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 83 Science Park Drive #04-02, The Curie, Singapore Science Park 1, Singapore 118258, Singapore
EC REP	MT Promedt Consulting GmbH Altenhofstrasse 80, 66386 St. Ingbert, Germany
CE	European Union Conformity
IVD	*In Vitro* Diagnostic medical device

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IFU-RTC-CD01-1004

Contents

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 virus. 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 result 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 decision.

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 assay.

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 79% similar to SARS-CoV-1 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 detection of the viral RNA directly from VTM / UTM or human saliva collected from infected patients. This kit also contains enzymes, oligonucleotide primers, dual-labelled hydrolysis probes and control material used in real-time RT-PCR assay for the *in vitro* qualitative detection of SARS-CoV-2. These oligonucleotide primers and probes 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' exonuclease 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 measured and recorded 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 PC 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

- Consumable
  - Personal protective equipment
  - Sterile filter pipette tips
  - 1.5 mL microcentrifuge tubes
  - Decontamination product

- 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

- 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 biohazard and 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 2 (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 amplification 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). Hence, 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 test 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.

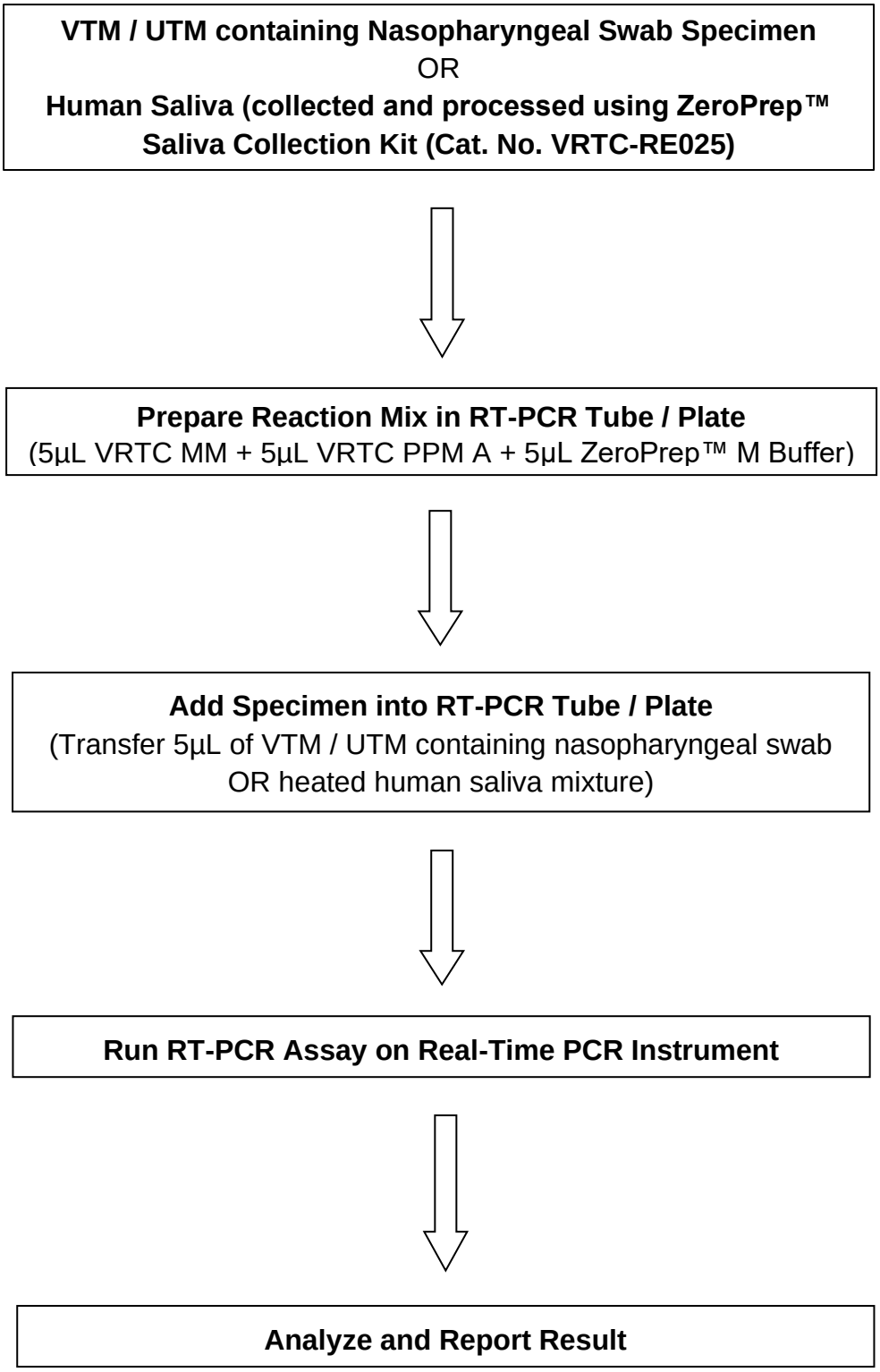
Additional Precautions when Handling Clinical Specimens

