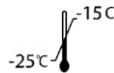




Kaira 2019-nCoV Detection Kit

IVD

REF RDM101-X



For Emergency Use Authorization (EUA) Only

For *in vitro* Diagnostic (IVD) Use

Rx Only

**The test has been validated but FDA's independent review of this validation is pending.
For use under the Emergency Use Authorization (EUA) only**



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Instructions for Use

Safety Warnings and Precautions

Please inquire OPTOLANE's Customer Service Center to obtain a copy of the Material Safety Data Sheet (MSDS) for this product.

Please read the Instructions for Use and check the integrity of all tubes, tips and other materials supplied with this kit prior to use.

Before, during and after use of this kit as described in this Instructions for Use, all potentially hazardous materials (i.e. materials that may have come in contact with clinical samples) including tubes, tips and materials should be processed and disposed of according to applicable and appropriate regulations of the municipality/ government in which this product is being used. Adhere to general clinical laboratory safety procedures during the experiment.

Warranty and Liability

All OPTOLANE's products are manufactured and tested under strict quality control protocols. OPTOLANE's guarantees the quality of all directly manufactured products until the expiration date printed on the label. If any issues are discovered relating to compromise in product quality, immediately contact OPTOLANE's Customer Service Center.

OPTOLANE does not assume liability for misuse of the product, i.e. usage of the product for any purposes other than its intended purpose as described in the appropriate and applicable Instructions for Use. OPTOLANE assumes liability under the condition that the user discloses all information related to the problem to OPTOLANE in written form within 30 days of occurrence.

Legal Disclaimer

Some applications that may be performed with this kit may infringe upon existing patents in certain countries. The purchase of this kit does not include or provide a license to perform patented applications. Users may be required to obtain a license depending on country and application. OPTOLANE does not condone nor recommend the unlicensed use of a patented application.

The use of the kit is only for qualified and well-trained users in handling of clinical specimens and molecular biological experiments. After testing, all wastes should be processed with the fulfillment of the regulation of the country.

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1. INTENDED USE

The *Kaira* 2019-nCoV Detection Kit is an *in vitro* diagnostic kit designed for the qualitative detection of SARS-CoV-2 in human samples such as nasopharyngeal swap, oropharyngeal swap, or sputum samples through reverse transcription real-time polymerase chain reaction (rt Real-Time PCR).

2. INTRODUCTION

Coronavirus (CoV) is a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV). 2019-nCoV (or COVID-19) is a new strain that was discovered in 2019 and has not been previously identified in humans. Coronaviruses are zoonotic, meaning they are transmitted between animals and people. Detailed investigations found that SARS-CoV was transmitted from civet cats to humans. Several known coronaviruses are circulating in animals that have not yet infected humans. To date, there is no vaccine and no specific antiviral medicine to prevent or treat 2019-nCoV. The only treatment for people affected is to relieve symptoms. Therefore, people with illness should be hospitalized. Currently, there are no possible vaccines and some specific drug treatments are under investigation which is being tested through clinical trials. Molecular test is one of the most effective way to diagnose the presence of 2019- nCoV in people who are thought to be infected. In particular, real-time reverse-transcription polymerase chain reaction (rt RT-PCR) assays are molecular tests that can be used to detect viral RNA in clinical samples. WHO's current case recommendation for laboratory confirmation of 2019-nCoV infection requires either a positive rt RT-PCR result for at least two specific genomic targets (RdRp and E gene) and a single positive target. OPTOLANE Technologies Inc. introduces *Kaira* 2019-nCoV Detection Kit to diagnose 2019-nCoV in human samples through reverse transcription Real-Time polymerase chain reaction (rt RT-PCR).

