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upE assay and ORF1b assay

Real-time rtPCR for Betacoronavirus (MERS-CoV), based on Rotterdam virus isolate (HCoV-EMC) by Ron Fouchier:

Example formulation: Invitrogen SuperScriptIII OneStep RT-PCR System with Platinum Taq DNA Polymerase

MasterMix:	**25µl** single rxn, µl
H_2O (RNAse free)	2.6
$MgSO_4$(50mM)	0.4
2x Reaction mix*	12.5
BSA (1 mg/ml)**	1
Fwd primer (10 µM)	1
Rev primer (10 µM)	1
Probe (10 µM)	0.5
SSIII/Taq EnzymeMix*	1
	20
Template RNA	5

Cycler:		
55°C	20’	
94°C	3’	
94°C	15’’	45x
58°C	30’’	
40°C	30’’	

' = minutes; " = seconds

*Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase

**non-acetylated. This component is only necessary if using glass capillary LightCycler. Can be replaced with water in plastic vessel machines such as ABI 7500, LC 480, etc.

Primers / probe:

No 1 Screening assay (upstream of E gene; upE assay):
upE-Fwd: GCAACGCGCGATTCAGTT
upE-Prb: FAM-CTCTTCACATAATCGCCCCGAGCTCG-TAMRA
upE-Rev: GCCTCTACACGGGACCCATA

No 2 Confirmatory assay (ORF 1b assay):
ORF1b-Fwd: TTCGATGTTGAGGGTGCTCAT
ORF1b-Prb: FAM-CCCGTAATGCATGTGGCACCAATGT-TAMRA
ORF1b-Rev: TCACACCAGTTGAAAATCCTAATTG

Positive Control:
upE-Assay: IVT-RNA

Reference:
Corman et. al; Eurosurveillance, Volume 17, Issue 39, 27 September 2012