- 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 biohazardous agent and potentially infectious which should be handled 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 used for setting up the assay reaction such as PCR plates, PCR strips, PCR plate seals, etc, which is at high risk of being contaminated with biohazardous agent should be properly decontaminated before being moved to a PCR machine for running.
- 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 for testing.
- Disposal of contaminated consumables such as gloves and tubes as according to your local authority's guidelines.

Quality Control

Under Veredus Laboratories 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 respective RNA and plasmid templates for quality control.

Workflow


VTM / UTM containing Nasopharyngeal Swab Specimen
OR
Human Saliva (collected and processed using ZeroPrep™ Saliva Collection Kit (Cat. No. VRTC-RE025)
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 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 conducting the assay for testing.

Specimen Type

Direct application of VTM / UTM containing nasopharyngeal swab specimen without the need for any RNA extraction. Five different brands of VTM / UTM listed below, have been validated for compatibility.

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 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

This kit has been validated using ZeroPrep™ Saliva Collection Kit (Cat. No. VRTC-RE025) for human saliva specimen.

Real-Time PCR Instrument

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

Assay Procedures

Note: It is highly advisable that the preparation of the reaction mix should be performed in a template-free hood / area / zone to minimize any possible cross-contamination with amplifiable SARS-CoV-2 or human sample template which may result in false positive results.

- In the template-free area, fully thaw the VRTC MM*, VRTC PPM A and ZeroPrep™ M Buffer[#] on ice or in cold-block. Keep the reagents cold during preparation and use. Thaw VRTC PC A separately and away from the template-free area to prevent any positive control contamination.
- Determine the number of assay reactions required. It is necessary to make excess Reaction Mix for controls and pipetting variations.
- 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 on ice or in the cold block.
- Prepare Reaction Mix according to the table below. Mix the Reaction Mix by pipetting up and down. DO NOT VORTEX.

* Upon first removal from the freezer, check if VRTC MM is still viscous by tilting the tube 2 times. Once fully-thawed to 4^{o}C, this reagent will be almost fluid-like.
[#] ZeroPrep™ M Buffer does not freeze and is fluid-like.

1. 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**	

^ replace the variable 'X+2' with the total number of reactions required on the real-time PCR plate together with an additional 2 reactions. This will account for any potential pipetting error in the process. Remember to factor in the number of reactions for No Template Control ("NTC"), Positive Control ("PC"), and replicates, if any.

2. Set up RT-PCR tube / plate.
3. 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

4. Transfer the RT-PCR tube / plate containing the reaction mix to the template area OR specimen handling area (BSL2 and above).
5. Put on the necessary PPE before handling template / specimen potentially containing live virus and biohazard agent.

6. 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 reaction 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 the designated reaction tube / well labeled as “NTC”.
- For Positive Control (“PC”), add 5 µL of VRTC PC A in place of the test sample into the designated reaction tube / well labeled as “PC”.

