Precision Biomonitoring Inc.
TripleLock ™ SARS-CoV-2 Test

Product Information and Instructions for Use v3.0

Release Date May 15, 2021

Document Number IFU-F000014

P/N: R000070

IVD

Precision Biomonitoring Inc.
5420 Highway 6 N.
Orchard Park Suite 226
Guelph, Ontario
N1H 6J2
Canada
1-888-444-7702
Support @
precisionbiomonitoring.com

EC REP
OBELIS S.A
Bd. Général Wahis, 53
1030 Brussels,
Belgium
+32.2.732.59.54
+32.2.732.60.03
mail@obelis.net
www.obelis.net

CE

Table of Contents

For instructions for use in other languages, please visit:
https://precisionbiomonitoring.com/triplelock-lab-test/

1 Device Description

1.1 Intended Use

The Precision Biomonitoring TripleLockTM SARS-CoV-2 tests are a qualitative RT-qPCR test for the detection of novel human coronavirus from clinical-collected nasopharyngeal swabs stored in viral transport media from patients who meet COVID-19 clinical and/or epidemiological criteria for testing. This device is not intended for use at the point of care (near-patient). This test requires the use of an RNA extraction kit and qPCR thermocycler, not provided. Results are for the identification of SARS-CoV-2 RNA, however, the assay does not differentiate between SARS-CoV-2 and SARS-CoV-1. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swabs during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infections or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories are required to report all positive results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. For laboratory use only.

1.2 Clinical Background Information

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). The novel coronavirus (SARS-CoV-2) is a new strain that has not previously been identified in humans. The current outbreak of SARS-CoV-2 has infected millions of individuals globally. The pathogen has high transmission rates and is spread through aerosolized droplets from the respiratory tract of infected patients. SARS-CoV-2 has extremely high virulence and infectivity.

The clinical impact ranges from asymptomatic infections to life-threatening illnesses. When present, signs of infection include respiratory symptoms such as cough, shortness of breath, difficulty breathing and fever as well as fatigue, rash, loss of taste or smell, and digestive distress. In more severe cases, pneumonia, kidney failure and death can occur.

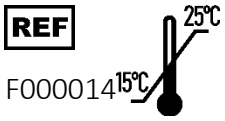
1.3 Intended User

The TripleLock^{TM} SARS-CoV-2 tests are intended for use by qualified laboratory personnel, who have received specific training on the use of this test from Precision Biomonitoring, for the *in vitro* qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal swabs from patients with or without signs and symptoms of infection who are suspected of COVID-19. This device is not intended for use at the point of care (near-patient).

Note: Other swab types, including nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with this test, but performance with these specimen types has not been established

1.4 Test Principle/Principles of Operation

The Precision Biomonitoring TripleLock™ SARS-CoV-2 tests is a multiplex RT-qPCR test. The device requires RNA extracted from viral transport media into which a nasopharyngeal swab has been collected and a qPCR thermocycler on which to run the test. The 96-well plate format of the test is sold by Precision Biomonitoring and has been evaluated on the CFX96.



Targets
E gene
UTR
CONTROL + RNaseP

Two viral targets are included to provide redundancy and reduce the likelihood of false negatives. More information on controls and targets is listed below.

1.5 Product Configuration

The Precision Biomonitoring TripleLock SARS-CoV-2 Assay 96-well plate format (F000014) consists of a pre-aliquoted PCR 96-well plate of reagents. Each well contains a single bead with a lyophilized triplex reaction containing all reagents to run the RT-qPCR reaction.

An RNA extraction and compatible real-time PCR thermocycler are required to run this test, as well as a 20uL pipette and sterile filter tips for loading/mixing samples into the plate. These are not provided by Precision Biomonitoring.

Note: Precision Biomonitoring does not provide external controls for use with the Precision Biomonitoring TripleLock™ SARS-CoV-2 Assay.

1.6 Assay Components

This assay targets: E Gene and UTR regions of the SARS-CoV-2 genome and RNaseP as a positive internal control. The RNase P gene is a single-copy human gene that encodes the RNA moiety for the RNase P enzyme, used as an endogenous human target in this assay to confirm the successful amplification of the sample in the absence of SARS-CoV-2 and that sufficient material was used for the RNA extraction. For thermocycler compatibility, the assay uses the following modifications on each probe respectively:

Probe modifications used in this device:

Name	Sequence with modifications
E Gene_P	5' TexRd-XN-/-BHQ2
UTR_P	5' FAM-/-BHQ1
RNase P_P	5' ATTO647NN-/-3IAbRQSp

2 Specifications

Key Performance Specifications for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay. Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument.

