[Emergency Use Authorization]

1. Intended Use

MolecuTech® Real-Time COVID-19 Test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in sputum, bronchial alveolar lavage fluid, oropharyngeal and nasopharyngeal swab samples from individuals with signs and symptoms of infection who are suspected of COVID-19. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in human sputum, bronchial alveolar lavage fluid, and oropharyngeal and nasopharyngeal swabs during the acute phase of infection. Positive results are indicative of active infection. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The **MolecuTech® Real-Time COVID-19** is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The **MolecuTech® Real-Time COVID-19** is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Summary

The respiratory disease has the highest prevalence of the virus, and has been reported in South Korea and the United States to account for half of all infectious diseases. Many types of viruses are directly or indirectly related to respiratory diseases and are commonly known as representative pathogens that cause colds, including influenza viruses, adenovirus, parainfluenza viruses (PIVs), respiratory syncytial virus (RSVs), rhinoviruses, and coronaviruses.

Influenza virus appears a lot in the early spring in the fall. Symptoms of influenza virus infection are characterized by high fever within 24 hours, as well as systemic symptoms such as headache, muscle pain and fatigue, sore throat, cough, sputum and rhinitis. A healthy person recovers within a few days, but can cause pneumonia in patients with a basal disease. Coronavirus, one of these influenza viruses, is accompanied by respiratory, gastrointestinal and neurological symptoms but is asymptomatic in 50%, and is known as a rare pathogen in childhood acute respiratory infections in South Korea. The WHO temporarily named the disease caused by the novel coronavirus in 2019 as the novel coronavirus acute respiratory infection 2019. Currently, novel coronavirus vaccines and antiviral drugs are actively developing at universities and Center for Disease Control (CDC) around the world, such as China, Hong Kong, and Australia. According to the WHO's official announcement, as of the end of January, the mortality rate by novel coronavirus is estimated to be about 3% so far, which is lower than the mortality rate of 9.6% for SARS (Severe Acute Respiratory Syndrome). As of February 2, 2020, no clear treatment has been established so far, and the treatment of patients is focused on relieving symptoms including fever, dry cough, and shortness of breath. In the current situation where there is no cure, it is a priority to prevent

the spread of infection through early diagnosis. YD DIAGNOSTIC Co., Ltd. developed a product that can qualitatively detect COVID-19 in a quick and accurate manner in single experiment by using real time one step RT- Multiplex PCR, from reverse transcription to real-time polymerase chain reaction.

3. Principle

The MolecuTech® Real-Time COVID-19 is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The 2019-nCoV primer and probe sets is designed to detect RNA from SARS-CoV-2 in human sputum, bronchial alveolar lavage fluid, and oropharyngeal and nasopharyngeal swab samples from patients with signs and symptoms of infection who are suspected of SARS-CoV-2. The principle of MolecuTech® Real-Time COVID-19 is based on real time reverse transcription polymerase chain reaction using TaqManTMprobe (hydrolysis probe) chemistry. Complementary DNA (cDNA) is synthesized through reverse transcription (RT) from extracted viral RNA and subsequently amplified using one of the recommended diagnostic instruments. During the PCR reaction, the probe hybridizes to the target DNA. With each PCR cycle, additional reporter dye molecules are cleaved from their respective probes, increasing fluorescence intensity. Fluorescence intensity is monitored by the diagnostic instrument. The PCR primer mixture includes RdRP, E gene, and human RPP30 primer and probe designed by YD DIAGNOSTICS with labeled fluorescence FAM (RdRP gene of SARS-CoV-2), CY5 (E gene of Coronavirus), and ROX (Huma RPP30 as IC), respectively.

4. Storage and Expiration date

- All reagents should be stored at -20℃ before open. Avoid repeated freezing and thawing to prevent the degradation of RT-PCR Premix activity.
- All reagents can be used until expiration date of manufacturer indicated on the kit label.

5. Kit contents

Contents	Cap color	Quantity (100T)
2X RT-PCR Premix	White	1000 ul
Primer/Probe Mixture	Brown	550 ul
Positive Control	White	50 ul
Ultra Pure Water	White	1500 ul

6. Biosafety Precaution

Wear appropriate personal protective equipment (e.g. gowns, gloves, eye protection) when working with clinical specimens. Specimen processing should be performed in a certified class II biological safety cabinet following biosafety level 2 or higher guidelines. For more information, refer to:

- Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV), https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
- Biosafety in Microbiological and Biomedical Laboratories 5th edition available at http://www.cdc.gov/biosafety/publications/.

