VereRT™ Duo
COVID-19 PCR Kit
Instructions for Use

REF	VCH3-CA200
Σ	200
	Store at -30°C to -10°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™ Duo 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. This test is suitable for use with extracted SARS-CoV-2 RNA from human 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™ Duo 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 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™ Duo COVID-19 PCR Kit contains specimen preparation reagent named as M Buffer, that facilitates amplification of the extracted viral RNA belonging to SARS-CoV-2. This kit also contains 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 as well as the ORF1a 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. In 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

Catalogue no.		**VCH3-CA200**
No. of Tests		**200**
Frozen Components	**(-30°C to -10°C)**	**Quantity**
VRTD MM	(Enzyme Master Mix)	1 tube
VRTD PPM	(Primer Probe Mix)	1 tube
VRTD PC	(Positive Control)	1 tube
VRTD M Buffer	(Sample Prep Solution)	1 tube

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

VRTD MM, VRTD PPM and VRTD M Buffer are to be kept at 2°C to 8°C after first use and the reagents are stable for at least 2 weeks in this condition.

Storage Condition

- Store all frozen kit components at -30°C to -10°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 filtered pipette tips
- 1.5 mL microcentrifuge tubes
- Decontamination product and equipment

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.
- 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 could occur if either the clinical specimen or the real-time 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 date 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.
- Extracted RNA should be maintained on cold block or on ice during test preparation and usage to ensure stability.
- Material Safety Data Sheet (MSDS) is available upon request.

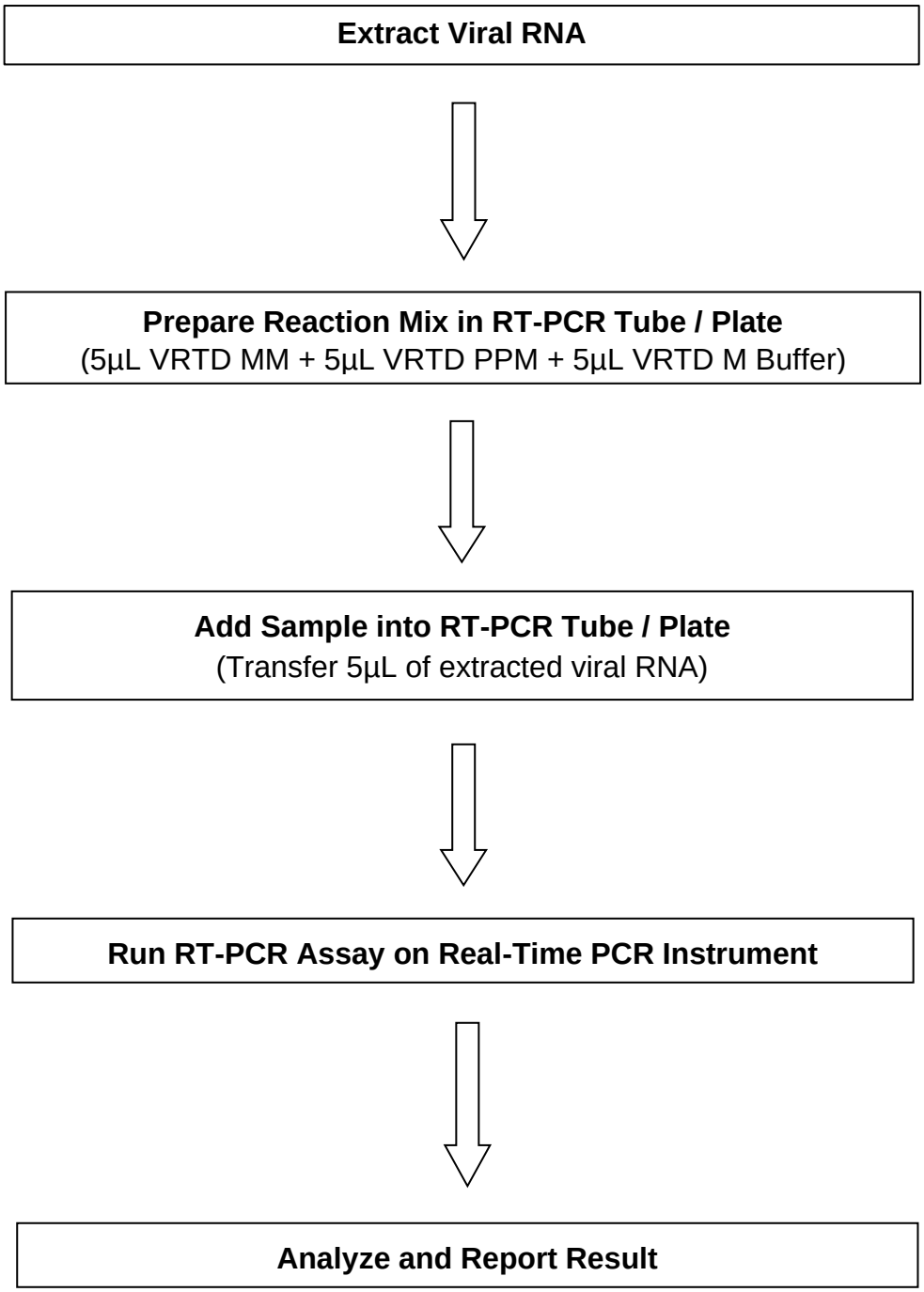
Additional Precautions when Handling Clinical Specimens

