# WHO Emergency Use Assessment SARS-CoV-2 IVDs
# PUBLIC REPORT

## Product: VitaPCR SARS-CoV-2 Gen 2 assay
## Manufacturer: Trentron Biomedical Ltd.
## EUL Number: EUL 0629-252-00
## Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance. This evaluation of limited scope is to verify critical analytical and performance characteristics.

VitaPCR SARS-CoV-2 Gen 2 assay with product code PCRAE0120 CE-mark regulatory version manufactured by Trentron Biomedical Ltd., New Taipei City, was listed as eligible for WHO procurement on 21 February 2023.

**Intended use:**

According to the claim of intended use from Trentron Biomedical Ltd., *"The VitaPCR SARS-CoV-2 Gen 2 Assay performed on the VitaPCR Instrument is a rapid molecular in vitro diagnostic test utilizing a real-time reverse transcription polymerase chain reaction (RT-PCR) amplification technology for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal (NP) or oropharyngeal (OP) swabs from patients who are suspected of COVID-19 by their healthcare providers.*
*Results are for the presumptive identification of SARS-CoV-2. The definitive identification of SARS-CoV-2 infection requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of SARS-CoV-2 infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the SARS-CoV-2.*

*The function of the assay is to aid in the diagnosis of COVID-19 disease. Rapid molecular assays that identify the target virus from patients infected with SARS-CoV-2 can aid in effective control of the global outbreak. SARS-CoV-2 infection is not precluded by negative results. Results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.*
*The VitaPCR SARS-CoV-2 Gen 2 Assay is intended to be performed by professionals in both laboratory and near-patient testing settings."*

**Specimen type(s) that were validated:**

Nasopharyngeal and oropharyngeal swab specimens.

**Assay Description:**

According to the claim of assay description from Trentron Biomedical Ltd., " *The VitaPCR SARS-CoV-2 Gen 2 Assay performed on the VitaPCR Instrument is a rapid molecular-based in vitro diagnostic test utilizing real-time reverse transcription polymerase chain reaction (real-time RT-PCR) technique. It is used for the qualitative detection and discrimination of SARS-CoV-2 viral RNAs in direct nasopharyngeal (NP) or oropharyngeal (OP) swab specimens from patients who are suspected of COVID-19 by their healthcare providers. The assay targets regions of the virus nucleocapsid (N) gene. It detects both specific SARS-CoV-2 RNA and universal SARS-like RNA (including SARS-CoV-2, SARS-CoV, bat SARS-like coronavirus); The assay includes artificial single stranded RNA (ssRNA) material as internal control (IC) to monitor the RT-PCR process.*
*To perform the test, NP or OP swab specimens are added to the Sample Collection Buffer (SCB) to solubilize the sample. Subsequently, 30µl of the SCB is then transferred into the Reagent Tube. There are 2 steps in the reaction process:*
*1. Lysis of the sample after swab specimen is added into the sample collection buffer.*
*2. One-step reverse transcription and PCR amplification with target primers and real-time detection with target specific probes Detection of target sequences is achieved through real-time measurement of the fluorescence signal emitted by the fluorophore released as a result of degradation of the specific SARS-CoV-2 target probes, universal SARS-like target probes, and internal control probes, following sequence amplification by the respective targets primer pairs. The test takes approximately 20 minutes to complete. In this respect, an operator can run 2 tests on the VitaPCR Instrument within one hour with ease. In an eight-hour shift, the operator would be able to handle 16 tests."*

**Test kit contents:**

| Component | 20 tests<br>(product code PCRAE0120) |
|---|---|
| VitaPCR Sample Collection Buffer_A | 4 mL x 20 vials |
| Reagent Tube | 20 vials |
| Reagent Tube Cap | 20 vials |
| Quick Reference Guide of VitaPCR SARS-CoV-2 Gen 2 Assay | 1 |

**Items required but not provided:**

- VitaPCR Instrument (Cat # PCRAC0101)
- Rack (Cat # PCRAC0101)
- Power Adaptor (Cat # PCRAC0101) (INPUT: AC 100-240V, 2.0A Max, 50-60Hz. OUTPUT: DC 12V, 5A)
- Nasopharyngeal Swab or Oropharyngeal Swab: For optimal test performance, ONLY use swabs which meet the CE directive requirements for medical devices. To avoid interference, please do not use swabs with wooden shafts or calcium alginate swabs since the reaction inhibitor might be contained. We strongly recommend using flocked swabs or synthetic fibre swabs with plastic shafts. Please refer to the list below for validated swab types:
  1. Puritan 25-3316-U BT; Puritan 25-3316-U
  2. Puritan 25-3317-U BT; Puritan 25-3317-U
  3. Puritan 25-3320-U BT; Puritan 25-3320-U; Puritan 25-3320-U EMB 100MM; Puritan 25-3320-U EMB 80MM
  4. Copan 503CS01; Copan 553C
  5. Copan 534CS01; Copan 534C
  6. Puritan 25-3306-U BT; Puritan 25-3306-U
  7. Puritan 25-1506 1PF BT
  8. Copan 520C; Copan 520CS01
  9. Copan 552C; Copan 502CS01
  10. Copan 519C; Copan 519CS01
- Transfer Pipette (Cat # PCREG0103)
- Adjustable Pipette & Filter Tip
- User Manual of VitaPCR Instrument Quick Reference Guide of VitaPCR Instrument
- VitaPCR SARS-CoV-2 Gen 2 External Control Set (Cat # PCRAE0121: 1 vial – 100 reactions) (Cat # PCRAE0135: 10 vials – single-use/one reaction per vial).

**Storage:**
The test kit should be stored at 5-25°C.

**Shelf-life upon manufacture:**
15 months

**Warnings/limitations:**
See attached instructions for use.

## Product dossier assessment

Trentron Biomedical Ltd. submitted a product dossier for VitaPCR SARS-CoV-2 Gen 2 assay as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and an external assessor appointed by WHO.

**Post listing Commitments for EUL:**

As a requirement to listing, the manufacturer is required to conduct a flex study assessing the possible influence of handling contamination from latex and powder gloves and provide the report by 31 March 2023. The report was provided and is being reviewed.

Risk-benefit assessment is acceptable.

## Quality Management Systems Review

To establish eligibility for WHO procurement, Trentron Biomedical Ltd. was asked to provide up-to-date information about the status of its quality management system.

Based on the review of the submitted quality management system documentation by WHO staff and external technical experts (assessors), it was established that sufficient information was provided by Trentron Biomedical Ltd. to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_ 347 "*.

Quality management documentation is acceptable.

## Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-prequalification activities are required to maintain the prequalification status:
1. Notification to WHO of any planned changes to a prequalified product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9).

Trentron Biomedical Ltd. is also required to report complaints related to the product. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensuring that post-emergency use listing safety, quality and performance monitoring activities are in place, per WHO guidance "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics."*[1]

**Scope and duration of procurement eligibility**

The VitaPCR SARS-CoV-2 Gen 2 assay with product code PCRAE0120 manufactured by Trentron Biomedical Ltd. is eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO-prequalified.

As part of the ongoing requirements for listing as eligible for WHO procurement, Trentron Biomedical Ltd. must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Trentron Biomedical Ltd. is required to notify WHO of any complaints, including adverse events related to the use of the product, within seven days and any changes made to the product.

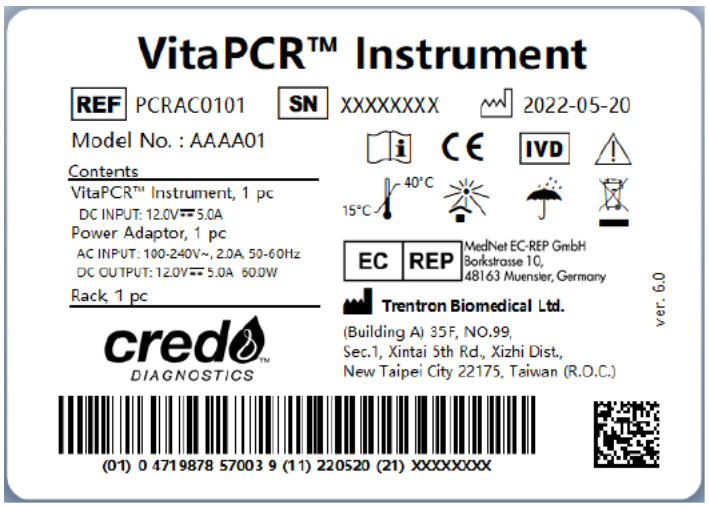
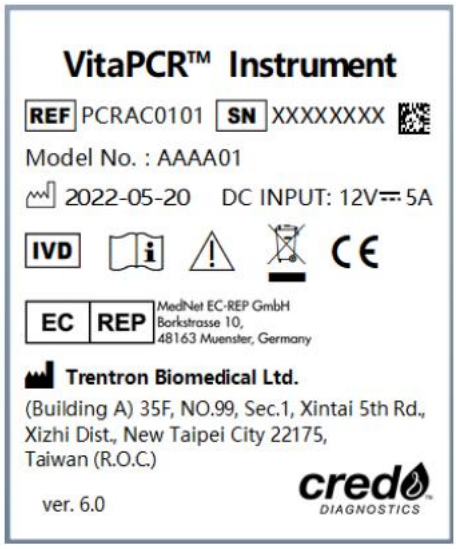
WHO reserves the right to rescind eligibility for WHO procurement if additional information on the safety, quality, and performance during post-market surveillance activities and if new data becomes available to WHO that changes the risk-benefit balance.

