User's Guide

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# *Kaira* 2019-nCoV Detection Kit

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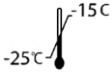
IVD

REF **RDM101-X**




**Please read all the information in booklet before using the unit**

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**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 User's Guide 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 User's Guide, 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 User's Guide. 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.

## TABLE OF CONTENTS

## 1. INTENDED USE

The Kaira 2019-nCoV Detection Kit is designed for qualitative detection of COVID-19 Virus (SARS-CoV-2) RNA in the specimens of suspected respiratory disease patients (sputum, oral swab, nasal swab) in in vitro settings, by Real-Time Polymerase Chain Reaction (RT-PCR), assisting diagnosis of COVID-19. For professional use only.

## 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 The 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

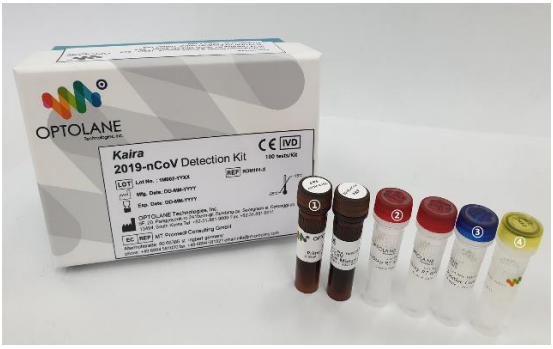


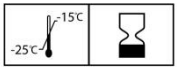

**Table 1. Contents of 2019-nCoV Detection Kit**

| Reagents | | Unit | Quantity |
|---|---|---|---|
| ① | COVID-19 Primer & Probe Mixture | 125ul/1via | 2 tube |
| ② | 2X OneStep RT qPCR Mixture | 625ul/1via | 2 tube |
| ③ | COVID-19 Positive control DNA | 100ul/1via | 1 tube |
| ④ | DEPC DW | 200ul/1via | 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 *User's Guide*.

## 5. STORAGE AND EXPIRATION DATE




The Kaira 2019-nCoV Detection Kit should be stored at -25℃ ~ -15℃ 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 *User's Guide* before use.
- DO NOT change the protocol as described in this *User's Guide*.
- 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℃. For long period of storage, samples should be stored at -20℃ ~ -80℃ in aliquot to avoid repeated freeze/thaw cycles

8.2.4 Interfering Substances

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

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 | 342umol/L |
| 3 | EDTA | 3.4umol/L |
| 4 | Hemoglobin | 2g/L |
| 5 | Heparin | 3,000U/L |

## 8.3. Procedure

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

8.3.1 Work Flow

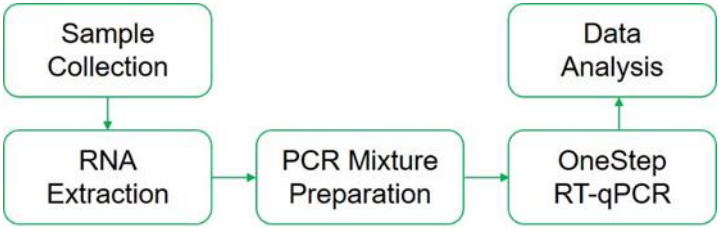



**Figure 2. Example of workflow for the detection of 2019-nCoV**

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 is used commercial RNA prep kits, it is recommended that the RNA prep kit 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 2X 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 4. 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.

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) |
|---|---|
| 2X 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 ㎕ of OneStep RT-qPCR Mixture to each PCR tube.

5) Add 10 ㎕ 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 ㎕ of PC to the tube assigned for PC.

7) Add 10 ㎕ 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 *User's Guide*.)

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.

**Table 6. Interpretation of results**

| Type | Target Result | | PCRC Result (Cy5)[1] | Results |
|---|---|---|---|---|
| | 2019-nCoV | | | |
| | RdRP(FAM) | E (HEX) | | |
| Positive Control | + | + | + | 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 ***User's Guide* 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.

