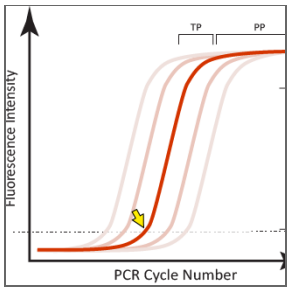

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MANUSCRIPT CITATION: Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance. 2020;25(3):2000045. https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045

Protocol status: Working
We use this protocol and it's working

Created: Feb 24, 2020

Last Modified: Feb 24, 2020

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR E gene 2020

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Public Health Virology, Forensic and Scientific Services | Coronavirus Method Development Community

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ABSTRACT

A real-time RT-PCR designed to amplify a portion of the envelope gene of sequences from the *Betacoronavirus* sub-genus *Sarbecovirus*.

The probe and primers were published by Corman *et al.*, and we have slightly modified the protocol, increasing the concentration of the reverse primer, using a different kit and different cycling conditions.

This test has identified clinical positive cases of coronavirus disease 2019 (COVID-19).

GUIDELINES

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol.

MATERIALS

MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #11732088**

STEP MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #11732088**

PROTOCOL MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #11732088**

In Materials, Materials, Step 2

PROTOCOL integer ID:
33441

Keywords: CoV, coronavirus, Wuhan, Real-time, RT-PCR, PCR, virus, China, pneumonia, seafood market, WSMPV, sarbecovirus, SARS-CoV-2, COVID-19

Oligonucleotides

1

	Oligo name	Sequence 5'-3'	Location*
	E_Sarbeco_F1	ACAGGTACGTTAATAGTTAATAGCGT	26269 - 26294
	E_Sarbeco_R2	ATATTGCAGCAGTACGCACACA	26381 - 26357
	E_Sarbeco_P1	6FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	26332 - 26357

*Based on numbering for GenBank accession NC_045512 Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1

Reagents

2

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #11732088**

Synthetic controls

3 Synthetic controls are produced using the binary synthetic template oligonucleotide positive control for in-house diagnostic real-time RT-PCR method.

The oligonucleotide sequences required to make controls for this assay are:

Probe control:
AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAAACACTAGCCATCCTTACTGCGCTTCGACAGTGTTCAGCAGG
TCCTGTTGAAAA

Primer control:
AAAATAATACGACTCACTATAGGGACAGGTACGTTAATAGTTAATAGCGTATGATCTGGCACGGGACCCTCCAATGTGTGCGTACTGCTG
CAATATAAAA

Reaction Set-up

4
- Assay has been designed to be used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs and a ABI 7500 Fast real-time machine.

- Total reaction volume is 20µL.
- Prepare sufficient for number of reaction plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

	Reagent	Volume (µl) x1	Final reaction concentration
	Nuclease-Free water	4.39	
	E_Sarbeco_F1	0.04	400nM
	E_Sarbeco_R2	0.09	900nM
	E_Sarbeco_P1	0.04	200nM
	2X Reaction mix*	10	
	Superscript III/Platinum Taq enzyme mix*	0.4	
	ROX reference dye (25uM)*	0.04	50nM
	TOTAL VOLUME	15	

*Superscript®III Platinum® One-Step qRT-PCR kit

Dispense 15µl to each reaction well.
Add 5µl of template, extracted RNA, controls or NTC (nuclease-free water).
Total reaction volume is 20µl.

Amplification

5 PCR Amplification

	1 cycle	40 cycles
	50°C 5 minutes	95°C 3 seconds
	95°C 2 minutes	60°C 30 seconds*

*Florescence acquisition step

Result analysis

6 The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A sigmoidal curve – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase
2. A suitable level of fluorescence intensity as measured in comparison to a positive control (y-axis)
3. A defined threshold cycle (C_T) value which the fluorescent curve has clearly exceeded (Fig.1 arrow) and which sits early in the log-linear phase and is <40 cycles
4. A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T value >40 cycles is considered a negative result
5. NTCs should not produce a curve.

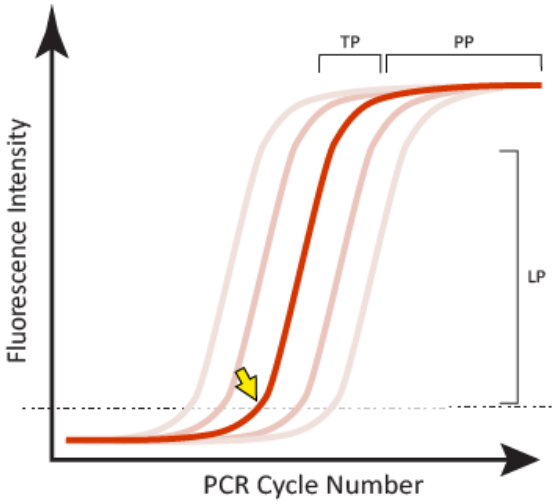

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.