For direct testing using human saliva

- Add 5 µL of heated saliva mixture (refer to IFU of ZeroPrep™ Saliva Collection Kit) into each sample reaction tube / well (e.g. “S1” to “S46”).
- For No Template Control (“NTC”), add 5 µL of nuclease-free water or ZeroPrep™ Saliva Buffer processed with equal volume of nuclease-free water in place of the sample into the designated reaction tube / well labeled as “NTC”.
- For Positive Control (“PC”), add 5 µL of VRTC PC A in place of the test sample into the designated reaction tube / well labeled as “PC”.

7. 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

8. 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

Note: It is not necessary to adjust any ramp rate when using Bio-Rad CFX96™ Touch Real-time PCR System.

Thermal cycling protocol for real-time PCR instruments such as QuantStudio™ / Applied Biosystems® 7500 Fast Real-time PCR Systems

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

* use a ramp rate of 100% when using Applied Biosystems® 7500 Fast Real-time PCR Systems

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

Recommended fluorophore detection configuration on different real-time PCR systems

	Fluorescence Detection Configurations of Different Real-Time PCR System			
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.

9. Passive reference detection: Optional but recommended, if available
10. Run mode: Standard
11. Reaction volume: 20 µL

Assay Controls

The following controls are included in the assay:

- Human Internal Control (HIC): This control is built into each reaction to not only monitor the quality of swab specimen in VTM / UTM or human saliva, as well as for testing potential RT-PCR inhibition.
- Positive Control (PC): VRTC PC A is included in the kit and should be used for each set of test run to monitor kit performance.
- No Template Control (NTC): VTM / UTM without swab, nuclease-free water or ZeroPrep™ Saliva Buffer treated with equal volume of nuclease-free water[@] should be included for each set of test run to monitor kit performance.

[@] In the event this control is used, please prepare in accordance to the IFU of ZeroPrep™ Saliva Collection Kit (Cat. No. VRTC-RE025)

Interpretation of Results

Baseline Threshold Adjustment

Baseline threshold judgement determined by the associated data analysis program of the real-time PCR instrument 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) for all targets. If NTC exhibits any amplification curves especially for SARS-CoV-2 N gene (FAM) that crosses the signal threshold before cycle 40, kit contamination with template 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 a partial RPP30 gene sequence from the human genome. 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 then the test results will be considered as valid for interpretation according to the following table:

No.	COV N Gene (FAM)	RPP30 Gene 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	Exponential amplification curve[1] and Ct ≤ 40	Ct > 40 or Ct undetermined[2]	Invalid Result[3]
4	Ct > 40 or Ct undetermined	Ct > 40 or Ct undetermined[2]	Invalid Result[3]

[1] It is critical 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, 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 assay test.
- Appropriate specimen collection, handling, storage and processing procedures are required for optimal performance of this assay.
- 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 virus.

Performance Characteristics

1. Analytical Sensitivity (Limit of Detection LOD)

The LOD of VereRT™ ZeroPrep™ COVID-19 PCR Kit was determined using commercially available inactivated SARS-CoV-2 (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 assay replicates tested were positive.

LOD of assay using VTM / UTM containing nasopharyngeal swab specimen as the direct input sample type

A test series of known concentration of inactivated SARS-CoV-2 were spiked into Viral Transport Medium (VTM) GeneXpert® Nasopharyngeal Sample Collection Kit (PN: SWAB/B-100) in the presence of nasopharyngeal swab specimen collected from healthy individual. The VTM samples, without undergoing RNA extraction, were directly tested over 20 replicate reactions using VereRT™ ZeroPrep™ COVID-19 PCR Kit. Based on the results, the LOD was determined to be **5 viral copies per reaction** OR **1 viral copy per µL of VTM / UTM**.

LOD of assay using human saliva specimen as sample type

Human saliva was collected from healthy individual using ZeroPrep™ Saliva Collection Kit before being spiked with a series of inactivated SARS-CoV-2 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 results, the LOD was determined to be **10 viral copies per reaction** OR **4 viral copies per µL human saliva**.

2. Analytical Sensitivity (Inclusivity)

SARS-CoV-2 is a single-stranded RNA virus, which is known for rapid mutation in its genomic sequence. To ensure that VereRT™ ZeroPrep™ COVID-19 PCR Kit maintains its performance to detect all known viral strains, especially those that World Health Organization (WHO) classifies as Variants of Concern (VOC) such as Alpha, Beta, Gamma, Delta and Omicron, *in silico* inclusivity analysis (BLAST) were performed against known SARS-CoV-2

viral genome sequences retrieved from both GISAID and NCBI databases to determine variant coverage.