[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 | |
|---|---|
| If the Cy 5 (PCRC) fluorescence signal was not detected in all wells (including controls). | • Extraction and/or PCR configuration error<br>☞ RNA extraction fellow the manufacture's protocol. Repeat the assay, if necessary.<br>See ***User's Guide* 8. PROTOCOL**<br><br>• Incorrect extraction or PCR kit use<br>☞ Make sure that you use proper kits for the intended tests.<br><br>• The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide* 5. STORAGE AND EXPIRATION DATE** |
| If the Cy 5 (PCRC) fluorescence signal was not detected in particular wells. | • Inhibition of PCR<br>☞ Clinical samples may contain a variety of PCR inhibitors. Repeat the assay from the sample pretreatment process which can reduce PCR inhibition.<br>☞ Make sure that you use the validated sample pretreatment method in accordance with the sample type.<br><br>• Low elution volume due to insoluble material of samples<br>☞ 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. |
| If the slope of PCRC is low or not appeared when the positive copy number is relatively high (e.g. Ct<25) | • 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.<br>☞ Regardless of the presence of PCRC, when the Ct value of the target is lower than 25 (Ct<25), the result is positive. |

| PC invalid results | |
|---|---|
| If the FAM/HEX (PC) fluorescence signal was undetermined. | • The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE**<br>• Re-use of reagents<br>☞ 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.<br>See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE, 7. General Precautions**<br>• PCR Protocol error<br>☞ Review your reaction preparation procedure. Confirm the amount of PC used in a single well.<br>See ***User's Guide*** **8.3.3 PCR Preparation**<br>• There may have been a pipetting error.<br>☞ 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.<br>☞ Make sure that workspace and instruments are decontaminated and repeat the assay.<br>• The kit may have spoiled, due to bad storage or expiration.<br>☞ Assess your storage conditions and review the expiration date. Repeat the assay with new reagents, if necessary.<br>See ***User's Guide*** **5. STORAGE AND EXPIRATION DATE**<br>• PCR Protocol error<br>☞ 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)).<br>See ***User's Guide*** **8.3.3 PCR Preparation**<br>• There may have been a pipetting error.<br>☞ Review the pipetting technique and calibration. |

## 10. Performance Analysis

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

10.1.1 Protocol

To generate RNA transcript of 2019-nCoV RNAs, a transcription plasmid (pBIC-A with 2019 nCoV sequence) that has T7 promoter with COVID-19 sequences was made. By using T7 RNA polymerase, COVID-19 specific RNA templates were generated. After transcribing RNA template, the concentration of RNAs was measured with Bio-analyzer (Agilent Technologies). Calculation of RNA copy number was performed using NeBioCalculator (New England Biolabs, MA, USA).

Start RNA concentration used
- E gene = 1.12 x $10^9$ copies/ul
- RdRP = 1.08 x $10^9$ copies/ul

Detection range test
- E gene = 1.12x$10^5$ ~ 1.12x$10^{-1}$ copies/ul
- RdRP = 1.08x$10^5$ ~ 1.08x$10^{-1}$ copies/ul

| Item | | Criteria |
|---|---|---|
| Result decision | Target: Ct value | 1 ~ 45 Ct |
| | | Not-detected (ND) |
| | PCRC: Must be detected in all samples tested | 25 ~ 35 Ct |
| Validity of the test | Linearity (Coefficient of determination, $R^2$) | ≥ 0.90 |