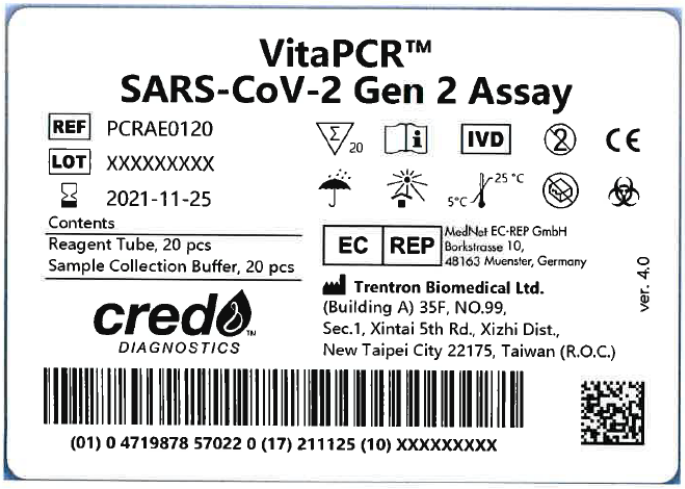
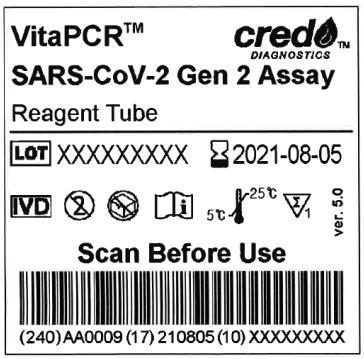
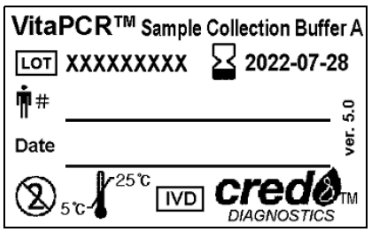
[1] https://www.who.int/publications/i/item/9789240015319

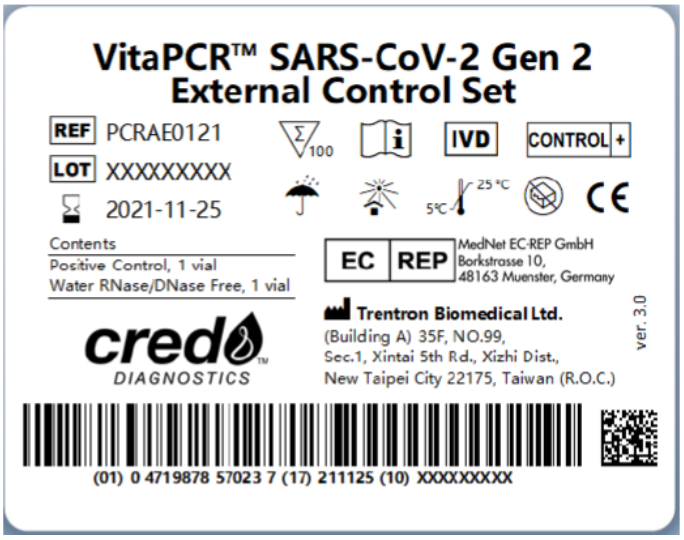
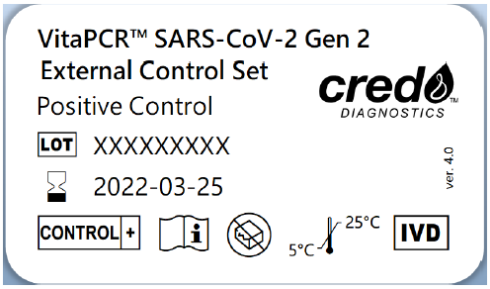
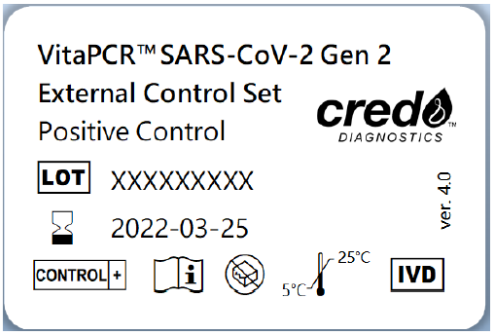
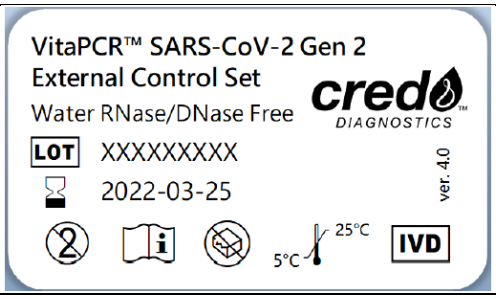
# Labelling

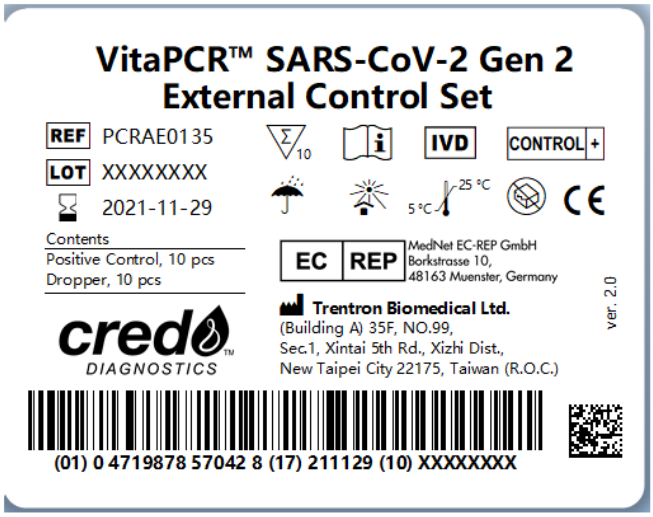
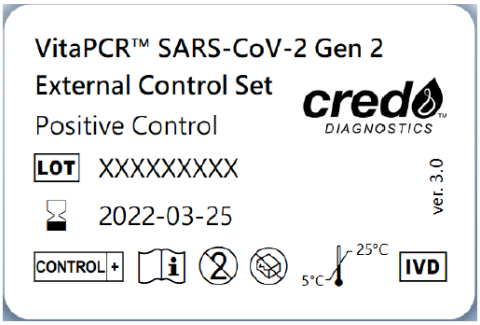
1. **Labels**
2. **Instructions for use**

## 1.0 Labels

| Product Name | Item | Content |
|---|---|---|
| VitaPCR™ Instrument | Box |  |
| | Instrument |  |

| Product Name | Item | Content |
|---|---|---|
| VitaPCR™ SARS-CoV-2 Gen 2 Assay | Box |  |
| | Reagent |  |
| | SCB |  |

| Product Name | Item | Content |
|---|---|---|
| VitaPCR™ SARS-CoV-2 Gen 2 External Control Set (1 vial) | Box |  |
| | Positive Control Tube |  |
| | Package of Positive Control Tube |  |
| | Water RNase Free Tube |  |

| Product Name | Item | Content |
|---|---|---|
| VitaPCR™ SARS-CoV-2 Gen 2 External Control Set (10 vials) | Box |  |
| | Package of Positive Control Tube |  |

## 2.0 Instructions for Use[2]

[2] English version of the IFU was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

VitaPCR™

# VitaPCR™ SARS-CoV-2 Gen 2 Assay

REF PCRAE0120

For use with the VitaPCR™ Instrument

For nasopharyngeal (NP) or oropharyngeal (OP) swab specimens

For *in vitro* diagnostic use only

IVD

## INTENDED USE

The VitaPCR™ SARS-CoV-2 Gen 2 Assay performed on the VitaPCR™ Instrument is a rapid molecular *in vitro* diagnostic test utilizing a real-time reverse transcription polymerase chain reaction (RT-PCR) amplification technology for the qualitative detection of SARS-CoV-2 RNA in nasopharyngeal (NP) or oropharyngeal (OP) swabs from patients who are suspected of COVID-19 by their healthcare providers.

Results are for the presumptive identification of SARS-CoV-2. The definitive identification of SARS-CoV-2 infection requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of SARS-CoV-2 infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the SARS-CoV-2.

The function of the assay is to aid in the diagnosis of COVID-19 disease. Rapid molecular assays that identify the target virus from patients infected with SARS-CoV-2 can aid in effective control of the global outbreak. SARS-CoV-2 infection is not precluded by negative results. Results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

The VitaPCR™ SARS-CoV-2 Gen 2 Assay is intended to be performed by professionals in both laboratory and near-patient testing settings.

## SUMMARY AND EXPLANATION

Refer to the WHO Health topics "Coronavirus disease (COVID-19)". COVID-19 is an acute respiratory illness caused by infection with the SARS-CoV-2, which was initially reported to WHO in Wuhan, China on December 31, 2019. The SARS-CoV-2 is from the same family of viruses as Severe Acute Respiratory Syndrome (SARS), and is spread from person to person. Virus-laden droplets from an infected person can transmit through nose, eyes, or mouth to another.

COVID-19 is associated with a variety of clinical outcomes, including asymptomatic infection and symptomatic infection. Symptoms of SARS-CoV-2 infection vary, it can cause mild illnesses including a runny nose, sore throat, cough, and fever. In severe cases, it can lead to pneumonia, breathing difficulties or death.

The VitaPCR™ SARS-CoV-2 Gen 2 Assay performed on the VitaPCR™ Instrument is a rapid molecular *in vitro* diagnostic test utilizing a real-time RT-PCR amplification technology for the qualitative detection of SARS-CoV-2. The product contains primers, fluorophore-labeled probes and control material used in real-time RT-PCR for the *in vitro* qualitative detection of specific SARS-CoV-2 RNA in respiratory specimens.

## PRINCIPLE OF THE TEST

The VitaPCR™ SARS-CoV-2 Gen 2 Assay performed on the VitaPCR™ Instrument is a rapid molecular-based *in vitro* diagnostic test utilizing real-time reverse transcription polymerase chain reaction (real-time RT-PCR) technique. It is used for the qualitative detection and discrimination of SARS-CoV-2 viral RNAs in direct nasopharyngeal (NP) or oropharyngeal (OP) swab specimens from patients who are suspected of COVID-19 by their healthcare providers. The assay targets regions of the virus nucleocapsid (N) gene. It detects both specific SARS-CoV-2 RNA and universal SARS-like RNA (including SARS-CoV-2, SARS-CoV, bat SARS-like coronavirus); The assay includes artificial single stranded RNA (ssRNA) material as internal control (IC) to monitor the RT-PCR process.