Specification	Details	Performance
Cross-reactivity	Common respiratory pathogens (bacterial and viral) were tested using the M1 prep kit and the TripleLock™ SARS-CoV-2 assay to determine any cross-reactivity that could cause a false positive for SARS-CoV-2	No cross-reactivity was observed with pathogens tested. There is a low likelihood of false positives from common respiratory pathogens.
Limit of detection	LOD was measured with contrived samples created from positive cell suspensions spiked into negative clinical samples in UTM.	The final calculated LOD for the assay is 1×10^4 copies/200 µL of initial sample input (corresponding to 2.12×10^2 assuming no loss during extraction). LOD is comparable for the 96-well plate format as well.
Clinical evaluation	33 positive and 34 negative clinical samples were tested with both the TripleLock™ SARS-CoV-2 assay and the CDC EUA test. An additional 10 positive and 10 negative samples were tested with the 96-well format.	100% concordance to the reference test in all positive and negative samples across all test formats.

Cross-Reactivity Data for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay. Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument.

Clinically confirmed specimen	5'UTR Cq	E gene Cq	RNaseP Cq
Viruses			
Negative nasal swab in UTM	0	0	35.90
Parainfluenza 1	0	0	26.03
Parainfluenza 2	0	0	27.16
Parainfluenza 3	0	0	25.91
Parainfluenza 4	0	0	29.74
Human Coronavirus 229E	0	0	23.81
Human Coronavirus HKU1	0	0	27.73
Human Coronavirus NL63	0	0	27.27
Human Coronavirus OC43	0	0	27.69
SARS-CoV2 (Pos control)	30.58	27.00	25.49
Adenovirus	0.00	0	28.29
Human Metapneumovirus	0	0	28.32
Influenza A – H1N1	0	0	27.18
Influenza A – H3N2	0	0	26.20
Influenza B	0.00	0.00	29.76
Enterovirus (not subtyped)	0.00	0.00	27.57
Respiratory syncytial virus	0.00	0.00	27.12
Rhinovirus	0.00	0.00	30.14
Haemophilus influenzae	0.00	0	26.96
Streptococcus pneumoniae	0	0	27.13
Streptococcus pyogenes	0	0	27.83
Candida albicans	0	0	28.25
Pseudomonas aeruginosa	0.00	0	27.05
Staphylococcus epidermis	0	0	27.28
Staphylococcus salivarius	0.00	0	27.46
Mycoplasma pneumoniae	0	0	27.43
Chlamydia pneumoniae	0	0	27.08
Legionella pneumophila	0	0	24.20
Bordatella pertussis	0	0	n/a
Pneumocystis jiroveci	0	0	n/a
MERS	0	0	29.20
SARS-CoV-1	16.38	11.17	0.00

Repeatability Data for the Precision Biomonitoring TripleLock™ SARS-CoV-2 assay. Results were generated using the Biomeme M1 prep kit, and the Franklin real-time PCR instrument.

A. Within run variation Cq values.

	Franklin D13AB6A254B2			Franklin CC92BD2871DB			Franklin C43440902003		
	UTR	E Gene	RNaseP	UTR	E Gene	RNase P	UTR	E Gene	RNase P
$\bar{x}$	28.98	26.88	27.28	29.06	26.95	27.39	29.03	26.96	27.76
σ	0.374797	0.336693	0.472211	0.588501	0.201179	0.478864	0.501677	0.442525	0.492146

B. Between run variation Cq values.

	UTR	E Gene	RNaseP
$\bar{x}$	29.02	26.93	27.48
σ	0.497045	0.343397	0.522158

C. Within day variation Cq values.

Day		UTR	E Gene	RNaseP
1	$\bar{x}$	28.91	26.84	28.91
1	σ	0.528507	0.528507	0.528507
2	$\bar{x}$	28.67	28.67	28.67
2	σ	0.453346	0.453346	0.453346
3	$\bar{x}$	29.07	29.07	29.07
3	σ	0.318276	0.318276	0.318276
4	$\bar{x}$	29.28	29.28	29.28
4	σ	0.450843	0.450843	0.450843
5	$\bar{x}$	29.19	29.19	29.19
5	σ	0.458395	0.458395	0.458395

D. Day to day variation Cq values.

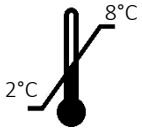
	UTR	E Gene	RNaseP
$\bar{x}$	29.02	26.93	27.48
σ	0.497045	0.343397	0.522158

E. Machine to machine variation Cq values.

	Franklin D13AB6A254B2	Franklin CC92BD2871DB	Franklin C43440902003
$\bar{x}$	27.72	27.80	27.92
σ	0.996085	1.013425	0.979807

3 Specimen Collection

Specimen collection is not included as part of TripleLock™ SARS-CoV-2. It is intended for use with RNA extracted from viral transport media from a nasopharyngeal swab collection made by a health care professional. Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result.