7. Sample Collection, Storage, and Transport

A. Specimens collection

- Respiratory specimens including: human sputum, bronchial alveolar lavage fluid, oropharyngeal and nasopharyngeal swab sample

B. Specimens collection method

a. Sputum

- The sputum from patient's oral cough collected in sterile tubes (minimum 300 ul)

b. Oropharyngeal Swab

- Open the patient's mouth and use a tongue depressor to depress the tongue.
- Hold the sterilized cotton swab in your right hand (or left) and swab the pharyngeal posterior wall with a cotton swab three to four times at 360 degrees. (To avoid patient nausea, do not touch the patient's uvula when smearing).

c. Nasopharyngeal Swab

- If the oropharyngeal smear is not easy, it can be replaced with a nasopharyngeal.
- For swabing the nasopharyngeal, use a sterile cotton swab to gently rotate the mucous membrane 3-4 times near the lower middle of the inferior turbinate.

C. Storage

- Specimens can be stored at 4℃ for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70℃ or lower.
- Extracted nucleic acids should be stored at -70℃ or lower.

D. Transport

a. Transportation of clinical samples must comply with local regulations for the transport of etiological agents.

b. Depending on the type of samples, utilize aUniversal Transport Media (UTM) container.

- Put nasopharyngeal swab and Throat swab in UTM container.
- Precaution: If there is no UTM container, add 1 mL of sterile saline to the sterile container and put a cotton swab (UTM container is recommended).

c. Keep the internal temperature at 4℃ during transportation.

E. Samples are inadequate under the following conditions

a. Samples are not stored at 2~4℃ or not stored at -70℃ or below.

b. Incorrect or improper labeling of samples

c. Not using recommended sample types

d. Insufficient sample volume

8. Reagent Preparation

- All reagents should be stored at -20℃ before open. Avoid repeated freezing and thawing to prevent the degradation of premix activity.
- Vortex and spin-down all reagents before using.
- Do not store reagents more than 1 hour at room temperature.Use all reagents in aliquots to avoid contamination which could affect the test results

A. 2X RT-PCR Premix: including but not limited to reverse transcriptase, dNTP, Taq polymerase, and reaction buffer

B. Primer/Probe Mixture: including primer and probe with labeled fluorescence set for amplifying COVID-19 RdRP, coronavirus E, and human RPP30 gene. The probe of the Primer/Probe Mixture is sensitive to light so it should be stored in the brown tube (supplied).

C. Positive Control (PC): The PC has been designed and validated to fluorescence and exhibit positive results with COVID-19 specific RdRP gene detection.

D. Ultra Pure Water: as the Ultra Pure Water is utilized as the negative control. It should beat room temperature (15 ~ 25℃) before use.

9. RNA extraction

- Different brands of RNA extraction kits are available and suitable for use with the MolecuTech® Real-Time COVID-19 Test. You may use your own extraction systems or the commercial kits based on the desired yield.
- The recommended RNA extraction kits is the QIAamp® Viral RNA mini (Cat No. 52906). Follow the manufacturer's instructions for RNA extraction
- Retain residual specimen and RNA nucleic extract, and store immediately at -70℃.
- Only thaw the number of specimen extracts that will be tested in a single day. Do not freeze/thaw extracts more than once before testing.

10. One Step Real-Time Reverse Transcription Polymerase Chain Reaction

A. Preparation of PCR mixture

Prepare the multiplex Real-time Reverse transcription Polymerase Chain Reaction (2X RT-PCR Premix) mixture as many as the number of the sample to be tested, the positive control solution (5 uL), and the negative control solution (5 uL) as shown in the table below.

Contents	Quantity (1 test)
2X RT-PCR Premix	10 ul
Primer/Probe Mixture	5 ul
Sample RNA*	5 ul
Ultra Pure Water	0 ul
Final volume	20 ul

* Negative control use a Ultra Pure Water (supplied) of same volume with sample

* We recommend using 50 ng/ul or more of sample RNA.

