# VereRT™ ZeroPrep™ COVID-19 PCR Kit Instructions for Use

| | |
|---|---|
| REF | VRTC-CD200 |
| Σ | 200 |
| | Store at -25°C to -15°C (frozen components) |
| | Veredus Laboratories Pte Ltd<br>83 Science Park Drive #04-02, The Curie,<br>Singapore Science Park 1,<br>Singapore 118258, Singapore |
| EC REP | MT Promedt Consulting GmbH<br>Altenhofstrasse 80,<br>66386 St. Ingbert, Germany |
| CE | European Union Conformity |
| IVD | *In Vitro* Diagnostic medical device |

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## Intended Use

VereRT™ ZeroPrep™ COVID-19 PCR Kit is a Reverse Transcription Polymerase Chain Reaction (RT-PCR)-based *In Vitro* Diagnostic (IVD) test intended for the qualitative detection of nucleic acid belonging to SARS-CoV-2. This test is suitable for use directly from either (1) Viral Transport Medium (VTM) / Universal Transport Medium (UTM) containing nasopharyngeal swab specimen; OR (2) Human Saliva specimen. Both specimen types do not require viral RNA extraction prior to testing.

The test results can be used as supplementary data for diagnosis. Negative result does not preclude SARS-CoV-2 infection and should not be used as a sole basis for treatment or other patient management decisions.

Testing with VereRT™ ZeroPrep™ COVID-19 PCR Kit is intended for use by trained laboratory professionals who are proficient in performing real-time RT-PCR assays.

## Summary and Explanation

Coronaviruses (CoV) are a family of viruses resulting in illnesses ranging from the common cold to the more severe disease such as Middle East Respiratory Syndrome (MERS-CoV), Severe Acute Respiratory Syndrome (SARS-CoV-1) and the most recent SARS-CoV-2, previously known as the 2019 novel coronavirus (2019-nCoV). Chinese authorities first identified SARS-CoV-2 and discovered it to be approximately 70% similar to SARS-CoV in genomic sequence.

Severe cases of infection may cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Since the outbreak, it is evident that SARS-CoV-2, especially with the emergence of virus variants, causes high incidences of transmission resulting in a pandemic situation, and as such, the need for an accurate and reliable test for surveillance and detection is essential.

## Principle of the Procedure

VereRT™ ZeroPrep™ COVID-19 PCR Kit contains specimen preparation reagent named as ZeroPrep™ M Buffer, that facilitate amplification of the viral RNA directly from VTM / UTM or human saliva. This kit also contains enzymes, oligonucleotide primers, dual-labelled hydrolysis probes and control material used in real-time RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2. These oligonucleotides were selected from two independent regions of the viral nucleocapsid (N) gene. An additional set of primer and probe to identify and detect the human RPP30 gene is also included in the primer-probe mix.

Viral RNA from SARS-CoV-2 is reverse transcribed to cDNA and subsequently amplified in the real-time PCR instrument. During this process, the probe anneals to specific target sequence located between the forward and reverse primers. During the extension phase of the Polymerase Chain Reaction (PCR) cycle, the 5’ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye and hence, generates a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, resulting in an increase in the fluorescence intensity which is monitored at every PCR cycle by a real-time PCR instrument.

## Kit Content

| **Catalog no.** | | **VRTC-CD200** |
|---|---|---|
| **No. of Tests** | | **200** |
| **Frozen Components** | **(-25 °C to -15 °C)** | **Quantity** |
| VRTC MM | (Enzyme Mix) | 1 tube |
| VRTC PPM A | (Primer Probe Mix) | 1 tube |
| VRTC PC A | (Positive Control) | 1 tube |
| ZeroPrep™ M Buffer | (Sample Prep Solution) | 1 tube |

**To prevent repeated freeze-thaw and avoid unnecessary carry-over contamination from VRTC PC A (Positive Control), it is recommended to prepare one-time use aliquots of VRTC PPM A in separate Nuclease-free tubes.**

**VRTC MM, VRTC PPM A and ZeroPrep™ M Buffer are to be kept at 2 °C to 8 °C after first use and the reagents are stable for up to 2 weeks in this condition.**

## Storage Condition

- Store all frozen kit components at -25°C to -15°C upon receipt.
- Keep kit components away from light until ready to use.

**NOTE**: **AVOID repeated thawing and freezing of frozen kit component. By doing so, it may affect overall performance of the assay**

- Keep all frozen components on ice block during usage.
- If left unopened, all kit components are stable until the expiration date indicated on respective component labels.
- DO NOT use kit components from different lots.

## Materials Required but Not Provided

a) Consumables
- Personal protective equipment
- Sterile filter pipette tips
- 1.5 mL microcentrifuge tubes
- Decontamination product

b) Equipment
- Real-time PCR Instrument (qPCR Thermal Cycler)
- Microcentrifuge for 1.5 mL tube
- Micropipettes (0.5-10 µL, 2-20 µL, 10-100 µL, 100-1000 µL)
- Freezer (-20°C)
- Refrigerator (4°C)
- Vortex Mixer

c) Additional Accessories
- Ice or cooler unit
- Tube rack / stand

## Warnings and Precautions

- For *In Vitro* Diagnostic use (IVD) use only.
- All specimens / samples should be treated as potentially infectious, unless otherwise proven.
- Specimen processing should be performed in accordance with national biological safety regulations.
- Perform all manipulations of live virus specimen / sample within a Class II (or higher) Biological Safety Cabinet (BSC).
- Wear appropriate Personal Protective Equipment (PPE), including (but not limited to) protective disposable gloves, laboratory coats and eye protection when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results may occur if either the clinical specimen or the PCR reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Clean and decontaminate work area and instruments, including pipettes, with commercially available decontamination products.
- A designated working area should be dedicated for processing specimens and to add specimens to RT-PCR Mix.
- Use sterile pipette tips with filters.
- Do not use kit or reagents beyond expiration dates shown on the respective label.
- Follow laboratory safety rules and procedures as defined by approved biohazard safety guidelines or regulations.
- Discard waste according to local safety regulations.
- Material Safety Data Sheets (MSDS) are available upon request.

