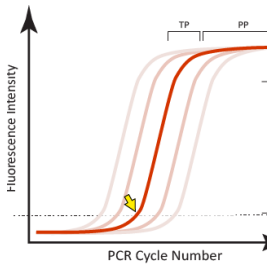
May 25, 2020

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) real-time RT-PCR CCDC-ORF1ab 2020



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We use this protocol and it's working

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Abstract

A real-time RT-PCR designed to amplify a portion of the ORF1ab gene of sequences from the Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

The probe and primers were published by the China CDC and are now included in the WHO in-house assay document.
This test has been modified for use with our standard reagents and instrumentation.

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

Oligonucleotides

1

	Oligo name	Sequence 5'-3'	Location *
	CCDC-ORF1ab-F	CCCTGTGGGTTTTACACTTAA	13342-13362
	CCDC-ORF1ab-R	ACGATTGTGCATCAGCTGA	13460-13442
	CCDC-ORF1ab-P	6FAM- CCGTCTGCGGTATGTGGAAAGGTTATGG -BHQ1	13377-13404

*Based on numbering for GenBank accession NC_045512.2 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:
AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAACCGTCTGCGGTAT
GTGGAAAGGTTATGGACAGTGTTCAGCAGGTCCTGTTGAAAA

Primer control:
AAAATAATACGACTCACTATAGGGCCCTGTGGGTTTTACACTTAAATGATCTGGCACGGGAC
CCTCCAATCAGCTGATGCACAATCGTAAAA

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	4.43	
	CCDC-ORF1ab-F (200nM)	0.05	500 nM
	CCDC-ORF1ab-R (200nM)	0.05	500 nM
	CCDC-ORF1ab-P (100nM)	0.03	150nM
	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	50 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.

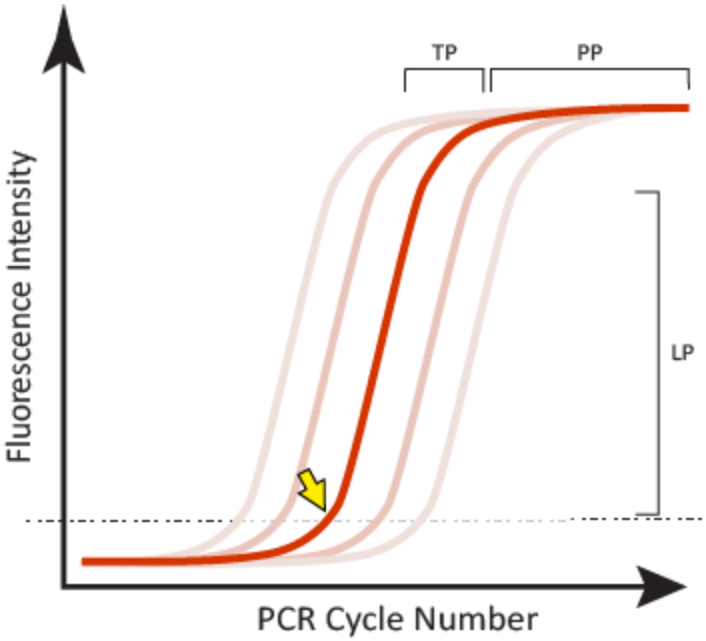

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) 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.