B. One step multiplex Real-time Reverse transcription Polymerase Chain Reaction is performed immediately with the following program (Total running time is about 2 hours)

	Status	Temp.	Time	Cycle
Step 1	cDNA synthesis	50℃	30 min	1 cycle
Step 2	Pre-denaturation	95℃	10 min	1 cycle
Step 3	Denaturation	95℃	15 sec	45 cycle
	Annealing/Extention (Acquisition)	58℃	30 sec	

C. Instrument

- It is recommended to use either of the following diagnostic instruments:

Manufacturer	Model
Bio-Rad	CFX 96 real-time PCR detection system (CFX 96)
ABI	Applied Biosystems 7500 Real-Time PCR Instrument System(ABI 7500)

D. Measurement of Results

a. The PCR reaction results are measured by a real-time PCR machine and the results are analyzed as Ct values.

b. When a reaction is finished, the threshold value of each machine (ABI 7500 or CFX 96) should be set as described in the table below:

CFX 96 & ABI 7500: Threshold values

Threshold / Model	Threshold		
	FAM (COVID-19)	**CY5 (Coronavirus)**	**ROX (IC*)**
CFX96	100	20	20
ABI7500	10,000	10,000	10,000
* IC is internal control.			
** When using the product for the first time, instrument setting may be necessary.			

11. Data analysis and Interpretation

A. The Ct values that determine a positive criteria for each device are shown in the table below.

Channel / Model	Ct value		
	FAM (SARS-CoV-2)	**CY5 (Coronavirus)**	**ROX (IC*)**
CFX96	Ct ≤ 40.0	Ct ≤ 40.0	Ct ≤ 40.0
ABI7500	Ct ≤ 40.0	Ct ≤ 40.0	Ct ≤ 40.0

B. The Ct values of positive control and negative control are shown in the table below.

CFX96/ ABI7500	**SARS-CoV-2 (FAM)**	**Coronavirus (CY5)**	**IC (ROX)**	**Data Interpretation**
Positive Control	Ct ≤ 30.0	Ct ≤ 30.0	Ct ≤ 30.0	Valid
Negative Control	-	-	-	Valid

* Use the threshold values suggested in 10.D. If the threshold values suggested in 10.D are not applied, a non-specific reaction occurs in the negative control, resulting in a Ct value.

C. The interpretation of possible sample results are shown in the table below.

Positive criteria: Ct ≤ 40.0	SARS-CoV-2 (FAM)	Coronavirus (CY5)	IC (ROX)	Data Interpretation
Sample 1	Ct ≤ 40.0	Ct ≤ 40.0 or > 40.0	Ct ≤ 40.0 or > 40.0	SARS-CoV-2 Positive
Sample 2	Ct > 40.0	Ct ≤ 40.0	Ct ≤ 40.0 or > 40.0	SARS-CoV-2 Negative
Sample 3	Ct > 40.0	Ct > 40.0	Ct ≤ 40.0	Negative
Sample 4	Ct > 40.0	Ct > 40.0	Ct > 40.0	Re-test

12. Quality Control

Control experiment should be performed simultaneously with all experimental samples.

A. Internal Control (IC): All samples should detect ROX channel below a Ct value 40.0. IC is a substance to check the PCR inhibitor. If there is no problem with RNA extraction and cDNA synthesis, IC should display Ct values below 40.0. If Ct values are not detected, perform a retest. For a sample with a high concentration of the virus, sometimes the Ct value of the IC is not detected, but it is a valid result if the Ct value is detected at channel of the target gene.

B. Negative Control (supplied Ultra Pure Water): Ct values should not be detected of FAM (SARS-CoV-2), CY5 (Coronavirus), and ROX (Human RPP30), respectively.

C. Positive Control: A Ct value less than or equal to 30 should be detected at the FAM (SARS-CoV-2), CY5 (Coronavirus), and ROX (IC) channel, respectively.

13. Specific Performance Characteristics

A. LoD (Limit of Detection)

LoD determines the lowest detectable concentration of SARS-CoV-2 at which ≥95% of all (true positive) replicates test positive.

Virus	Target Gene	LoD (copy/reaction)
SARS-CoV-2	Wuhan seafood market pneumonia virus RdRP gene (Accesion No. LR757998.1)	10
Coronavirus	Coronavirus E protein gene (Accesion No. MK211377.1)	100
IC	Human RPP gene (Accession No. NM_001104546.2)	10

B. Analytical specificity

a. Inclusivity

Since MolecuTech® Real-Time COVID-19 is a diagnostic kit capable of detecting 2019 Novel Coronavirus and Coronavirus, the in silico analysis determines whether the test inclusively detects genetically diverse coronavirus subtype strains. Inclusivity was demonstrated by comparing the MolecuTech® Real-Time COVID-19 primers and probes to alignment of all SARS-CoV-2 sequences available in NCBI and GISAID database as of March 18, 2020. In silico analysis multiple alignment was generated by NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi database.)

