JN Medsys, Revision: 06 Effective date: 7th Jul 2022

ProTect™ COVID-19 RT-qPCR kit 2.0

100 tests (Cat No. 10029)

Instructions for Use

For In-vitro Diagnostic (IVD) Use

CE
IVD

Contact Details

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EC REP

Obelis s.a.
Bd. Général Wahis 53 B-1030 Brussels, Belgium

This assay has received Provisional Authorisation from the Health Sciences Authority in Singapore

Contents

INTENDED USE

The ProTect™ COVID-19 real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) kit 2.0 is developed for qualitative detection of RNA from the SARS-CoV-2 virus. The ProTect™ COVID-19 RT-qPCR kit 2.0 is validated to detect for SARS-CoV-2 RNA in upper respiratory specimens (e.g. nasopharyngeal, oropharyngeal, nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate swabs) collected from suspected infected individuals.

Positive results indicate the presence of SARS-CoV-2 RNA but do not rule out other infections (bacteria and other viruses) and the presence of SARS-CoV-2 RNA may not be the definite cause of disease. Negative results of the SARS-CoV-2 infection should not be used as the sole basis for treatment or other patient management decisions and must be combined with clinical observations, patient history, and epidemiological information.

Testing with the ProTect™ COVID-19 RT-qPCR kit 2.0 is intended for use by trained laboratory personnel who has the proper skills to run RT-qPCR assays. The ProTect™ COVID-19 RT-qPCR kit 2.0 is currently for use under the provisional authorisation of the Health Sciences Authority of Singapore.

The ProTect™ COVID-19 RT-qPCR kit 2.0 by JN Medsys provides all necessary reagents for the *in vitro* qualitative detection of SARS-CoV-2 from upper respiratory nasopharyngeal specimens and does not include reagents for the extraction and purification of RNA from the SARS-CoV-2 virus. The ProTect™ COVID-19 RT-qPCR kit 2.0 is validated using Applied Biosystems® QuantStudio® 3 Real-Time PCR System and should also work on other Real-Time Systems with similar specifications. The test is compatible with the US CDC protocol, targeting SARS-CoV-2 N1 and N2 genes and the human RNase P control gene.

SUMMARY AND EXPLANATION

Coronavirus disease 2019 (COVID-19) is caused by a novel coronavirus now called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; formerly called 2019-nCoV).

SARS-CoV-2, which is the causative agent of the pneumonia outbreak in Wuhan City, Hubei Province, China, was reported to World Health Organization (WHO) on December 31, 2019. This novel coronavirus was later identified, although it had already resulted in thousands of confirmed human infections in multiple provinces throughout China and many countries subsequently including Singapore. SARS-CoV-2 is known to be capable of asymptomatic infection, mild illness, severe illness, and cause death.

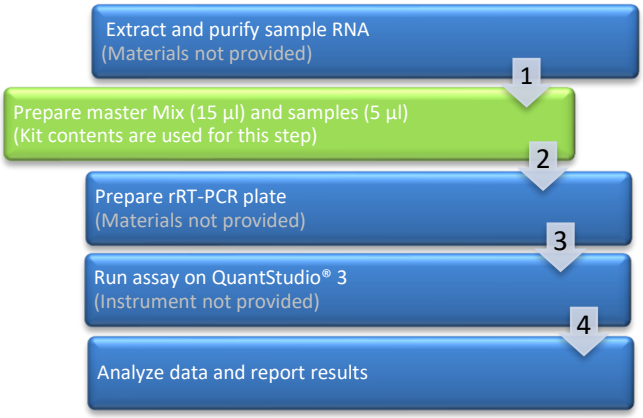
The ProTect™ COVID-19 RT-qPCR kit 2.0 is a molecular *in vitro* test that detects for SARS-CoV-2 viral RNA. The detection of the viral RNA will aid in the diagnosis of SARS-CoV-2 and is based on RT-qPCR technology. The product contains oligonucleotide primers and dual-labelled hydrolysis probes (TaqMan®) and control materials used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.