To perform the test, NP or OP swab specimens are added to the Sample Collection Buffer (SCB) to solubilize the sample. Subsequently, 30µl of the SCB is then transferred into the Reagent Tube. There are 2 steps in the reaction process:
1. Lysis of the sample after swab specimen is added into the sample collection buffer
2. One-step reverse transcription and PCR amplification with target primers and real-time detection with target specific probes

Detection of target sequences is achieved through real-time measurement of the fluorescence signal emitted by the fluorophore

released as a result of degradation of the specific SARS-CoV-2 target probes, universal SARS-like target probes, and internal control probes, following sequence amplification by the respective targets primer pairs. The test takes approximately 20 minutes to complete. In this respect, an operator can run 2 tests on the VitaPCR™ Instrument within one hour with ease. In an eight hour shift, the operator would be able to handle 16 tests.

## REAGENTS AND MATERIALS

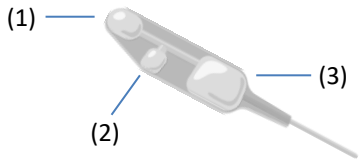
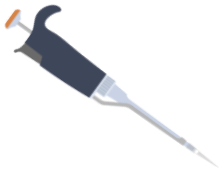
### Materials Provided

The VitaPCR™ SARS-CoV-2 Gen 2 Assay contains sufficient reagents to process 20 specimens or quality control samples.
The kit contains the following:

| VitaPCR™ SARS-CoV-2 Gen 2 Assay | |
|---|---|
|  | **VitaPCR™ Sample Collection Buffer_A**: Screw capped plastic tube containing 4 mL of sample collection buffer (SCB). |
|  | **Reagent Tube**: Transparent test tube with lyophilized reagents for the targeted amplification of RNA of SARS-CoV-2. |
|  | **Reagent Tube Cap**: A plastic stopper used to seal reagent tube after addition of sample. |
| Quick Reference Guide of VitaPCR™ SARS-CoV-2 Gen 2 Assay | |

### Materials Required but not Provided

| | |
|---|---|
|  | VitaPCR™ Instrument (Cat # PCRAC0101) |
|  | Rack (Cat # PCRAC0101) |
|  | Power Adaptor (Cat # PCRAC0101)<br>(INPUT: AC 100-240V, 2.0A Max, 50-60Hz. OUTPUT: DC 12V, 5A) |
|  | **Nasopharyngeal Swab** or **Oropharyngeal Swab**: For optimal test performance, ONLY use swabs which meet the CE directive requirements for medical devices. To avoid interference, please do not use swabs with wooden shafts or calcium alginate swabs since the reaction inhibitor might be contained. We strongly recommend the use of flocked swabs or synthetic fiber swabs with plastic shafts.<br>**Please refer to the list below for validated swab types:**<br>1. Puritan 25-3316-U BT; Puritan 25-3316-U<br>2. Puritan 25-3317-U BT; Puritan 25-3317-U<br>3. Puritan 25-3320-U BT; Puritan 25-3320-U; Puritan 25-3320-U EMB 100MM; Puritan 25-3320-U EMB 80MM<br>4. Copan 503CS01; Copan 553C<br>5. Copan 534CS01; Copan 534C<br>6. Puritan 25-3306-U BT; Puritan 25-3306-U<br>7. Puritan 25-1506 1PF BT<br>8. Copan 520C; Copan 520CS01<br>9. Copan 552C; Copan 502CS01<br>10. Copan 519C; Copan 519CS01 |

| | |
|---|---|
|  | **Transfer Pipette (Cat # PCREG0103):**<br>Single-use, disposable plastic component with three air bulbs to transfer the sample elution from the Sample Collection Buffer to the Reagent Tube.<br>The **top bulb (1)** is used to draw and release the solution.<br>The **middle bulb (2)** is used for pipetting action.<br>The **bottom bulb (3)** serves as an overflow chamber. |
|  | **Adjustable Pipette & Filter Tip:**<br>It is used to transfer the sample extract from the Sample Collection Buffer to the Reagent Tube.<br>(No Cat # is listed for the adjustable pipette & Filter Tip as this is a consumables/ tools that are commonly found in the laboratory.) |
| User Manual of VitaPCR™ Instrument<br>Quick Reference Guide of VitaPCR™ Instrument | |

## Materials available but purchase separately

| External Control | |
|---|---|
| VitaPCR™ SARS-CoV-2 Gen 2 External Control Set<br>(Cat # PCRAE0121: 1 vial – 100 reactions)<br>(Cat # PCRAE0135: 10 vials – single-use/one reaction per vial) | External Control is available, but not provided in this assay. Please contact your local distributor if needed. |

## PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Only use with the VitaPCR™ Instrument.
3. Wear protective gloves and other personal protective equipment before running the test.
4. Proper sample collection, storage, and transport are essential for correct results. Please only use validated specimen types described in the Intended Use.
5. Please only use dry swab specimens and do NOT use the swabs previously stored in VTM or UTM.
6. Treat all biological specimens, including used VitaPCR™ reagent tubes, caps, sample collection buffer, and transfer pipettes, as if capable of transmitting infectious agents. Because it is often impossible to know which specimens might be infectious, all biological specimens should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention and the Clinical and Laboratory Standards Institute.
7. All kit materials are single-use items. Do not apply to multiple samples.
8. Do NOT leave the swab inside the SCB after rotating the swab inside the buffer. Remove and discard the swab from the SCB immediately afterward. Proceed with the remaining steps outlined in the TEST PROCEDURE section.
9. For virus inactivation, keep the SCB tightly closed and leave it on the rack for a minimum of 5 minutes.
10. Do NOT touch the heads of swabs. Contamination may occur and interfere with the performance of the test.
11. Do NOT use the kit after its expiration date.
12. Do NOT open the Reagent Tubes foil before running the test.
13. Avoid eye and skin contact with sample collection buffer (SCB) (e.g. Hydrochloric Acid) that may cause irritation after exposure. If contacted, rinse cautiously with water for several minutes. Seek medical treatment if necessary.
14. If SCB is spilled while opened, clean the work area as per the instructions provided in the VitaPCR™ User Manual. Restart the test with a new SCB.
15. If any assay material is dropped, cracked, found to be damaged, or opened when received, DO NOT USE and discard. Do not use scissors or sharp objects to open foil pouches as damage to test pieces can occur.
16. Do not leave anything on rack and device after testing. Please immediately discard Reagent Tube and do NOT try to open Reagent Tube Cap when test is done. According to the removal instructions described in the device User Manual, follow your country, state, and local regulations to dispose of items.
17. The used items may contain infectious waste, chemical waste, or general waste, if the country or regional regulations do not provide clear direction on proper disposal, all used items should be disposed of as per WHO [World Health Organization] guidance documents on Healthcare waste.

18. Visibly bloody samples must NOT be used with VitaPCR™ SARS-CoV-2 Gen 2 Assay.
19. Rarely, tested samples might contain inhibitors that fail the test. The failure rate is a case-by-case result.
20. Previous positive samples left around work area may cause false positive results. Handle samples with the protocol set forth by the lab. Clean your device and surrounding as the User Manual instructions.

## QUALITY CONTROL

### Internal Control (IC)

Internal control included in each Reagent Tube monitors the whole RT-PCR process and verifies the validity of RT-PCR reactions associated with the sample. Among all general results, internal control shows signal constantly. In some particular circumstances, targets may be detected without internal control signal due to PCR competition.

### External Positive and Negative Controls

- The positive control consists of an RNA transcript of the SARS-CoV-2 N gene segment which sequence is used for both universal primer/probe and specific primer/probe set target.
- Refer to the instructions of VitaPCR™ SARS-CoV-2 Gen 2 External Control Set and follow the Test Procedure immediately to perform the positive control test.
- SCB can be used as the negative control and follow the Test Procedure to perform the negative control test.
- The controls are used for quality control testing and each time a new shipment of kits is received or when training a new operator; or in accordance with local regulations, accrediting groups, or laboratory's standard quality control procedures.
- If external QC testing fails, repeat the test again or contact your local distributor.

| Quality Control | | | | | |
|---|---|---|---|---|---|
| **Control Type** | **Control Type External Control Name** | **Used to Monitor** | **Universal SARS-like** | **Specific SARS-CoV-2** | **Internal Control** |
| Positive Control | SARS-CoV-2 N gene segment | Substantial reagent failure including detection primer and probe integrity | + | + | + |
| Negative Control | Sample Collection Buffer | Reagent and/or environmental contamination | - | - | + |

## STORAGE AND STABILITY

Store the reagent kit at 5-25°C. The VitaPCR™ SARS-CoV-2 Gen 2 Assay is stable before the expiration date marked on the outer packaging and containers. Ensure all test materials have reached room temperature before use.

## SPECIMEN COLLECTION AND HANDLING

Use freshly collected specimens for optimal test performance. Inadequate specimen collection or improper sample handling/storage/transport may yield wrong results.

For optimal test performance, ONLY use swabs which meet the CE directive requirements for medical devices. To avoid interference, please do not use swabs with wooden shafts or calcium alginate swabs since the reaction inhibitor might be present. We strongly recommend the use of flocked swabs or synthetic fiber swabs with plastic shafts. Place nasopharyngeal or oropharyngeal swabs collected from patient immediately into SCB.

***NOTE:***

***The swab samples from patients who have taken oral or nasal medicine recently or before the test may have a high probability of generating invalid results.***

***Insufficient collection of samples may lead to false negative results, while overcollection of samples may introduce PCR inhibitors which may interfere with PCR efficiency, leading to invalid results.***

**Nasopharyngeal swab (NP swab)**

Carefully insert the swab into the nostril and pass the swab directly backwards without tipping the swab head up or down. Using gentle rotation, insert the swab into the anterior nares parallel to the palate advancing the swab into the nasopharynx, leave in place for a few seconds, and then slowly rotate the swab as it is being withdrawn. To ensure proper collection, the swab should travel a length that is halfway of that from the nose to the tip of the ear. DO NOT USE FORCE while inserting the swab.