As a result of blasting the PCR target region of the 2019-Novel Coronavirus subtype RdRP gene, it was confirmed that the subtype demonstrated 100% homology (64 sequences). Also, as a result of blasting the PCR target region of the E gene, it was confirmed that the subtype was 99.99% identical (100 sequence). Through this inclusivity study, results of in silico analysis for RdRP and E gene targeting primer/probe confirmed 100% homology and inclusivity.

b. Cross-reactivity

In Silico Analysis

In silico analysis was performed to analyze homology between the selected probe/primer and the sequence of various pathogenic disease agents or microorganisms.

The conclusion of this analysis is that there is very limited opportunity for cross-reactivity to influence the performance of detecting SARS-CoV-2 through *in silico* analysis.

For the RdRP gene detecting SARS-CoV-2 target, the 5'-forward primer has 90% homology in many microorganisms including isolated Bat coronaviruses, but has 0% homology in the 3'-reverse primer. In the case of isolated Bat coronaviruses, the 5'-forward primer will bind, but the probe will not be amplified per in silico analysis. Mismatches in the 3' end of the primers makes extension unlikely. For many organisms, only one primer has > 80% homology and probe is unlikely to bind for any of the hits (<80% homology).

Therefore, the results of the in silico analysis predict that there will be no significant cross-reactivity or microbial interference.

Laboratory Testing

The cross-reactivity of MolecuTech® Real-Time COVID-19 was evaluated using 16 respiratory viruses, 1 respiratory sample, 12 respiratory fungi, and 23 microbes and 22 Mycobacteria (including TB). The concentrations for respiratory viruses and fungi were evaluated as 2X105 TCID50 / mL, and the concentrations for other bacteria were 2x106 copy/ul. The material for each concentration was run at 5ul / reaction. This was evaluated for 9 times by repeating 3 tests x 3 times by 1 tester.

Recommended List of Organisms to be analyzed *in silico* and by Wet Testing*

Microorganism	Concentration	Results (No. Pos./No. Tested)	Final Result
Corumebacterium ammoniagenes	$2x10^6$ CFU/ml	0/9	Neg.
Pseudomonas spp.	$2x10^6$ CFU/ml	0/9	Neg
Clostridium spp.	$2x10^6$ CFU/ml	0/9	Neg
Staphylococcus aureus	$2x10^6$ CFU/ml	0/9	Neg
Staphylococcus epidermidis	$2x10^6$ CFU/ml	0/9	Neg
Enterobacter spp.	$2x10^6$ CFU/ml	0/9	Neg
Klebsiella spp.	$2x10^6$ CFU/ml	0/9	Neg
Escherichia coli	$2x10^6$ CFU/ml	0/9	Neg
Proteus spp.	$2x10^6$ CFU/ml	0/9	Neg
Fusobacterium spp.	$2x10^6$ CFU/ml	0/9	Neg
Bacteroides spp.	$2x10^6$ CFU/ml	0/9	Neg
Lactobacillus acidophilus	$2x10^6$ CFU/ml	0/9	Neg
Streptococcus faecalis	$2x10^6$ CFU/ml	0/9	Neg
Bifidobacterium spp.	$2x10^6$ CFU/ml	0/9	Neg
Streptococcus pyogenes	$2x10^6$ CFU/ml	0/9	Neg
Peptostreptococcus spp.	$2x10^6$ CFU/ml	0/9	Neg
Candida albicans	$2x10^6$ CFU/ml	0/9	Neg
Streptococcus agalactiae	$2x10^6$ CFU/ml	0/9	Neg
Cytomegaol virus	$2x10^6$ CFU/ml	0/9	Neg
Shigella flexneri	$2x10^6$ CFU/ml	0/9	Neg
Epstein-barr virus	$3x10^5$ CFU/ml	0/9	Neg
Chlamydia trachomatis	$2x10^6$ CFU/ml	0/9	Neg
Neisseria gonorrhoeae	$2x10^6$ CFU/ml	0/9	Neg
ADENOVIRUS 4	$3x10^5$ TCID50/mL	0/9	Neg
CORONAVIRUS	$3x10^5$ TCID50/mL	0/9	Neg
INFLUENZA A	$3x10^5$ TCID50/mL	0/9	Neg
INFLUENZA B	$3x10^5$ TCID50/mL	0/9	Neg