PRINCIPLES OF THE PROCEDURE

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from regions of the virus nucleocapsid (N) gene. The kit is designed for the specific detection of 2 regions on the SARS-CoV-2 (two primer/probe sets) N gene. An additional primer/probe set is also included in the kit to detect for the human RNase P gene (RP) in control samples and clinical specimens.

The viral RNA is first extracted and purified from upper respiratory specimens using nasal swabs or oropharyngeal specimens using throat swabs. The purified RNA is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems® QuantStudio® 3 Real-Time PCR System. In the process, the probe first anneals to its specific target sequence by base pairing and designed to be located in the region between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe which is already bound to the specific sequence, causing the reporter dye to separate from the quencher dye, resulting in a fluorescent signal. With each amplification cycle, additional reporter dye molecules are liberated, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle and will result in a cycle threshold (ct) value. This ct value will be used to determine whether the target is present or not.

Summary of testing process



RECOMMENDED KITS AND EQUIPMENT

Items	Details
Nasopharyngeal nasal swabs	3ml Universal Transport Medium™ (Copan; Cat No: 305C) Refer to manufacturer's instructions on packaging
Viral RNA extraction and purification	QIAamp Viral RNA Mini Kit (Cat No: 52904) Refer to manufacturer's instructions: https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa72-8989420e3018&lang=en
qPCR instrument	Applied Biosystems® QuantStudio® 3 Real-Time PCR System Refer to manufacturer's instructions: https://www.thermofisher.com/order/catalog/product/A28567#/A28567

WARNINGS AND PRECAUTIONS

- Patient specimens and positive controls should assumed to be potentially infectious and handled properly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Maintain separate areas for assay setup and handling of nucleic acids.

- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Always check the expiration date and do not use expired reagents.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Use and change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Allocate separate equipment and supplies for assay setup and for handling extracted RNA.
    - Wear a clean lab coat and new powder-free disposable gloves when setting up assays.
    - Gloves should be changed between samples or whenever contamination is suspected.
    - Reagents and reaction tubes should be capped or covered as much as possible.
    - Primers, probes, and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
    - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products (e.g., 10% bleach, “DNAZap™” or “RNase AWAY®”) before every test to minimize risk of nucleic acid contamination.
    - RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
    - Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

SPECIMEN COLLECTION, HANDLING, AND STORAGE (MATERIALS NOT PROVIDED)

- Improper specimen collection, storage, and/or transport are likely to affect test results. Specimens should be collected by trained personnel due to the importance of specimen quality.

Collecting the Specimen

- Follow manufacturer instructions on specimen collection device for proper collection methods.
- Swab specimens should be collected using only swabs with a synthetic tip. Do not use Calcium alginate swabs and cotton swabs with wooden shafts are not recommended. After collection, place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

Storing Specimens

- Specimens can be stored at 2-8^{o}C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70^{o}C or lower.
- Extracted nucleic acid should be stored at -70^{o}C or lower.