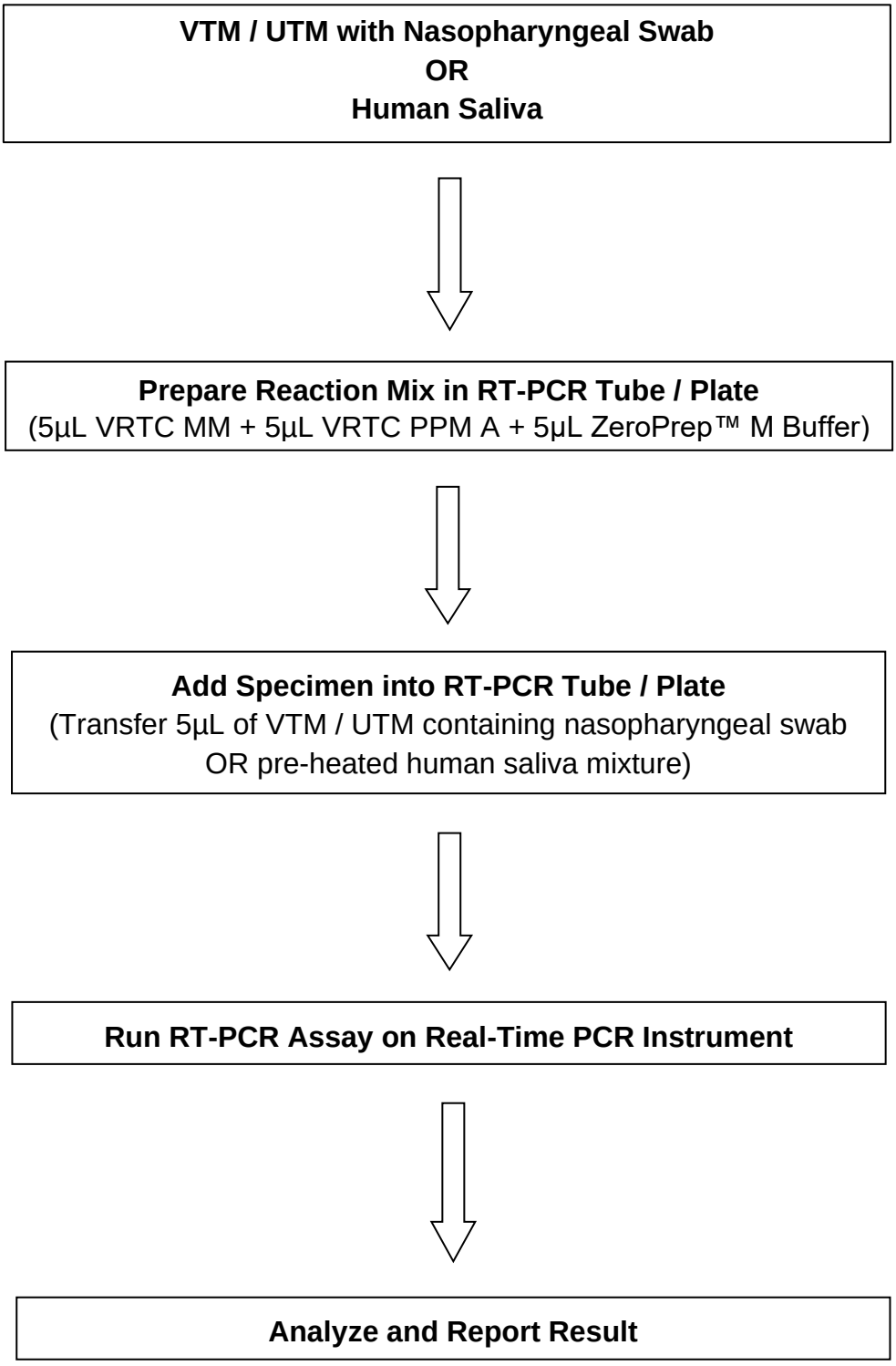
## Additional Precautions when Handling Clinical Specimens

- Designate a dedicated working area for handling of clinical specimens.
- Change gloves when moving between working areas to prevent cross-contamination.
- Treat all patient specimens as potentially infectious and handle with caution.
- Handle all specimens using safe laboratory practices. Refer to your local authority for guidelines. (For more information, you may also refer to: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html).
- Patient specimens should be processed in accordance with national biological safety regulations.
- Personal protective equipment such as gloves, eye protection, and lab coats must be worn when handling clinical specimens.
- Procedures that involve generating aerosols, e.g. aliquoting of specimens, mixing (or vortexing) of specimens should be performed in at least a BSL2 or higher certified biological safety hood.
- The exterior of consumables such as PCR plates, PCR strips, PCR plate seals, etc, at high risk of being contaminated with pathogens should be properly decontaminated before being moved to a PCR machine.
- Disinfect work area and instruments thoroughly with disinfecting solution such as 70%(v/v) ethanol before and after use.
- It is recommended to wear double or more layers of gloves while working with clinical specimens.
- Dispose of contaminated consumables such as gloves and tubes according to your local authority's guidelines.

## Quality Control

Under Veredus quality assurance program, the performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit is monitored routinely to ensure consistent product quality. Sampling is done on every manufactured lot and tests carried out via amplification of the control templates.

## Workflow


VTM / UTM with Nasopharyngeal Swab
OR
Human Saliva
Prepare Reaction Mix in RT-PCR Tube / Plate
(5µL VRTC MM + 5µL VRTC PPM A + 5µL ZeroPrep™ M Buffer)
Add Specimen into RT-PCR Tube / Plate
(Transfer 5µL of VTM / UTM containing nasopharyngeal swab
OR pre-heated human saliva mixture)
Run RT-PCR Assay on Real-Time PCR Instrument
Analyze and Report Result

## Specimen Collection, Handling and Storage

Specimens should be collected, handled and stored following the user institution's standard procedures. Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results. Training in specimen collection is highly recommended to protect the quality of specimens before testing.

## Specimen Type

1) Direct use of VTM / UTM containing nasopharyngeal swab specimen without the need for any RNA extraction

   For direct testing using VTM / UTM containing nasopharyngeal swab specimen, this kit has been validated with 5 different brands of VTM / UTM for compatibility usage as listed in the table below.

| **Compatible VTM/ UTM** | | |
|---|---|---|
| **Product Brand** | **Product Name** | **Catalog Number** |
| Cepheid® | Nasopharyngeal Collection and Transport System Xpert® Sterile | SWAB/B-100 |
| Precision Medical Instruments | Disposable Virus Sampling Tube (non-inactivating) | D-3ml |
| Copan | UTM: Viral Transport | 330C |
| Mantacc | Nasal Flocked Swab with Transport Medium | MSC-96000<br>MCP-010D |
| Citotest Labware Manufacturing Co Ltd | Citoswab® Collection and Transport Kit | 2118-1504-99 |

**If the VTM/ UTM to be used is not within the recommended list, it is advisable that the user validates its compatibility with the assay.**

2) Human saliva

   For direct testing using human saliva specimen, this kit has been validated for use with human saliva collected using ZeroPrep™ Saliva Collection Kit (Cat. No. VRTC-RE025).

## Real-Time PCR Instruments

The following real-time PCR instruments are recommended*:

- Bio-Rad CFX96 Series of Real-Time PCR Detection System
- Applied Biosystems™ QuantStudio™ Series of Real-Time PCR Systems
- Applied Biosystems™ 7500 Fast Real-Time PCR Systems

*It is advisable for the user to validate the real-time PCR instrument should the user utilize an instrument that is not within the abovementioned recommended list.

## Assay Setup Procedures

**Note:** The preparation of the reaction mix should preferably be performed in a template-free hood / area / zone to prevent possible cross-contamination with amplifiable SARS-CoV-2 or human specimen template.

i) In the template-free workspace, fully thaw the VRTC MM, VRTC PPM A, VRTC PC A and ZeroPrep™ M Buffer on ice or cold-block. Keep cold during preparation and use.
ii) Determine the number of reactions required. It is necessary to make excess Reaction Mix for controls and pipetting variations.
iii) Perform a quick centrifugation for VRTC MM, VRTC PPM A, VRTC PC A and ZeroPrep™ M Buffer to collect content at the bottom of each tube. Place tubes back in the cold block.
iv) Prepare Reaction Mix according to table below. Mix the Reaction Mix by pipetting up and down. DO NOT VORTEX.