3. FEATURES AND PRINCIPLE OF THE TEST

Real-time PCR involves the selective amplification of a target sequence while monitoring the progress of amplification in real-time through a visualizing agent such as a fluorescent dye. The specificity is provided by a pair of specific primers, along with a hydrolysis probe which is also sequence specific. Monitoring amplified product is conducted by labeling the hydrolysis probe with a matched pair of fluorescent dyes (5'-Fluorescent reporter; 3'- Quencher). However, upon cleavage by the 5' – 3' exonuclease activity of the DNA polymerase during PCR, the fluorescent reporter molecule will emit a specific wavelength of light within the visible spectrum when cleaved after binding to the amplicon.

4. CONTENTS AND RELATED INSTRUMENTS

4.1. Contents of the Kit



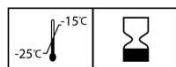
Table 1. Contents of 2019-nCoV Detection Kit

	Reagents	Unit	Quantity
①	COVID-19 Primer & Probe Mixture	125 μ L/1 tube	2 tube
②	2 \times OneStep RT qPCR Mixture	625 μ L/1 tube	2 tube
③	COVID-19 Positive control DNA	100 μ L/1 tube	1 tube
④	DEPC DW	200 μ L/1 tube	1 tube

4.2. Related Instruments

This kit is optimized for use with QuantStudio 5 Real-Time PCR system (Thermo-Fisher Scientific), ABI 7500 Real-Time PCR system (Applied Biosystems co.), or CFX96 Dx System (Bio-Rad Laboratories). For detailed operating instructions of Thermo-Fisher Scientific QuantStudio 5 Real-Time PCR, ABI 7500 Real-Time PCR systems, or Bio-Rad CFX96 Dx system, please refer to the instrument *Instructions for Use*.

5. STORAGE AND EXPIRATION DATE



The *Kaira* 2019-nCoV Detection Kit should be stored at -25°C to -15°C away from UV/sunlight. The kit is guaranteed stable until the expiration date printed on the label. Repeated thawing and freezing (more than four times) of the components should be avoided, as this may reduce assay performance.

6. REQUIRED MATERIALS AND EQUIPMENT (NOT PROVIDED IN THE KIT)

- Disposable powder-free gloves
- Appropriate volume pipette set
- Sterilized pipette tips with filters
- 1.5 mL micro tubes or 0.2 mL PCR tubes
- Equivalent protocol and materials necessary to extract nucleic acid
- QuantStudio 5 Real-Time PCR system (Thermo-Fisher Scientific), ABI 7500 Real-Time PCR system (Applied Biosystems co.), or CFX96 Dx system(Bio-Rad Laboratories)

7. GENERAL PRECAUTIONS



- Real-Time PCR through this kit should be performed using QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system.

- Always wear gloves and a mask when handling specimens and kit reagent.
- DO NOT mix reagents from different production lots.
- DO NOT use a kit after its expiration date.
- This kit is for use only with human nasopharyngeal swap, oropharyngeal swap, or sputum specimens. This kit is not intended for use with other types of specimens.
- Avoid microbial contamination of kit components when preparation of specimens.
- Please read this *Instructions for Use* before use.
- DO NOT change the protocol as described in this *Instructions for Use*.
- Always use sterile, filtered pipette tips.
- Clinical samples and their derivatives should be stored in a separate location/freezer from where the rest of the kit components are stored.
- Briefly vortex and spin-down all kit components after thawing to ensure optimum results

8. PROTOCOL

8.1. Preparation

We recommend that several precautionary measures be taken for the safety of user and laboratory and for the prevention of laboratory environmental contamination.

8.1.1 Proper Use of a Biosafety Cabinet

When handling clinical samples, all related works (i.e. decapping, pipetting, capping of clinical samples and containers) should be conducted within a negative pressure biosafety cabinet (**Class III**). Negative pressure biosafety cabinet sends air from the laboratory space outside. In other words, air flows inward. This airflow prevents dangerous substances from contaminating the laboratory environment.

Positive pressure biosafety cabinet is a workspace where filtered air flows outward, thus keeping a clean environment within the workspace.