EXTRACTION PROCEDURE

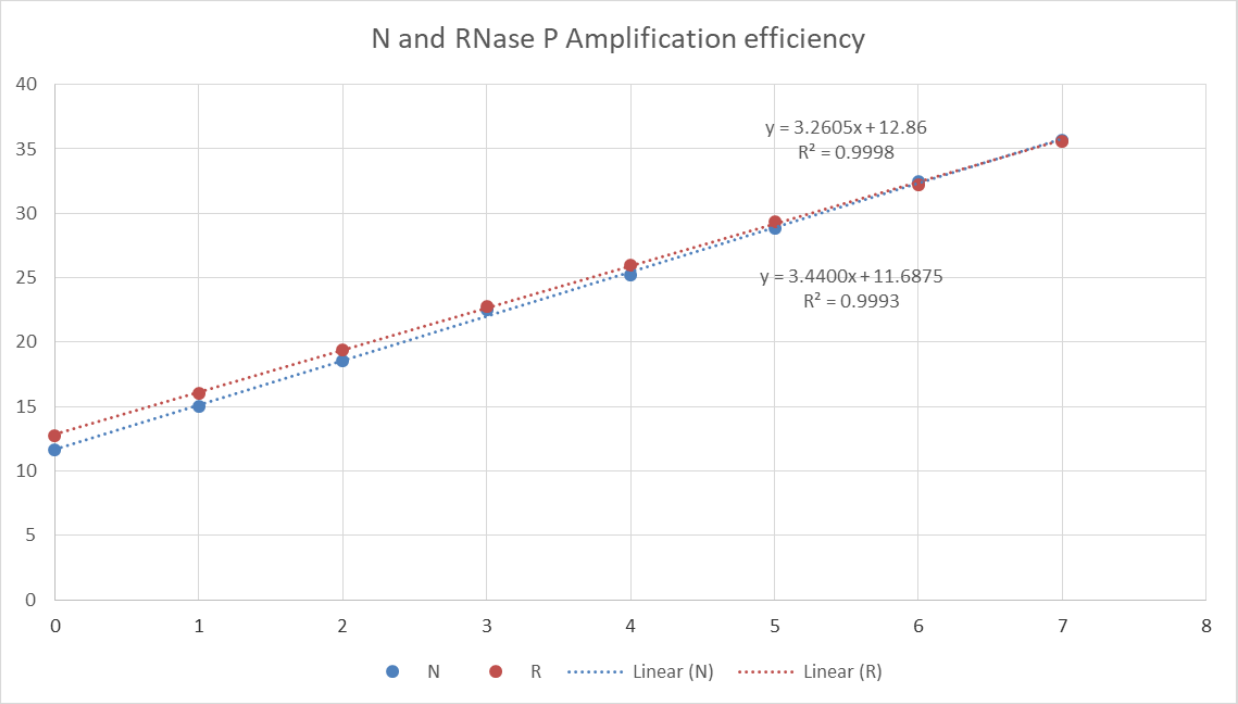
Performance of the ProTect™ COVID-19 RT-qPCR kit 2.0 is dependent on the quantity and quality of template viral RNA purified from human specimens. QIAamp Viral RNA Mini Kit (Cat No: 52904) was used to extract template viral RNA from nasopharyngeal swabs (Instruction for use: https://www.qiagen.com/us/resources/resourcedetail?id=c80685c0-4103-49ea-aa728989420e3018&lang=en) and have been qualified and validated for use with this kit.

KIT FEATURES

Test Principle	One-step RT-qPCR (TaqMan®-based detection)
Targets	N1, N2 (SARS-CoV-2, FAM) and RNase P (Human, HEX)
Number of Tests	100/kit
Compatible Specimen Type	Upper respiratory nasopharyngeal specimens (i.e. nasopharyngeal, oropharyngeal, nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate swabs)
Limit of Detection	5 copies RNA per reaction
Amplification efficiency	N >95%, RNase P > 99%
Precision	<2%
Specificity	Detects only SARS-CoV-2 based on *in silico* sequence validation

PERFORMANCE CHARACTERISTICS

Amplification efficiency:



Target	Amplification Efficiency (%)
N	95.30
RNase P	102.61

Limit of Detection (LoD):

LoD studies determine the lowest detectable concentration of SARS-CoV-2 at which approximately 95% of all replicates test positive. The LoD was determined to be 5 copies/reaction based on limiting dilution studies. The results are summarised as follow:

Target	Concentration (copies/ reaction)	Number of replicates tested positive	Mean Ct	Standard deviation	RU (%)
N	10	3/3	32.10	0.15	0.46
	5	3/3	33.11	0.63	1.91
	2.5	1/3	-	-	-
	1.25	0/3	-	-	-

** Relative uncertainty = Standard deviation/Mean*

Target	Concentration (copies / reaction)	Replicates Detected
N	5	20/20
	2.5	9/20

Precision

Precision studies determine the reproducibility and robustness of the test. Four independent tests were conducted for N assay in 10 replicates from 2 test and 3 replicates from 2 test. The results demonstrate that these assays achieved a relative uncertainty of <2%.