- Designate a dedicated working area for handling clinical specimens.
- Change gloves when moving between working areas to prevent cross-contamination.
- Treat clinical 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).
- Clinical specimens should be processed in accordance with national biological safety regulations.
- Personal protective equipment such as gloves, eye protection, and lab coat must be worn when handling clinical specimens.
- Procedures that involve generating aerosols, e.g. aliquoting of specimen, mixing (or vortexing) of specimen should be performed in at least a BSL2 or higher certified biological safety hood.
- The exterior of consumables such as PCR plate, PCR strip, PCR plate seal, 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™ Duo 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.

Workflow


Extract Viral RNA
Prepare Reaction Mix in RT-PCR Tube / Plate
(5µL VRTD MM + 5µL VRTD PPM + 5µL VRTD M Buffer)
Add Sample into RT-PCR Tube / Plate
(Transfer 5µL 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 because of the importance of specimen quality.

Sample Preparation

VereRT™ Duo COVID-19 PCR Kit is only 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/ extraction systems are recommended*:

- CommaXP® Virus DNA/RNA Extraction Kit (Cat. No. MNP027-1)
- MagMAX™ Viral/ Pathogen II Nucleic Acid Isolation Kit (Cat. No. A48383)
- MagNA Pure 24 Total NA Isolation Kit (Cat. No. 50548300)
- KingFisher™ Flex Purification System (Cat. No. 24074441)
- MagNA Pure 24 System (Cat. No. 07290519001)

* It is advisable for the user to validate the viral RNA extraction process should the user select a nucleic acid extraction kit/ extraction system that is not within the abovementioned recommended list.

Real-Time PCR Instrument

The following real-time PCR instruments are recommended*:

- Bio-Rad CFX96™ Touch Real-Time PCR System
- QuantStudio™ Series of 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.

Reagent and Controls Preparation

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

i) In the template-free area, thaw the VRTD MM, VRTD PPM and VRTD 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 VRTD MM, VRTD PPM, VRTD M Buffer and VRTD PC to collect content at the bottom of each tube. Place tubes in a cold rack.
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^
VRTD PPM	5 µL	(5 x 'X+2') µL
VRTD MM	5 µL	(5 x 'X+2') µL
VRTD 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 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 starting from 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	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn	Rxn

	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	Mix	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 containing the reaction mix to the template area OR specimen handling area (BSL II and above).

viii) Carefully pipette extracted RNA sample / NTC / PC into the designated tube / well (refer to the next diagram for example):

- Add 5 µL of extracted RNA into each sample tube / well (e.g. “S1” to “S10”).
- For No Template Control (“NTC”), add 5 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 5 µL of VRTD PC in place of the extracted RNA sample into the designated tube / well labelled as PC.

ix) Final volume for 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 following thermal cycling protocol:

- Thermal cycling protocol for Bio-Rad CFX96™ Touch Real-time PCR System

Bio-Rad CFX96™ Touch Real-time PCR 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 instrument such as QuantStudio™ with fast-temperature ramping option

QuantStudio™ (or related) Real-Time PCR Instrument			
Step	**Cycle**	**Temp**	**Time**
Adjust ramp rate (heating / cooling) to 1.5ºC / sec			
UNG Incubation	1	25 ºC	2 min
Adjust ramp rate (heating) to 1.5ºC / sec			
RT Incubation	1	50 ºC	15 min
Adjust ramp rate (heating) to 1.5ºC / sec			
Enzyme Activation	1	95 ºC	2 min
Amplification	45	**Adjust ramp rate (heating) to 1.5ºC / sec**	
		95 ºC	3 sec
		Adjust ramp rate (cooling) to 1.5ºC / 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™**	**Quencher Dye**
SARS-CoV-2 N gene	FAM	FAM	none
SARS-CoV-2 ORF1a gene	CAL Fluor® Red 610	JUN™	none
Human RPP30 gene Human Internal Control (HIC)	HEX	VIC™	none
Passive reference dye	N.A.	Mustang Purple	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 the quality of the sample extraction efficacy and RT-PCR inhibition.
ii) Positive Control (PC): VRTD PC 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 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 all 3 fluorophore detection channels for FAM (channel 1), HEX / VIC, (channel 2) and CAL Fluor® Red 610 / JUN (channel 3).