8.2. Specimen



All samples should be treated as potential biohazards. For the best results, we recommend RNA extracted from nasopharyngeal swap, oropharyngeal swap, or sputum (however induction of sputum is not recommended) samples

8.2.1 Specimen Collection

The *Kaira* 2019-nCoV Detection Kit is optimized for RNA extracted from nasopharyngeal swap, oropharyngeal swap, or sputum samples. All samples should be kept in preservative-free containers

8.2.2 Specimen Transport

All samples should be transported in a shatterproof transport container to prevent potential infection from sample leakage. Samples should be transported according to local/national guidelines regarding biohazard transportation.

8.2.3 Specimen Storage

The isolated nasopharyngeal swap, oropharyngeal swap, or sputum can be stored up to 7 days at 2-8°C. For long period of storage, samples should be stored at -20°C to -80°C in aliquot to avoid repeated freeze/thaw cycles

8.2.4 Interfering Substances

Heparin (≥ 10 IU/mL) is a known inhibitor of PCR. Samples that have been collected in tubes

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containing heparin should not be used. In addition, samples from heparin-treated patients should not be used. Clinical samples may contain substances which interfere with PCR. For efficient PCR, such inhibitors must be removed during the RNA extraction and purification process.

The breakdown products of heme, such as bilirubin, as well as bile salts also can inhibit PCR in clinical samples (Kreader, 1996).

EDTA is found in several elution buffers of purification kits for preservation of DNA, but at certain concentrations, it may deplete magnesium ions and thus inhibit DNA polymerase activity (Schrader et al., 2012)

Hemoglobin is one of the major PCR inhibitors where the first affects amplification through a direct effect on the DNA polymerase activity and quenches the fluorescence of free dye molecules, and the latter binds to single-stranded genomic DNA, hindering DNA polymerization in the first few PCR cycles (Sidstedt et al. 2018). According to the recommendation in Interference testing in clinical chemistry; approved guideline EP7-A2, potential interference substances were tested with indicate concentrations (Table 2).

Table 2. Potential interfering substances and concentrations used for the test.

No.	Potential interference substance	Conc. used
1	Albumin	5g/dL
2	Bilirubin	342 μ mol/L
3	EDTA	3.4 μ mol/L
4	Hemoglobin	2g/L
5	Heparin	3,000U/L

8.3. Procedure

The *Kaira* 2019-nCoV Detection Kit is designed for use with QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system.

8.3.1 Work Flow

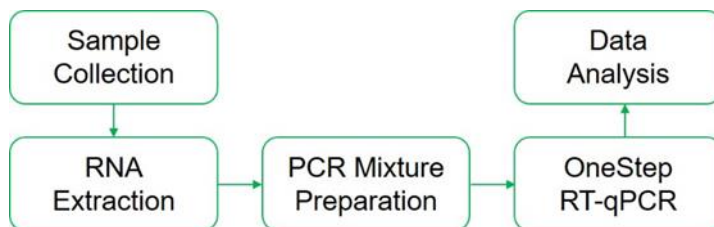


Figure 2. Example of workflow for the detection of 2019-nCoV

The *Kaira* 2019-nCoV Detection Kit is designed for use with QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system. Schematic workflow is shown in Fig. 2.

8.3.2 Nucleic Acid Extraction

This *Kaira* 2019-nCoV Detection Kit uses commercial RNA prep kits, it is recommended that the RNA prep kits indicated in the Table 3. Prepare the total RNA from samples of patients by referring to the instruction manual of the kit that you want to use.

Table 3. List of commercially available RNA extraction kits

Specimen	Kit Name	Manufacture	Cat. No.
Nasopharyngeal swap, oropharyngeal swap, sputum	QIAamp Viral RNA mini kit	Qiagen	52904/52906
	MagMAX™ Viral RNA Isolation Kit	Applied Biosystems	AM1939

8.3.3 PCR Preparation

1) The appropriate number of controls and extracted nucleic acids should be thawed for at least 10 minutes at room temperature (if frozen). PCR 2 × Master Mixture and Primer & Probe Mixture should be prepared prior to the test. 2 extra reactions are needed for experimental controls (NTC and PC). *Kaira* 2019-nCoV Detection Kit uses commercial RNA prep kits. Examples of commercially available kits used for *Kaira* 2019-nCoV Detection Kit are indicated in Table 3. Prepare the total RNA from samples of patients according to the instruction of manufacturers of the kits.