Test	N			
	No. of Repeats	Mean Ct	Standard Deviation	Relative Uncertainty (%)
1	10	32.13	0.10	0.32
2	3	32.25	0.13	0.40
3	3	32.51	0.27	0.82
4	10	32.46	0.35	1.08
Inter-run		32.34	0.17	0.54

** Relative uncertainty = Standard deviation/Mean*

IN SILICO ANALYSIS OF PRIMER AND PROBE SEQUENCES

An alignment was performed with the oligonucleotide primer and probe sequences of the ProTect™ COVID-19 RT-qPCR kit 2.0 with all publicly available complete, high coverage, nucleic acid sequences for SARS-CoV-2 in www.GISAID.org as of 15 June 2020 to demonstrate that the ProTect™ COVID-19 RT-qPCR kit 2.0 was able to detect all known SARS-CoV-2 viral mutations. All the alignments show that ProTect™ COVID-19 RT-qPCR kit 2.0 was able to detect 100% of the available SARS-CoV-2 sequences with set criteria of detection of N target.

KIT CONTENTS

Each kit includes reagents sufficient to perform 100 tests (Cat No. 10029). Each test include 1 duplex RT-qPCR assays, which target the N1, N2 on FAM, and RNase P on Hex.

Reagents Supplied	**Tube Cap**	**100 Tests (10029)** **Volume (µL)**
ProTect™ Probe qPCR Mastermix (2X)	Red-labelled	1 x 1000
ProTect™ RT Mix (50X)	Green-labelled	1 x 45
ProTect™ Primer & Probe Mix	Purple-labelled	1 x 510
ProTect™ Positive Control^*	Yellow-labelled	1 x 110
Nuclease Free Water	Blue cap	1 x 2000

^Sufficient for 20 runs

** The ProTect™ Positive Control is made up of plasmid consisting of N1, N2 and RNase P sequences. This serves as a control for the N1, N2 and RNase P tests.*

STORAGE AND STABILITY

The ProTect™ COVID-19 RT-qPCR kit 2.0 should be stored at -20°C upon receipt. Avoid repeated freezing and thawing of kit contents. The kit is stable through the expiry date indicated on the kit label (6 months shelf life).

ASSAY SETUP

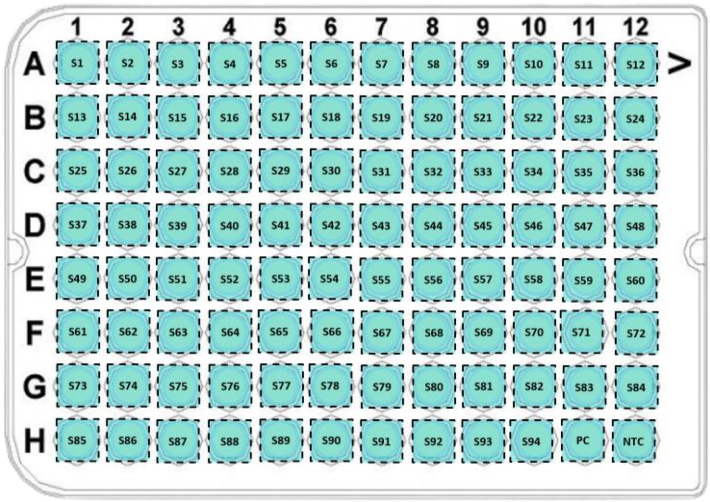
1. Thaw reagents at room temperature and maintain reagents on ice when thawed. Mix reagents gently and briefly centrifuge to collect contents at the bottom of the tubes.
2. Prepare each reaction mix as shown in the table below:

No.	Reagents	Volume (µL)
1	ProTect™ Probe qPCR Mastermix (2X) [Red-labelled]	10
2	ProTect™ RT Mix (50X) [Green-labelled]	0.4
3	ProTect™ Primer & Probe Mix [Purple-labelled]	4.6
4	RNA Sample/ ProTect™ Positive Control [Yellow-labelled] / Water [Blue cap] (NTC)	5*
	Total Vol	20

❗ Positive and no template controls should be included in each run

* Added straight into wells containing 15 µL of the reaction mix

3. Pipette 15 µL of the reaction mix into the required reaction tube strip or 96-well plate. (Table below shows an example of run setup) and add 5 µL of the sample

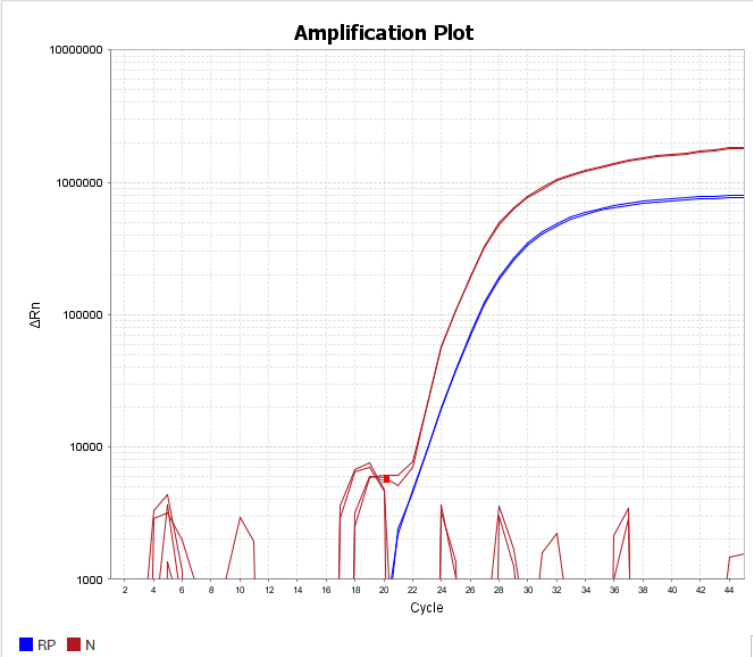


NTC: No Template Control
S: Samples
PC: Positive Control

4. Centrifuge to collect contents at the bottom of the tube strip/plate.
5. Transfer tube strip/plate to qPCR instrument
6. For QuantStudio® 3 Real-Time PCR System, refer to user manual for machine operation and experimental setup (https://www.thermofisher.com/order/catalog/product/A28567#/A28567). *Do not set reference dye setting. The kit **does not** contain reference dye (e.g. ROX).
7. Perform one-step RT-qPCR according to the following protocol. Fluorescence data for FAM and HEX should be collected during the 55°C annealing & extension step.
8. Analyze results from the plot. PC curves should be smooth and NTC should not result in any ct values.

Step	Cycle	Temperature	Time
Reverse Transcription	1	45°C	15 min
Reverse Transcriptase Inactivation & DNA Polymerase Activation	1	95°C	2 min
Denaturation	40	95°C	3 sec
Annealing & Extension		55°C	30 sec

https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-detection-instructions.html



DATA ANALYSIS AND INTERPRETATION

Interpretation of ProTect™ COVID-19 RT-qPCR kit 2.0 test result should take into consideration the CT values, as well as the shape of the amplification curve.

Extraction and Positive Control Results and Interpretation

1. No Template Control (NTC)

   The NTC consists of using nuclease-free water in the RT-qPCR reactions instead of RNA sample. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and use new reagents. Repeat the assay with strict adherence to the guidelines.

