iDetect™ SARS-CoV-2 Detection kit

1. Product Indication

- Category: IVD reagent for infectious disease marker (Detection of high-risk infectious pathogens)
- In-vitro diagnostic medical device

2. Intended Use

iDetect™ SARS-CoV-2 Detection kit is an in-vitro medical device based on a reverse transcription loop-medicated isothermal amplification(RT-LAMP) reaction intended for the qualitative detection of RdRp and N gene for SARS-CoV-2 in nasopharyngeal and oropharyngeal swabs, sputum, nasopharyngeal aspirates and bronchoalveolar lavage (BAL) specimens.

3. Kit Contents [Packing Unit: 100 tests / kit]

Reagents	Descriptions	Volume/ Quantity
RT-LAMP Premix	RTase/DNA polymerase	1,000 ㎕/ 2 tubes
RdRp primer mix	RdRp gene primer	500 ㎕ / 1 tube
N primer mix	N gene primer	500 ㎕ / 1 tube
Positive control	Positive control	200 ㎕ / 1 tube
Negative control	Negative control	200 ㎕ / 1 tube

4. Test Procedure

1) Preparation and Storage

① Applicable specimens are the upper respiratory tract(nasopharyngeal/oropharyngeal swabs) and lower respiratory tract(sputum and bronchoalveolar lavage) fluids.

② Specimens stored in containers should be processed within 48 hours during refrigerated storage(2-8℃). For long period of storage, specimens should be stored at -70℃. Specimens stored in shipping containers should be stored at refrigerated storage(2-8℃) until processing can proceed. If a delay in testing or shipping is expected to exceed 2~3 days, specimens can be stored at-70℃.

③ Avoid repeated freeze/thaw samples as it degrades nucleic acids and can reduce sensitivity.

2) Preparation Procedure

① Completely thaw all regents and samples on ice before use.

② Prepare a commercial viral RNA extraction kit for extracting RNA
Example 01) Ribospin™ vRD,
Example 02) NX-48 Viral NA kit-NV-NV111

③ Instruments can be used with test
A) QuantStudio™ 6 Flex Real-Time PCR System
B) 7500 Real-Time PCR Instrument System

3) RNA extraction

- Extraction of RNA using the RNA kit should be performed following the manufacturer's instructions.

4) RT-LAMP Reaction Setup

① Prepare the Mastermix according to the following table.

Mastermix component	Volume (㎕)
RT-LAMP premix	10
Each primer mix	5

② Vortex and spin down briefly.

③ Pipet 15㎕ of Mastermix into PCR tube.

④ Pipet 5㎕ of the RNA sample, positive control and negative control to the tube, respectively.

Component	Volume (㎕)
Mastermix	15
RNA sample, positive control and negative control	5

⑤ Place tubes into the Real-Time PCR instrument and start the run according to the cycling conditions table below.

[Gene and Fluorescence Conditions]

Gene	Fluorescence
RdRp	FAM
N	FAM

[PCR Instrument with the Cycling Conditions]

Temperature	Time	Cycles
65℃	3 min	1
65℃	30 sec**	40
** : for collecting FAM signals every 30 sec		

5. Result Interpretation

① Set Threshold values, baseline start and end values for each target according to the following table.

Instrument	Target	Threshold	Baseline start	Baseline end
Instrument A / B	RdRp	1.0	5	10
	N	1.0	5	10

② Interpretation criteria of the results is in table below.

Results	Ct Values
Positive (+)	$10 < Ct \leq 36$
Negative (-)	N/D† or $C_T \leq 10$ or $C_T > 36$
† N/D : Not detected	

③ Result interpretation

Case	PC†	NC†	Gene		Results
			RdRp	N	
1	+	-	+	+	SARS-CoV-2 detected
2	+	-	-	-	SARS-CoV-2 not detected
3	+	-	+	-	SARS-CoV-2 detected
4	+	-	-	+	SARS-CoV-2 detected
5	+	+	+/-	+/-	Invalid / retest
6	-	+	+/-	+/-	Invalid / retest
7	-	-	+/-	+/-	Invalid / retest
†PC : positive control, NC : negative control					

6. Quality Control

1) Confirm the results of positive control and negative control as described below.
2) Retest with the product of the same lot if the controls are not valid. If the repeat result remains invalid, contact the supplier.