The design of VereRT™ ZeroPrep™ COVID-19 PCR Kit encompasses two independent sets of primer and probe responsible for the detection of two different sites of the viral nucleocapsid (N) gene of SARS-CoV-2. The primer and probe sequence of both sets were matched against all genomic sequences in the aforementioned databases and as of May 2022, amongst an estimate of 4.9 million isolates of SARS-CoV-2, there are 9 and 26 isolates within Delta and Omicron variant, respectively, with mutation(s) in both target regions that may escape detection using VereRT™ ZeroPrep™ COVID-19 PCR Kit.

The dual targets design on the viral N gene dramatically enhanced the tolerance of any mutations in the viral genome, as only when both target regions are mutated simultaneously in a particular viral strain, the mutations may then result in drastic decrease of the Tm of primers and probes, in which the detection of this strain could potentially be affected. Out of 4,934,475 genome sequences, the analysis identified 3,031 sequences having mismatch within both N gene target regions that lowered the Tm below 57°C. All detected mutations corresponding to primers and probes used in the kit were consolidated and their corresponding mutant amplicons were synthesized.

The functional validation with those synthesized mutant amplicon sequences was done and results showed that all mutants pertaining to the 3,031 sequences identified through the *in silico* analysis could still be successfully detected with 100% strain coverage. The adjusted detection coverage is summarized in the table below.

Database	GISAID	NCBI
Genome Count	3,885,504	1,048,971
In silico Strain Coverage	99.93%	99.99%
Adjusted Strain Coverage	100%*	100%*

* Adjusted based on functional validation data

3. Analytical Specificity (Cross-reactivity)

To further substantiate that VereRT™ ZeroPrep™ COVID-19 PCR Kit is specific for detecting SARS-CoV-2 in clinical specimen, 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, as well as other respiratory pathogens such as Influenza A and B, and commensals in the human respiratory tract such as *Staphylococcus aureus* and *Candida albicans*.

A total of 36 pathogens and 81 representative genomes were analyzed. From *in silico* analysis, there is one genome of *Mycobacterium tuberculosis* that has homologous 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 result due to cross-reactivity. The result of this analysis 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

Additional verification was conducted to validate the assay for specificity to SARS-CoV-2 and cross-reactivity with other common respiratory pathogens, especially phylogenetically similar pathogens such as other Coronaviruses. Both sample types: VTM / UTM and human saliva were spiked with inactivated respiratory pathogens from the NATtrol™ Respiratory Verification Panel 2.1 (P/N: NATRVP2.1-BIO) before being tested for non-specific signal which might be indicative for cross reactivity with SARS-CoV-2 using VereRT™ ZeroPrep™ COVID-19 PCR Kit. 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, and not with other test organisms or even phylogenetically-related Coronaviruses. For a full list of the respiratory pathogens tested in the validation study, please refer to the following table.

Panel Member	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 VereRT™ ZeroPrep™ COVID-19 PCR Kit were validated by performing 3 rounds of testing on 3 different days by 3 different operators with 3 different lots of reagent and respective sample type from 3 different healthy donors. Four copies of inactivated virus per μl of VTM from GeneXpert or saliva was used as sample for 2 sample types. The RT-PCR reaction was conducted in Bio-Rad CFX96™ Real-Time PCR System.

Study using VTM / UTM containing nasopharyngeal swab specimen as sample type

Twenty replicate reactions were run in each round of sample 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 assay 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 determined to be consistently repeatable and reproducible when tested with VTM containing nasopharyngeal swab.

Sample	Operator	SARS-CoV-2 (FAM)			HIC (HEX / VIC)		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
20 virus copies/ reaction	1	32.30	0.51	1.58	31.28	0.50	1.61
	2	31.28	0.44	1.42	31.65	0.81	2.56
	3	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**

Study using human saliva as sample type

For saliva sample, 10 replicate reactions 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 test reactions giving expected positive results across 3 different runs.