Prepare the Reaction Mix accordingly:

| Reagent | Volume for Single Reaction | Volume required for 'X+2' Reactions^ |
|---|---|---|
| VRTC PPM A | 5 µL | (5 x 'X+2') µL |
| VRTC MM | 5 µL | (5 x 'X+2') µL |
| ZeroPrep™ M Buffer | 5 µL | (5 x 'X+2') µL |
| **Total** | **15 µL** | |

^ Variable X refers to the total number of reactions to be tested on the real-time PCR plate, thus variable 'X+2' is the total number of reactions plus an additional 2 reactions. This will account for potential pipetting error in the process. Reminder to factor in the number of PCR reactions for No Template Control ("NTC"), Positive Control ("PC"), and replicates if any.

v) Set up RT-PCR tube / plate.

vi) Dispense Reaction Mix into each reaction tube / well going across the row as shown below for example.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| B | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| C | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| D | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| E | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| F | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| G | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |
| H | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix | Rxn Mix |

vii) Transfer the RT-PCR tube / plate to the template area OR specimen handling area (BSL II and above).

viii) Don the necessary PPE before handling template / specimen potentially containing live virus.

ix) Carefully pipette template / specimen and controls into designated tube / well accordingly:

- For direct testing using VTM / UTM containing nasopharyngeal swab specimen
  - Mix the VTM / UTM gently by pipetting up and down with a pipette set at about 50% of total volume of VTM / UTM in the tube. Add 5 µL of VTM / UTM into each sample tube / well (e.g. “S1” to “S46”).
  - For No Template Control (“NTC”), add 5 µL of nuclease-free water OR VTM / UTM without swab specimen into designated tube / well labeled as “NTC”.
  - For Positive Control (“PC”), add 5 µL of VRTC PC A in place of the sample into designated tube / well labeled as “PC”.

- For direct testing using human saliva
  - Add 5 µL of pre-heated saliva mixture (refer to IFU of ZeroPrep™ Saliva Collection Kit) into each sample tube / well (e.g. “S1” to “S46”).

- For No Template Control ("NTC"), add 5 µL of nuclease-free water or ZeroPrep™ Saliva Buffer mixed with equal volume of nuclease-free water in place of the sample into designated tube / well labeled as "NTC".
- For Positive Control ("PC"), add 5 µL of VRTC PC A in place of the sample into designated tube / well labeled as "PC".

x) Total volume of each reaction tube / well would be 20 µL.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | PC |
| B | NTC | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | PC |
| C | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 | S19 | S20 | S21 | S22 |
| D | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 | S19 | S20 | S21 | S22 |
| E | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | S31 | S32 | S33 | S34 |
| F | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | S31 | S32 | S33 | S34 |
| G | S35 | S36 | S37 | S38 | S39 | S40 | S41 | S42 | S43 | S44 | S45 | S46 |
| H | S35 | S36 | S37 | S38 | S39 | S40 | S41 | S42 | S43 | S44 | S45 | S46 |

xi) Prepare the thermal cycling protocol on the real-time PCR instrument:

- Thermal cycling protocol for Bio-Rad CFX96 Real-Time Detection System:

| **Bio-Rad CFX96 Real-time Detection System** | | | |
|---|---|---|---|
| **Step** | **Cycle** | **Temp** | **Time** |
| UNG Incubation | 1 | 25 ºC | 2 min |
| RT incubation | 1 | 50 ºC | 15 min |
| Enzyme activation | 1 | 95 ºC | 2 min |
| Amplification | 45 | 95 ºC | 3 sec |
| | | 55 ºC (Data Collection) | 30 sec |

- Thermal cycling protocol for real-time PCR instruments with fast-temperature ramping option (E.g., QuantStudio™):

| QuantStudio™ (or related) Real-Time PCR Instrument | | | |
|---|---|---|---|
| **Step** | **Cycle** | **Temp** | **Time** |
| **Adjust ramp rate (heating / cooling) to 2.7ºC / sec** | | | |
| UNG Incubation | 1 | 25 ºC | 2 min |
| **Adjust ramp rate (heating) to 2.7ºC / sec** | | | |
| RT Incubation | 1 | 50 ºC | 15 min |
| **Adjust ramp rate (heating) to 2.7ºC / sec** | | | |
| Enzyme Activation | 1 | 95 ºC | 2 min |
| Amplification | 45 | **Adjust ramp rate (heating) to 2.7ºC / sec** | |
| | | 95 ºC | 3 sec |
| | | **Adjust ramp rate (cooling) to 2.1ºC / sec** | |
| | | 55 ºC (Data Collection) | 30 sec |

QuantStudio™ instruments may have a default ramping rate that is different from the recommended protocol. Please adjust the ramp rate accordingly.

xii) Recommended fluorophore detection configurations on different real-time PCR systems.

| | **Fluorescence detection configurations of different Real-Time PCR Systems** | | | |
|---|---|---|---|---|
| **Targets** | **CFX96** | **QuantStudio™** | **7500 Fast** | **Quencher Dye** |
| SARS-CoV-2 N gene | FAM | FAM | FAM | none |
| Human RPP30 gene Human Internal Control (HIC) | HEX | VIC™ | HEX | none |

| | | | | |
|---|---|---|---|---|
| Passive reference dye | N.A. | ROX | CY5 | N.A. |

xiii) Passive reference detection: Optional but recommended, if available.
xiv) Run mode: Standard
xv) Reaction volume: 20 µL

## Assay Controls

The following controls are included in the assay

i) Human Internal Control (HIC): This control is built into each reaction to monitor for the quality of the swab / saliva specimen (if applicable), sample extraction efficacy (if applicable) as well as RT-PCR inhibition.
ii) Positive Control (PC): VRTC PC A is included in the kit and should be used for each set of test run to monitor the kit performance.
iii) No Template Control (NTC): VTM without sample, nuclease-free water or ZeroPrep™ Saliva Buffer mixed with equal volume of nuclease-free water should be included in place of the sample for each set of test run to monitor kit contamination.

## Interpretation of Results

**Baseline threshold adjustment**

Baseline threshold judgement by associated data analysis program used by the real-time PCR instrument used should be adjusted to 'Auto' for the 2 fluorophore detection channels, FAM (channel 1) and HEX / VIC™ (channel 2).

**No Template Control (NTC)**

NTC should be negative (Ct undetermined or Ct > 40). If NTC exhibits an amplification curve that crosses the signal threshold before cycle 40, kit contamination may have occurred. Invalidate the run and repeat the test assay with strict adherence to the sample and reagent handling guidelines. Determine the cause of failed NTC and implement appropriate corrective actions.

**Positive Control (PC)**

PC consists of plasmids containing the nucleocapsid (N) gene sequence from SARS-CoV-2 and partial RPP30 gene sequence from human. PC should be positive (exhibits exponential amplification curve and Ct ≤ 40) for both signal channels (FAM and HEX / VIC™). If not, invalidate the run and repeat the test assay with strict adherence to the sample and reagent handling guidelines. Determine the cause of failed PC and implement appropriate corrective actions.

Only when both the NTC and PC exhibit the expected performance can the test results be interpreted according to the following table:

| No. | COV (FAM) | HIC (HEX / VIC™) | Diagnostic Outcome |
|---|---|---|---|
| 1 | Ct > 40 or Ct undetermined | Exponential amplification curve[1] and Ct ≤ 40[2] | Negative for SARS-CoV-2 |
| 2 | Exponential amplification curve[1] and Ct ≤ 40 | Exponential amplification curve[1] and Ct ≤ 40[2] | Positive for SARS-CoV-2 |
| 3 | Ct > 40 or Ct undetermined | Ct > 40 or Ct undetermined[2] | Invalid Result[3] |

[1] It is important to ensure that the amplification curve is exponential, not linear, before proceeding to determine any Ct value. Repeat the test assay if amplification curve is not exponential.
[2] For testing on human specimen, HIC shall produce amplification curve of Ct ≤ 40 to indicate the presence of human cells and no significant inhibition of RT-PCR. If HIC produces amplification of Ct > 40 or Ct is undetermined, it is advisable to repeat the test assay. Upon retest and if HIC remains at Ct > 40 or Ct is undetermined, it is advisable to repeat specimen collection from the patient with strict adherence to the specimen collection instructions.
[3] May be due to failed specimen collection, unsuccessful viral RNA extraction or RT-PCR Inhibition. Users are advised to repeat the test assay. If test result is still invalid, repeat specimen collection from the patient with strict adherence to the specimen collection instructions.

