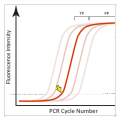

Feb 02, 2020

Novel coronavirus (2019-nCoV) real-time RT-PCR N gene 2020 (Wuhan-N; 2019-nCoV-related screening test) V.2

Judy A. Northill[1], Ian Mackay[1]

[1]Public Health Virology, Forensic and Scientific Services

In Development dx.doi.org/10.17504/protocols.io.bb3piqmn

Public Health Virology, Forensic and Scientific Services

Judy A. Northill
Public Health Virology, Forensic and Scientific Services

ABSTRACT

- A real-time RT-PCR to detect the "novel Wuhan" betacoronavirus. Based on sequence MN908947 made available by Professor Yong-Zhen Zhang, Fudan University, Shanghai, China.
- The target region encodes the nucleocapsid (N).
- Not tested on wild-type virus (as of 25Jan2020), it is expected to be capable of detecting Wuhan virus, bat-like SARS and SARS virus.
- Limit of detection not yet determined.
- A single 1 mismatch at probe-binding site identified with the *BetaCoV/USA/CA1/2020|EPI_ISL_406034* variant of 2019-nCoV (as of 29JAN2020).
- Probe is in the 3'-5' (reverse complement) direction.

Notes:

1. Assay is fully optimised (as of 24Jan2020).
2. A final name for this virus has not been decided (as of 25Jan2020).

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.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
SuperScript™ III Platinum™ One-Step qRT-PCR Kit	11732088	Life Technologies

Mix

1 Oligonucleotides

Oligo Name	Sequence 5'-3'	Location based on NC_045512*
Wuhan-TM2020For	TCGTGCTACAACTTCCTCAAG	28648-28668
Wuhan-TM2020Probe	6FAM-CCGCCTCTGCTCCCTTCTGC-BHQ1	28714-28695
Wuhan-TM2020Rev	CTGCCWGGAGTTGAATTTCTTG	28780-28759

*GenBank accession NC_045512 Wuhan seafood market pneumonia virus isolate Wuhan-Hu-1

2 Reagents

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

3 Synthetic controls

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:
AAAATAATACGACTCACTATAGGGTGAAGAGAATCCACAAGGAATTGAACCGCCTCTGCTCCCTTCTGCACAGTGTTCAGCAGGTCCTGTTGAAAA
Primer control:
AAAATAATACGACTCACTATAGGGTCGTGCTACAACTTCCTCAAGATGATCTGGCACGGGACCCTCCAACAAGAAATTCAACTCCAGGCAGAAAA

4 **Reaction Set-up**

- Assay has been designed to be used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs and an 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 (ul) X1	Final reaction concentration
Nuclease free water	4.41	
Wuhan-TM2020F (200uM)	0.05	500nM
Wuhan-TM2020R (200uM)	0.09	900nM
Wuhan-TM2020Probe (100uM)	0.01	50nM
2 X Reaction mix*	10	1X
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 5min	95°C 3 seconds
95°C 2min	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 (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

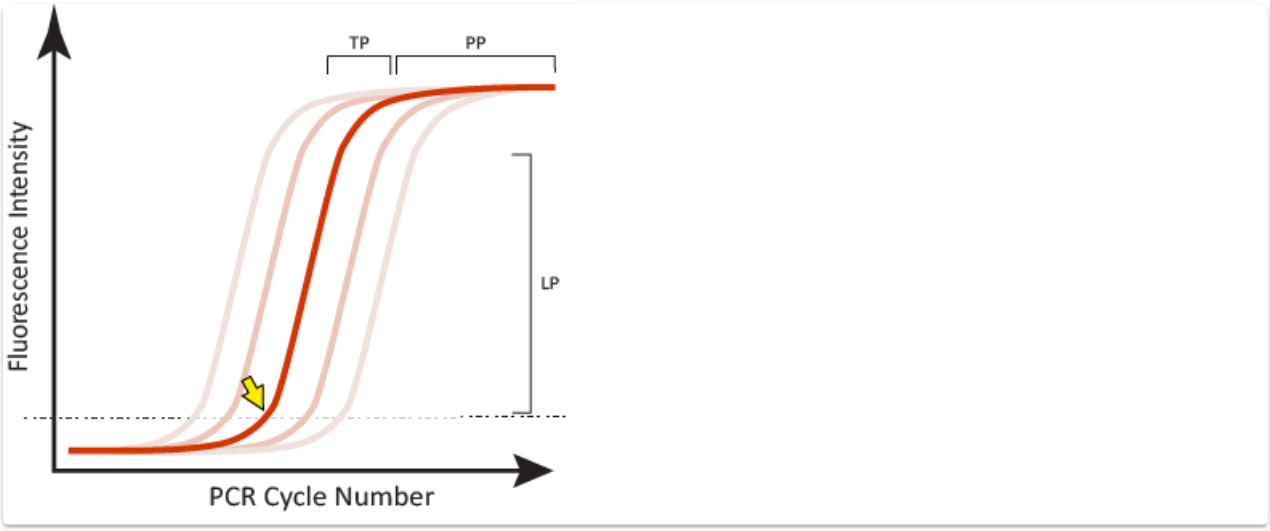

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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