**Oropharyngeal swab (OP swab, e.g. throat swab)**

Swab both the posterior pharynx and tonsils, avoiding the tongue.

## SPECIMEN TRANSPORT AND STORAGE

Collected specimens on swab should be tested as soon as possible. If immediate testing is not possible, refer to the following guidelines for transport and storage:

1. Specimen on swab stored at 2°C to 8°C up to 24 hours.
2. Specimen eluted in the Sample Collection Buffer (SCB) can be stored at:

- 4°C to 25°C for 3 days
- 2°C to 8°C in the refrigerator for 7 days
- For long-term storage, specimens should be frozen at -80°C.

**Patient specimen swabs that were previously stored in VTM or UTM are not recommended to be used with the assay as it will invalidate the test.**

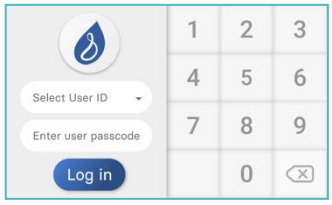
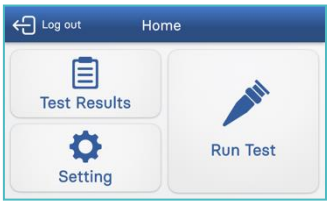
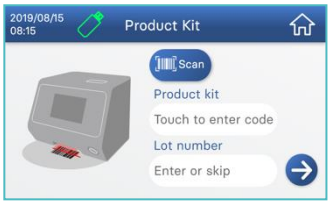
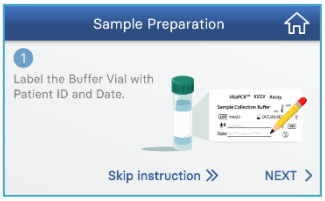
***NOTE: Keep the specimen at the temperature as indicated above. Do not freeze and thaw the specimen repeatedly.***

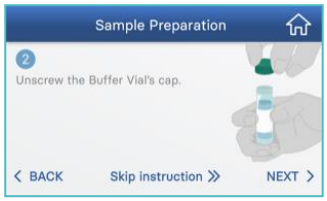
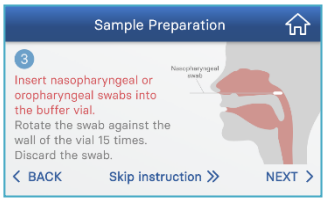
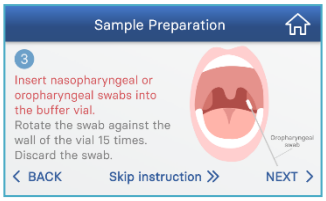
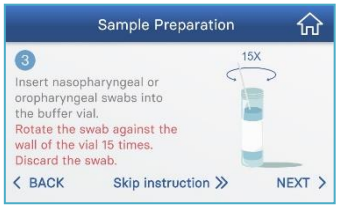
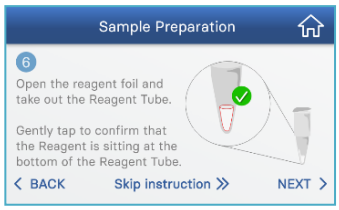
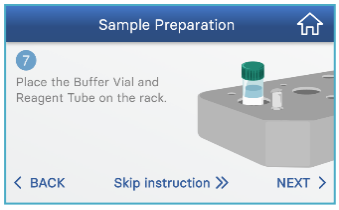
## TEST PROCEDURE

Before testing with VitaPCR™ SARS-CoV-2 Gen 2 Assay:

- Allow all samples to reach room temperature.
- Allow all test materials to reach room temperature.

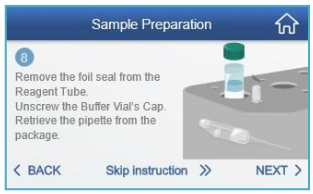
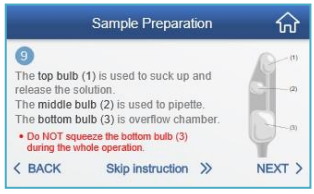
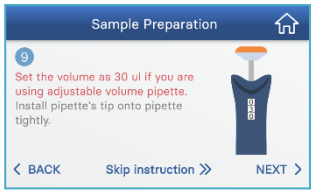
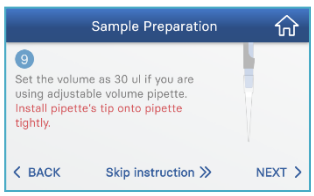
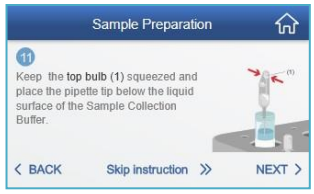
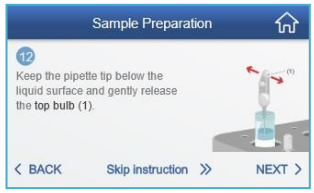
For best results, direct nasopharyngeal or oropharyngeal swabs should be tested immediately after collection.

| | |
|---|---|
| Place VitaPCR™ Instrument on a flat surface.<br>Turn on VitaPCR™ Instrument by pressing the power button at the front of the instrument. |  |
| Select User ID.<br><br>Enter User Passcode.<br><br>Press 'Log in'.<br><br>** Ensure proper log in of individual user accounts for proper data traceability.* |  |
| Press 'Run Test'. |  |
| Scan the barcode on the reagent package using the built-in barcode scanner on the lower front side of VitaPCR™ Instrument.<br><br>** It is strongly recommended that a USB is correctly plugged into the USB port (located at the back of the VitaPCR™ Instrument). This helps to collect vital raw data useful to check a test. Only USB with 3.0 flash drive in FAT32 format is compatible.* |  |
| Scan or key in Patient ID.<br>Confirm the Product Kit and Patient ID.<br><br>** When entering Patient ID, please follow local regulations and do not include any personal information that may allow the individual to be identified.* |  |
| 1. Label the Buffer Vial with patient ID and date. |  |

| | |
|---|---|
| 2. Unscrew the Buffer Vial's cap. |  |
| 3. Insert nasopharyngeal or oropharyngeal swabs into the buffer vial. Rotate the swab against the wall of the vial **at least 15 times**. Discard the swab.<br><br>***Please remove and discard the swab immediately after rotating.*** |  <br>  <br>  |
| 4. Screw the Buffer Vial's cap back. |  |
| 5. Gently invert the Buffer Vial upside down **10 times**. Place the Buffer Vial on the rack. |  |
| 6. Open the reagent foil and take out the Reagent Tube. Gently tap to confirm that the Reagent is sitting at the bottom of the Reagent Tube. |  |
| 7. Place the Buffer Vial and Reagent Tube on the rack. |  |

**Choose the one you use .**

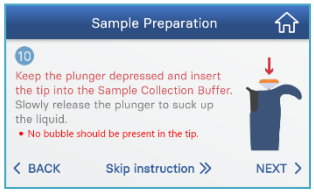

** The precipitated inside the Reagent Tube are one common factor affecting the VitaPCR™ reaction. If there are visible impurities in the Sample Collection Buffer after it is spiked with the swab sample, please ensure that the transferred liquid is clear.*

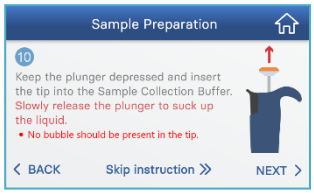
| Adjustable Pipette & Filter Tip | | Transfer Pipette | |
|---|---|---|---|
| 8. Remove the foil seal from the Reagent Tube. Unscrew the Buffer Vial's Cap. |  | 8. Remove the foil seal from the Reagent Tube. Unscrew the Buffer Vial's Cap. Retrieve the pipette from the package.<br>9. The **top bulb (1)** is used to draw and release the solution. The **middle bulb (2)** is used for pipetting action. The **bottom bulb (3)** serves as an overflow chamber.<br>***Do NOT squeeze the bottom bulb (3) during the whole operation.*** |  <br>  |
| 9. Set the volume as 30 µL if you are using adjustable volume pipette.<br><br>Install pipette's tip onto pipette tightly. |  <br>  | 10. Firmly squeeze the **top bulb (1)** and do not release.<br>11. Keep the **top bulb (1)** squeezed and place the pipette tip below the liquid level of the **Sample Collection Buffer**.<br>12. Keep the pipette tip below the liquid level and gently release the **top bulb (1)**.<br>13. Check the pipette stem is full of liquid without bubbles. |  <br>  <br>  <br>  |

10. Keep the plunger depressed and insert the tip into the Sample Collection Buffer.

    Slowly release the plunger to draw the liquid.

    ***No bubble should be present in the tip.***



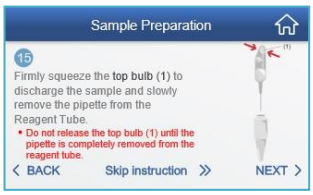

14. Place the pipette tip into the **Reagent Tube.**

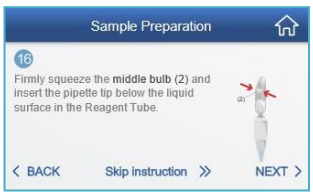
15. Firmly squeeze the **top bulb (1)** to discharge the sample and slowly remove the pipette from the Reagent Tube.

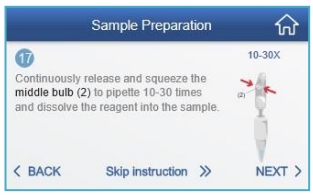
    ***Do NOT release the top bulb (1) until the pipette is completely removed from the reagent tube.***

16. Firmly squeeze the **middle bulb (2)** and re-insert the pipette tip into the Reagent Tube. Ensure that the end of the tip is below the liquid level.

17. Continuously release and squeeze the **middle bulb (2)** to pipette **10-30 times** to dissolve the reagent in the sample solution.