In the case of QuantStudio™ Real-time PCR Systems, baseline threshold for both FAM channel (N gene) and JUN channel (ORF1a gene) should be adjusted to **0.15** when the passive reference using Mustang Purple is used OR **48,000** when passive reference is NOT used. The result should only be analysed after threshold adjustment. Failure to adjust the threshold may potentially result in false positive result interpretation.

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 and non-structural protein 10 (NSP10) from SARS-CoV-2, as well as partial RPP30 gene sequence from human. PC should be positive (exhibits exponential amplification curve and Ct ≤ 40) for all 3 signal channels (FAM, CAL Fluor® Red 610 / JUN™ and HEX / VIC™). If not, invalidate the run and repeat the test assay with strict adherence to the sample and reagent guidelines. Determine the cause of failed PC and implement appropriate corrective actions.

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

No.	COV N gene (FAM)[3]	COV ORF1a gene (CAL Fluor® Red 610 / JUN)[3]	RPP30 gene HIC (HEX / VIC)[3]	Diagnostic Outcome
1	Ct > 40 or Ct undetermined	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	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	Exponential amplification curve[1] and Ct ≤ 40[2]	Positive for SARS-CoV-2
4	Ct > 40 or Ct undetermined	Exponential amplification curve[1] and Ct ≤ 40	Exponential amplification curve[1] and Ct ≤ 40[2]	Positive for SARS-CoV-2

5	Ct > 40 or Ct undetermined	Ct > 40 or Ct undetermined	Ct > 40 or Ct undetermined	Invalid Result[2]

[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 specimen that is human in origin, HIC shall produce amplification 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 undetermined, user is advised to repeat the test assay. Upon retest and if HIC remains at Ct > 40 or Ct undetermined, user is advised to repeat specimen collection from the patient.

[3] Threshold setting for FAM as well as JUN channels need to be set to **0.15** for test conducted using QuantStudio™ Real-time PCR System. The threshold for VIC channel can be set to 'AUTO' on the same system. For CFX96™ Touch Real-time PCR System, threshold for all 3 channels can be set to 'AUTO'.

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 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. Both CFX96™ Touch Real-time PCR System and QuantStudio™ 7 Pro Real-time PCR System were used to determine the LOD, which is 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 the LOD of VereRT™ Duo COVID-19 PCR Kit was determined to be **2 viral RNA copies per test / assay / reaction**. This also equates to **0.4 viral RNA copy per µL of extracted RNA**.

2. 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 the VereRT™ Duo COVID-19 PCR Kit maintains its performance in detecting all known viral strains, especially those that the World Health Organization (WHO) classifies as Variants of Concern such as Alpha, Beta, Gamma, Delta and Omicron, *in silico* inclusivity analyses were proactively being performed against known SARS-CoV-2 viral genome sequences based on BLAST analysis using both GISAID and NCBI databases to determine SARS-CoV-2 virus variant coverage by VereRT™ Duo COVID-19 PCR Kit.

The design of VereRT™ Duo COVID-19 PCR Kit encompasses two independent sets of primer and probe responsible for the detection of two different viral target N and ORF1a genes. Primer and probe sequences of both sets were matched against all genomic sequences in the aforementioned databases and to-date, amongst an estimate of 5.5 million isolates of SARS-CoV-2, there are 91 isolates within the Alpha, Beta and Delta

variants with mutation(s) in both target regions, that may escape detection using VereRT™ Duo COVID-19 PCR Kit.

3. Analytical Specificity (Cross-reactivity)

To ensure that VereRT™ Duo COVID-19 PCR Kit is specific for detecting SARS-CoV-2 in clinical specimens, the primer and probe sequences of VereRT™ Duo 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™ Duo 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 Coronaviruses, VereRT™ Duo 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. 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 VereRT™ Duo COVID-19 PCR Kit were validated by performing three rounds of testing using three different manufacturing lots of the kit over three different days by three different operators. Each round of testing included 20 replicates from a unique lot of VereRT™ Duo COVID-19 PCR Kit run on both CFX96™ Touch Real-time PCR System and QuantStudio™ 7 Pro Real-time PCR System. The variation of Ct values within each round represents test repeatability and the overall variation of Ct values across three rounds represents test reproducibility.

It is evident from the results on both instruments that the percent Coefficient of Variation (%CV) for the detection of N and ORF1a gene targets of SARS-CoV-2 and human

RPP30 gene for the HIC is no more than 2.56% conducted by 3 operators. The Ct SD (Standard Deviation) are below value of 1. Comparing the results between operators, the overall %CV is up to 1.94% for the test done using CFX96™ Touch Real-time PCR System and 2.09% for that performed using QuantStudio™ 7 Pro Real-time PCR System.

