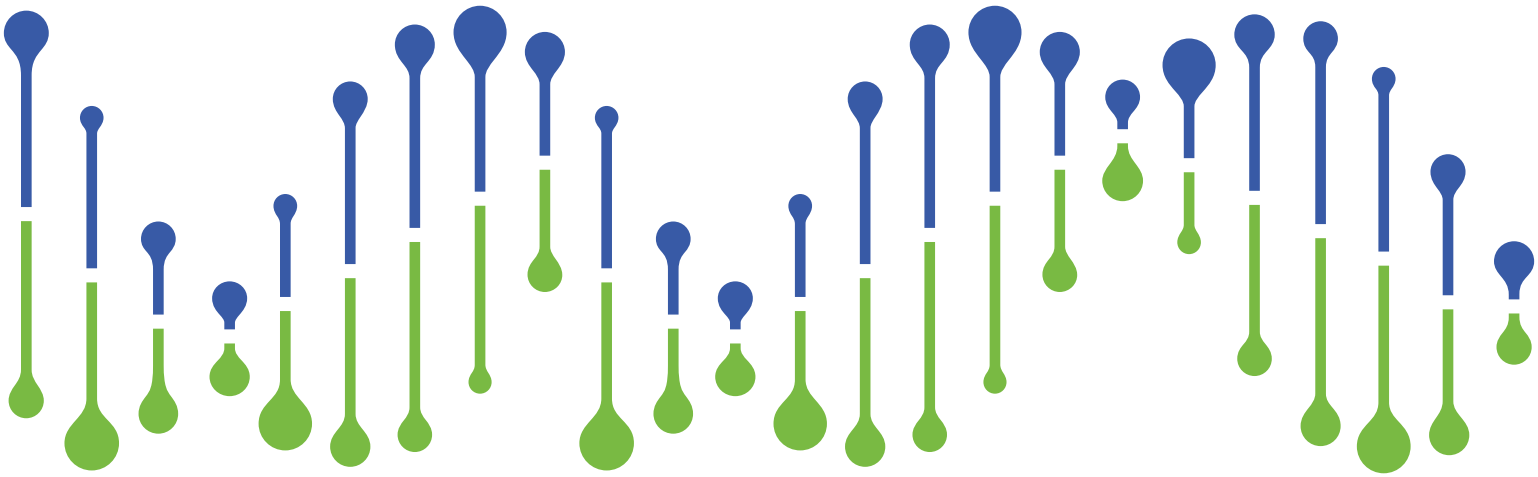
Nonacus Protocol Guide v1.0.2

VirPath Sars-CoV-2 qRT-PCR Protocol



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Intended use

The VirPath Sars-CoV-2 qRT-PCR kit is intended to be used for the detection of Sars-CoV-2 genomic RNA extracted from biological samples derived from lower or upper respiratory tract specimens. The inhibitor tolerant qRT-PCR mix provided in the kit is a one-step solution designed for amplification of the Sars-CoV-2 RNA targets and does not contain an internal reference dye. Included in the kit are the N1 and N2 primer / probe assays targeting the nucleocapsid gene of Sars-CoV-2; and the RP primer / probe assay to be used as internal RNA extraction control. Two plasmid controls are also included in the kit to confirm functionality of the assays and the qRT-PCR reaction: the RPP30 Negative Control and the 2019-nCoV nucleocapsid gene Positive Control.

Kit contents

Reagent	Volume (1,000 rxns)	Volume (10,000 rxns)	Storage
VirPath qRT-PCR Master Mix	1.5 ml	2x 30 ml	- 20°C
N1 primer / probe mix *	2x 0.9 ml	1x 18 ml	- 20°C
N2 primer / probe mix *	2x 0.9 ml	1x 18 ml	- 20°C
RP primer / probe mix *	2x 0.9 ml	1x 18 ml	- 20°C
RPP30 Negative Control	1x 0.5 ml	2x 1 ml	- 20°C
2019-nCoV nucleocapsid gene Positive Control	1x 0.5 ml	2x 1 ml	- 20°C

* All primer / probe mix assay tubes contain a 6.7 µM concentration of each primer (ie forward and reverse) and a 1.7 µM concentration of probe. Once added to the reaction mix, primers will be present at a working concentration of 500 nM and the probe at a working concentration of 125 nM.

Required equipment

- Class II Biological safety cabinet
- Single and/or multichannel pipettes (10, 100, 200, 1000 ul)
- PCR-clean filtered tips
- 1.5 / 2 ml cold block (or access to ice)
- 96 well cold block (or access to ice)
- qPCR Instrument (4 colour)
- 96 well plate and optical seal compatible with qPCR instrument

Additional user supplied consumables

- Molecular biology grade water

Storage and handling

Upon receipt, store all reagents at -20°C.

