VereRT™ COVID-19 PCR Kit
Instructions for Use

REF	VRTC-CB200
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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Contents

Intended Use

VereRT™ COVID-19 PCR Kit is a one-step 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 in nasopharyngeal swab specimens.

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™ 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-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™ COVID-19 PCR Kit 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. The 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-CB200
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

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 and VRTC PPM A 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 products

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 (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 samples within a Class II (or higher) Biological Safety Cabinet (BSC).
- Wear appropriate personal protective equipment, including (but not limited to) protective disposable gloves, laboratory coats and eye protection when handling samples 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 extracted samples to RT-PCR Mix.
- Use sterile pipette tips with filters.
- Do not use kit or reagents after the expiry dates shown on the respective labels.
- 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.

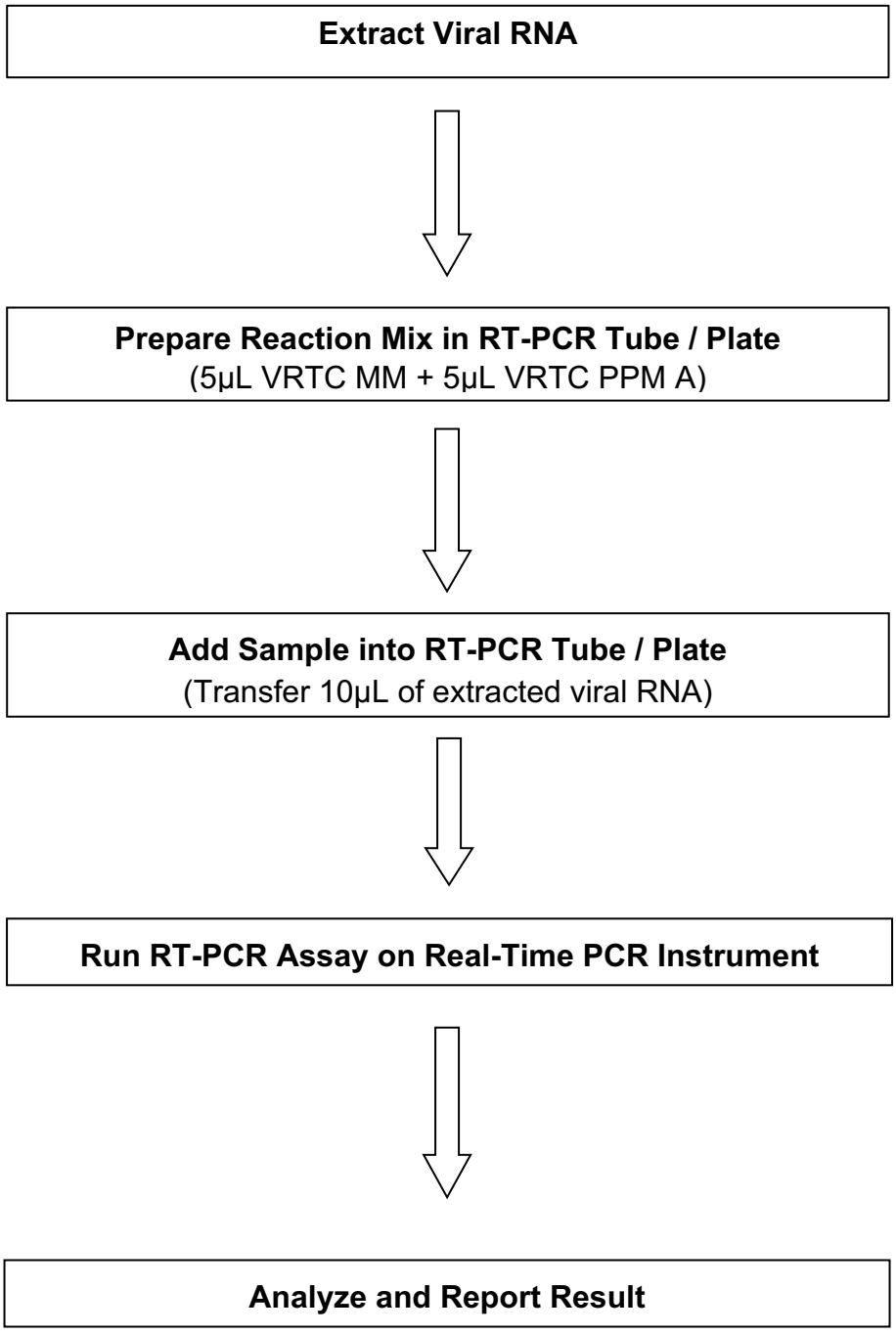
Additional Precautions when Handling RNA Samples

- Designate a separate working area for RNA work only.
- Clean work area and instruments, including pipettes, with 100% ethanol and/or commercially available RNase inactivation reagents.
- Always wear gloves while working with RNA. Avoid touching surfaces and equipment that are not decontaminated.
- Change gloves when moving between working areas to prevent cross-contamination.
- Use only sterile and RNase-free disposable plastic consumables.
- Use only nuclease-free water.
- Work quickly on ice or cold block during test preparation as RNA samples are prone to degradation.