2) Vortex more than 10 seconds and briefly spin-down all reagents, controls and samples prior to use.

Instructions for Use

3) Make a PCR pre-mix. For detailed components of pre-mix, refer to Table 4. Make the mixture for at least two more reactions to compensate some loss. Vortex and spin-down the mixture for more than 10 seconds before use.

Table 4. components and volumes of reaction mixture

Component	Volume (µL)
2× OneStep RT-qPCR Mixture	12.5
2019-nCoV Primer & Probe Mixture	2.5
RNA specimen, PC, or NTC	10
Nuclease free water	0
Total Reaction volume	25

4) Add 12.5 µL of OneStep RT-qPCR Mixture to each PCR tube.

5) Add 10 µL of DEPC DW into the tube assigned for NTC. Please seal the tubes with cap to prevent contamination from other tubes.

6) Move the tubes to a separated location before proceeding to prevent contamination. Add 10 µL of PC to the tube assigned for PC.

7) Add 10 µL of extracted nucleic acids of clinical samples to tubes assigned.

8) Seal the tubes with cap completely.

Note: In order to avoid contaminations and invalid results, seal all the tubes accordingly.

9) To mix the tubes thoroughly, vortex for 5 sec and centrifuge at 1500 rpm for 2 min.

10) Place the mixed tubes in the QuantStudio 5 Real-Time PCR system, ABI 7500 Real-Time PCR system, or CFX96 Dx system and run PCR immediately. (Please refer to each instrument *Instructions for Use*.)

8.3.4 OneStep RT-qPCR setting

For instrument software setting, refer to the user manual for each instrument. The PCR condition of 2019-nCoV detection kit is in Table 5.

Table 5. PCR conditions for Kaira 2019-nCoV detection kit

STEP	Temperature	Running Time	Cycle Number
Reverse transcription	50°C	10 minutes	1 cycle
Pre-Denaturation	95°C	10 minutes	1 cycle
Denaturation	95°C	10 seconds	45 cycles
Annealing & Extension *	60°C	30 seconds	
END			

* This step scanned the fluorescence signal.

8.4. Data Analysis

8.4.1 Cut-off Ct value for Positive

The test uses 2 wells of each NTC and PC to determine the validity of the experiment, and each reaction includes PCRC in wells of samples as well as NTC and PC to check the validity of PCR. Interpretation criteria of the PCR results is in Table 6.

Table 6. Interpretation of results

Type	Target Result		PCRC Result (Cy5) ^[1]	Results
	2019-nCoV			
	RdRP(FAM)	E (HEX)		
Positive Control (PC)	+	+	+	Valid
NTC	-	-	+	Valid
Sample case 1	+	+	+/- ^[2]	2019-nCoV Positive
Sample case 2	+	-	+/- ^[2]	2019-nCoV Positive
Sample case 2	-	+	+/- ^[2]	2019-nCoV Negative
Sample case 3	-	-	+	
Sample case 4	-	-	-	Invalid ^[3]

NTC: to determine whether the sample is contaminated in the process of sample pretreatment, nucleic acid extraction, and PCR preparation (prevent false-positive error)

PC: to determine whether target plasmid DNA is properly amplified (prevent false-negative error)

PCRC: to check whether PCR is inhibited by the sample and to determine the amplification of nucleic acids in each well. High concentrations of target RNA can lead to a reduced or absent fluorescence signal of PCRC due to PCR competition.