Thaw the VirPath qRT-PCR Master Mix on ice and keep on ice at all times. Thaw the RP, N1 and N2 Assay Primer / Probe Mixes at room temperature in the dark and then keep on ice throughout the qRT-PCR setup preparation. After thawing, ensure that all reagents are mixed by briefly by vortexing and then spun down. Avoid repeated freeze / thawing whenever possible.

Handling of plasmid controls

The RPP30 Negative Control and 2019-nCoV nucleocapsid gene Positive control consist of plasmids containing the RPP30 gene and the nucleocapsid gene from SARS-CoV-2 respectively and are provided at a concentration of 200 copies/µl. When thawed for the first time, it is recommended to aliquot out the entire amount of both controls in single use aliquots in order to minimize freeze / thaw cycles. For each qRT-PCR run, 15 µl of each control are used (ie 5 µl per reaction = 1000 copies per reaction).

qRT-PCR setup procedure

Table 1. outlines the required volumes of sample / control and reagents needed to set up the qRT-PCR reaction for one assay. Prepare three reaction mixes separately; one reaction mix for assay N1, one for assay N2 and one for assay RP. Each sample / control requires one replicate for each assay. Each qRT-PCR run should include the No Template Control (ie molecular biology grade water), the HPP30 negative control and the 2019-nCoV Nucleocapsid gene positive control. All procedures should be carried out in a sterile environment, ideally a Class II biosafety cabinet. Thaw reagents as described above and setup the reaction on ice (or on cold block).

Table 1. qRT-PCR reaction mix setup volumes for a single reaction. When preparing a mix for multiple reactions, include a 5% overage for each reagent.

Reagent	Volume
VirPath qRT-PCR Master Mix	5 µL
Primer and Probe Mix	1.5 µL
Template	5 µL
Water	8.5 µL
Total	**20 µL**

NOTE: the volume of water and template can be adjusted to include more template in the reaction mix as required.

qRT-PCR setup and cycling conditions

Set up the qPCR instrument using manufacturers guidelines. Where possible, choose the Quantitation by Comparative Ct (ΔΔCt) method with TaqMan or "Other" reagents (do not add a melt curve option). For qPCR instruments with "FAST" blocks, select the Standard ramp speed. Select the FAM filter for the N1, N2 and RP targets. If possible, select NFQ-MGB as quencher; or alternatively leave this field empty. Do not select a dye as quencher (such as TAMRA). Select Program cycling conditions as shown in table 2. below. Set reaction volume to 20 µl.

Table 2. qRT-PCR Program cycling conditions

Step	Cycles	Temperature	Time
1	1	50 °C	10 min
2	1	95 °C	2 min
3	45	95 °C	5 s
4		55 °C	30 s

NOTE: fluorescence acquisition is performed at step 4.

qRT-PCR data analysis and interpretation

Please note data analysis may vary between qPCR machines and thresholds must be determined empirically by the end user or laboratory. We recommend setting the Baseline start cycle at 5 and the end cycle at 15; and the threshold at 200 RFU or 0.02 ΔCt.

As per CDC guidelines, Ct values that fall below the 40 cycles threshold are considered positive signals. Refer to Table 3. below for interpretation of results from control and patient samples.

Table 3. Interpretation of results from control and patient samples

Sample	RP Result	N1 Result	N2 Result
RPP30 Negative Control	+	-	-
2019-nCoV N gene Positive Control	-	+	+
Positive patient sample	+	+	+
	-	+	+
Negative patient sample	+	-	-
Inconclusive patient sample	+	+	-
	+	-	+
	-	+	-
	-	-	+
Failed patient sample	-	-	-

Kit specification and performance

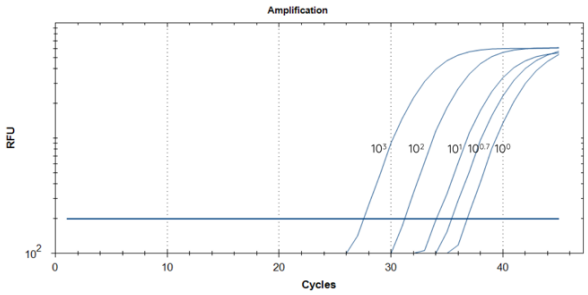
Application	Qualitative PCR test for detection of SARS-CoV-2 Nucleocapsid gene
Type of Detection	Ribonucleic acid (RNA) of SARS-CoV-2
Sample Type	Lower respiratory tract specimens (e.g bronchoalveolar lavage, tracheal aspirate) and upper respiratory tract specimens (e.g nasaopharygeal fluids, nasal swab) and other biological fluids.
qRT-PCR Limit of Detection	$1x10^0$

