VISION COVID19 EASYPREP FAST REAL TIME PCR TEST KIT

INSTRUCTION FOR USE

REF V2004F190

-20 °C

CE IVD

Important!
The instructions for use must be read carefully prior to use and followed strictly to achieve reliable results. Any deviations from the instructions will have a significant impact on the end result.

Storage and Transportation Conditions
Protect from light during storage and transportation.

CERTIFICATIONS

ISO 13485:2016

ISO 9001 2015 CERTIFIED

Vision by Agebock

Vision Biotechnology was founded purpose of test kits development, which is based on Real-Time PCR particularly for medical diagnosis in Istanbul in 2020. Our company completed the process of certifications of ISO 13485 Medical Devices Quality Management System and TS EN ISO 9001:2015 Quality Management System successfully. Moreover, our firm has been performing procedure of

1. *INTRODUCTION*

For use with Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX 96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4-Channel+HRM

"Vision Covid19 Easyprep Fast Kit" Real-Time RT-PCR Diagnostic Panel is a molecular in vitro diagnostic test that aids in the detection and diagnosis 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in rRT-PCR for the in vitro qualitative detection of 2019-nCoV RNA in respiratory specimens.

1.1 Intended Use

"Vision Covid19 Easyprep Kit" is based on real-time Reverse Transcriptase Polymerase Chain Reaction technology, for the qualitative detection of SARSCoV- 2 specific RNA in nasal pharyngeal swab samples. A positive result from the test may indicate the presence of SARS-CoV-2 specific RNA in the test sample.

2. Limitations, Warnings and Precautions

"Vision Covid19 Easyprep Kit" described here have not been systematically verified for platforms or chemicals other than those mentioned in this instructions. Read the Instructions for Use carefully before using the product.

2.1 Acceptable Specimens

Nasal pharyngeal swab specimens.

2.2 On Specimen Handling

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

2.3 Handling

Follow specimen collection device manufacturer instructions for proper collection methods. Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

3. Product Description

On January 11, 2020, Chinese health authorities preliminarily identified more than 40 human infections with a novel coronavirus in an outbreak of pneumonia under investigation in

Wuhan City, Hubei Province, China. The Chinese authorities identified a new type of coronavirus (novel coronavirus, named as 2019-nCoV), which was isolated on 7 January 2020.

Coronaviruses are a large family of viruses, some causing illness in human and others circulating among animals such as camels, cats and bats. 2019-nCoV is a novel coronavirus. The primer and
probe design for this kit is based on the newly released strain (2019-nCoV) (GeneBank accession: MN908947) and covers 6 2019-nCoV strains sequences (EPI_ISL_402119, EPI_ISL_402120, EPI_ISL_402121, EPI_ISL_402122, EPI_ISL_402123, EPI_ISL_402124 included). The kit contains a specific ready-to-use system for the detection of Novel Coronavirus (2019-nCoV) by Reverse transcription Polymerase Chain Reaction (RT-PCR) in the real-time PCR system. The master contains a Oligo Mix for the specific amplification of virus RNA. The reaction is done in one step real time RT-PCR. The first step is a reverse ranscription (RT), during which the virus RNA is transcribed into cDNA.

Afterwards, a thermostable DNA polymerase is used to amplify the specific gene fragments by means of polymerase chain reaction (PCR). Fluorescence is emitted and measured by the real time systems optical unit during PCR. The detection of amplified virus DNA fragment is performed in fluorimeter channel FAM.

4. Storage and Transportation

- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity ofthe assay.
- Cool all reagents during the working steps.
- Oligo Mix should be stored in the dark

5. Material and Devices Required But Not Provided

- Biologic Safety Cabinet
- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µL, 10 or 100 µL and 100 or 1000 µL)
- Multichannel micropipettes (5-50 µl)
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold blocks
- Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX 96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4-Channel+HRM Extraction systems or test kits
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 96 well plates or 0,1/0,2 ul strips
- Plate Seal

6. Warning and Precautions

For in vitro diagnostic use (IVD).
For emergency use only.

Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.

Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.

Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.

If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.

Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection.

Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).

Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats.

Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment 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.

Maintain separate areas for assay setup and handling of nucleic acids.

Change aerosol barrier pipette tips between all manual liquid transfers.

7. Procedure

7.1 RNA Sample Preparation

These kits dont need to isolation of RNA steps.