The validity of PCRC is determined by Ct value of PCRC signal. If its Ct value is within the specified range, it is valid. If the Ct value is out of the specified range, it is invalid. The validity of PC and NTC is determined by Ct value of target signal. If the assay is valid, target Ct will be 'undetermined' in NTC well and PC Ct value will be within its specified range. If the control results are invalid, take measures according to **Instructions for Use 9. Trouble shooting**.

The result of PCRC determines the validity of the test, and Ct value of the target signal determines whether the target is 'Detected' or 'Non-detected'.

Cut-off value: to classify results as positive or negative. The cut-off value is determined utilizing statistical technique, probability analysis. The low value of confidence interval (CI) of LoD is converted into Ct value derived from the LoD test. The cut-off Ct value determines the target RNA detection results as positive with 97.5% probability.

Instructions for Use

^[1] PCRC detection results is not required for determining 2019-nCoV positive or negative. In case of heavy viral load of positive specimen, PCRC fluorescence signal could be lower by the PCR reaction competition.

^[2] When the copy numbers of RdRP or E gene in clinical samples are extremely high, sometimes the amplified curve of PCRC may be very low or not appeared. In this case, the result is considered as 2019-nCoV positive.

^[3] The validity of PCRC is determined by the Ct (cycle threshold) of PCRC signal (Cy5 dye) defined by the manufacturer. When PCRC is valid, the validity of NTC or PC is determined by the Ct of target signal which is defined by the manufacturer.

Criteria for cut-off of Kira 2019-nCoV detection kit are summarized in Table 7.

Table 7. Cut-off table of Kaira 2019-nCoV Detection Kit

Analyte	Fluorophore	Cut-off Ct value for positive
2019-nCoV RdRP	FAM	Ct ≤ 37.5
2019-nCoV E gene	HEX(VIC)	Ct ≤ 36.0
PCRC	Cy5	25 < Ct < 35

9. TROUBLESHOOTING

Comments and suggestions

PCR Control (PCRC) invalid results	
<p>If the Cy 5 (PCRC) fluorescence signal was not detected in all wells (including controls).</p>	<ul style="list-style-type: none"> • Extraction and/or PCR configuration error <ul style="list-style-type: none"> ☞ RNA extraction follow the manufacture's protocol. Repeat the assay, if necessary. See Instructions for Use 8. PROTOCOL • Incorrect extraction or PCR kit use <ul style="list-style-type: none"> ☞ Make sure that you use proper kits for the intended tests. • The kit may have spoiled, due to bad storage or expiration. <ul style="list-style-type: none"> ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary. See Instructions for Use 5. STORAGE AND EXPIRATION DATE
<p>If the Cy 5 (PCRC) fluorescence signal was not detected in particular wells.</p>	<ul style="list-style-type: none"> • Inhibition of PCR <ul style="list-style-type: none"> ☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition. ☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type. • Low elution volume due to insoluble material of samples <ul style="list-style-type: none"> ☞ Yield of nucleic acid can be affected by sample conditions (viscosity etc.). Repeat the assay from the sample pretreatment process which can make the sample more soluble.
<p>If the slope of PCRC is low or not appeared when the positive copy number is relatively high (e.g. Ct<25)</p>	<ul style="list-style-type: none"> • This is common phenomenon for all the assays when the concentration of target template is too high. In this case, the resources for PCR is used up and therefore the reaction of the PCR control may be weak. <ul style="list-style-type: none"> ☞ Regardless of the presence of PCRC, when the Ct value of the target is lower than 25 (Ct<25), the result is positive.

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PC invalid results

If the FAM/HEX (PC) fluorescence signal was undetermined.

- The kit may have spoiled, due to bad storage or expiration.
 - ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.
See **Instructions for Use 5. STORAGE AND EXPIRATION DATE**
- Re-use of reagents
 - ☞ Make sure not to re-use reagents. Re-use or repeated freeze/thaw cycles of reagents may affect the kit quality and the results of assay conclusively. Repeat the assay with new reagents, if necessary.
See **Instructions for Use 5. STORAGE AND EXPIRATION DATE, 7. General Precautions**
- PCR Protocol error
 - ☞ Review your reaction preparation procedure. Confirm the amount of PC used in a single well.
See **Instructions for Use 8.3.3 PCR Preparation**
- There may have been a pipetting error.
 - ☞ Review the pipetting technique and calibration.