## Limitations of the Test

- Use of this kit should be limited only to trained personnel.
- This test is a qualitative assay and does not provide a quantitative value for the detected pathogen in the specimen collected.
- Strict compliance with this IFU is required for optimal results. Modifications to the instructions may alter performance of this test.
- Appropriate specimen collection, handling, storage and processing procedures are required for optimal performance of this test.
- Results from this test should be interpreted with other laboratory data and clinical information made available.
- Although this kit is highly specific and sensitive, a low incidence of false results may occur. A negative result from this test does not preclude the possibility of existence

of the target organism in the specimen collected. Other available tests are required if questionable results are obtained.

- A specimen yielding a negative result from this test may contain viruses other than SARS-CoV-2.
- Mutation(s) within the target regions covered by primers and/or probes used in this test may result in failure to detect the target organism.
- The prevalence of infection will affect the predictive value of this test.
- False negative results may occur due to presence of sequence variant in the viral target, procedural error, RT-PCR inhibitor in specimen, or inadequate viral nucleic acid for amplification.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids, amplicon, or from non-specific signal in this test.
- Viral nucleic acids may persist *in vivo* independent of virus viability. Detection of analyte target(s) do not imply that the corresponding virus is infectious.
- Inclusivity to target strains was evaluated by *in silico* analysis only. Due to the high genetic diversity of *Coronaviridae* and high rate of mutation, some viral strains may not be detected or may be detected with reduced sensitivity.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.

## Performance Characteristics

### 1. Analytical Sensitivity (Limit of Detection LOD)

The LOD of the test was determined using commercially available inactivated SARS-CoV-2 virus (PN: VR-1986HK), of which the viral copy number was quantified in-house against characterized stock of synthetic SARS-CoV-2 N gene standard. The LOD was defined as the lowest detectable concentration of SARS-CoV-2 at which 95% of all 20 replicates tested were positive.

<u>To determine LOD using VTM / UTM containing nasopharyngeal swab specimen as the input material</u>

A series of known concentrations of inactivated SARS-CoV-2 virus were spiked into Viral Transport Medium (VTM) GeneXpert® Nasopharyngeal Sample Collection Kit (PN: SWAB/B-100) in the presence of SARS-CoV-2 virus negative human nasopharyngeal swab specimen to mimic a clinical sample. The VTM samples, without undergoing RNA

extraction, were directly tested over 20 replicates using the VereRT™ ZeroPrep™ COVID-19 PCR assay. Based on the study results, the LOD was determined to be the equivalent of **10 virus copies per test assay reaction** OR **2 virus copies per µL of VTM / UTM**.

To determine LOD using human saliva specimen as the input material

SARS-CoV-2 negative human saliva were collected using ZeroPrep™ Saliva Collection Kit before being spiked with a series of inactivated SARS-CoV-2 virus of known concentrations. The contrived saliva samples were heated in accordance to the saliva collection kit's IFU. The heated contrived saliva samples were directly tested over 20 replicates using the VereRT™ ZeroPrep™ COVID-19 PCR Kit. Based on the study results, the LOD of the kit using saliva as a sample type was determined to be **10 virus copies per test assay reaction** OR **4 virus copies per µL human saliva**.

## 2. Analytical Sensitivity (Inclusivity) Analysis

SARS-CoV-2 is a single-stranded RNA virus, which is known for rapid mutation in its genomic sequence. In order to ensure that VereRT™ ZeroPrep™ COVID-19 PCR Kit maintains its performance to detect all known viral strains, especially those that the World Health Organization (WHO) classifies as Variants of Concern (VOC) such as Alpha, Beta, Gamma and Delta. *In silico* inclusivity analyses based on BLAST analysis were performed against known SARS-CoV-2 viral genome sequences retrieved from both GISAID and NCBI databases to determine SARS-CoV-2 virus variant coverage by VereRT™ ZeroPrep™ COVID-19 PCR Kit.

The design of VereRT™ ZeroPrep™ COVID-19 PCR Kit encompasses two independent sets of primers and probe responsible for the detection of two different sites of the viral nucleocapsid (N) gene, the same sets of primers and probe as VereRT™ COVID-19 PCR Kit. Primer and probe sequences of both sets were matched against all genomic sequences in the aforementioned databases and as of November 2021, amongst an estimate of 1.9 million isolates of SARS-CoV-2, there is 1 isolate within Delta variant with mutation(s) in both target regions, that may escape detection using VereRT™ ZeroPrep™ COVID-19 PCR Kit.

The dual target design on the viral N gene dramatically enhanced the tolerance of any mutation in viral genome, as only when both target regions are mutated simultaneously in a viral strain and the mutations result in drastic decrease of the Tm of the primers and probes, the detection of the strain could be potentially affected. Out of 1,905,947 sequences, the analysis identified 1,091 sequences having mismatch within both N gene target regions that lowers the Tm below 57°C. All detected mutations corresponding to

the primers and probes used in the kit are consolidated with their corresponding mutant amplicons synthesized. The functional validation was performed using VereRT™ COVID-19 PCR Kit in place of VereRT™ ZeroPrep™ COVID-19 PCR Kit because it was expected that mutation tolerance between the 2 assays would be very similar given that both assays' core components comprising primers, probes, reverse transcriptase, and polymerase are exactly the same, except that the latter contains ZeroPrep™ M Buffer. The validation study has conclusively shown that all mutants pertaining to the 1,091 sequences could be successfully detected by the kit. The adjusted detection coverage is summarized in the table below.

| Database | GISAID | NCBI |
|---|---|---|
| Date of Analysis | 10 Nov 2021 | 10 Nov 2021 |
| Genome Count | 1,493,830 | 412,117 |
| *In silico* Strain Coverage | 99.93% | 99.99% |
| Adjusted Strain Coverage | 100%* | 100%* |

*: Adjusted based on functional validation data

Summary of BLAST and functional validation results

## 3. Analytical Specificity (Cross-Reactivity) Analysis

To further substantiate that VereRT™ ZeroPrep™ COVID-19 PCR Kit is specific for detecting SARS-CoV-2 in clinical specimens, the primer and probe sequences of VereRT™ ZeroPrep™ COVID-19 PCR Kit were matched against representative genomic sequences of closely related coronaviruses such as SARS-CoV-1 and MERS-CoV, other respiratory pathogens such as Influenza A and B, and commensals in human respiratory tract such as *Staphylococcus aureus* and *Candida albicans*. A total of 36 pathogens and 81 representative genomes were analyzed. From this *in silico* analysis, there is one genome of *Mycobacterium tuberculosis* that has homology sequence to one of the two primer-probe sets. However, the homologous regions are more than 1 million bases apart from each other in the genome. Therefore, VereRT™ ZeroPrep™ COVID-19 PCR Kit is unlikely to give rise to false positive results due to cross-reactivity. The analysis results are summarized in the following table.