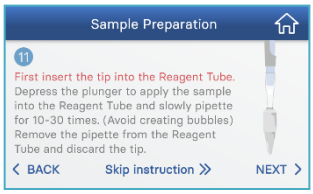



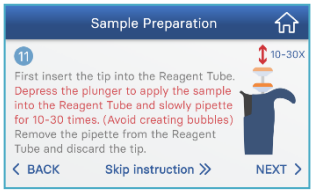

11. Insert the transfer pipette into the Reagent Tube.

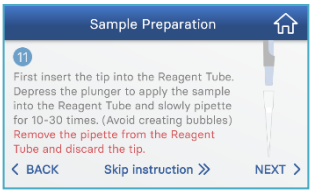
    Depress the plunger to discharge the sample into the Reagent Tube and slowly pipette for 10-30 times.

    Remove the pipette from the Reagent Tube and discard the tip.

    ** Avoid Creating Bubbles*

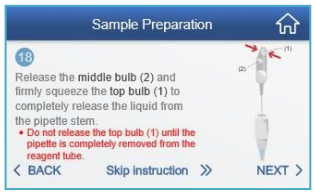


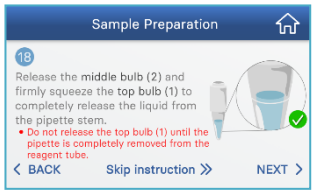




18. Release the **middle bulb (2)** and firmly squeeze the **top bulb (1)** to completely release the liquid from the pipette stem.

    ***Do not release the top bulb (1) until the pipette is completely removed from the reagent tube.***



12. Retrieve the Reagent Cap. Close the tube with the Cap. (press the Cap until you hear the “click”)

** Make sure there’s **no gap** between the Cap and the Reagent Tube.*

13. Swing the tube in a quick, sharp downwards movement **three times** to remove bubbles.


19. Retrieve the Reagent Cap. Close the tube with the Cap. (press the Cap until you hear the “click”)

**Make sure there is **no gap** between the Cap and the Reagent Tube.*

20. Swing the tube in a quick, sharp downwards movement **three times** to remove bubbles.

**Also, you may tap the tube a few times to avoid bubbles at the bottom.*

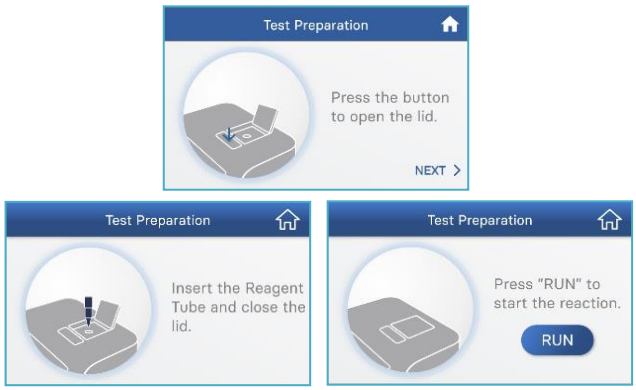

Press the button to open the lid.

Insert the Reagent Tube and close the lid.

Press ‘Run’ to start the reaction.

** Ensure that the lyophilized reagent is fully dissolved and mixed evenly once the sample is transferred from the Sample Collection Buffer.*

** Ensure that the sample volume is as close to 30µL as possible when transferring to Reagent Tube. There is a "line", which is set at 30µL, imprinted on the Reagent Tube. A great deviation of sample volume may cause heating discrepancies during PCR, leading to erroneous results.*



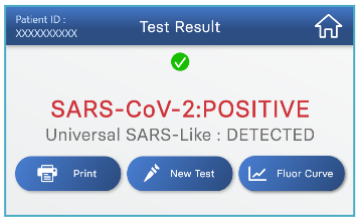

When the reaction is running correctly, the progress bar on the VitaPCR™ screen turns blue while the percentage number will increase steadily.

***Note:***

- ***The screen will continue to display the percentage until 100% when the entire test is completed. The VitaPCR™ will beep at the end of the completion of a run.***
- ***The lid will be locked during reaction. Do not try to re-open the VitaPCR™ lid until the display indicates that the test is completed.***
- ***To abort the test, press ‘End Run’ and wait for the instruction on the screen.***
- ***Do not move or unplug the VitaPCR™ while the test is processing.***

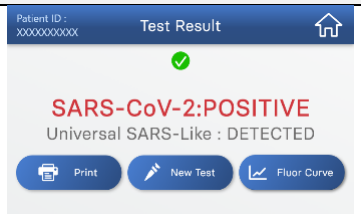
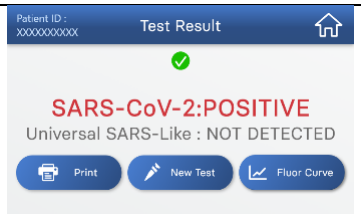
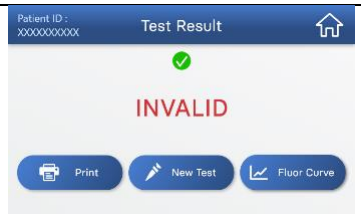

The VitaPCR™ will beep at the end of test processing, and the result will display on the screen automatically.

To start a new test, press "New Test".



## INTERPRETATION OF RESULTS AND REPORTING

The table below lists the expected results for The VitaPCR™ SARS-CoV-2 Gen 2 Assay.

| Detection of Universal SARS-like | Detection of Specific SARS-CoV-2 | Internal Control | Result | Interpretation |
|---|---|---|---|---|
| + | + | ± |  | SARS-CoV-2 RNA Detected.<br>(Both specific SARS-CoV-2 RNA and universal SARS-like RNA are detected.) |
| - | + | ± |  | SARS-CoV-2 RNA Detected.<br>(The universal SARS-like RNA not detected might be caused by low viral load in the specimen or the accumulation of mutation over time.) |
| + | - | ± |  | Presumptive Positive for SARS-CoV-2 RNA.<br><br>(The specific SARS-CoV-2 RNA not detected might be caused by low viral load in the specimen or the accumulation of mutation over time.)<br><br>Sample should be retested. For samples with a repeated Presumptive Positive result, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other SARS-like coronaviruses currently unknown to infect humans, for epidemiological purposes or clinical management. |
| - | - | + |  | SARS-CoV-2 RNA Not Detected. |
| - | - | - |  | RT-PCR inhibition or reagent failure.<br>Collect a new sample and Repeat testing. |

**NOTE:**
Due to the molecular evolution of SARS-CoV-2, there is an inherent risk for any PCR based test system that accumulation of mutations over time may lead to false negative results.

## DEVICE CLEANING

We recommend cleaning the VitaPCR™ Instrument each day after use.

**Procedure:**

1. Unplug the power cord from the wall outlet and VitaPCR™ Instrument .
2. Close the lid.
3. Using 70% ethanol or a germicidal disposable wipe, gently wipe the outer surfaces of VitaPCR™ Instrument, removing any dust.

***NOTE: Do not press the wipe against the open vents of VitaPCR™ Instrument.***

4. Using a new dry cloth, wipe the front of VitaPCR™ Instrument twice top to bottom, then twice left to right. Follow this step for the back, top and bottom of VitaPCR™ Instrument.
5. Do not let any liquid to gather around any opening. Make sure no liquid enters your device.
6. Allow the unit to dry for at least 10 minutes and check it's all dry before re-connecting the power cord for the AC Adapter.

## LIMITATIONS

- The performance of the VitaPCR™ SARS-CoV-2 Gen 2 Assay is determined by the procedures described in this document. Failure to follow the instruction may alter test performance.
- The VitaPCR™ SARS-CoV-2 Gen 2 Assay is for use with nasopharyngeal or oropharyngeal swab specimens.
- Improper collection, storage or transport of specimens may lead to false negative results.
- Test results should also be considered with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- As with other tests, negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for patient management decisions.
- False negative results may occur if the levels of viruses are lower than the detection limit.
- False negative results may occur if there are mutations in the regions targeted by the test.
- The presence of inhibitors in the sample can lead to invalid results.

## PERFORMANCE CHARACTERISTICS

### *In Silico* Analysis of Analytical Reactivity (Inclusivity)

BLASTN analysis was performed with the primers and probes for detection of Specific SARS-CoV-2 RNA and Universal SARS-like RNA in the VitaPCR™ SARS-CoV-2 Gen 2 Assay against all publicly available nucleic acid sequences in GISAID, for 3 months between May 01 and July 31, 2022. The search parameters automatically adjust for short sequences and the expected threshold is 1000. The match and mismatch scores are 1 and -3 respectively. The penalty for creating and extending a gap is 5 and 2, respectively.

The sequences were aligned to complete sequences using the BLASTN to the database, which contains 210,353 complete and high coverage sequences among 5,456,297 total sequences.
Analysis of the forward and reverse primer and probe sequences of Specific SARS-CoV-2 RNA showed 98.43% match to almost all the available SARS-CoV-2 nucleic acid sequences.

Results of *in silico* inclusivity analysis against Specific SARS-CoV-2 RNA

| Analysis of Specific SARS-CoV-2 RNA | | | | |
|---|---|---|---|---|
| Oligonucleotide | Forward Primer | Reverse Primer | Probe | All targeted region[2] |
| Sequences available[1] | 210,353 | | | |
| Perfect match | 209,631 | 209,435 | 208,675 | 207,065 |
| with 1 mismatch | 656 | 904 | 1,672 | 3,204 |
| with ≧2 mismatches or indel(s) | 66 | 14 | 6 | 84 |
| Homology[3] | | | | 98.43% |

[1]Sequences were selected from GISAID between May 01 and July 31, 2022.
[2]Count three oligonucleotides binding sites together.
[3]The homology was calculated with the sequences with the perfect match.