7.2 Reaction Setup

Reaction Mix 1,8 ml X2	Oligo Mix N1 150 ul	Oligo Mix N2 150 ul	Oligo Mix RP 150 ul	Negative Control N1-N2 100 ul
Nuclease Free Water 400 ul	Control Positive N1-N2 100 ul	Control Positive RP 50 ul	Negative Control RP 50 ul	Rox Dye 100 ul

1. In the reagents set-up room clean hood, place Reaction Mixes, Oligomixes and Controls on ice or cold-block. Keep cold during preparation and use.

2. Before starting, thaw the mixtures.

3. Reaction mix and Oligo Mixes (ON1,ON2 and ORP) by inversion 5 times.

4. Centrifuge Reaction Mix and Oligomixes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.

5. Label one 1.5 mL microcentrifuge tube for each target(N1,N2,RP).

6. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the Negative Controls, Positive Controls and RP reactions and for pipetting error. Use the following guide to determine N:

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• If number of samples (n) including controls equals 1 through 14, then N = n + 1
• If number of samples (n) including controls is 15 or greater, then N = n + 2

7. For each target, calculate the amount of each reagent to be added for each reaction mixture (N = # of reactions).
8. Dispense reagents into each respective labeled 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down DO NOT VORTEX.
9. Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
10. Set up reaction strip tubes or plates in a 96-well cooler rack.
11. Dispense 15 µL of each PCR Mix into the appropriate wells going across the row as shown below (Figure3

STEP	Reagent	Vol. Of Reagent Added per Reaction	Advice
1	Oligomix	1.5 µL	Prepare the reagents 1 test more against to pipetting mistakes for 10 tests PCR runnings.
2	Reaction Mix	10.0 µL	
3	Nuclease Free Water	3.50 µL	For high volume Rox dye needs instrument: 1 µL volume Rox Dye add and decrease Nuclease free Water volume 2,5 µL For low volume Rox Dye needs instrument: 0,50 µL volume Rox Dye add decrease the Nuclease Free Water volume 05 µL
4	Rox Dye	1 µL/ 0.5 µL	
	Total Volume	15.0 µL	

12.Prior to moving to the nucleic acid handling area, prepare the Negative Control and positive control. If you choose A1 for negative control, you should use A12 for positive control because of contamination risk.

13. Pipette 3,5 µL of nuclease-free water into the NTC sample wells (A1). Securely cap NTC wells before proceeding.

Nucleic Acid Template Addition

1.Gently vortex nucleic acid sample tubes for approximately 5 seconds.

2.Centrifuge for 5 seconds to collect contents at the bottom of the tube.

3.After centrifugation, place extracted nucleic acid sample tubes in the cold rack.

4. Samples should be added to columns 2-11 (column 1 and 12 are for controls) to the specific assay that is being tested as illustrated in Figure 2. Carefully pipette 5.0 µL of the first sample into all the wells labeled for that sample (i.e. Sample "S1" down column #2). Keep other sample wells covered during addition. Change tips after each addition.

5.Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.

6.Change gloves often and when necessary to avoid contamination.

7.Repeat steps #4 and #5 for the remaining samples.

8.Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

Assay Control Solutions

1. Pipette 5 µL of positive control solition to the sample wells of column 12 (Figure 2). Securely cap wells after addition of the control RNA. NOTE: If using 8-tube strips, label the TAB of each strip to indicate sample position.

	1	2	3	4	5	6	7	8	9	10	11	12
A	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1	N1
B	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2	N2
C	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP
D												
E												
F												
G												

7.3 Real-Time PCR Instrument

Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX 96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4-Channel+HRM

7.4 Programming the Real-Time PCR Instrument

	VISION COVID19 EASYPREP		
	Temp. °C	Duration	Cycles
Reverse Transcription	90	3 Min	1
Initial activation	60	5 Min.	1
Denaturation	95	5 Sec.	40
Annealing/Extension*	55	30 Sec.	