Quality Control

Under Veredus quality assurance program, the performance of VereRT™ 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


Extract Viral RNA
Prepare Reaction Mix in RT-PCR Tube / Plate
(5µL VRTC MM + 5µL VRTC PPM A)
Add Sample into RT-PCR Tube / Plate
(Transfer 10µL of extracted viral RNA)
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.

Sample Preparation

VereRT™ COVID-19 PCR Kit is compatible with extracted viral RNA from SARS-CoV-2. To ensure maximal performance, it is important to establish the viral RNA extraction process for compatibility with the assay. Some naturally occurring substances, such as heme, melanin, and polysaccharides could potentially act as PCR inhibitors and interfere with the assay performance. Please refer to the respective manufacturer's handbook for detailed extraction procedures.

The following nucleic acid extraction kits/ systems are recommended*:

- QIAamp® Viral RNA Mini Kit (Cat. No. 52904/52906)
- CommaXP® Virus DNA/RNA Extraction Kit (Cat. No. MNP027-1)
- MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383)
- KingFisher™ Flex Purification System (Cat. No. 24074441)

* It is advisable to validate the viral RNA extraction process should the nucleic acid extraction kit is not within the abovementioned recommended list.

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
- Agilent AriaMx Real-time PCR System

* 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 viral RNA or human specimen template.

i) In the template-free workspace, fully thaw the VRTC MM, VRTC PPM A and VRTC PC A on ice or a 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 and VRTC PC A 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
Total	**10 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. Be reminded 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.

viii) Carefully pipette 10 µL of extracted RNA sample / NTC / PC into the designated tube / well as listed below:

- Add 10 µL of extracted RNA into each sample tube / well (e.g. “S1” to “S46”).
- For No Template Control (“NTC”), add 10 µL of nuclease-free water OR elution buffer (from nucleic acid extraction kit) in place of the extracted RNA sample into designated tube / well labelled as NTC.
- For Positive Control (“PC”), add 10 µL of VRTC PC A in place of the extracted RNA sample into the designated tube / well labelled as PC.

ix) Total volume of each reaction tube / well is 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

x) 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.

xi) 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.

xii) Passive reference detection: Optional but recommended, if available.
xiii) Run mode: Standard
xiv) 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 sample extraction efficacy 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): Elution buffer used for the RNA extraction process or Nuclease-free water should be included in place of the sample for each set of test run to monitor for any template 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	Invalid Result[2,3]

[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 test 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 the 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 acids for amplification.
- False positive results may occur due to cross-contamination by target organisms, their nucleic acids, amplicons, or from non-specific signals in the 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

Analytical Sensitivity (Limit of Detection, LOD)

The LOD of the test was determined using viral RNA extracted from inactivated SARS-CoV-2 virus (P/N: VR-1986HK) using CommaXP® Virus DNA/RNA Extraction Kit (P/N: NP027-1). The RNA was previously quantified against a characterized stock of synthetic SARS-CoV-2 N gene standard using VereRT™ COVID-19 PCR Kit. Bio-Rad CFX96 Real-time PCR Detection System was used to determine the LOD, which can be defined as the lowest detectable concentration of SARS-CoV-2 at which 95% of all replicates were tested positive.

In conclusion, the analytical sensitivity or LOD for VereRT™ COVID-19 PCR Kit is determined to be **2 viral RNA copies per test / assay / reaction**, OR **0.2 viral RNA copies per µL of extracted RNA**.

Analytical Sensitivity (Inclusivity)

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™ 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, Delta and Omicron. *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™ COVID-19 PCR Kit.

The design of VereRT™ 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. 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™ COVID-19 PCR Kit.

Analytical Specificity (Cross-reactivity)

To ensure that VereRT™ COVID-19 PCR Kit is specific for detecting SARS-CoV-2 in clinical specimens, the primer and probe sequences of VereRT™ 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™ COVID-19 PCR Kit is unlikely to give rise to false positive results due to cross-reactivity.

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 other Coronaviruses, VereRT™ 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. Thus, this supports the assay's specificity to only SARS-CoV-2 virus, and no 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

Repeatability and Reproducibility

The repeatability and reproducibility performance of VereRT™ COVID-19 PCR Kit were validated by conducting three rounds of testing using three different manufactured kit lots over three different days and by three different operators. Each round included 20 replicate testing of the positive control, VRTC PC A, from each of the 3 unique lots of VereRT™ COVID-19 PCR Kit using the Bio-Rad CFX96 Real-time PCR Detection System. The calculated variation of Ct values within each round represents assay repeatability and the overall variation of Ct values across the three rounds represents assay reproducibility.

As shown in the table below, it is evident that the Percent Coefficient of Variation (%CV) for the detection of N gene targets of SARS-CoV-2 and human RPP30 gene (HIC) is no more than 0.88% as conducted by 3 operators. The Ct SD (Standard Deviation) for each of the tests is also below value of 1. Comparing the results between operators, the overall %CV is up to 1.47% for the test done using CFX96 Real-time PCR Detection System.

