCGAGAAGTAGTACGTRGC
			SAT1/I/P	FAM-CAGGAYTGCGCCCACCA-BHQ1
SAT2	IV	unnamed	SAT2/IV/FP	CRATCCGCGGTGAYCG
			SAT2/IV/RP	CGCTTCATYCTGTAGTARACGTC
			SAT2/IV/P	FAM-TTTGGACAYGTGACCGCCG-BHQ1
SAT2	VIII, IX, X, XII	unnamed	SAT2/EAfr/FP	CCATYCGTGGCGAYAG
			SAT2/EAfr/RP	CGYTTCATYCGGTAGTAAACGTC
			SAT2/EAfr/P	FAM-TTCGGKTTYGTGACCGCCG-BHQ1

Assembly of the assay:

Each assay is assembled according to the same protocol, as follows:

REAGENT	
FP^ (10µM)	2 µl
RP^ (10µM)	2 µl
P^ (5µM)	1.5 µl
SuperScript III RT/Platinum *Taq* Mix*	0.5 µl
2x Reaction Mix*	12.5 µl
Nuclease free water	1.5 µl
RNA	5 µl
total volume	25 µl

^relates to listed above were FP= forward primer, RP= reverse primer, P=probe.

*Part of SuperScript III/ Platinum Taq One-Step qRT-PCR Kit (Invitrogen).

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.

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