Interpretation	Control Type	Results
Valid	Positive control	$10 < Ct \leq 36$
	Negative control	N/D† or $C_T \leq 10$ or $C_T > 36$
Invalid	Positive control	N/D† or $C_T \leq 10$ or $C_T > 36$
	Negative control	$10 < Ct \leq 36$
† N/D : Not detected		

7. Storage and Shelf Life

1) All components of the kit are valid for 12 months if reagents are un-opened and stored below -20℃.
2) All components of the kit are valid for 12 days at 4℃ once opened.

8. Assay Performance

1) Limit of Detection(LoD) – Analytical Sensitivity

The experiments for determining LoDs for each target were prepared by diluting the SARS-CoV-2 RNA from 1,000 to 0 copies and confirmed by testing 24 replicates. The LoD was determined by calculating the SARS-CoV-2 RNA copies that can be detected at 95% confidence using a prohit analysis.

Gene	Probability	LoD
RdRp	95%	115 copies
N	95%	256 copies

2) Cut-off

Threshold adjust 1.0 to remove the noise; baseline range is set to 5~10 cycles to include the initial cycles. The cut-off is set to 36 cycles where the LoD was observed at 30%.

3) Analytical Specificity (Cross-Reactivity)

iDetect™ SARS-CoV-2 Detection kit was tested with $2.99x10^7$~$1.29x10^8$/ reaction for 15 respiratory related pathogens. There was no cross-

reactivity observed for any of the tested pathogens.

4) Analytical Specificity (Interfering Substances Testing)

The results of hemoglobin 40/80μg, mucin 60ng, human serum 1/10% and nasal spray 0.1/1.0% confirmed that they were not affected the iDetect™ SARS-CoV-2 Detection kit assay results.

5) Precision (Repeatability)

Repeatability was accessed by using 3 different concentrations (high, medium and low). The test was performed by one operator using a single lot with 3 runs a day, 2 repeats per run for 6 consecutive days. The %CV of Ct values was within 9.2~14.7% and showed repeatability.

6) Precision (Reproducibility)

Test was performed by 2 operators using 3 lots with 2 repeats per run, 2 runs a day for 5 consecutive days. The %CV of Ct of 3 lots was 4.8~15.0%%, Ct between two operators was 5.6~14.8%. The %CV of the standard and total Ct value was found to be within 7.1~14.5% and showed reproducibility.

7) Precision (Inter-instrument Reproducibility)

Inter-instrument reproducibility was measured by one operator using 1 lots with 3 repeats a day for each of high, medium and low concentrations. The %CV between the 2 instruments was 0.9~12.0% and showed reproducibility.

8) Clinical Evaluation (Sensitivity/Specificity/Agreement/Running Time)

A clinical evaluation study was performed to evaluate the performance of the clinical sensitivity, specificity, agreement and test time. The clinical sensitivity and specificity were 97.14% (95% CI: 85.08~ 100.0%) and 100.00% (95% CI: 92.89~100.00%), respectively, and overall agreement was 98.82(95% CI: 93.62~99.97%). The longest test time from the sample preparation to the final report took 58 minutes and confirmed that the test was possible within 1 hour.

9. Warnings and Precautions

1) Repeated testing with the same sample may not be guaranteed statistical significance due to that this kit was designed for a qualitative test.
2) For in vitro diagnostic use only.
3) Please read this user guide carefully before use.
4) Wear disposable gloves, protective clothing, eye protection and masks while handling samples and reagents to avoid contact with the skin and eyes. If skin and eyes contact occurs, immediately flush with water and seek medical attention.
5) Care should be taken to avoid cross-contamination with amplified products.
6) Clean and disinfect laboratory equipment and space using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectants.
7) Do not reuse of the amplified product after the reaction, and dispose of waste in a designated place.
8) All test samples shall be regarded as infectious substances. The operation of sample and waste for each laboratory shall meet the requirements of relevant laws and regulations.
9) Improper storage may hinder the ability of assay to detect.
10) Do not use a kit after its expiration date, and do not mix reagents from different lots or the same lot with other packaging.
11) The results of this kit should not be used as the sole basis for treatment or other management decisions. Results must be combined with clinical observations based upon other testing methods or expert judgements.

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