Results of *in silico* inclusivity analysis against Universal SARS like RNA

| Analysis of Universal SARS-like RNA | | | | |
|---|---|---|---|---|
| Oligonucleotide | Forward Primer | Reverse Primer | Probe | All targeted region2 |
| Sequences available[1] | 210,353 | | | |
| Perfect match | 207,749 | 209,452 | 209,151 | 205,738 |
| with 1 mismatch | 2,590 | 890 | 1,197 | 4,586 |
| with ≧2 mismatches or indel(s) | 14 | 11 | 5 | 29 |
| Homology[3] | | | | 97.8% |

[1]Sequences were selected from GISAID between May 01 and July 31, 2022.
[2]Count three oligonucleotides binding sites together.
[3]The homology was calculated with the sequences with the perfect match.

Additional Analysis for the emerging SARS-CoV-2 Variants.
During late 2020, the emergence of variants that posed an increased risk to global public health prompted the characterization of specific Variants of Concern (VOCs) by WHO, in order to prioritize global monitoring and research, and ultimately to inform the ongoing response to the COVID-19 pandemic. According to the published information on March 2022 by WHO, VOC Omicron (known as B.1.1.529) was currently circulating and additionally analyzed in this study. These sequences have been included in our analysis already. In consideration of the importance per emerging variants, we isolated the outcome from 155,268 sequences.

| WHO label | Pango lineage | Total No. sequences identified in GISAID | Homology to Specific SARS-CoV-2 | Homology to Universal SARS-like |
|---|---|---|---|---|
| Omicron | B.1.1.529 | 155,268 | 98.7% | 97.8% |

### Analytical Reactivity (Inclusivity)

Analytical reactivity was assessed with wet test performed with five additional SARS-CoV-2 variants – Alpha, Beta, Gamma, Delta, and Omicron which were purchased from ZeptoMetrix. All of them were detected by the VitaPCR™ SARS-CoV-2 Gen 2 Assay. The Ct values have no significant difference to the control virus (USA-WA1/2020).

| SARS-CoV-2 | Positive Result of 3x LoD SRAS-CoV-2 virus |
|---|---|
| USA-WA1/2020 | 3/3 |
| Variant Alpha | 3/3 |
| Variant Beta | 3/3 |
| Variant Gamma | 3/3 |
| Variant Delta | 3/3 |
| Variant Omicron | 3/3 |

## Analytical Sensitivity (Limit of Detection)

Individual native nasopharyngeal swab (NPS) and oropharyngeal swab (OPS) specimens collected from healthy donors with nasopharyngeal swab and oropharyngeal swab were eluted in 4 mL SCB. Swab eluates were combined and mixed to make a pool of negative matrix for NPS and OPS.

SARS-CoV-2 dilutions were prepared using WHO International Standard for SARS-CoV-2 RNA (20/146: NIBSC, UK), Concentration: 7.70 $Log_{10}$ IU/mL (5.00E+07 IU/mL) diluted in the negative natural matrix. Contrived SARS-CoV-2 positive NPS and OPS were prepared by adding 10 µL of each SARS-CoV-2 dilution onto each swab. The WHO SARS-CoV-2 dilutions in the negative matrix were dispensed onto each swab by lightly scratching the swab surface with the pipette tip as virus dilution was pipetted to ensure liquid was absorbed into the swab tip. The following SARS-CoV-2 predicated concentrations in SCB were used in building the initial LoD range finding study: 1250 IU/mL, 125 IU/mL, 62.5 IU/mL, and 12.5 IU/mL. All contrived positive swabs were tested following the instructions for use of the VitaPCR™ SARS-CoV-2 Gen 2 Assay. The lowest concentration at which all three replicates tested positive for both N-gene targets was treated as the initial LoD.
The final LoD is the concentration of WHO International Standard ( IU/mL ) that is successfully detected with at least a 95% positivity rate. To determine the final LoD, 24 replicate swabs were tested at the initial LoD concentration and at a higher concentration 2-fold above the initial LoD. Results for the LoD confirmation study are shown in the table below.

NPS results of virus serial dilution (WHO International Standard )

| International Standard for SARS-CoV-2 RNA Concentration (IU/mL) | Universal SARS-like | | | Specific SARS-CoV-2 | | | Internal Control | | | SARS-CoV-2 Positive Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate Detection | Mean Ct | Standard Deviation | Replicate Detection | Mean Ct | Standard Deviation | Replicate Detection | Mean Ct | Standard Deviation | |
| 125 | 21/24 | 35.57 | 0.93 | 24/24 | 36.17 | 1.17 | 24/24 | 33.46 | 0.78 | 24/24 |
| 62.5 | 10/24 | 36.20 | 0.79 | 18/24 | 37.06 | 0.73 | 24/24 | 33.42 | 0.88 | 18/24 |

OPS results of virus serial dilution (WHO International Standard)

| International Standard for SARS-CoV-2 RNA Concentration (IU/mL) | Universal SARS-like | | | Specific SARS-CoV-2 | | | Internal Control | | | SARS-CoV-2 Positive Results |
|---|---|---|---|---|---|---|---|---|---|---|
| | Replicate Detection | Mean Ct | Standard Deviation | Replicate Detection | Mean Ct | Standard Deviation | Replicate Detection | Mean Ct | Standard Deviation | |
| 125 | 22/24 | 35.68 | 0.84 | 24/24 | 36.96 | 1.04 | 24/24 | 33.58 | 0.78 | 24/24 |
| 62.5 | 13/24 | 36.23 | 1.01 | 13/24 | 37.15 | 1.14 | 24/24 | 33.25 | 0.68 | 13/24 |

This data demonstrates that the VitaPCR™ SARS-CoV-2 Gen 2 Assay detects 125 IU/mL (233 copies/mL) of both NPS and OPS specimen with a confidence ≥95%. This concentration therefore serves as the confirmed limit of detection for testing direct swab specimens.

## *In Silico* Analysis of Analytical Specificity (Cross-Reactivity)

BLASTn analysis was performed with the primers and probes of the VitaPCR™ SARS-CoV-2 Gen 2 Assay against all publicly available nucleic acid sequences in NCBI as of August 17, 2022. The nucleotide collection consists of GenBank, EMBL, DDBJ, PDB, and RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100 Mb. The search parameters automatically adjust for short sequences and the expected threshold is 1000. The match and mismatch scores are 1 and -3, respectively. The penalties for creating and extending a gap are 5 and 2, respectively.

Regarding Recommended List of Organisms in the EUA interactive review template, the potential cross-reactivity with all primers and probes in VitaPCR™ SARS-CoV-2 Gen 2 Assay was also verified. The probe sequence for detection of Specific SARS-CoV-2 RNA showed 90 % homology with *Pseudomonas aeruginosa*. The probe sequence for detection of Universal SARS-like RNA showed 86 % homology with *Bordetella pertussis* and *Staphylococcus epidermidis*.

In conclusion, the *in silico* test showed that all possible combinations of primers and probes in VitaPCR™ SARS-CoV-2 Gen 2 Assay no cross-reactivity with the selected organisms listed in the table below was observed, except *Pseudomonas aeruginosa*, *Bordetella pertussis*, and *Staphylococcus epidermidis*. In three cases, there is > 80 % homology with the probe alone, without substantial homologous primers, the microbial DNA cannot be amplified by PCR, and thus it will not affect detection.

| **Pathogen** | | | |
|---|---|---|---|
| Enterovirus A | Enterovirus B | Enterovirus C | Enterovirus D |
| Enterovirus D68 | Human Adenovirus B1 | Human Adenovirus C | Human coronavirus 229E |
| Human coronavirus HKU1 | Human coronavirus NL63 | Human coronavirus OC43 | Human Metapneumovirus (hMPV) |
| Human parechovirus 1 | Human parechovirus 2 | Human parechovirus 3 | Human parechovirus 4 |
| Human parechovirus 5 | Human parechovirus 6 | Human rhinovirus 85 | Human rhinovirus B97 |
| Human rhinovirus C | Influenza A | Influenza B | Influenza C |
| MERS coronavirus | Parainfluenza virus 1 | Parainfluenza virus 2 | Parainfluenza virus 3 |
| Parainfluenza virus 4 | Respiratory syncytial virus | SARS coronavirus | *Bacillus anthracosis* (Anthrax) |
| *Bordetella pertussis* | *Candida albicans* | *Chlamydia pneumoniae* | *Chlamydia psittaci* |
| *Corynebacterium diphtheriae* | *Coxiella burnetii (Q-Fever)* | *Haemophilus influenzae* | *Legionella non-pneumophila* |
| *Legionella pneumophila* | *Leptospira borgpetersenii* | *Leptospira interrogans* | *Leptospira santarosai* |
| *Moraxella catarrhalis* | *Mycobacterium tuberculosis* | *Mycoplasma pneumoniae* | *Neisseria meningitidis* |
| *Pneumocystis jirovecii* (PJP) | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *Staphylococcus epidermidis* |
| *Streptococcus pneumoniae* | *Streptococcus pyogenes* | *Streptococcus salivarius* | |

## Analytical Specificity (Cross-Reactivity)

Cross reactivity performance of the VitaPCR™ SARS-CoV-2 Gen 2 Assay was evaluated by testing the whole organisms or appropriate representative samples listed in the table below. These organisms were tested in three replicates in this study. High levels of these organisms (i.e., $10^6$ CFU/mL for bacteria and $10^5$ $TCID_{50}$/mL for viruses, if available) were added into the SCB containing negative clinical matrix, and then was tested with the VitaPCR™ SARS-CoV-2 Gen 2 Assay in triplicate. Negative clinical matrix was created from oropharyngeal swab specimens collected from individual subjects, stored at -20°C in a clean, dry, tightly sealed plastic tube for up to 24 hours before use. The native oropharyngeal swab specimens were eluted in 4 mL of SCB and gently mixed to generate a negative clinical matrix.