8. Data Analysis

8.1 CT Determination By Single Threadshold

The recommended threshold for this assay on the above described realtime

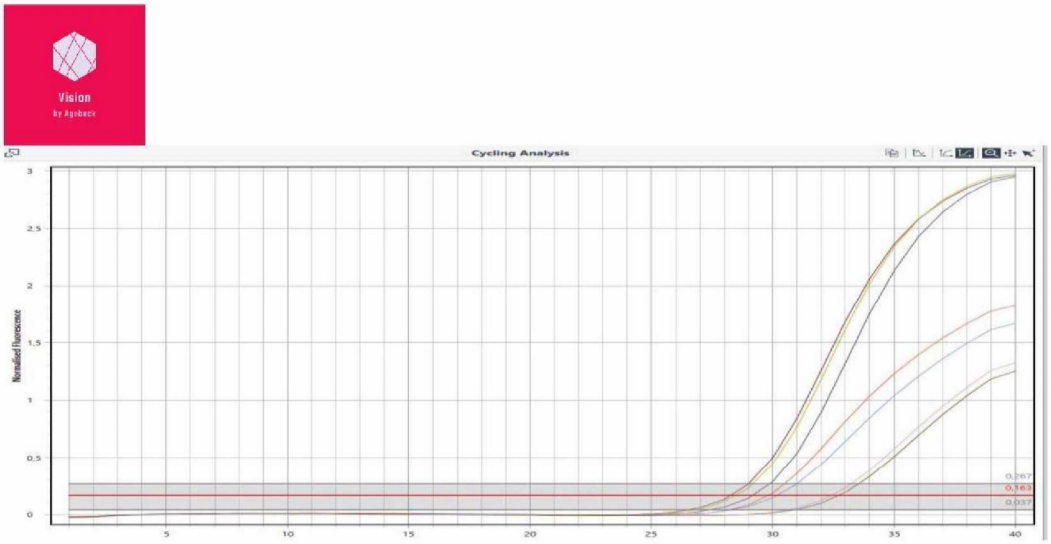
PCR systems is 50 for FAM channels, based on a Relative Fluorescence Units (RFU) range of 0-18 (ΔRn) for the FAM channel.

For potential instrument-to-instrument variation in the fluorescence signal saturation range, instrument calibration might be required to set the appropriate threshold for individual real-time PCR system.

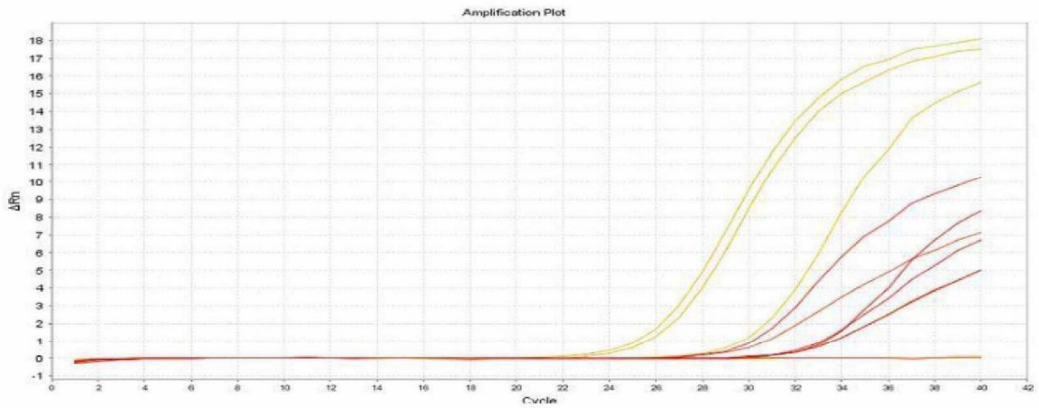
All CT values reported in this IFU is based on the recommended threshold settings at 50 for FAM channels as described above. CT values are subject to changes with different thresholds set by the users.

Interpretataion of Vision Covid19 EasyPrep test result should take into consideration **the CT values,** as well as **the shape of the amplification curve**, as shown in Figure below.

Interpretatation for BioMolecular Systems Bio 4- Channel+HRM:



Interpetation for Applied Biosystems SteponePlus



8.2 Interpretation of Test Results

The table below lists the expected results for the 2019-nCoV rRT-PCR Diagnostic Panel. If a laboratory obtains unexpected results for assay controls or if inconclusive or invalid results are obtained and cannot be resolved through the recommended re-testing, please contact Goverment Health Minister for consultation and possible specimen referral. See pages 10 and 35 for referral and contact information.