| | | | Target 1 | | | Target 2 | | |
|---|---|---|---|---|---|---|---|---|
| | | | Forward 1 | Reverse 1 | Probe 1 | Forward 2 | Reverse 2 | Prob 2 |
| **Pathogen** | **Strain** | **GenBank Acc#** | **% Homology** | **% Homology** | **% Homology** | **% Homology** | **% Homology** | **% Homology** |
| Human coronavirus 229E | 229E | NC_002645.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Human coronavirus OC43 | OC43 | NC_006213.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Human coronavirus HKU1 | HKU1 | NC_006577.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Human coronavirus NL63 | NL63 | NC_005831.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| SARS-coronavirus | Tor2 | NC_004718.3 | 0 | 91.67 | 91.67 | 100 | 0 | 0 |
| MERS-coronavirus | England 1 | NC_038294.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | HCoV-EMC | NC_019843.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| Adenovirus (e.g. C1 Ad. 71) | Type 1 subgroup C | AF534906.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Human Metapneumovirus (hMPV) | 00-1 | NC_039199.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Parainfluenza virus 1-4 | Washington 1964 | NC_003461.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Influenza A | A/New York/392/2004(H3N2) | NC_007366.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007367.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007368.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007369.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007370.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007372.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007373.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_007371.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | A/Alabama/03/2019(H1N1) | MK630774.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630773.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630772.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630771.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630770.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630769.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630768.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | MK630767.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Influenza B | B/Lee/1940 | NC_002204.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002211.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002210.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002209.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002208.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002207.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002206.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | NC_002205.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Enterovirus (e.g. EV68) | Fermon | NC_038308.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Respiratory syncytial virus | S2 ts1C | NC_001803.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Rhinovirus | ATCC VR-1559 | NC_038311.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chlamydia pneumonia* | CWL029 | NC_000922.1 | 0 | 0 | 0 | 0 | 66.67 | 0 |
| *Haemophilus influenzae* | Rd KW20 | NC_000907.1 | 70 | 0 | 0 | 85 | 66.67 | 0 |
| *Legionella pneumophila* | Philadelphia 1 | NC_002942.5 | 80 | 66.67 | 66.67 | 70 | 0 | 56.52 |
| *Mycobacterium tuberculosis* | H37Rv | NC_000962.3 | 75 | 0 | 0 | 80 | 72.22 | 86.96 |
| *Streptococcus pneumonia* | R6 | NC_003098.1 | 80 | 0 | 0 | 60 | 0 | 65.22 |
| *Streptococcus pyogenes* | M1 GAS | NC_002737.2 | 75 | 54.17 | 0 | 70 | 0 | 0 |
| *Bordetella pertussis* | Tohama I | NC_002929.2 | 75 | 0 | 66.67 | 60 | 72.22 | 0 |
| *Mycoplasma pneumoniae* | M129 | NC_000912.1 | 0 | 0 | 54.17 | 0 | 0 | 0 |
| Influenza C | C/Ann Arbor/1/50 | NC_006312.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006310.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006309.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006308.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006307.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006306.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | C/Ann Arbor/1/50 | NC_006311.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Parechovirus | EV22, Harris | NC_038319.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gregory | NC_001897.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Candida albicans* | SC5314 | NC_032089.1 | 0 | 0 | 54.17 | 65 | 0 | 0 |
| | SC5314 | NC_032090.1 | 65 | 0 | 0 | 70 | 0 | 0 |
| | SC5314 | NC_032091.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | SC5314 | NC_032092.1 | 0 | 0 | 0 | 0 | 0 | 56.52 |
| | SC5314 | NC_032093.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | SC5314 | NC_032094.1 | 65 | 0 | 66.67 | 0 | 0 | 0 |
| | SC5314 | NC_032095.1 | 0 | 0 | 0 | 80 | 0 | 0 |
| | SC5314 | NC_032096.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Corynebacterium diphtheriae* | NCTC11397 | NZ_LN831026.1 | 70 | 54.17 | 0 | 0 | 72.22 | 56.52 |
| *Bacillus anthracis(Anthrax)* | Ames | NC_003997.3 | 0 | 62.5 | 0 | 65 | 0 | 0 |
| *Moraxella cararrhalis* | BBH18 | NC_014147.1 | 70 | 54.17 | 0 | 65 | 0 | 78.26 |
| *Neisseria elongata and miningitidis* | MC58 | NC_003112.2 | 85 | 54.17 | 0 | 0 | 0 | 0 |
| | ATCC 29315 | NZ_CP007726.1 | 80 | 0 | 0 | 65 | 0 | 0 |
| *Pseudomonas aeruginosa* | PAO1 | NC_002516.2 | 0 | 54.17 | 54.17 | 0 | 0 | 73.91 |
| *Staphylococcus epidermis* | ATCC 12228 | NC_004461.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ATCC 12228, plasmid | NC_005008.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ATCC 12228, plasmid | NC_005007.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ATCC 12228, plasmid | NC_005006.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| | ATCC 12228, plasmid | NC_005005.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Streptococcus salivarius* | NCTC 8618 | NZ_CP009913.1 | 0 | 0 | 0 | 75 | 0 | 0 |
| *Leptospirosis* | 56601 | NC_004342.2 | 65 | 0 | 0 | 65 | 72.22 | 0 |
| | 56601 | NC_004343.2 | 0 | 0 | 0 | 65 | 0 | 0 |
| *Chlamydia psittaci* | 6BC | NC_017287.1 | 75 | 0 | 0 | 0 | 0 | 0 |
| | 6BC, plasmid | NC_017288.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Coxiella burnetii (Q-Fever)* | RSA 493 | NC_002971.4 | 0 | 83.33 | 0 | 0 | 0 | 69.57 |
| | RSA 493, plasmid | NC_004704.2 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Streptococcus aureus* | NCTC 8325 | NC_007795.1 | 70 | 54.17 | 0 | 65 | 0 | 0 |

Result summary of *in silico* cross reactivity analysis

To validate the assay for specificity to SARS-CoV-2 virus and cross reactivity with other common respiratory pathogens, especially phylogenetically similar pathogens such as Coronaviruses, VereRT™ ZeroPrep™ COVID-19 PCR Kit was challenged with nucleic acids extracted from 22 inactivated respiratory pathogens which formed part of the NATtrol™ Respiratory Verification Panel 2.1 (P/N: NATRVP2.1-BIO). The validation study showed that the assay did not cross-react with any of the 22 respiratory pathogens, especially Coronaviruses HKU-1, NL63, OC43, and 229E, providing support for the assay's specificity to only SARS-CoV-2 virus, and not other organisms or even phylogenetically related Coronaviruses. For a full list of the pathogens, please refer to the following table:

| Panel Members | Strain |
|---|---|
| Adenovirus 1 | N/A |
| Adenovirus 3 | N/A |
| Adenovirus 31 | N/A |
| *B. parapertussis* | A747 |
| *B. pertussis* | A639 |
| *C. pneumoniae* | CWL-029 |
| Coronavirus 229E | N/A |
| Coronavirus HKU-1 | Recombinant |
| Coronavirus NL63 | N/A |
| Coronavirus OC43 | N/A |
| Influenza A H1N1pdm | A/NY/02/09 |
| Influenza AH1 | A/New Caledonia/20/99 |
| Influenza AH3 | A/Brisbane/10/07 |
| Influenza B | B/Florida/02/06 |
| *M. pneumoniae* | M129 |
| Metapneumovirus 8 | Peru6-2003 |
| Parainfluenza 1 | N/A |
| Parainfluenza 2 | N/A |
| Parainfluenza 3 | N/A |
| Parainfluenza 4 | N/A |
| Rhinovirus 1A | N/A |
| Respiratory Syncytial Virus A | N/A |
| SARS-CoV-2 | USA-WA1/2020 |

## 4. Repeatability and Reproducibility

The repeatability and reproducibility of the test kit were validated by performing 3 rounds of testing on 3 different days by 3 different operators with 3 different lots of reagents and saliva from 3 different healthy donors. 4 copies of inactivated virus per μl of VTM from GeneXpert or saliva was used as sample for the 2 sample types. The RT-PCR reaction was conducted in Bio-Rad CFX96™ Real-Time PCR System.