10.1.2 Sensitivity result (measurement range)

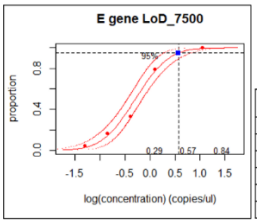
Table 8. Measurement ranges by instruments

| E gene | | | | RdRp | | | |
|---|---|---|---|---|---|---|---|
| Conc. (copy/ul) | 7500 | QS5 | CFX96 | Conc. (copy/ul) | 7500 | QS5 | CFX96 |
| 1.12x$10^5$ | 18.85 | 18.54 | 18.12 | 1.08x$10^5$ | 20.40 | 20.83 | 20.57 |
| | 18.84 | 18.50 | 18.11 | | 20.32 | 20.88 | 20.64 |
| | 18.87 | 18.60 | 18.10 | | 20.45 | 20.78 | 20.68 |
| 1.12x$10^4$ | 22.25 | 22.36 | 21.72 | 1.08x$10^4$ | 24.30 | 25.02 | 24.51 |
| | 22.17 | 22.20 | 21.73 | | 24.24 | 24.99 | 24.50 |
| | 22.17 | 22.35 | 21.70 | | 24.00 | 24.95 | 24.52 |
| 1.12x$10^3$ | 25.77 | 25.68 | 25.19 | 1.08x$10^3$ | 27.91 | 28.29 | 27.90 |
| | 25.72 | 25.66 | 25.25 | | 27.89 | 28.24 | 27.94 |

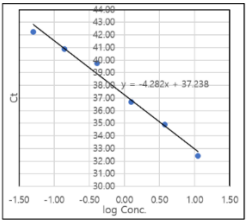
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 25.72 | 25.59 | 25.18 | | 27.80 | 28.15 | 27.88 |
| $1.12x10^2$ | 29.17 | 29.08 | 28.84 | $1.08x10^2$ | 31.52 | 31.90 | 31.60 |
| | 29.10 | 29.05 | 28.94 | | 31.38 | 31.90 | 31.54 |
| | 29.11 | 29.08 | 28.84 | | 31.10 | 31.87 | 31.61 |
| $1.12x10^1$ | 31.80 | 32.59 | 32.19 | $1.08x10^1$ | 34.97 | 35.33 | 35.44 |
| | 31.98 | 32.60 | 32.24 | | 34.88 | 35.31 | 35.61 |
| | 31.70 | 32.62 | 32.08 | | 34.44 | 35.15 | 35.34 |
| $1.12x10^0$ | 35.06 | 36.89 | 36.01 | $1.08x10^0$ | 38.28 | 38.94 | 38.56 |
| | ND | 37.04 | 36.38 | | ND | 40.06 | ND |
| | 35.34 | ND | ND | | ND | ND | 39.07 |
| $1.12x10^{-1}$ | ND | ND | ND | $1.08x10^{-1}$ | ND | ND | ND |
| | ND | ND | ND | | ND | ND | ND |
| | ND | ND | ND | | ND | ND | ND |
| **Linearity ($R^2$)** | **0.9987** | **0.9923** | **0.9995** | **Linearity ($R^2$)** | **0.9977** | **0.9997** | **0.9986** |

Measurement ranges for the three instruments were within $1.12x10^5$ ~ $1.12x10^0$ copies/ul for E-gene and $1.08x10^5$ ~ $1.08x10^0$ copies/ul for RdRp ($R^2$: 0.9923 ~ 0.9997). The lowest concentration was selected as $1.12x10^1$ copies/ul (E-gene) and $1.08x10^1$ copies/ul (RdRp). With the lowest concentration as the start point, limit of detection (LoD) for 2019-nCoV Detection Kit was determined.

To verify the LOD, 6 different concentration points were selected at near the lowest concentration and prepared by 1/3 dilution from the selected starting concentration. Test were performed 24 replicates per each point. The results were analyzed by probit analysis using RStudio software (version 1.2.5033). Based on the result, cut-off value was selected



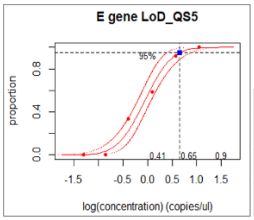

| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 11.20 | 24/24 | 100.0 |
| 3.73 | 23/24 | 95.8 |
| 1.24 | 19/24 | 79.2 |
| 0.41 | 8/24 | 33.3 |
| 0.14 | 4/24 | 16.7 |
| 0.05 | 1/24 | 4.2 |