In conclusion, as shown from the repeatability and reproducibility study results, it is evident that the functional testing conducted by each operator is highly repeatable because the %CV for the detection of N and human RPP30 gene targets by the VereRT™ COVID-19 PCR Kit is well below 5%. The overall %CV for each of the targets is no more than 1.47% (below the threshold of 5%), hence lending support to the assay's high reproducibility even when conducted by different individuals.

Operator	Fluorophore	Target	Ct Mean	Ct SD	%CV
Bio-Rad CFX96™ Real-time PCR System					
1	FAM	N	32.06	0.26	0.82
	HEX	RPP30	30.90	0.25	0.81
2	FAM	N	31.74	0.20	0.61
	HEX	RPP30	29.98	0.23	0.76
3	FAM	N	31.72	0.28	0.88
	HEX	RPP30	30.39	0.24	0.79
Overall	**FAM**	**N**	31.84	0.29	0.92
	HEX	**RPP30**	30.42	0.45	1.47

Interference Study

10 common endogenous and exogeneous substances were evaluated for any possible interference with the detection of SARS-CoV-2 and the Human Internal Control (HIC) using VereRT™ COVID-19 PCR Kit. To address this, the 10 potential interference substances were added to Viral Transport Medium (VTM) / Universal Transport Medium (UTM) containing pre-collected nasopharyngeal swab samples. The resultant samples were extracted before being spiked with known concentration of SARS-CoV-2 synthetic N gene RNA at 3X as well as 50X the Limit of Detection (LOD) of the assay. VereRT™ COVID-19 PCR Kit was able to confidently detect for the presence of SARS-CoV-2 virus as well as HIC despite the presence of the interference substance.

In conclusion, there was no compelling evidence to suggest that the 10 potential interference substances (as listed below), comprising endogenous substances like human whole blood and saliva, as well as exogenous substances exhibited significant interference to impact the detection of SARS-CoV-2 and the HIC using the VereRT™ COVID-19 PCR Kit.

No.	Substances	Active Ingredient
1	Nasonex™ Aqueous Nasal Spray	Mometasone furoate
2	Afrin® Nasal Spray	Oxymetazoline
3	Nazal Spray	Naphazoline HCl, Chlorpheniramine Maleate
4	Flixonase™ Aqueous Nasal Spray	Fluticasone propionate
5	Axe Brand Universal Oil	Menthol crystals, eucalyptus oil, methyl salicylate, camphor, essential oil
6	Eye Mo® Regular	Benzalkonium chloride, boric acid, sodium borate
7	Vicks® Inhaler	Menthol, camphor, methyl salicylate
8	Tiger Balm® White Ointment	Camphor, dementholized mint oil, cajuput oil, menthol, clove oil, paraffin and petrolatum
9	Human saliva	Mucin
10	Human whole blood	Hemoglobin/ plasma

Clinical Evaluation

The clinical performance of VereRT™ COVID-19 PCR Kit assay was validated by an independent 3rd party laboratory. The evaluation was conducted using viral RNA samples extracted from a total of 946 nasopharyngeal swab specimens collected in VTM / UTM. The 946 specimens comprise of a combination of prospective and retrospectively identified test subject specimens. 1.27% of the total clinical specimens tested were excluded from the performance analysis calculation due to the presence of invalid data. In summary, VereRT™ COVID-19 PCR Kit was evaluated with a total of 361 positive and 573 negative specimens against comparator assay, TaqPath™ COVID-19 Combo PCR Kit. The clinical performance of VereRT™ COVID-19 PCR Kit has been summarized in a 2 x 2 agreement table below.

		TaqPath™ COVID-19 Combo Kit (Comparator)		
		Positive	**Negative**	**Total**
VereRT™ COVID-19 PCR Kit (Test under evaluation)	**Positive**	359	4	363
	Negative	0	571	571
	Total	359	575	934

Performance Indicator	Calculation	Outcome	95% Confidence Interval
Clinical Sensitivity	359 / (359+0) x 100%	100.00%	98.98-100.00%
Clinical Specificity	571 / (4+571) x 100%	99.30%	98.23-99.81%
Positive Predictive Value (PPV)	359 / (359+4) x 100%	98.90%	97.13-99.58%
Negative Predictive Value (NPV)	571 / (0+571) x 100%	100.00%	100.00%

In conclusion, the clinical trial study has evaluated VereRT™ COVID-19 PCR Kit to demonstrate good concordance with the comparator assay and can be conservatively claimed to have a clinical sensitivity and specificity of 100.00% and at least 99.30%, respectively. Other clinical performance indicators, as represented by Positive Predictive Value (PPV) is at least 98.90% while the Negative Predictive Value (NPV) has been determined to be 100.00% in this cohort of test subjects.

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.

For information and technical assistance, please contact us via:

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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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