For VTM samples, 20 replicates were run in each round of testing. The variation of Ct values within each round represents the repeatability of the test and the overall variation of Ct values across the 3 rounds represent the reproducibility of the test. Ct mean, Ct standard deviation (SD) and coefficient of variation (%CV) are summarized in the table below. VereRT™ ZeroPrep™ COVID-19 PCR Kit is consistently repeatable and reproducible with VTM samples.

| Sample | Operator | Test Date | SARS-CoV-2 (FAM) | | | HIC (HEX/VIC) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ct Mean | Ct SD | %CV | Ct Mean | Ct SD | %CV |
| 20 virus copies/ reaction | 1 | 29/07/2020 | 32.30 | 0.51 | 1.58 | 31.28 | 0.50 | 1.61 |
| | 2 | 30/07/2020 | 31.28 | 0.44 | 1.42 | 31.65 | 0.81 | 2.56 |
| | 3 | 31/07/2020 | 32.64 | 0.37 | 1.13 | 32.51 | 0.48 | 1.47 |
| **Overall** | | | **32.08** | **0.73** | **2.27** | **31.81** | **0.80** | **2.51** |

Result summary of repeatability and reproducibility validation for VTM

For saliva samples, 10 replicates were run in each round of testing. The variation of Ct values within each round represents the repeatability of the test. Reproducibility of the test was calculated by percentage of the samples giving expected positive results across 3 different runs as the saliva samples were from different donors, variation of Ct values across the 3 rounds were comparable. The test results are similarly summarized in the table below. VereRT™ ZeroPrep™ COVID-19 PCR kit is consistently repeatable and reproducible with saliva samples collected and processed using ZeroPrep™ Saliva Collection Kit.

| Sample | Operator | Test Date | SARS-CoV-2 (FAM) | | | HIC (HEX/VIC) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ct Mean | Ct SD | %CV | Ct Mean | Ct SD | %CV |
| 10 virus copies/ reaction | 1 | 05/10/2020 | 32.67 | 0.85 | 2.61 | 22.10 | 0.19 | 0.85 |
| | 2 | 07/10/2020 | 30.35 | 0.24 | 0.78 | 26.79 | 0.26 | 0.96 |
| | 3 | 08/10/2020 | 34.74 | 1.22 | 3.51 | 28.90 | 0.18 | 0.62 |
| Overall Reproducibility | | | 100% | | | | | |

Result summary of repeatability and reproducibility validation for saliva samples

## 5. Validation of VTM / UTM

Validation was performed on 5 different brands of VTM / UTM (refer to table below). In summary, inactivated SARS-CoV-2 virus was spiked in each of the VTM / UTM containing human nasopharyngeal swab samples, at viral copy number of 2 copies per μl of VTM / UTM. The VTM sample is subjected to VereRT™ ZeroPrep™ COVID-19 PCR Kit assay in 20 replicates.

| Product Brand | Product Name | Catalog Number |
|---|---|---|
| Cepheid | Nasopharyngeal Collection and Transport System Xpert® Sterile | SWAB/B-100 |
| Citotest Labware Manufacturing Co Ltd | Citoswab® Collection and Transport Kit | 2118-1504-99 |
| Precision Medical Instruments | Disposable Virus Sampling Tube (non-inactivating) | D-3ml |
| Copan | UTM: Viral Transport | 330C |
| Mantacc | Nasal Flocked Swab with Transport Medium | MSC-96000 MCP-010D |

As summarized in the table below, the Ct standard deviation (SD) and coefficient of variation (%CV) for both detection channels within a round of test are less than 1 and 3% respectively, and overall Ct SD and %CV across all three rounds of test are not greater than 1.6 and 5% respectively. Hence, detection of 10 copies of virus collected in the 5 different brands of VTM / UTM containing nasopharyngeal specimens using VereRT™ ZeroPrep™ COVID-19 PCR Kit has been validated to be reproducible.

| Sample | VTM/ UTM | SARS-CoV-2 (FAM) | | | HIC (HEX/VIC) | | |
|---|---|---|---|---|---|---|---|
| | | Ct Mean | Ct SD | %CV | Ct Mean | Ct SD | %CV |
| 10 virus copies/ reaction | Cepheid® | 32.06 | 0.32 | 0.99 | 31.32 | 0.64 | 2.05 |
| | Precision | 32.16 | 0.46 | 1.42 | 31.79 | 0.49 | 1.55 |
| | Mantacc | 31.8 | 0.30 | 0.95 | 32.77 | 0.95 | 2.90 |
| | Copan | 31.42 | 0.51 | 1.62 | 29.52 | 0.52 | 1.76 |
| | Citotest | 32.33 | 0.67 | 2.08 | 33.68 | 0.87 | 2.58 |
| **Overall** | | **31.95** | **0.56** | **1.76** | **31.81** | **1.58** | **4.97** |

## 6. Interference Studies

In order to rule out detection interference of SARS-CoV-2 virus in samples tested using the VereRT™ ZeroPrep™ COVID-19 PCR kit, VTM and saliva samples containing drugs/ substances commonly administered nasally and/or orally were tested. The substances tested for VTM and saliva samples are listed in the tables below.

| Substances | Active Ingredient | Amount tested in VTM (Actuation) |
|---|---|---|
| Nasonex Aqueous Nasal Spray | Mometasone furoate | 100μg (2) |
| Nazolin Nasal Spray | Oxymetazoline | 50μg (2) |
| Seretide EVOHALER | Salmeterol xinafoate | 50μg (2) |
| | Fluticasone propionate | 250μg (2) |
| Salbuair inhaler | Salbutamol | 200μg (2) |
| Saliva | Mucin | ~60μg/mL (NA) |
| Whole blood | Haemoglobin/ plasma | 0.25%(v/v) (NA) |

List of Substances tested in VTM samples.

| Substances | Active Ingredient | Amount tested in saliva |
|---|---|---|
| Nasonex Aqueous Nasal Spray | Mometasone furoate | 1%(v/v) |
| Seretide EVOHALER | Salmeterol xinafoate<br>Fluticasone propionate | 1%(v/v)<br>1%(v/v) |
| Salbuair inhaler | Salbutamol | 1%(v/v) |
| Whole blood | Haemoglobin/ plasma | 0.25%(v/v) |
| Mouth wash | Sodium fluoride | 0.5%(v/v) |

List of substances tested in the saliva samples

For VTM samples, nasopharyngeal swabs containing the recommended dosing of each potential interfering substances were inoculated into VTM before being spiked with

inactivated SARS-CoV-2 virus at concentration of 2 viral copies per µl of VTM. The resulting samples in the presence of the various potential interfering substances were tested using VereRT™ ZeroPrep™ COVID-19 PCR kit.