The cross-reactivity wet testing data is presented in the table below.

| Organisms | Concentration | Detection rate of Universal SARS-like | Detection rate of Specific SARS-CoV-2 |
|---|---|---|---|
| | | Result (x/3) | Result (x/3) |
| Enterovirus (EV68) | $1.26x10^6$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Human Adenovirus type 1 | $5.62x10^8$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Human Coronavirus 229E | $2.00x10^7$ copies/mL | 0/3 | 0/3 |
| Human Coronavirus HKU1 RNA | $5.40x10^8$ copies/mL | 0/3 | 0/3 |
| Human Coronavirus NL63 | $1.17x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Human Coronavirus OC43 | $1.00x10^7$ copies/mL | 0/3 | 0/3 |
| Human Metapneumovirus (hMPV) (hMPV3 type B1) | $1.00x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Influenza A/California/7/2009 (H1N1) | $3.16x10^6$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Influenza A/Wisconsin/67/2005 (H3N2) | $3.16x10^7$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Influenza B/Malaysia/2506/2004 (B/Victoria) | $3.16x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Middle East Respiratory Syndrome-related coronavirus | $1.70x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Parainfluenza virus 1 | $1.00x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Parainfluenza virus 2 | $1.00x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Parainfluenza virus 3 | $1.00x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Parainfluenza virus 4 | $1.60x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Respiratory syncytial virus (long A) | $3.00x10^6$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Rhinovirus (type 1A) | $1.41x10^5$ $TCID_{50}$/mL | 0/3 | 0/3 |
| Severe Acute Respiratory Syndrome coronavirus | $1.80x10^6$ copies/mL | 3/3 | 0/3 |
| *Bordetella pertussis* | $1.90x10^8$ CFU/mL | 0/3 | 0/3 |
| *Chlamydia pneumonia* | $1.00x10^8$ CFU/mL | 0/3 | 0/3 |
| *Haemophilus influenzae* | $4.70x10^8$ CFU/mL | 0/3 | 0/3 |
| *Legionella pneumophilia* | $1.20x10^9$ CFU/mL | 0/3 | 0/3 |
| *Mycobacterium tuberculosis* | $1.00x10^8$ CFU/mL | 0/3 | 0/3 |
| *Mycoplasma pneumoniae* | $1.00x10^6$ CFU/mL | 0/3 | 0/3 |
| *Pseudomonas aeruginosa* | $9.70x10^8$ CFU/mL | 0/3 | 0/3 |
| *Staphylococcus epidermis* | $8.30x10^8$ CFU/mL | 0/3 | 0/3 |
| *Streptococcus pneumonia* | $1.20x10^8$ CFU/mL | 0/3 | 0/3 |
| *Streptococcus pyogenes* | $1.10x10^8$ CFU/mL | 0/3 | 0/3 |

| | | | |
|---|---|---|---|
| *Streptococcus salivarius* | 1.20x10$^8$ CFU/mL | 0/3 | 0/3 |
| *Candida albicans* | 2.00x10$^8$ CFU/mL | 0/3 | 0/3 |
| Pooled human nasal wash | - | 0/3 | 0/3 |

No unexpected cross-reactivity was observed among the organisms at the concentrations tested with the VitaPCR™ SARS-CoV-2 Gen 2 Assay. As expected, all three replicates of the SARS-CoV sample tested positive with the universal SARS-like assay, as designed. SARS-CoV and other bat SARS-like coronaviruses are not known to be currently circulating in the human population, therefore are very unlikely to be present in patient respiratory samples during the current emergency.

## Microbial Interference Study

Based on the *in silico* analysis results, potential microbial interference from specimens that are co-infected with *Pseudomonas aeruginosa* or *Bordetella pertussis* with SARS-CoV-2 should be assessed. The heat-inactivated SARS-CoV-2 virus (at 3x LoD) was tested to investigate the interference effects with a high concentration of interfering *Pseudomonas aeruginosa* and *Bordetella pertussis* in triplicates. The testing data is presented in the table below and there was no interference observed.

| Organism | Concentration (CFU/mL) | SARS-CoV-2 detection result (Detected/Tested) | |
|---|---|---|---|
| | | Non-Spike SARS-CoV-2 | Spike 3xLoD SARS-CoV-2 |
| *Pseudomonas aeruginosa* | 5.0 x 10$^7$ | 0/3 | 3/3 |
| *Bordetella pertussis* | 1.9 x 10$^8$ | 0/3 | 3/3 |

No unexpected false-negative SARS-CoV-2 result was observed among the two co-present organisms at the concentrations tested with the VitaPCR™ SARS-CoV-2 Gen 2 Assay.

## Substance Interference

The performance of the VitaPCR™ SARS-CoV-2 Gen 2 Assay was evaluated with potentially interfering substances in respiratory specimens. This study was conducted to evaluate the potential interference effects of 18 interfering substances. The potential interfering substances in respiratory specimens may contain natural material and nasal/throat medication, such as mucin, blood, antiviral drug, and nasal medication. Each interfering substance was tested in the presence and absence of heat-inactivated SARS-CoV-2 virus at 3x LoD.

The substance interference study summary results are presented in the tables below. This study verified that no false positive or false negative results were obtained in 18 potential interference substances tested at listed concentrations

| Potential Interfering Substance | Adsorbed Concentration | Eluted Concentration | SARS-CoV-2 Detected / Tested | |
|---|---|---|---|---|
| | | | Negative Sample | Positive Sample (3x LoD SARS-CoV-2) |
| Mucin | 5 mg/mL | 62.5 µg/mL | 0/3 | 3/3 |
| Blood | 1% | 0.0125% | 0/3 | 1/3 |
| | 0.5% | 0.00625% | 0/3 | 3/3 |
| Normal saline | 100% | 1.25% | 0/3 | 3/3 |
| Antimicrobial (Tobramycin) | 2.5 mg/mL | 31.25 µg/mL | 0/3 | 3/3 |
| Anti-inflammatory drug (Dexamethasone) | 0.5 mg/mL | 6.25 µg/mL | 0/3 | 3/3 |
| Throat lozenges (Menthol) | 5 mg/mL | 62.5 µg/mL | 0/3 | 3/3 |

| | | | | |
|---|---|---|---|---|
| Zicam Extreme Congestion Relief | 2.5% | 0.03125% | 0/3 | 2/3 |
| | 1.25% | 0.015625% | 0/3 | 2/3 |
| | 0.5% | 0.00625% | 0/3 | 3/3 |
| Antiviral drug (Tamiflu) | 2.5 mg/mL | 31.25 µg/mL | 0/3 | 3/3 |
| Antiviral drug (Relenza) | 0.5 mg/mL | 6.25 µg/mL | 0/3 | 3/3 |
| Nasal corticosteroids (Fluticasone furoate) | 5% | 0.0625% | 0/3 | 3/3 |
| Nasal spray (Oxymetazoline) | 5% | 0.0625% | 0/3 | 3/3 |
| Antibiotic, nasal ointment (Mupirocin) | 5 mg/mL | 62.5 µg/mL | 0/3 | 3/3 |
| Betadine sore throat spray | 5% | 0.0625% | 0/3 | 3/3 |
| Difflam Forte throat spray | 5% | 0.0625% | 0/3 | 3/3 |
| LISTERINE COOL MINT Antiseptic mouthwash | 5% | 0.0625% | 0/3 | 3/3 |
| Cough syrup (SECORINE SYRUP) | 5% | 0.0625% | 0/3 | 3/3 |
| Tobacco | 0.03 mg/mL | 0.375 µg/mL | 0/3 | 3/3 |
| Toothpaste (Colgate Total) | 0.5% | 0.00625% | 0/3 | 3/3 |

## Clinical Performance

The performance of the VitaPCR™ SARS-CoV-2 Gen 2 Assay was evaluated using clinical nasopharyngeal (NP) swab specimens in the SCB. Two NPS specimens were collected from each subject. One NPS was eluted in SCB and another one was eluted into Universal Transport Medium (UTM). A total of 671 NP swab specimens were tested with VitaPCR™ SARS-CoV-2 Gen 2 Assay side by side with a comparator which is a CE-marked SARS-CoV-2 RT-PCR test.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of the VitaPCR™ SARS-CoV-2 Gen 2 Assay correlative to the results of the comparator and one invalid result was excluded. As the result, VitaPCR™ SARS-CoV-2 Gen 2 Assay demonstrated a PPA and NPA of 92.31% and 100% for SARS-CoV-2, respectively.

| | | **Comparator Method** | | |
|---|---|---|---|---|
| | **Result** | **Positive** | **Negative** | **Total** |
| VitaPCR™ SARS-CoV-2 Gen 2 Assay | Positive | 156 | 0 | 156 |
| | Negative | 13[a] | 501 | 514 |
| | Total | 169 | 501[b] | 670 |
| PPA (95% CI) | | 92.31% (87.28%-95.45%) | | |
| NPA (95% CI) | | 100.00% (99.24%-100.00%) | | |
| OPA (95% CI) | | 98.06% (96.71%-98.86%) | | |

a. Testing results by a secondary comparator test: 5 of 13 were negative; 8 of 13 were positive

b. One sample was excluded due to the invalid/error issue.