Specimen Storage before RNA extraction: 

 72 h

4 Instructions for Use

Precision Biomonitoring SARS-CoV-2 Tests 96-well Plate Format with BioRad CFX-96.

Each Precision Biomonitoring SARS-CoV-2 Test contains everything needed for testing a nasopharyngeal swab collected from a patient and stored in viral transfer media. These can be run on a compatible thermocycler. The test has been evaluated on the Bio-Rad CFX-96.

You will need:

- Lyophilized qPCR reagents for the PBI SARS-CoV-2 Tests in 96-well plate format (Precision Biomonitoring REF F000014)
- CFX-96 (BioRad) or other compatible thermocycler
- RNA extraction kit
- 
- 
-  10% bleach then distilled water

To reconstitute the Precision Biomonitoring SARS-CoV-2 Tests for use, add 20 µL of purified RNA extract, and pipette up and down 3-5 times to mix fully. Change tips and repeat for each sample being tested. Ensure there are no bubbles. A plate spinner can be used if desired to remove bubbles. Once samples are prepared, apply an optical adhesive seal to the plate and load it into the thermocycler.

Enter cycling conditions as below:

55° C x 10 mins
95° C x 2 mins
45 cycles
95° C x 5 secs
60° C x 20 secs

Choose the following fluorophore channels for each target:
UTR – FAM
E Gene – Texas Red
RNaseP – Quasar 670

Label all samples in the run, as well as tracking these in a second location.

- Do not deviate from this protocol, edit or adjust it in any way

Ensure that sample ID corresponds patient data. Do not include any personal identifiers.

Approximately 1 hour

5 Results Interpretation

After run completion, data should be interpreted using the table below.

E Gene	UTR	RNase P	Result
+	+	±	Positive
If only one target is positive		+	Inconclusive, repeat testing to confirm positive
If only one target is positive		-	Inconclusive, repeat testing to confirm positive
-	-	-	Invalid*
-	-	+	Negative

*If the RNaseP target is negative and the other targets are also negative, this means that there was an error in either extraction or cycling. If all the samples in the run have this result, it is likely a cycling error. If it is individual samples only, this indicates either a failed RNA extraction, or an insufficient amount of starting sample. Repeat your test.

Test results are considered negative for a target if the Cq value is above 38 cycles.

Quality control requirements should be performed according to applicable regulations and the laboratory's standard quality control procedures.

6 Caution & Warnings

- Always use appropriate safety equipment and PPE. Consult SDS sheets for additional information.
  - Dispose of any potentially biohazardous or otherwise harmful materials according to the appropriate provincial and federal regulations in your region.
  - Negative results do not preclude infection with the SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
  - Laboratories are required to report all positive results to the appropriate public health authorities
  - Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
  - False-negative results may arise from:
    - Improper sample collection or storage
    - Specimen collection after nucleic acid can no longer be found in the specimen matrix
    - Using unauthorized assay reagents
    - The presence of RT-PCR inhibitors
    - Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use
  - False-positive results may arise from:
    - Cross-contamination during specimen handling or preparation
    - Cross-contamination between patient samples
    - Specimen mix-up
    - RNA contamination during product handling
  - This assay does not differentiate between SARS-CoV-2 and SARS-CoV-1
  - The performance of this device has not been assessed in a population vaccinated against COVID-19.

7 Symbols Glossary

IVD In vitro diagnostic medical device	Do not re-use, single-use only.	Manufacturer	CE Mark	REF Catalogue number
CONTROL Control	CONTROL – Negative control	CONTROL + Positive control	Use-by date	LOT Batch Code
Appropriate waste disposal	Disinfectant for work surfaces	Sample identification number	Wait time	Warning
Telephone	Fax	E-mail	Website	Date of manufacture
Keep Dry	Consult Instructions for Use	Temperature limit	EC REP Authorized representative in the European Community	
 Appropriate personal protective equipment: gloves, lab coat, face/eye protection				

EC REP
OBELIS S.A
Bd. Général Wahis, 53
1030 Brussels,
Belgium
+32.2.732.59.54
+32.2.732.60.03
mail@obelis.net
www.obelis.net

Precision Biomonitoring Inc.
5420 Highway 6 N.
Orchard Park Suite 226
Guelph, Ontario
N1H 6J2
Canada
1-888-444-7702
support@
precisionbiomonitoring.com