The table below summarizes the performance of the assay in the presence of interfering substances. It is evident from the table that there is minimal interference to the detection of SARS-CoV-2 virus in the presence of commonly used potential interfering substances.

| Sample | Interfering substances | SARS-CoV-2 (FAM) | | | HIC (HEX/VIC) | | |
|---|---|---|---|---|---|---|---|
| | | Ct Mean | Ct SD | %CV | Ct Mean | Ct SD | %CV |
| 10 virus copies/ reaction | Mometasone | 31.94 | 0.51 | 1.60 | 31.28 | 0.38 | 1.22 |
| | Oxymetazoline | 32.19 | 0.21 | 0.65 | 31.73 | 0.38 | 1.21 |
| | Salmeterol/ Fluticasone | 32.34 | 0.86 | 2.65 | 31.45 | 0.05 | 0.16 |
| | Salbutamol | 32.79 | 0.89 | 2.72 | 30.87 | 0.98 | 3.17 |
| | Mucin | 33.49 | 0.23 | 0.69 | 24.77* | 0.50 | 2.01 |
| | Whole blood | 34.03 | 0.39 | 1.14 | 31.43 | 1.17 | 3.72 |
| | None | 33.01 | 0.62 | 1.89 | 31.36 | 0.54 | 1.73 |
| **Overall** | | **32.83** | **0.86** | **2.63** | **31.35** | **0.64** | **2.05** |

Result summary of interference study for VTM samples.

** HIC Ct values from Mucin were excluded in overall statistics as saliva introduced more HIC templates in the samples.*

Saliva samples were collected using the ZeroPrep™ Saliva Collection Kit and then spiked with potential interfering substances together with inactivated viruses. Each test was performed using 50 viral copies per reaction, which translates to 20 viral copies per µl of saliva. The resulting samples in the presence of the various potential interfering substances were tested using VereRT™ ZeroPrep™ COVID-19 PCR Kit.

The table below summarizes the performance of the assay in the presence of interfering substances. It is evident from the table that there is minimal interference to the detection of SARS-CoV-2 virus in the presence of commonly used potential interfering substances.

| Sample | Interfering substances | SARS-CoV-2 (FAM) | | | HIC (HEX/VIC) | | |
|---|---|---|---|---|---|---|---|
| | | Ct Mean | Ct SD | %CV | Ct Mean | Ct SD | %CV |
| 50 virus copies/ reaction | Mometasone | 28.06 | 0.15 | 0.50 | 27.18 | 0.02 | 0.08 |
| | Salmeterol/ Fluticasone | 27.69 | 0.29 | 1.06 | 27.25 | 0.07 | 0.24 |
| | Salbutamol | 28.43 | 0.31 | 0.95 | 27.32 | 0.19 | 0.68 |
| | Whole blood | 28.51 | 0.44 | 1.51 | 27.06 | 0.12 | 0.45 |
| | Sodium fluoride | 28.45 | 0.19 | 0.55 | 27.15 | 0.09 | 0.33 |
| | None | 28.22 | 0.14 | 0.22 | 27.38 | 0.05 | 0.16 |
| **Overall** | | **29.24** | **28.28** | **0.31** | **27.22** | **0.14** | **0.52** |

Result summary of interference study for saliva samples

## 7. Clinical Evaluation

Direct testing of VTM / UTM containing nasopharyngeal swab specimen

The clinical performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit assay was validated by a 3rd party laboratory. This study was conducted using 30 positive swab specimens in VTM / UTM and 30 negative (uninfected) swab specimens in VTM / UTM. Two replicates of RT-PCR reaction were set up for every positive and negative specimen. Using VereRT™ COVID-19 PCR Kit (Cat. No. VRTC-CB200) as comparison, the concordance rate of VereRT™ ZeroPrep™ COVID-19 PCR Kit is 100% as summarized in the table below.

| Sample ID | VereRT™ COVID-19 PCR Kit (Positive/Total) | VereRT™ ZeroPrep™ COVID-19 PCR Kit (Positive/Total) | Concordance Rate |
|---|---|---|---|
| Positive 1 | 2/2 | 2/2 | 100% |
| Positive 2 | 2/2 | 2/2 | 100% |
| Positive 3 | 2/2 | 2/2 | 100% |
| Positive 4 | 2/2 | 2/2 | 100% |
| Positive 5 | 2/2 | 2/2 | 100% |
| Positive 6 | 2/2 | 2/2 | 100% |
| Positive 7 | 2/2 | 2/2 | 100% |
| Positive 8 | 2/2 | 2/2 | 100% |
| Positive 9 | 2/2 | 2/2 | 100% |
| Positive 10 | 2/2 | 2/2 | 100% |
| Positive 11 | 2/2 | 2/2 | 100% |
| Positive 12 | 2/2 | 2/2 | 100% |
| Positive 13 | 2/2 | 2/2 | 100% |

| | | | |
|---|---|---|---|
| Positive 14 | 2/2 | 2/2 | 100% |
| Positive 15 | 2/2 | 2/2 | 100% |
| Positive 16 | 2/2 | 2/2 | 100% |
| Positive 17 | 2/2 | 2/2 | 100% |
| Positive 18 | 2/2 | 2/2 | 100% |
| Positive 19 | 2/2 | 2/2 | 100% |
| Positive 20 | 2/2 | 2/2 | 100% |
| Positive 21 | 2/2 | 2/2 | 100% |
| Positive 22 | 2/2 | 2/2 | 100% |
| Positive 23 | 2/2 | 2/2 | 100% |
| Positive 24 | 2/2 | 2/2 | 100% |
| Positive 25 | 2/2 | 2/2 | 100% |
| Positive 26 | 2/2 | 2/2 | 100% |
| Positive 27 | 2/2 | 2/2 | 100% |
| Positive 28 | 2/2 | 2/2 | 100% |
| Positive 29 | 2/2 | 2/2 | 100% |
| Positive 30 | 2/2 | 2/2 | 100% |
| | | | |
| Negative 1 | 0/2 | 0/2 | 100% |
| Negative 2 | 0/2 | 0/2 | 100% |
| Negative 3 | 0/2 | 0/2 | 100% |
| Negative 4 | 0/2 | 0/2 | 100% |
| Negative 5 | 0/2 | 0/2 | 100% |
| Negative 6 | 0/2 | 0/2 | 100% |
| Negative 7 | 0/2 | 0/2 | 100% |
| Negative 8 | 0/2 | 0/2 | 100% |
| Negative 9 | 0/2 | 0/2 | 100% |
| Negative 10 | 0/2 | 0/2 | 100% |
| Negative 11 | 0/2 | 0/2 | 100% |
| Negative 12 | 0/2 | 0/2 | 100% |
| Negative 13 | 0/2 | 0/2 | 100% |
| Negative 14 | 0/2 | 0/2 | 100% |
| Negative 15 | 0/2 | 0/2 | 100% |
| Negative 16 | 0/2 | 0/2 | 100% |
| Negative 17 | 0/2 | 0/2 | 100% |
| Negative 18 | 0/2 | 0/2 | 100% |
| Negative 19 | 0/2 | 0/2 | 100% |
| Negative 20 | 0/2 | 0/2 | 100% |
| Negative 21 | 0/2 | 0/2 | 100% |
| Negative 22 | 0/2 | 0/2 | 100% |
| Negative 23 | 0/2 | 0/2 | 100% |
| Negative 24 | 0/2 | 0/2 | 100% |
| Negative 25 | 0/2 | 0/2 | 100% |
| Negative 26 | 0/2 | 0/2 | 100% |
| Negative 27 | 0/2 | 0/2 | 100% |
| Negative 28 | 0/2 | 0/2 | 100% |
| Negative 29 | 0/2 | 0/2 | 100% |
| Negative 30 | 0/2 | 0/2 | 100% |