NOVEL INFLUENZA A H1N1	$3x10^5$ TCID50/mL	0/9	Neg
PARAINFLUENZA 1	$3x10^5$ TCID50/mL	0/9	Neg
PARAINFLUENZA 2	$3x10^5$ TCID50/mL	0/9	Neg
PARAINFLUENZA 3	$3x10^5$ TCID50/mL	0/9	Neg
PARAINFLUENZA 4	$3x10^5$ TCID50/mL	0/9	Neg
RESPIRATORY SYNCYTIAL VIRUS (subtype A)	$3x10^5$ TCID50/mL	0/9	Neg
RESPIRATORY SYNCYTIAL VIRUS (subtype B)	$3x10^5$ TCID50/mL	0/9	Neg
MERS CORONAVIRUS	$3x10^5$ TCID50/mL	0/9	Neg
Mycoplasma pneumoniae	$2x10^6$ CFU/ml	0/9	Neg
Chlamydophila pneumoniae	$2x10^6$ CFU/ml	0/9	Neg
Legionella pneumophila	$2x10^6$ CFU/ml	0/9	Neg
Bordetella pertussis	$2x10^6$ CFU/ml	0/9	Neg
Bordetella parapertussis	$2x10^6$ CFU/ml	0/9	Neg
Streptococcus pneumoniae	$2x10^6$ CFU/ml	0/9	Neg
Haemophilus influenzae	$3x10^5$ CFU/ml	0/9	Neg
Mycobacterium tuberculosis	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium avium	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium intracellulare	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium scrofulaceum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium abscessus	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium massiliense	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium chelonae	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium fortuitum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium ulcerans	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium marinum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium kansasii	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium gastri	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium haemophilum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium genavense	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium simiae	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium terrae	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium nonchromogenicum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium celatum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium gordonae	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium szulgai	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium mucogenicum	$2x10^6$ CFU/ml	0/9	Neg
Mycobacterium aubagnense	$2x10^6$ CFU/ml	0/9	Neg
SARS-coronavirus	$2x10^6$ TCID50/mL	0/9	Neg
Human Metapneumovirus (hMPV)	$2x10^6$ TCID50/mL	0/9	Neg
Enterovirus	$2x10^6$ TCID50/mL	0/9	Neg
Rhinovirus	$2x10^6$ TCID50/mL	0/9	Neg
Legionella pneumophila	$2x10^6$ CFU/ml	0/9	Neg
Normal human sputum	$2x10^6$ CFU/ml	0/9	Neg
Pseudomonas aeruginosa	$2x10^6$ CFU/ml	0/9	Neg
Streptococcus salivarius	$2x10^6$ CFU/ml	0/9	Neg
Bordetella pertussis	$2x10^6$ CFU/ml	0/9	Neg
Haemophilus influenzae	$2x10^6$ CFU/ml	0/9	Neg

14. Clinical Performance Evaluation

A clinical evaluation study was performed to evaluate the performance of the MolecuTech® Real-Time COVID-19 kit using lower respiratory tract specimens (sputum). A total of 90 contrived positive specimens at approximately 1XLOD, 2XLOD and 20x LOD were tested (30 tests each). Samples were contrived by spiking

known concentrations of recombinant virus containing COVID-19 RNA sequences into negative patient specimens. In addition to the contrived positive specimens, 30 negative specimens were tested. All negative samples were confirmed as negative. All results were valid and included in the analysis.

Table: Results of the Clinical Evaluation of the MolecuTech® Real-Time COVID-19

Target	Transcripts	SARS-CoV-2 (FAM)			Coronavirus (CY5)			IC (ROX)		
		Number Tested	Number Detected	% Detection	Number Tested	Number Detected	% Detection	Number Tested	Number Detected	% Detection
COVID-19 (RdRP)	1XLoD (10copy/reaction)	30	30	100 (30/30)	30	0	0 (0/30)	30	30	100 (30/30)
	2XLoD (20copy/reaction)	30	30	100 (30/30)	30	0	0 (0/30)	30	30	100 (30/30)
	20XLoD (200copy/reaction)	30	30	100 (30/30)	30	0	0 (0/30)	30	30	100 (30/30)
	No spiked	30	0	0 (0/30)	30	0	0 (0/30)	30	30	100 (30/30)
Coronavirus (E gene)	1XLoD (10copy/reaction)	30	0	0 (0/30)	30	30	100 (30/30)	30	30	100 (30/30)
	2XLoD (20copy/reaction)	30	0	0 (0/30)	30	30	100 (30/30)	30	30	100 (30/30)
	20XLoD (200copy/reaction)	30	0	0 (0/30)	30	30	100 (30/30)	30	30	100 (30/30)
	No spiked	30	0	0 (0/30)	30	0	0 (0/30)	30	30	100 (30/30)