N1	N2	RP	Result Interpretation	Actions
+	+	±	2019-nCoV detected	Report.
±	Inconclusive Result		Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR.	
-	-	+	2019-nCoV not detected	Report results to sender. Consider testing for other respiratory viruses
-	-	-	Invalid Result	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

9. Performance Evaluation

Vision Covid19 Easyprep Test Kit performance evaluation has been generated on the Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX96Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4- Channel+HRM instruments for analytical sensitivity (LoD).

9.1 Analytical Sensitivity

The Limit of detection (LoD) was defined as the lowest concentration of analyte that could be reliably detected at least 95% of the time. First a tentative LoD was detected by testing five limiting dilutions, prepared by spiking quantified SARS-CoV-2 whole viral RNA supplied by the European Virus Archive-Global (Cat: 026N-03889) into oropharyngeal matrix negative for SARSCoV-2. The LoD was confirmed by testing single replicates of the 20 extraction eluates on the these instruments: Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4- Channel+HRM.

StepOnePlus Real-Time PCR System					
SARS-CoV2Mean Concentration (copies/µl)	Overall Mean Concentration (copies/rxn)	Positive calls/Total no. results included in analysis	% Replicate Detection	Mean Cq	Cq Standard Deviation
0.35	2.70	20/20	100	32.5	0.98

9.2 Analytical Specificity

	LightCycler 480 II (Roche®)	BioMolecular Systems Bio 4-Channel+HRM	CFX96 (BioRad®)
SARS-CoV-2 Mean Concentration (copies/µl)		% Replicate Detection	
0.38	100% (20/20)	100% (20/20)	100% (20/20)

Alternative Instrument Testing The tentative LoD was also confirmed by testing 20 replicates on the Roche LightCycler 480 Instrument II, Applied Biosystems 7500 Fast, Biorad CFX96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio4+Channel+HRM.

10. Assay Limitations

The Vision COVID19 Easyprep Real-Time PCR test kit has been validated for use with oropharyngeal swab samples run on the Roche LightCycler 480 Instrument II, Applied

Biosystems 7500 Fast, Biorad CFX96 Realtime PCR, Applied Biosystems SteponePlus, BioMolecular Systems Bio 4- Channel+HRM

• All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.

• Performance of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel has only been established in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate).

• Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.

• A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.

• Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.

• Do not use any reagent past the expiration date.

• If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.

• Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and, during the course of infection, when these specimens are most likely to contain levels of viral RNA that can be readily detected.

• Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.

• The performance of this test has not been established for monitoring treatment of 2019-nCoV infection.

• The performance of this test has not been established for screening of blood or blood products for the presence of 2019-nCoV.

• This test cannot rule out diseases caused by other bacterial or viral pathogen.

11. Quality Control

Negative control, and positive control must be performed correctly, otherwise the sample results are invalid.

No Template Control (NTC)

The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all oligomixes should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

Positive Control (nCoVPC)

The nCoVPC consists of in vitro transcribed RNA. The nCoVPC will yield a positive result with the following N1, N2 and RP Positive Controls.
If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (<35.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and / or RNA degradation.

- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.

- Improper assay set up and execution.

- Reagent or equipment malfunction.
If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the 2019-nCoV N1, N2 and are positive even in the absence of a positive RP, the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.

- If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

2019-nCoV Markers (N1, N2)

• When all controls exhibit the expected performance, a specimen is considered negative if all 2019-nCoV marker (N1, N2,) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (<35.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (<35.00 Ct).

• When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (<35.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.

• When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2,) and the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (<35.00 Ct), the result is invalid. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

• When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 or but not each markers) crosses the threshold line within 40.00 cycles (<35.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the same result is obtained, the laboratory should coordinate transfer of the specimen to CDC for further analysis.

	N1	N2	RP	Expected Ct Values
Positive Control	Positive	Positive	Positive	>35.00
Negative Control	Negative	Negative	Negative	None Detected (<35.00 Ct)

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12. Technical Assistance

For technical advice, please contact Technical Support:
5.1.5 **@visionbiotechnology.com**

13. Disclaimer

VISION COVID19 EASYPREP REAL TIME PCR TEST KITshould only be used for the intended purpose and
in accordance with the Instructions for Use.
Accelerate Technologies Pte Ltd (DxD Hub) is not liable for any damage or loss that may result from your use of the test.