Large Cohort Clinical Trial

The clinical performance of detecting SARS-CoV-2 virus using VereRT™ ZeroPrep™ COVID-19 PCR Kit has been validated by 3rd party laboratories using both Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System and Hi-Media's Insta Q 96 Real-Time PCR System. The presence of viral RNA was determined using RNA extracted from nasopharyngeal swab specimens collected in VTM/ UTM before being tested using one of the three ICMR (Indian Council of Medical Research) approved SARS-CoV-2 virus qPCR detection kits: CoviPath™ COVID-19 RT-PCR Kit (Thermo Fisher Scientific), PathoDetect™ COVID-19 Qualitative PCR Kit (Mylab Discovery Solutions Pvt.Ltd.), or COVISure COVID-19 Real Time PCR Kit (Genetix Biotech Asia Pvt Ltd.) as the comparator test. Corresponding VTM specimens from the same subjects were directly evaluated without RNA extraction using VereRT™ ZeroPrep™ COVID-19 PCR Kit in accordance to the IFU. The validation outcomes from the study are summarized in the table below.

| | | Comparator Kits | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **VereRT™ ZeroPrep™ COVID-19 PCR Kit** (Test under evaluation) | **Positive** | 307 | 0 | 307 |
| | **Negative** | 3 | 515 | 518 |
| | **Total** | 310 | 515 | 825 |

| **Clinical Performance Indicators** | **Calculations** | **Outcomes** | **95% Confidence Intervals** |
|---|---|---|---|
| Clinical Sensitivity | 307 / (307+3) x 100% | 99.03% | 97.20% to 99.80% |
| Clinical Specificity | 515 / (0+515) x 100% | 100.00% | 99.29% to 100.00% |
| Positive Predictive Value (PPV) | 307 / (307+0) x 100% | 100.00% | N.A. |
| Negative Predictive Value (NPV) | 515 / (3+515) x 100% | 99.42% | 98.24% to 99.81% |

Determining the performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit against comparator tests.

In conclusion, it is evident from the large cohort clinical trial that VereRT™ ZeroPrep™ COVID-19 PCR Kit can be claimed to have a clinical sensitivity of 99.03% and clinical specificity of 100.00%. The clinical performance as described by PPV is 100%, while the NPV has been determined to be 99.42%.

Direct testing of human saliva specimen

The clinical performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit assay was validated by a 3rd party laboratory. The retrospective validation study was conducted with blinded subject studies of nasopharyngeal swabs and saliva specimens tested using Da An Gene Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) and VereRT™ ZeroPrep™ COVID-19 PCR Kit respectively. The concordance rate of VereRT™ ZeroPrep™ COVID-19 PCR Kit is 94.1% as summarized in the table below.

| **Subject number** | **Da An Gene Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing) (WHO EUL 0493-141-00)** | **VereRT™ ZeroPrep™ COVID-19 PCR Kit** | **Agreement (Yes/No)** |
|---|---|---|---|
| S01 | - | - | Yes |
| S02 | + | + | Yes |
| S03 | - | - | Yes |
| S04 | - | - | Yes |
| S05 | + | + | Yes |
| S06 | - | + | No |
| S07 | + | + | Yes |
| S08 | + | + | Yes |
| S09 | + | + | Yes |
| S10 | - | - | Yes |
| S11 | - | - | Yes |
| S12 | + | + | Yes |
| S13 | + | + | Yes |
| S14 | - | - | Yes |
| S15 | - | - | Yes |
| S16 | - | - | Yes |
| S17 | + | + | Yes |

Clinical sensitivity for SARS-CoV-2 was determined through the calculation of the positive predictive value (PPV) and clinical specificity for SARS-CoV-2 was determined through the calculation of the negative predictive value (NPV). True positives and true negatives were determined using the results from Da An Gene Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing). Clinical sensitivity, expressed as PPV, and clinical specificity, expressed as NPV, of the VereRT™

ZeroPrep™ COVID-19 PCR Kit with blind-tested clinical samples were 88.9% and 100% respectively.

Large Cohort Clinical Trial

The clinical performance of VereRT™ ZeroPrep™ COVID-19 PCR kit using human saliva as a sample type has been validated by 3rd party laboratories using Applied Biosystems™ 7500 Fast Real-Time PCR System, Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System and Hi-Media's Insta Q 96 Real-Time PCR System.

Both nasopharyngeal swab specimen as well as saliva specimen were collected from each enrolled subject. The presence or absence of SARS-CoV-2 viral RNA from extracted nasopharyngeal swab specimens collected in VTM/ UTM were determined using one of four comparator kits: 1) Da An Gene Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA (PCR-Fluorescence Probing), 2) CoviPath™ COVID-19 RT-PCR Kit (Thermo Fisher Scientific), 3) PathoDetect™ COVID-19 Qualitative PCR Kit (Mylab Discovery Solutions Pvt. Ltd.), or 4) COVISure COVID 19 Real Time PCR Kit (Genetix Biotech Asia Pvt Ltd.). Comparator kit 1 has received the WHO EUL Listing, while comparator kits 2-4 have been approved by ICMR (Indian Council of Medical Research). In comparison, saliva specimens from the same subjects were also collected using ZeroPrep™ Saliva Collection Kit before being processed in accordance to its IFU. The presence or absence of SARS-CoV-2 virus in the saliva specimens were determined using VereRT™ ZeroPrep™ COVID-19 PCR Kit according to the IFU. The validation results are summarized in the table below.

| | | Comparator Kits | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | **Total** |
| **VereRT™ ZeroPrep™ COVID-19 PCR Kit** (Test under evaluation) | **Positive** | 290 | 2 | 292 |
| | **Negative** | 12 | 532 | 544 |
| | **Total** | 302 | 534 | 836 |

| Clinical Performance Indicators | Calculations | Outcomes | 95% Confidence Intervals |
|---|---|---|---|
| Clinical Sensitivity | 290 / (290+12) x 100% | 96.03% | 93.16% to 97.93% |
| Clinical Specificity | 532 / (2+532) x 100% | 99.63% | 98.65% to 99.95% |
| Positive Predictive Value (PPV) | 290 / (290+2) x 100% | 99.32% | 97.32% to 99.83% |
| Negative Predictive Value (NPV) | 532 / (12+532) x 100% | 97.79% | 96.22% to 98.72% |

Determining the performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit against comparator tests.

In conclusion, it is evident from the clinical study that VereRT™ ZeroPrep™ COVID-19 PCR Kit possesses a clinical sensitivity and clinical specificity of 96.03% and 99.63%, respectively. The clinical performance as described by PPV is 99.32%, while the NPV has been evaluated to be 97.79%.

## Disposal

Dispose of hazardous or biologically contaminated materials according to local safety regulations.

## Technical Assistance

If you have any questions or technical issues regarding the use of the kit, or any other Veredus products, please contact our technical support department.

## Contact

Your opinions, comments, questions or feedback are important to us and all Veredus customers. Please contact us if you have any suggestions about product performance or new applications and techniques.

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## Understanding the Symbols

| Symbol | Meaning |
|---|---|
|  | Catalog number |
|  | Lot number |
|  | Contains sufficient for <n> tests |
|  | Manufacturer |
|  | Temperature limitation |
|  | Use-by date (YYYY-MM-DD) |
|  | Consult Instructions for Use |
|  | *In Vitro* Diagnostic medical device |
|  | European Union Conformity |
|  | Authorized representative in the European Community |

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