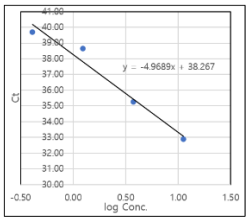

| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.05 | -1.30 | 42.29 |
| 0.14 | -0.85 | 40.92 |
| 0.41 | -0.39 | 39.79 |
| 1.24 | 0.09 | 36.68 |
| 3.73 | 0.57 | 34.88 |
| 11.20 | 1.05 | 32.42 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 3.72 | 0.57 | 34.79 |
| LoD CI | 1.95 | 0.29 | 36.00 |
| | 6.92 | 0.84 | 33.64 |

For ABI 7500, LOD (95% CI) of E-gene is $10^{0.57}$ ($10^{0.29}$ ~ $10^{0.84}$) copies/ul (= 3.72 (1.95 ~ 6.92) copies/ul). Ct cut-off value based on the LOD result is 36.00.



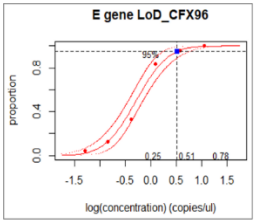

| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 11.20 | 24/24 | 100.0 |
| 3.73 | 22/24 | 91.7 |
| 1.24 | 14/24 | 58.3 |
| 0.41 | 8/24 | 33.3 |
| 0.14 | 0/24 | 0.0 |
| 0.05 | 0/24 | 0.0 |



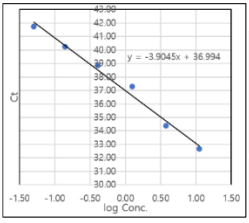

| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.05 | -1.30 | ND |
| 0.14 | -0.85 | ND |
| 0.41 | -0.39 | 39.69 |
| 1.24 | 0.09 | 38.64 |
| 3.73 | 0.57 | 35.25 |
| 11.20 | 1.05 | 32.89 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 4.47 | 0.65 | 35.04 |
| LoD CI | 2.57 | 0.41 | 36.23 |
| | 7.94 | 0.90 | 33.80 |

For ABI QS5, LOD (95% CI) of E-gene is $10^{0.65}$ ($10^{0.41}$ ~ $10^{0.90}$) copies/ul (= 4.47 (2.57 ~ 7.94) copies/ul). Ct cut-off value based on the LOD result is 36.23



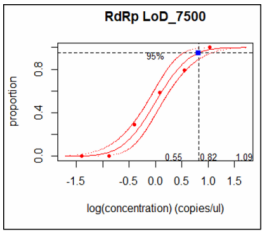

| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 11.20 | 24/24 | 100.0 |
| 3.73 | 23/24 | 95.8 |
| 1.24 | 20/24 | 83.3 |
| 0.41 | 8/24 | 33.3 |
| 0.14 | 3/24 | 12.5 |
| 0.05 | 1/24 | 4.2 |



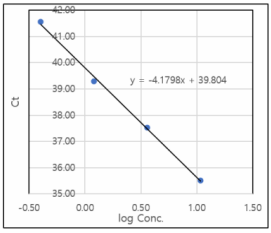

| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.05 | -1.30 | 41.73 |
| 0.14 | -0.85 | 40.23 |
| 0.41 | -0.39 | 38.88 |
| 1.24 | 0.09 | 37.30 |
| 3.73 | 0.57 | 34.38 |
| 11.20 | 1.05 | 32.67 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 3.24 | 0.51 | 35.00 |
| LoD CI | 1.78 | 0.25 | 36.02 |
| | 6.03 | 0.78 | 33.95 |

For Bio-Rad CFX96, LOD (95% CI) of E-gene is $10^{0.51}$ ($10^{0.25}$ ~ $10^{0.78}$) copies/ul (= 3.24 (1.78 ~ 6.03). Ct cut-off value based on the LOD result is 36.02.