No template Control (NTC) invalid results

If the FAM/HEX (PC) fluorescence signal was detected in NTC well.

- Contamination may have occurred.
 - ☞ Make sure that workspace and instruments are decontaminated and repeat the assay.
- The kit may have spoiled, due to bad storage or expiration.
 - ☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.
See **Instructions for Use 5. STORAGE AND EXPIRATION DATE**
- PCR Protocol error
 - ☞ Review your reaction preparation procedure. Confirm whether controls and samples are loaded in proper wells which are assigned through S/W protocol (especially NTC well(s)).
See **Instructions for Use 8.3.3 PCR Preparation**
- There may have been a pipetting error.
 - ☞ Review the pipetting technique and calibration.

10. Performance Analysis

10.1. Analytical sensitivity (Limit of Detection (LoD))

10.1.1 Protocol

Serially diluted in vitro transcribed RNA fragment was used to determine the LoD.

Concentration of in vitro transcribed RNA was measured by BioAnalyzer 2100 (Agilent Technologies Inc.). RNA (1.12×10^7 copies/mL) was serially diluted with negative clinical matrices (nasal swabs & sputa) obtained from a reference laboratory, Seoul Clinical Laboratory (SCL) and mixed with RNA inhibitors followed by sample preparation using QIAmp Viral miniprep kit (Qiagen). The linearity of the targets; E gene & RdRp were determined first and the initial LoD was determined as 1.12×10^3 for E gene and 1.08×10^3 for RdRp gene using Applied Biosystems 7500 Real-Time PCR System, QuantStudio 5 (QS5) Real-Time PCR and Bio-Rad CFX96 Dx System. At each concentration, tests were repeated 3 times (Table 8).

Table 8. Result for initial LoD determination by instruments (ABI 7500, QS5, and CFX96)

E gene				RdRp gene			
Conc. (copies/mL)	7500	QS5	CFX96	Conc. (copies/mL)	7500	QS5	CFX96
1.12×10^7	3/3	3/3	3/3	1.08×10^7	3/3	3/3	3/3
1.12×10^6	3/3	3/3	3/3	1.08×10^6	3/3	3/3	3/3
1.12×10^5	3/3	3/3	3/3	1.08×10^5	3/3	3/3	3/3
1.12×10^4	3/3	3/3	3/3	1.08×10^4	3/3	3/3	3/3
1.12×10^3	3/3	3/3	3/3	1.08×10^3	3/3	3/3	3/3
1.12×10^2	3/3	2/3	2/3	1.08×10^2	2/3	1/3	2/3
Linearity(R ²)	0.9999	1	0.9999		0.9995	0.9999	0.9985

The final LoD was confirmed by testing 24 replicates of 3-fold diluted samples (1120 copies/mL, 746.7 copies/mL, 373.3 copies/mL) in three different instruments (ABI QS5, ABI 7500, and CFX 96). The LoD values for E gene were 746.7, 373.3, and 373.3 copies/mL for ABI QS5, ABI 7500, and CFX 96, respectively. The LoD values for RdRp were 720 copies/mL for the three instruments. The results are summarized in Table 9.

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Table 9. LOD for E gene and RdRp gene by instruments (ABI QS5, 7500, and Bio-Rad CFX96)

Target		E gene			RdRp		
RNA (copies/mL)		1120.0	746.7	373.3	1080.0	720.0	360.0
QS5	positive/Test	24/24	23/24	22/24	24/24	23/24	17/24
	rate (%)	100.0	95.8	91.7	100.0	95.8	70.8
CFX96	positive/Test	24/24	24/24	23/24	24/24	24/24	19/24
	rate (%)	100.0	100.0	95.8	100.0	100.0	79.2
7500	positive/Test	24/24	24/24	23/24	24/24	24/24	20/24
	rate (%)	100.0	100.0	95.8	100.0	100.0	83.3

10.2. Cross-reactivity

For cross-reactivity, a total of 11 species of bacteria and virus total RNA was tested for potential cross reactivity in the use of *Kaira* 2019-nCoV Detection Kit. Three replicates of each RNA were run for the evaluation. No interference was observed for the microorganisms listed in Table 10.

