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Confirmatory real-time RT-PCR assay (1A assay)

Real-time rtPCR for Betacoronavirus (MERS-CoV):

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

<u>MasterMix:</u>	<u>25µl</u> single rxn, µl	<u>Cycler:</u>	
H ₂ O (RNase free)	2.6	55°C	20'
MgSO ₄ (50mM)	0.4	94°C	3'
2x Reaction mix*	12.5		
BSA (1 mg/ml)**	1	94°C	15''
Fwd primer (10 µM)	1	58°C	30''
Rev primer (10 µM)	1		45x
Probe (10 µM)	0.5	40°C	
SSIII/Taq EnzymeMix*	1		
	20		
Template RNA	5	' = 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:

1A assay
EMC-Orf1a-Fwd: CCACTACTCCCATTTCGTCAG
EMC-Orf1a-Prb: FAM-TTGCAAATTGGCTTGCCCCACT-TAMRA
EMC-Orf1a-Rev: CAGTATGTGTAGTGCGCATATAAGCA

Positive Control:

ORF1A assay: IVT-RNA

Reference:

Corman/Müller et al.; Eurosurveillance, Volume 17, Issue 49, 06 December 2012