Although the saliva samples were contributed from different donors, but the variation of Ct values across the 3 rounds were largely comparable, the test results are summarized in the table below. VereRT™ ZeroPrep™ COVID-19 PCR Kit is determined to be consistently

repeatable and reproducible when tested with human saliva collected and processed using ZeroPrep™ Saliva Collection Kit.

Sample	Operator	SARS-CoV-2 (FAM)			HIC (HEX / VIC)		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
10 viral copies per reaction	1	32.67	0.85	2.61	22.10	0.19	0.85
	2	30.35	0.24	0.78	26.79	0.26	0.96
	3	34.74	1.22	3.51	28.90	0.18	0.62
Overall		**100%**					

5. Validation of VTM / UTM

Validation test was performed on 5 different brands of VTM / UTM (refer to table below) for compatibility with VereRT™ ZeroPrep™ COVID-19 PCR Kit. In summary, intact inactivated SARS-CoV-2 was spiked into each type of VTM / UTM containing human nasopharyngeal swab specimen, at 2 viral copies per μl of VTM / UTM. The VTM sample is then subjected to testing using VereRT™ ZeroPrep™ COVID-19 PCR Kit in 20 replicate reactions.

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 percentage coefficient of variation (%CV) for detection of both FAM and HEX / VIC channels within a round of testing are less than 1% and 3%, respectively. The overall Ct SD and %CV across all three rounds of test are not greater than 1.6% and 5%, respectively. Hence, the detection of 10 viral copies in 5 different brands of VTM / UTM containing nasopharyngeal swab using VereRT™ ZeroPrep™ COVID-19 PCR Kit was demonstrated to be both repeatable and reproducible. The study confirms that 5 different brands of VTM are compatible for direct use with VereRT™ ZeroPrep™ COVID-19 PCR Kit.

Sample	VTM / UTM	SARS-CoV-2 (FAM)			HIC (HEX / VIC)		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
10 viral copies per 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

To rule out the possibility of exogenous interference substance affecting the performance of VereRT™ ZeroPrep™ COVID-19 PCR Kit, interference study was conducted using the VereRT™ ZeroPrep™ COVID-19 PCR Kit for SARS-CoV-2 detection on direct VTM containing nasopharyngeal swab and human saliva that had been added with a panel of 6 different drugs / substances that are commonly administered nasally and/or orally by patients for testing. The substances tested for VTM and saliva samples are listed in both tables below.

Substance	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)

Substance	Active Ingredient	Amount Tested in Saliva
Nasonex Aqueous Nasal Spray	Mometasone furoate	1% (v/v)
Seretide EVOHALER	Salmeterol xinafoate Fluticasone propionate	1% (v/v) 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)

Study using VTM / UTM containing nasopharyngeal swab specimen as sample type

Nasopharyngeal swabs containing the recommended dosing of each potential interfering substance were inoculated into VTM before being spiked with inactivated SARS-CoV-2 at concentration of 2 viral copies per μl of VTM. The resulting test samples, in the presence of each potential interfering substance, were tested using VereRT™ ZeroPrep™ COVID-19 PCR Kit for SARS-CoV-2 detection.

The table below summarizes the performance of using VereRT™ ZeroPrep™ COVID-19 PCR Kit in the presence of interfering substances. It is evident demonstrated from the test results that there is minimal interference from these exogenous substances to impact the detection of SARS-CoV-2 in VTM by VereRT™ ZeroPrep™ COVID-19 PCR Kit, hence confirming the assay remains robust when commonly used interfering substances are present in VTM / UTM containing nasopharyngeal swab.

Sample	Interfering Substance	SARS-CoV-2 (FAM)			HIC (HEX / VIC)		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
10 viral copies per 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**

^ Mucin is an inherent component in human saliva. Ct mean for HIC is lower as compared to those with other interference substances, thus indicating a higher concentration of human RNaseP, resulting in an artificially skewed Ct mean.

* Due to the exogeneous introduction of mucin from saliva in this study, the Ct values from the test using mucin had to be excluded from the overall statistics so that a more representative statistics can be derived.