Table 10. Material list of cross-reactivity test

No.	Pathogens*	Conc.(copies/ μ L)	<i>Kaira</i> COVID-19 Detection Kit		
			RdRP	E gene	PCR
1	Corona 229E	4.44×10^5	ND	ND	ND
2	Inf A H1N1	1.54×10^5	ND	ND	ND
3	Inf A H3N2	4.4×10^5	ND	ND	ND
4	Inf B	1.03×10^5	ND	ND	ND
5	EAEC	10,000	ND	ND	ND
6	EEE	12,500	ND	ND	ND
7	KPN	10,000	ND	ND	ND
8	OTS	10,000	ND	ND	ND
9	STLEV	12,500	ND	ND	ND
10	TRA	10,000	ND	ND	ND
11	WEEV	12,500	ND	ND	ND

* Corona 229E: Human coronavirus 229E, Inf A H1N1: Influenza A virus subtype H1N1, Inf A H3N2: Influenza A virus subtype H3N2, Inf B: Influenza B virus, EAEC: Enterococcal Aggregative Escherichia coli, EEE: Eastern equine encephalitis; KPN: Klebsiella pneumoniae; OTS: *Orientia tsutsugamushi*; STLEV: ST Louis encephalitis virus; TRA: Trypanosoma rangeli; WEEV: Western equine encephalitis virus;

Blast homology search with primers and probes of the *Kaira* 2019-nCoV Detection Kit showed no homology for the organisms listed in the Table 11.

Instructions for Use

Table 11. *In silico* analysis of cross-reactivity

Pathogen	Strain	GenBank Acc#	% Homology Test Forward Primer	% Homology Test Reverse Primer	% Homology Test Probe
Human coronavirus HKU1	All	All	0	0	0
Human coronavirus NL63	All	All	0	0	0
SARS-coronavirus	All	All	0	0	0
MERS-coronavirus	All	All	0	0	0
Human Adenovirus	All	All	0	0	0
Human Metapneumovirus	All	All	0	0	0
Parainfluenza virus 1	All	All	0	0	0
Parainfluenza virus 2	All	All	0	0	0
Parainfluenza virus 3	All	All	0	0	0
Parainfluenza virus 4	All	All	0	0	0
Enterovirus D68	All	All	0	0	0
Respiratory syncytial virus	All	All	0	0	0
Rhinovirus	All	All	0	0	0
<i>Chlamydia pneumoniae</i>	All	All	0	0	0
<i>Haemophilus influenzae</i>	All	All	0	0	0
<i>Legionella pneumophila</i>	All	All	0	0	0
<i>Mycobacterium tuberculosis</i>	All	All	0	0	0
<i>Streptococcus pneumoniae</i>	All	All	0	0	0
<i>Streptococcus pyogenes</i>	All	All	0	0	0
<i>Bordetella pertussis</i>	All	All	0	0	0
<i>Mycoplasma pneumoniae</i>	All	All	0	0	0
<i>Pneumocystis jirovecii</i>	All	All	0	0	0
<i>Candida albicans</i>	All	All	0	0	0
<i>Pseudomonas aeruginosa</i>	All	All	0	0	0
<i>Staphylococcus epidermis</i>	All	All	0	0	0
<i>Staphylococcus salivarius</i>	All	All	0	0	0

10.3. Specificity Test (Interfering Substances)

Interference test was performed to determine whether potential interfering substances would affect the 2019-nCoV Real-Time RT-PCR assay results. Substances of potentially interfering with 2019-nCoV

Instructions for Use

were spiked with high, middle, low, and negative levels and changes of Ct were measured. Three replicates were tested for each substance. No interference was observed for the substances listed in Table 2.

