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Real-Time PCR protocol to screen for SARS-COV-2 variants of concern (B.1.1.7, P.1 and B.1.1.35)

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ABSTRACT

The recently emerged variants of concern (VOC) B.1.1.7 in UK, B.1.1.35 in South Africa and P.1 in Brazil have increased transmissibility and are rapidly spreading worldwide. These variants have several substitutions that allow them to bypass the immune responses and are up to 10 times more contagious than the previous circulating SARS-COV-2 lineages. While in some regions P.1 predominates (as several estates in Brazil), in other countries B.1.1.7 or B.1.1.35 are most prevalent. Therefore, real-time surveillance of VOC is the key for rapid public health responses. In this matter, RT-PCR protocols that are capable of detecting VOCs easily and rapidly are needed. We developed and validated a protocol that detect simultaneously the 3 most widespread VOC. Although this protocol does not discriminate among the VOCs, it can reliably detect mixed infection. Importantly, our protocol successfully identified a co-infection by two SARS-COV-2 lineages (P.1 and B.1), confirmed through complete genome sequencing.

PROTOCOL CITATION

Camila M Romano, Jaqueline De Jesus, Alvina Clara Felix, Anderson V de Paula, Pâmela S Andrade, Franciane M de Oliveira, Darlan Cândido, Nuno Faria, William M de Souza, Ester C Sabino 2021. Real-Time PCR protocol to screen for SARS-COV-2 variants of concern (B.1.1.7, P.1 and B.1.1.35) . **protocols.io** https://protocols.io/view/real-time-pcr-protocol-to-screen-for-sars-cov-2-va-bszbnf2n

KEYWORDS

SARS-COV-2, variants of concern, RT-PCR

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GUIDELINES

This protocol was not set for diagnoses purpose.

MATERIALS TEXT

Here are the sequences of primers and probes.

A	B	C
Name	**Definition (primer/probe)**	**sequence**
NSP6_Forw	primer F	GTGATGCGTATTATGACATGGT
NSP6_Rev	primer R	GATTAGTAACACTACAGCTGATGC
NSP6_Wild	probe for non-VOC	FAM-TGTCTGGTTTTAAGCTAAAAGACTGTG-BHQ
NSP6_Delete	probe that detects VOC	HEX-GGATATGGTTGATACTAGTTTGAAGCT-BHQ

We recommend the **4x TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems)**

DISCLAIMER:

1 Reconstitute the oligos/probes to 100uM with TE or nuclease-free water. Vortex briefly and allow to sit for about 2 minutes.

2 Prepare 10uM working solutions (primers and probes). Suggestion: if the original concentration is 100uM, add 10ul of the reagents in 90ul of ultrapure nuclease-free water.

3 The reaction was not adjusted for multiplex use, so prepare the reactions separately. We recommend running the probes NSP6 Wild and NSP6 Delete in the same plate/experiment for better results.

4 Prepare your Master mix as the following (use primers and probes at 10uM)

A	B	C
Reagents	**Volume 1 reaction (ul)**	**x100**
4x TaqMan Fast Virus 1-Step Master Mix	5	500
NSP6_Forw (300nM/reaction)	0.5	50
NSP6_Rev (300nM/reaction)	0.5	50
Probe (300nM/reaction)	0.5	50
RNA	8.5	-
Total	15	-

5 Add 6.5ul of the mix to each well and 8.5ul of the RNA template. Don't forget using positive and negative controls.

6 In case of bubbles, spin the plate before cycling.

7 Run the following cycling at the Real time machine:

A	B	C
50°C	2 min	
95°C	10 min	
95°C	15 sec	40x
60°C	1 min	

Set the thermocycler for the proper FAM and HEX fluorophores.

8 Interpreting results:

A	B	C
NSP6_Wild	**NSP6_Delete**	**Result**
CT ≤ 38	CT > 39/undetected	non- VOC
CT > 39 /undetected	CT ≤ 38	VOC (B.1.17; P.1 or B.1.1.35)
CT ≤ 38	CT ≤ 38	mixed infection
CT > 39/undetected	CT > 39/undetected	invalid/negative