## CONTACT INFORMATION, ORDERING, AND PRODUCT SUPPORT

For technical and product support, contact email：service@credodxbiomed.com

## SYMBOLS

| | | | |
|---|---|---|---|
|  | Do Not Re-Use |  | Manufacturer |
|  | Consult Instructions for Use |  | Catalogue Number |
|  | Caution |  | Contains sufficient for <n> tests |
|  | Temperature Limit |  | Patient Number |
|  | Use-By Date |  | Keep away from sunlight |
|  | Batch Code |  | Keep Dry |
|  | *In vitro* diagnostic medical device |  | Authorized Representative in the European Community |
|  | Do not use if package is damaged |  | Biological risks |
|  | CE mark | | |

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CE

EC REP
MedNet EC-REP GmbH
Borkstrasse 10,
48163 Muenster,
Germany

## REVISION HISTORY

**Version Changes**: Version 6.0 to 7.0

| Document Version | Date | Revision |
|---|---|---|
| 5.0 | Oct 2022 | 1. Added the catalog number of transfer pipette<br>2. Updated the summary and explanation with clinical outcomes<br>3. Updated the "Materials Required but Not Provided" section<br>4. Updated the external control and internal control information<br>5. Updated the "Specimen Transport and Storage" section<br>6. Updated the description of "Test procedure"<br>7. Updated the "Interpretation of Results and Reporting" section<br>8. Updated the "Performance Characteristics" section<br>9. Updated the harmonized symbol page |
| 6.0 | Mar 2023 | 1. Updated the "PRECAUTION" section for the viral inactivation step. |
| 7.0 | May 2023 | 1. Updated the "INTENDED USE" section for stating the qualitative detection of SARS-COV-2 RNA, the function and the intended use setting.<br>2. Updated the "SUMMARY AND EXPLANATION" section for adding the reference.<br>3. Updated the "PRINCIPLE OF THE TEST" section for the description of the extraction step.<br>4. Updated the "REAGENTS AND MATERIALS" section for more details about the materials.<br>5. Updated the "PRECAUTIONS" section for the specific hazard. Giving instructions that users can use to contain the effects of the exposure and to ensure that the items are disposed of correctly.<br>6. Updated the "SPECIMEN TRANSPORT AND STORAGE" section for the claim of the temperature range. |

VitaPCR™

# VitaPCR™ SARS-CoV-2 Gen 2 External Control Set

REF PCRAE0135

For use with the VitaPCR™ System

For *in vitro* diagnostic use only

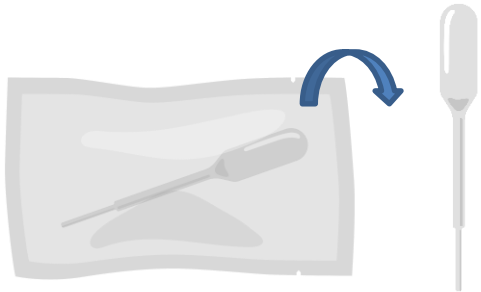
IVD

## INTENDED USE

The VitaPCR™ SARS-CoV-2 Gen 2 External Control Set performed on the VitaPCR™ Instrument is intended to be used for quality control testing. This external Positive Control for use with a series of VitaPCR™ Assays (exclude VitaPCR™ SARS-CoV-2 Assay, Cat # PCRAE0114) contains noninfectious *in vitro* transcribed RNA in a lyophilized state and must be reconstituted before use.

External Control Set is used for quality control testing and each time a new shipment of kits is received or when training a new operator; or in accordance with local state, and/or federal regulations, accrediting groups, or laboratory's standard quality control procedures.

## CONTENTS

| VitaPCR™ SARS-CoV-2 Gen 2 External Control Set | |
|---|---|
|  | **Positive Control** (with foil sealed): Single-use only, consisting of RNA transcripts representing the fragment of the SARS-CoV-2 N gene.<br><br>Quantity: 10 Vials |
|  | **Dropper:** Single-use only, a disposable plastic tool used for transferring and pipetting the external control solution.<br><br>Quantity: 10 PCS |

## WARNINGS AND PRECAUTIONS

1. Single use only.
2. In order to obtain the optimal outcome, it is recommended to use the external control immediately after you tear the foil.
3. This product should be utilized with VitaPCR™ Assays and VitaPCR™ Instrument.

## STORAGE AND STABILITY

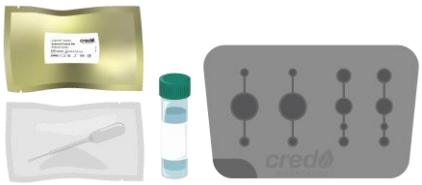
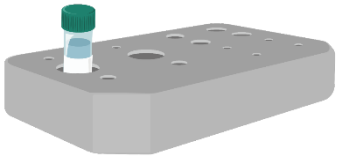
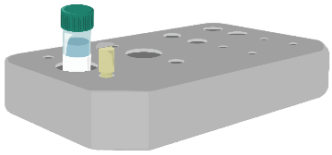
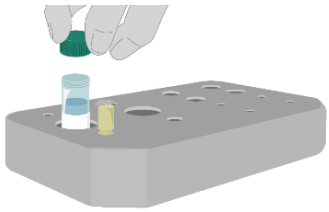
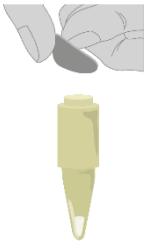
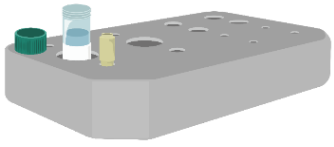
Store the External Control Set at 5-25°C. The VitaPCR™ SARS-CoV-2 Gen 2 External Control Set is stable until the expiration date marked on the outer packaging and containers. Ensure all test materials have reached room temperature before use.

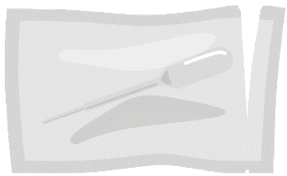
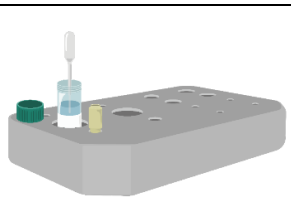
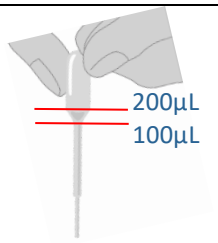
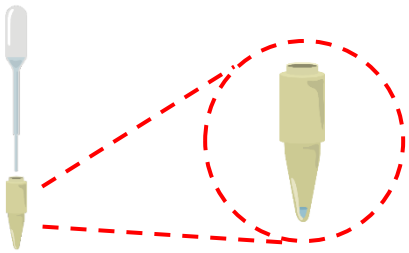
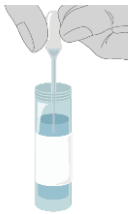
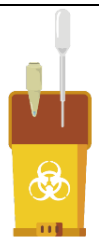
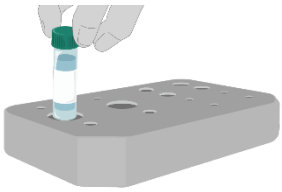
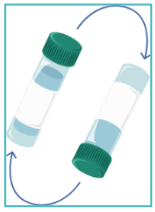
## OPERATING PROCEDURE

### Before using the External Control Set, please make sure to prepare the following items:

- VitaPCR™ Assay and VitaPCR™ Instrument
- Personal Protective Equipment (PPE) for safety issues

### Positive Control preparation

| | |
|---|---|
| Prepare all materials required:<br>1. Positive Control and Dropper from VitaPCR™ SARS-CoV-2 Gen 2 External Control Set.<br>2. VitaPCR™ Sample Collection Buffer (SCB) from VitaPCR™ Assay.<br>3. Rack from VitaPCR™ Instrument. |  |
| Place SCB Vial on the rack. |  |
| Open the Positive Control package.<br>Carefully take out the Positive Control Tube.<br>***NOTE: Before opening, please ensure that the Positive Control seal is not broken. If the seal is found broken, please discard and change to another new Positive Control.*** |  |
| Place the Positive Control Tube on the rack. |  |
| Unscrew the SCB Vial's cap. Place the opened SCB Buffer Vial back on the rack. |  |
| Gently tap to confirm that the Positive Control powder is sitting at the bottom of the Positive Control Tube.<br>Carefully remove the foil seal on the top of the Positive Control Tube. Ensure that the Positive Control powder is not spilled. |  |
| Place the opened Positive Control Tube on the rack. |  |

| | |
|---|---|
| Open the Dropper package. |  |
| Draw up the buffer solution from SCB Vial into the Dropper to a level between the two red lines as indicated in the figure on the right. (Around 100-200 µL) |    |
| Verify that the buffer volume in the Dropper is at the prescribed level. |  |
| Transfer all the drawn buffer solution from the Dropper to the Positive Control Tube. |  |
| Insert the Dropper tip below the liquid surface in the Positive Control Tube.<br>Continuously release and squeeze the Dropper bulb to pipette 10-30 times to fully reconstitute the Positive Control. |  |
| Transfer all mixed solutions from Positive Control Tube to SCB Vial.<br>***NOTE: Few drops of solution left in the Positive Control Tube would not affect the reaction and results.*** |  |
| Immediately discard the used Positive Control Tube and Dropper. |  |
| Recap the SCB Vial tightly and invert it 10 times to mix the solution thoroughly.<br>Please continue the assay testing by following the VitaPCR™ Assay protocol.<br>***NOTE: After Positive Control testing, please change gloves immediately and clean the equipment and working area to prevent cross-contamination.*** |   |

## Negative Control preparation

Take one new tube of Sample Collection Buffer (SCB) from VitaPCR™ Assay kit as the negative control.

## INTERPRETATION OF RESULTS AND REPORTING

The table below lists the expected results for the VitaPCR™ SARS-CoV-2 Gen 2 External Control test.

| Control Type | Composition | Used to Monitor | SARS-CoV-2 |
|---|---|---|---|
| Positive Control | SARS-CoV-2 N gene (*in vitro* transcribed RNA) | Reagent performance, Individual operator variation, and/or Device performance | + |
| Negative Control | Sample Collection Buffer (SCB) | Reagent and/ or environmental contamination | - |

## LIMITATIONS

1. For *in vitro* diagnostic use.
2. This External Control Set is intended for use in a molecular testing methodology only.

## CONTACT INFORMATION, ORDERING, AND PRODUCT SUPPORT

For technical and product support, please contact：service@credodxbiomed.com

## SYMBOLS

| | | | |
|---|---|---|---|
|  | Do Not Re-Use |  | Manufacturer |
|  | Consult Instructions for Use |  | Positive Control |
|  | Caution |  | Catalogue Number |
|  | Temperature Limit |  | Contains sufficient for <n> tests |
|  | Use-By Date |  | Keep away from sunlight |
|  | Batch Code |  | Keep Dry |
|  | *in vitro* diagnostic medical device |  | Authorized Representative in the European Community |
|  | Do not use if package is damaged |  | CE mark |

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## REVISION HISTORY

**Version Changes**: Version 2.0 to 3.0

| Document Version | Date | Revision |
|---|---|---|
| 3.0 | Oct 2022 | Revised section format |