Study using human saliva as sample type

Similarly, human saliva samples were collected using the ZeroPrep™ Saliva Collection Kit before being spiked with each of the potential interfering substances together with inactivated SARS-CoV-2. Each test was performed using 50 viral copies per reaction, which translates to 20 viral copies per µl of saliva. The resulting saliva test samples, in the presence of each potential interfering substance, were tested using VereRT™ ZeroPrep™ COVID-19 PCR Kit for SARS-CoV-2 detection.

The table below summarizes the performance of using VereRT™ ZeroPrep™ COVID-19 PCR Kit in the presence of interfering substances. It was evidently shown in the test results that there is minimal interference from exogenous substances tested to impact the detection of SARS-CoV-2 in human saliva by VereRT™ ZeroPrep™ COVID-19 PCR Kit, hence confirming the assay remains robust when commonly used interfering substances are present in human saliva.

Sample	Interfering Substance	SARS-CoV-2 (FAM)			HIC (HEX / VIC)		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
50 viral copies per reaction	Mometasone	28.06	0.15	0.55	27.18	0.02	0.08
	Salmeterol/ Fluticasone	28.00	0.29	1.04	27.25	0.07	0.24
	Salbutamol	28.43	0.31	1.11	27.32	0.19	0.68
	Whole blood	28.51	0.44	1.56	27.06	0.12	0.45
	Sodium fluoride	28.45	0.19	0.65	27.15	0.09	0.33
	None	28.22	0.14	0.50	27.38	0.05	0.16
Overall		**28.28**	**0.31**	**1.09**	**27.22**	**0.14**	**0.52**

7. Clinical Evaluation

Direct testing of VTM / UTM containing nasopharyngeal swab specimen as sample type

The clinical performance of detecting SARS-CoV-2 in nasopharyngeal swab specimen collected in VTM / UTM as a direct sample type using VereRT™ ZeroPrep™ COVID-19 PCR Kit was evaluated in a clinical trial site. This was in comparison to VTM / UTM samples from the same subject, from which viral RNA were extracted and tested by comparator test kit, before test sample was deemed to be positive or negative for SARS-CoV-2. The sample size for the trial was 847, of which 825 (97.40%) of the data were valid, and were used to determine the clinical performance of the assay to detect SARS-CoV-2 as summarized in the table below.

		Comparator Kit		
		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 Indicator	Calculation	Outcome	95% Confidence Interval
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%

In conclusion, the clinical trial study demonstrated that VereRT™ ZeroPrep™ COVID-19 PCR Kit possessed a **clinical sensitivity of 99.03%** and **clinical specificity of 100.00%** to confidently detect SARS-CoV-2 directly from the VTM / UTM without the need for viral RNA extraction. The **PPV and NPV** of the assay, as determined from the clinical trial results, were **100% and 99.42%**, respectively.

Direct testing of human saliva as sample type

The clinical performance of detecting SARS-CoV-2 in human saliva, after being collected and processed using ZeroPrep™ Saliva Collection Kit (Cat No. VRTC-RE025), by VereRT™ ZeroPrep™ COVID-19 PCR Kit was evaluated in two independent clinical trial sites. The performance was evaluated in comparison to nasopharyngeal swab specimens collected from the same subject in VTM / UTM before viral RNA were extracted and tested by comparator test kit to determine if test samples were positive or negative for SARS-CoV-2. The total sample size for both trials was 842, of which 836 (99.29%) of the data were valid, and were therefore used to determine the clinical performance of the assay to detect SARS-CoV-2 using human saliva as summarized in the table below.

		Comparator Kit		
		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 Indicator	**Calculation**	**Outcome**	**95% Confidence Interval**
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%

In conclusion, it was evident from both clinical trials that VereRT™ ZeroPrep™ COVID-19 PCR Kit demonstrated a **clinical sensitivity of 96.03%** and **clinical specificity of 99.93%** to confidently detect for SARS-CoV-2 in human saliva samples without the need for viral RNA extraction. The **PPV and NPV** of the assay, as derived from the clinical trial results, were **99.32% and 97.79%**, respectively.

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
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	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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