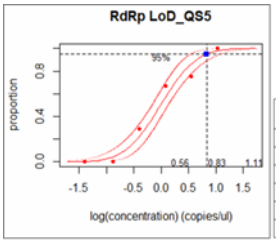

| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 10.80 | 24/24 | 100.0 |
| 3.60 | 19/24 | 79.2 |
| 1.20 | 14/24 | 58.3 |
| 0.40 | 7/24 | 29.2 |
| 0.13 | 0/24 | 0.0 |
| 0.04 | 0/24 | 0.0 |



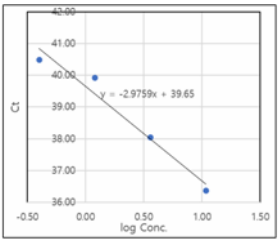

| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.04 | -1.40 | ND |
| 0.13 | -0.89 | ND |
| 0.40 | -0.40 | 41.57 |
| 1.20 | 0.08 | 39.30 |
| 3.60 | 0.56 | 37.52 |
| 10.80 | 1.03 | 35.52 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 6.61 | 0.82 | 36.38 |
| LoD CI | 3.55 | 0.55 | 37.50 |
| | 12.30 | 1.09 | 35.25 |

For ABI 7500, LOD (95% CI) of RdRP is $10^{0.82}$ ($10^{0.55}$ ~ $10^{1.09}$) copies/ul (= 6.61 (3.55 ~ 12.30) copies/ul). Ct cut-off value based on the LOD result is 37.50.



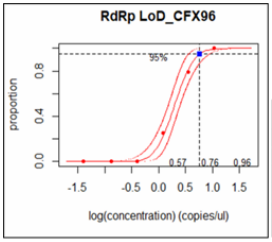

| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 10.80 | 24/24 | 100.0 |
| 3.60 | 18/24 | 75.0 |
| 1.20 | 16/24 | 66.7 |
| 0.40 | 7/24 | 29.2 |
| 0.13 | 0/24 | 0.0 |
| 0.04 | 0/24 | 0.0 |



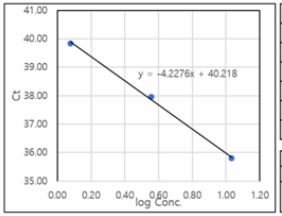

| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.04 | -1.40 | ND |
| 0.13 | -0.89 | ND |
| 0.40 | -0.40 | 40.48 |
| 1.20 | 0.08 | 39.93 |
| 3.60 | 0.56 | 38.05 |
| 10.80 | 1.03 | 36.37 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 6.76 | 0.83 | 37.18 |
| LoD CI | 3.63 | 0.56 | 37.98 |
| | 12.88 | 1.11 | 36.35 |

For ABI QS5, LOD (95% CI) of RdRP is$10^{0.83}$ ($10^{0.56}$ ~ $10^{1.11}$) copies/ul (= 6.76 (3.63 ~ 12.88) copies/ul). Ct cut-off value based on the LOD result is 37.98.




| conc. (copies/ul) | positive | ratio (%) |
|---|---|---|
| 10.80 | 24/24 | 100.0 |
| 3.60 | 19/24 | 79.2 |
| 1.20 | 6/24 | 25.0 |
| 0.40 | 0/24 | 0.0 |
| 0.13 | 0/24 | 0.0 |
| 0.04 | 0/24 | 0.0 |




| conc. (copies/ul) | log conc. | Ave. Ct |
|---|---|---|
| 0.04 | -1.40 | ND |
| 0.13 | -0.89 | ND |
| 0.40 | -0.40 | ND |
| 1.20 | 0.08 | 39.84 |
| 3.60 | 0.56 | 37.95 |
| 10.80 | 1.03 | 35.81 |

| | X (conc.) | logX | Y (Ct) |
|---|---|---|---|
| LoD | 5.75 | 0.76 | 37.01 |
| LoD CI | 3.72 | 0.57 | 37.81 |
| | 9.12 | 0.96 | 36.16 |

For Bio-Rad CFX96, LOD (95% CI) of RdRP is $10^{0.76}$ ($10^{0.57}$ ~ $10^{0.96}$) copies/ul (= 5.75 (3.72 ~9.12) copies/ul). Ct cut-off value based on the LOD result is 37.81.