Limit of Detection (LOD) testing

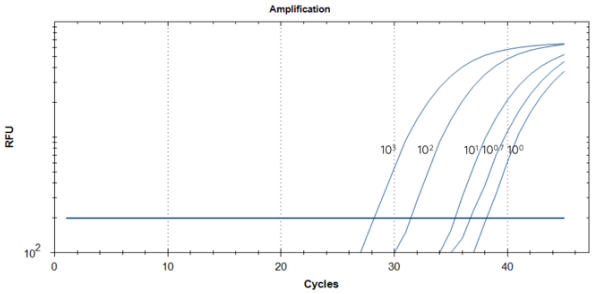
To understand the limit of detection (LOD) for the VirPath Sars-CoV-2 qRT-PCR kit we undertook the following experiment to calculate experimentally the minimum copies of viral template RNA which can be detected.

Synthetic SARS-CoV-2 RNA: ORF, E, N (ATCC® VR-3276SD™) was used in a serial dilution experiment to establish a limit of detection of VirPath qRT-PCR Master Mix using N1 and N2 primer and probe mixes on a BioRad CFX96.

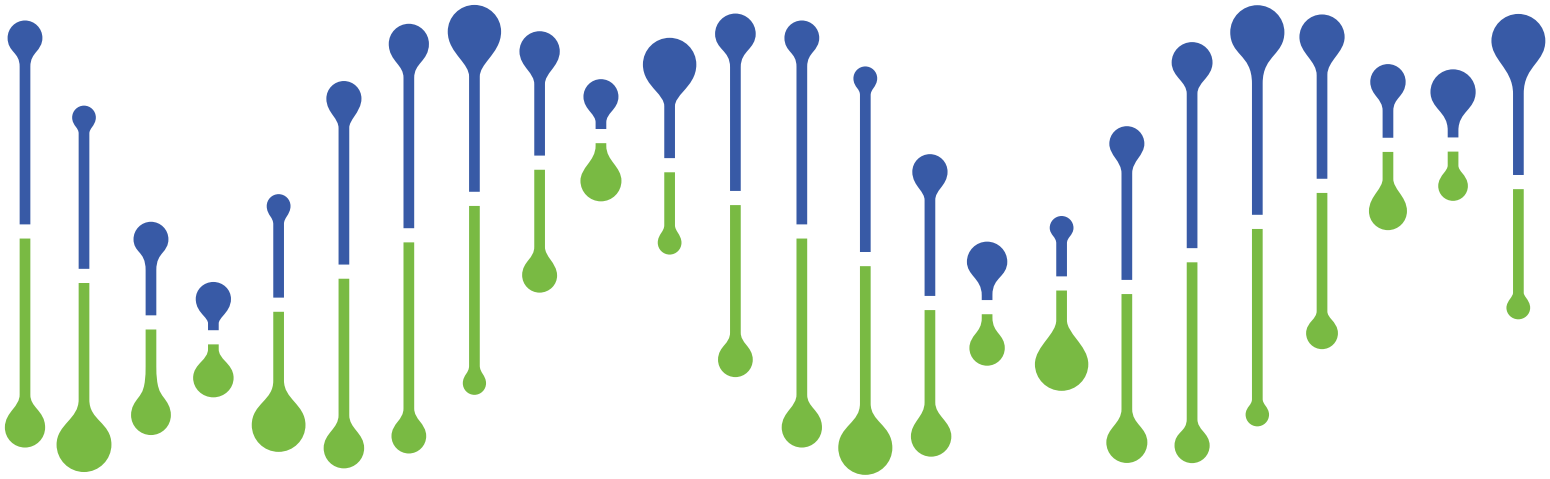
N1 Assay



N2 Assay



Sample	Copies	Type	N1 Cq	N1 Cq StDev	N2 Cq	N2 Cq StDev
$1x10^0$	1	Synthetic RNA	37.01	1.16	37.45	0.93
$1x10^{0.7}$	5	Synthetic RNA	35.61	0.31	36.77	1.22
$1x10^1$	10	Synthetic RNA	34.92	0.76	35.18	0.22
$1x10^2$	100	Synthetic RNA	31.00	0.31	31.46	0.32
$1x10^3$	1000	Synthetic RNA	27.58	0.10	28.05	0.12



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