15. Precautions

A. For In vitro diagnostic use only.
B. All reagents should be stored at the appropriate storage conditions before and after use.
C. Do not store reagents with the reagent lid open.
D. This product is designed to be used separately according to the type of samples obtained from individual patients, such as upper and lower latitudes.
E. Specimens may cause infection and unknown disease, so they should be handled with caution.
F. Each laboratory should follow their own guidelines regarding quality control substances.
G. Operators must wear disposable gloves before performing tests during handling of clinical specimens.
H. To prevent contamination, all specimens should be handled on a clean bench.
I. Do not pipette any reagent into your mouth.
J. Do not smoke, eat, ord drink when handling specimens.
K. All reagents should be tapped or vortexed prior to use and then should be used after quick-spin.
L. For efficiency of PCR activity, minimize standing time at room temperature. The 2X RT-PCR Premix activity will be reduced when it is allowed to stand at room temperature for 1 hour or more.
M. If the Primer/Probe Mixture and 2X RT-PCR Premix have excessive exposure to light or are thawed and frozen repeatedly then its activity may decrease. The aliquot should be used all at once to avoid re-freezing and thawing.
N. When dispensing reagent, be careful of microorganism contamination. Using a sterilized disposable filter-tip is recommended.
O. PCR conditions were set based on CFX 96 (Bio-Rad) and ABI7500 (Applied Systems). When using other devices, PCR conditions and Ct values may change.
P. Follow the manufacturer's operating instructions for the PCR diagnostic instrument (caution: Using

consumables that are not suitable for the analyzere may cause device malfunction and may affect the results).

Q. Use before the expiration date of the product.

R. Liquid or solid waste should be stored in a liquid or solid waste container and controlled by the "Waste and Wastewater Management Regulations".

S. This product can not completely exclude the possibility of false positive or false negative results due to various factors. Be careful when interpreting the results. Final results should be assisted by other test methods and the findings of the clinical specialist.

T. IC DNA may not amplify when there is a high concentration of template DNA or interferences in the PCR mixture. In this case, we recommend the retest. DNA can be diluted 10-100 times with 1X TE buffer.

U. Do not mix together products from different Lots.

V. When analyzing the final results of samples, it is necessary to check whether the amplification curve appearing on the instrument shows sigmoid or linearity, and if it shows linearity from the beginning, it should not be read positive result because it is non-specific amplification.

16. Reference

A. Novel Coronavirus (COVID-19): Situation Report - 10 (30 January 2020) World Health Organization (WHO, Report).

B. “Experts explain the latest bulletin of unknown cause of viral pneumonia”. 《Wuhan Municipal Health Commission》.

C. Hui DS, I Azhar E, Madani TA, Ntoumi F, Kock R, Dar O, Ippolito G, Mchugh TD, Memish ZA, Drosten C, Zumla A, Petersen E. The continuing COVID-19epidemic threat of novel coronaviruses to global health – The latest 2019 novel coronavirus outbreak in Wuhan, China. Int J Infect Dis. 2020 Jan 14;91:264–266.

D. “Surveillance case definitions for human infection with novel coronavirus (nCoV)”. 《www.who.int》.

E. “Coronavirus: Thailand has apparent treatment success with antiviral drug cocktail”.South China Morning Post.

F. “Novel coronavirus (COVID-19), Wuhan, China”. cdc.gov.

G. “Why snakes probably aren't spreading the new China virus”. Callaway, Ewen; Cyranoski, David. Nature.doi:10.1038/d41586-020-00180-8.

H. “Transmission of COVID-19Infection from an Asymptomatic Contact in Germany”. 2020. Rothe, Camilla 《NEJM》: Correspondence to the Editor Page.doi:10.1056/NEJMc2001468

I. “A novel coronavirus outbreak of global health concern”.(2020) Wang, Chen; Horby, Peter W; Hayden, Frederick G; Gao, George F 《The Lancet》S0140673620301859.doi:10.1016/S0140-6736(20)30185-9.

17. Manufacturer

YD DIAGNOSTICS CORP.

76, Seori-ro, Idong-myeon , Cheoin-gu, Yongin-si, Gyeonggi-do, 17127, Republic of Korea

TEL: 82-31-329-2000 / FAX: 82-31-329-2002

Rev.00/2020-02-20