10.1.3 Conclusion

In order to determine the sensitivity of *Kaira* 2019-nCoV Detection Kit, three different instruments (Applied Biosystems 7500, QS5, and BIO-RAD CFX96) were tested. The LOD of each instrument was shown in table 10. Taken together, the cut-off values were selected as 36.00Ct for E-gene and 37.65 Ct for RdRP.

**Table 10. LOD and Cut-off values for three instruments**

| Instrument | ABI 7500 | | ABI QS5 | | BIO-RAD CFX96 | |
|---|---|---|---|---|---|---|
| Target | LoD (95% CI) | Cut-off value | LoD (95% CI) | Cut-off value | LoD (95% CI) | Cut-off value |
| E gene | 3.72 copies/ul (1.95 ~ 6.92) | 36.00 | 4.47 copies/ul (2.57 ~ 7.94) | 36.23 | 3.24 copies/ul (1.78 ~ 6.03) | 36.02 |
| RdRP | 6.61 copies/ul (3.55 ~ 12.30) | 37.50 | 6.76 copies/ul (3.63 ~ 12.88) | 37.98 | 5.75 copies/ul (3.72 ~ 9.12) | 37.81 |

### 10.2. Specificity Test (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 11.

**Table 11. Material list of cross-reactivity test**

| No. | Pathogens* | Conc.(cp/ul) | Kaira COVID-19 Detection Kit | | |
|---|---|---|---|---|---|
| | | | RdRP | E-gene | PCRC |
| 1 | Corona 229E | $4.44X10^5$ | ND | ND | ND |
| 2 | Inf A H1N1 | $1.54X10^5$ | ND | ND | ND |
| 3 | Inf A H3N2 | $4.4X10^5$ | ND | ND | ND |
| 4 | Inf B | $1.03X10^5$ | 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: Enteroaggregative 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;

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

### 10.4. 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.

### 10.5. 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%.

### 10.6. 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℃. 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℃.

### 10.7. Clinical Evaluation

From the clinical sample the total 63 clinical samples were tested with Kaira 2019-nCoV Detection Kit. 28 specimens from COVID-19 cases and 30 negative specimens from symptomatic individuals were included in the specimen set. Results of individual primer & Probe Mixture testing are compared to this reference product in the table below.

**Table 12. Results of the 58 analyzed clinical samples with *Kaira* 2019-nCoV Detection Kit**

| Specimen category | Tested | *Kaira* 2019-nCoV Detection Kit | |
|---|---|---|---|
| | | RdRP Positive | E gene Positive |
| 2019-nCoV Positive | 28 | 28/28 | 28/28 |
| 2019-nCoV Negative | 30 | 0/30 | 0/30 |
| Positive percent agreement | | 100% (28/28) 95% CI: 87.94%-100% | |
| Negative percent agreement | | 100% (0/30) 95% CI: 88.65%-100% | |
| 100% (58/58) 95% CI: 100% | | | |

## 11. REFERENCES

Munster, VJ et al. A Novel Coronavirus Emerging in China — Key Questions for Impact Assessment. N Engl J Med 2020; 382:692-694.

Mackay IM. (2004) Real-time PCR in the microbiology laboratory. Clin. Microbiol. Infect. 10:190-212.

CLSI. Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition. CLSI document EP7-A2.

CLSI. Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition. CLSI document EP5-A2.

NCCLS. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. NCCLS document EP6-A.

NCCLS. Quantitative Molecular Methods for Infectious Diseases; Approved Guideline. NCCLS document MM6-A.

NCCLS. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. NCCLS document EP17-A.

## 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 |  | Date of Manufacture |
|  | Caution, consult accompanying documents. |  | Keep away from sunlight |

# User's Guide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