In conclusion, as shown from the repeatability and reproducibility study results, it is evident that the functional testing conducted by each operator using CFX96™ Touch Real-time PCR System and QuantStudio™ 7 Pro Real-time PCR System is highly repeatable because the %CV for the detection of N, ORF1a and human RPP30 gene targets by the VereRT™ Duo COVID-19 PCR Kit is well below 5%. The overall %CV for each of the targets is no more than 2.09% (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
CFX96™ Touch Real-time PCR System					
1	FAM	N	33.16	0.59	1.79
	CalRed	ORF1a	34.38	0.52	1.51
	HEX	RPP30	33.81	0.40	1.17
2	FAM	N	33.67	0.63	1.88
	CalRed	ORF1a	35.08	0.48	1.36
	HEX	RPP30	33.43	0.50	1.49
3	FAM	N	33.84	0.55	1.64
	CalRed	ORF1a	34.31	0.51	1.49
	HEX	RPP30	34.00	0.30	0.89
Overall	**FAM**	**N**	**33.56**	**0.65**	**1.94**
	CalRed	**ORF1a**	**34.59**	**0.61**	**1.75**
	HEX	**RPP30**	**33.75**	**0.47**	**1.38**
QuantStudio™ 7 Pro Real-time PCR System					
1	FAM	N	32.88	0.32	0.98
	CalRed	ORF1a	34.47	0.74	2.14
	HEX	RPP30	34.10	0.35	1.03
2	FAM	N	32.94	0.38	1.16
	CalRed	ORF1a	34.32	0.49	1.42
	HEX	RPP30	34.05	0.29	0.86
3	FAM	N	32.33	0.39	1.20
	CalRed	ORF1a	34.60	0.89	2.56
	HEX	RPP30	33.88	0.33	0.97
Overall	**FAM**	**N**	**32.72**	**0.45**	**1.38**
	CalRed	**ORF1a**	**34.46**	**0.72**	**2.09**
	HEX	**RPP30**	**34.01**	**0.33**	**0.98**

5. Interference Study

10 common endogenous and exogeneous substances were evaluated for any possible interference with the detection of SARS-CoV-2 virus and the Human Internal Control (HIC) using VereRT™ Duo COVID-19 PCR Kit. To address this, the 10 substances were to VTM/ UTM which contained pre-collected nasopharyngeal swab samples. The resultant samples were extracted before being spiked with known concentration of SARS-CoV-2 genomic RNA at level close to the Limit of Detection (LOD) of the assay. VereRT™ Duo COVID-19 PCR Kit was still able to confidently detect for SARS-CoV-2 virus as well as HIC.

There was no compelling evidence to suggest that the 10 substances, comprising endogenous substances like human whole blood and saliva, as well as other exogenous substances listed in the table below, exhibited severe interference with the detection of its targets by VereRT™ Duo 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

6. Clinical Evaluation

The clinical performance of VereRT™ Duo COVID-19 PCR Kit assay, as validated by a 3rd party clinical laboratory, was conducted based on RNA samples extracted from a total of 929 nasopharyngeal swab specimens collected in VTM / UTM comprising a combination of prospective and retrospectively identified subject samples. VereRT™ Duo COVID-19 PCR Kit was evaluated based on a total of 349 positive and 580 negative samples against TaqPath™ COVID-19 Combo PCR Kit as a comparator. The clinical performance of VereRT™ Duo COVID-19 PCR Kit has been summarized in an agreement table below.

		TaqPath™ COVID-19 Combo Kit (Comparator)		
		Positive	**Negative**	**Total**
VereRT™ Duo COVID-19 PCR Kit (Test under evaluation)	**Positive**	349	4	353
	Negative	0	576	576
	Total	349	580	929

Performance Indicators	**Calculations**	**Outcomes**	**95% Confidence Interval**
Clinical Sensitivity	349 / (349+0) x 100%	100.00%	98.95% to 100.00%
Clinical Specificity	576 / (4+576) x 100%	99.31%	98.24% to 99.81%
Positive Predictive Value (PPV)	349 / (349+4) x 100%	98.87%	97.05% to 99.57%
Negative Predictive Value (NPV)	576 / (0+576) x 100%	100.00%	N.A.

In conclusion, it is evident from the clinical trial that VereRT™ Duo COVID-19 PCR Kit can be conservatively claimed with a clinical sensitivity and specificity of 100.00% and 99.31%, respectively. The clinical performance as described by PPV is at least 98.87% while the NPV has been determined to be 100.00% in this cohort of 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 and 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
	Catalogue 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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