2. SARS-CoV-2 Positive Control (PC)

   The PC consists of plasmid consisting of N1 and N2 target sequences and plasmid containing RNase P sequence. The PC will yield a positive result with the following primer and probe sets: ProTect™ Primer & Probe Mix

3. RNase P (Extraction Control)

   All clinical samples is expected to contain the RNase P RNA and should have a ct value of less than 40 cycles (< 40 ct). If RNase P is not detected in any clinical specimens, it may be due to:

   1. Improper collection resulting in loss of specimen integrity
   2. Improper storage before extraction
   3. Improper extraction of nucleic acid from clinical samples
   4. Improper assay set up and execution.
   5. Improper use of reagents or equipment malfunction.

   If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

   − If the SARS-CoV-2 N are positive and RP is negative, the result should be considered valid. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.

   − If all SARS-CoV-2 N target AND RP are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

4. SARS-CoV-2 Marker (N)

When all controls exhibit the expected performance, a specimen is considered positive if N cycle threshold is less than 40 cycles (< 40 ct). The RNase P may or may not be positive as described above, but the SARS-CoV-2 result is still valid.

When all controls exhibit the expected performance and the SARS-CoV-2 N AND the RNase P marker are more than 40 cycles (> 40 ct), the result is invalid. The test should be re-run:

1. The extracted RNA from the specimen should be re-tested.
2. If residual RNA is not available, re-extract RNA from residual specimen and re-test.
3. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

When all controls exhibit the expected performance, a specimen is negative for SARS-CoV-2 when SARS-CoV-2 target N cycle threshold are more than 40.00 cycles (> 40) and the cycle threshold of RP is less than 40 cycles (< 40 ct).

Summary

1. No Template Control - No fluorescence signal should be detected
2. Positive Control – Fluorescence signal should be detected with Ct value below 30
3. Results for the respective targets may be interpreted as follow:

N (FAM)	RNase P (HEX)	Outcome
+	±	SARS-CoV-2 detected
-	+	SARS-CoV-2 not detected
-	-	Invalid result. Repeat test

QUALITY CONTROL

a. Quality control procedures are in place for reagent monitoring and to inspect assay performance.

b. Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.

c. A positive extraction control is recommended to be included in each nucleic acid isolation batch in concordance with Good laboratory practice (cGLP)

d. Always include a negative control (NTC), and the appropriate positive control provided (PC) in each amplification and detection run. All clinical samples should be tested for human RNase P gene for interpretations listed above

LIMITATIONS

• The kit is intended to be used by trained personnel as this procedure requires technical skills to perform. They should be able to perform the test and interpret the results independently.

• Performance of the ProTect™ COVID-19 RT-qPCR kit 2.0 has only been established in upper respiratory specimens (nasopharyngeal swabs).

• Negative results should not be used as the sole basis for treatment or other patient management decisions.

• False negative results may occur if qPCR inhibitors are present in the sample.

• Do not use any reagent past the expiration date.

• If the virus mutates in the RT-qPCR target region, performance of the kit may be affected

• Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.

• The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.

• The performance of this test has not been established for screening of lower respiratory, blood or blood products for the presence of SARS-CoV-2.

• This test cannot determine diseases caused by other bacterial or viral pathogens.

Revision History

Revision	Effective Date	Description of Change
01	30 June 2020	1. Initial release for use
02	20 July 2020	1. Included CE mark and EU Rep Details
03	01 Dec 2020	1. Included colour codes for reagents tubes (pages 10 & 11) 2. Included page numbers 3. Removed “For Research Use” on page 5
04	05 Oct 2021	1. Included oropharyngeal clinical matrix
05	03 Nov 2021	1. Included nasal mid-turbinate, anterior nasal, oropharyngeal + nasal mid-turbinate clinical matrix
06	07 Jul 2022	1. Uprevision of the footer and added document number