10.4. Inclusivity

In silico analysis using NCBI Blast web-based search tool, primer and probe sets for RdRp and E gene showed 100% match against 485 complete sequences of SARS-CoV-2 (taxid:2697049) as of April 13th. Request results of this search (RID 984J3EKY016) was saved as a text file and XML format. These files can be provided upon request..

10.5. Precision (Reproducibility)

Precision was assessed by using 3 lots of *Kaira* 2019-nCoV reagents. Tests were performed with 3 different concentrations of COVID-19, 9 repeats per run, 2 runs a day for 3 consecutive days for 2 genes (E gene and RdRP) with 3 different instruments. Each run was performed by independent operator. A total of 1458 tests were performed.

The results indicated that the SD was lower than 1 with the CV% value of less than 5%, which is the reproducibility of *Kaira* 2019-nCoV Detection Kit was high.

10.6. Precision (Repeatability)

In order to access the precision (repeatability) of *Kaira* 2019-nCoV Detection Kit, repeatability was tested for indicated period by one operator using three instruments.

Repeatability was accessed by using 4 different concentrations (negative, low, middle, high positive) of 2019-nCoV RNA transcripts. The test was performed by one operator using 3 lots with 9 repeats per run, 2 runs a day for 3 days for 3 instruments. The results showed that *Kaira* 2019-nCoV Detection Kit was highly repeatable with the SD value of less than 1 and CV% of less than 5%.

10.7. Stability tests (accelerated)

The periodical stability was assessed to ensure the quality of *Kaira* 2019-nCoV Detection Kit (accelerated / long period). Accelerated test was performed duplicates using 3 different lots for 1, 2, and 4 days with the product stored at 25°C. Based on the results of accelerate test, we assigned 12 months for the expiration of *Kaira* 2019-nCoV Detection Kit when stored at -20± 5°C.

10.8. Clinical Evaluation

To evaluate the clinical performance of *Kaira* 2019-nCoV Detection Kit, a clinical investigation with human-derived samples was carried out at Seoul Clinical Laboratories in Korea. The clinical evaluation was designed for randomized, single-blinded comparative clinical evaluation.

Instructions for Use

Leftover specimens including nasopharyngeal swab, oropharyngeal swab and sputum from COVID-19 patients were used for clinical evaluation. The extracted nucleic acid (RNA) is stored frozen, and only samples without repetition of freeze and thaw are use. For tests, Bio-Rad CFX96 System was used.

Clinical sensitivity and specificity of 120 samples were evaluated through a comparative investigation of Allplex™ 2019-nCoV Assay (Cat.No. RP10243X) from Seegene company. This product was authorized under the Korea Centers for Disease Control and Prevention (KCDC) emergency use authorization. Allplex™ 2019-nCoV Assay has similar measurement principles and items to *Kaira* 2019-nCoV Detection Kit. The results of 60 positive and 60 negative samples tested below showed that overall agreement is 100.0%, clinical sensitivity 100.0% (95% CI, 94.04 - 100.0%), and clinical specificity is 100.0% (95% CI, 94.04 - 100.0%). The *Kaira* 2019-nCoV Detection Kit developed by our company did not show any false positives and false negatives for the detection of COVID-19 in comparison with the licensed product.

Table 12. Clinical performance of *Kaira* 2019-nCoV Detection Kit

		(Allplex™ 2019-nCoV Assay)		Total
		Positive	Negative	
(Kaira 2019-nCoV Detection Kit)	Positive	60	0	60
	Negative	0	60	60
Total		60	60	120

11. REFERENCES

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12. SYMBOLS



Catalog number



Temperature limitation



In vitro diagnostic medical device



Contains sufficient for test



Manufacturer



Caution, consult accompanying documents



Batch code



Expiration date



Consult instructions for use

Instructions for Use



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