

**STANDARD M**  
**nCoV** Real-Time Detection kit



SARS-like coronavirus (SARS-CoV-2) Real-Time RT-PCR Kit



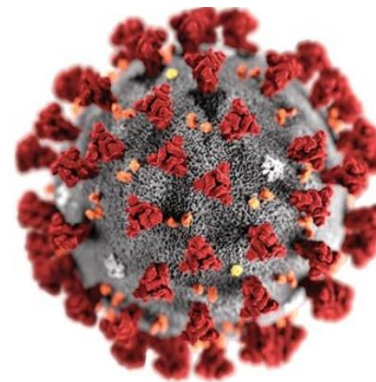
**SD BIOSENSOR**



# I. Introduction of COVID-19

# Outlook of COVID -19

- **Original finding** December 2019 in Wuhan China
- **Pathogen** SARS-like coronavirus (SARS-CoV-2)
- **Symptom** Fever, cough, sore throat, dyspnea, diarrhea, infiltration of the lungs on chest X-ray etc.
- **Transmission** Animal → Human / Human → Human  
Transmission between human via droplet (respiratory secretion),  
second transmission within healthcare settings
- **Window Period** 2-14 days (assumption)



SARS-CoV-2

According to WHO

The disease caused by  
Novel Coronavirus, SARS-CoV-2

is now officially called

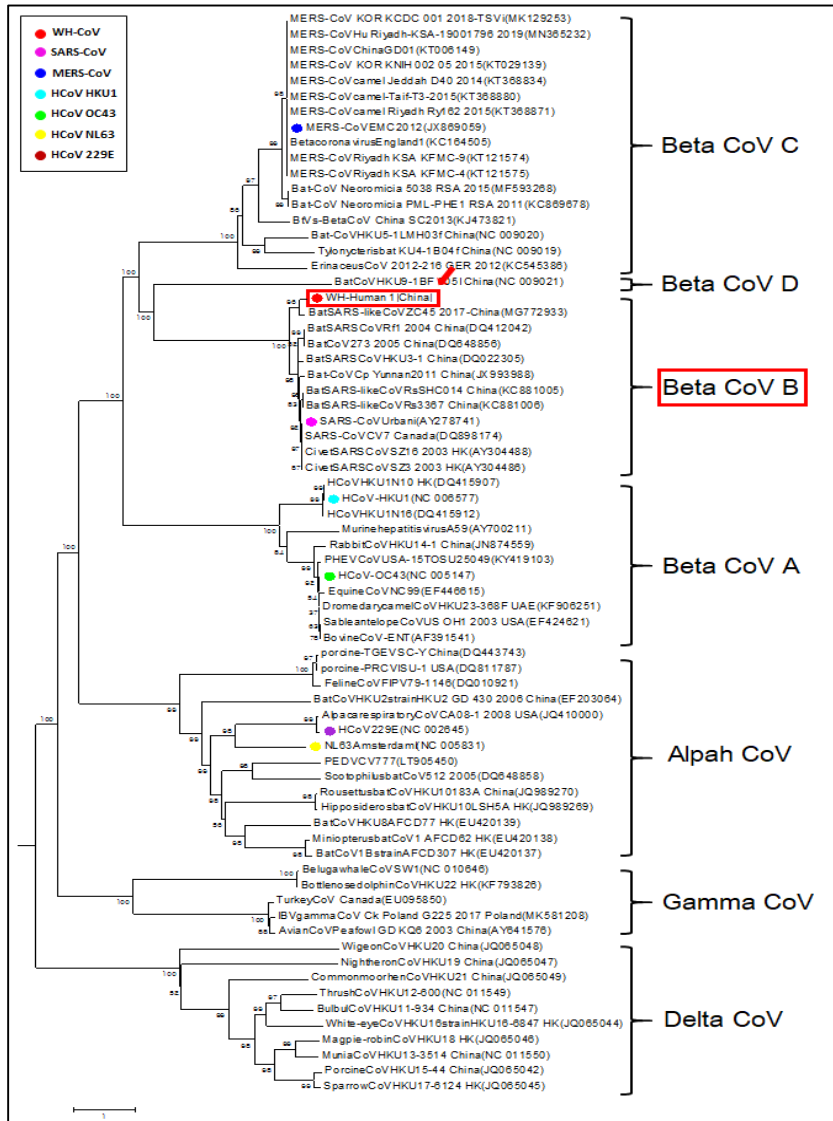
**COVID-19**

CO - Corona  
VI - Virus  
D - Disease

[www.microbenotes.com](http://www.microbenotes.com)



# Phylogenetic Systematics of SARS-CoV-2



Source : Korean CDC

- **Corona Virus**
  - Alpha, Beta
  - Gamma, Delta
- **Beta coronavirus is divided into A~D lineages**
  - A: OC43, HKU1
  - B: SARS-CoV (Severe acute respiratory syndrome coronavirus)
  - C: MERS-CoV (Middle East respiratory syndrome coronavirus)
- **SARS-CoV-2: a member of the subgenus Sarbecovirus (Beta-CoV lineage B)**

# Genetic Association Analysis of Human Coronavirus

| Genus             | Human coronavirus (HCoV) | NCBI GenBank Register no. | Homology(%) |
|-------------------|--------------------------|---------------------------|-------------|
| Alpha Coronavirus | HCoV 229E                | NC002645                  | 39.3        |
|                   | HCoV NL63                | NC005831                  | 40.2        |
| Beta Coronavirus  | HCoV OC43                | NC005147                  | 42.7        |
|                   | HCoV HKU1                | NC006577                  | 43.6        |
|                   | <b>SARS-CoV</b>          | <b>AY278741</b>           | <b>77.5</b> |
|                   | MERS-CoV                 | JX869059                  | 50.0        |

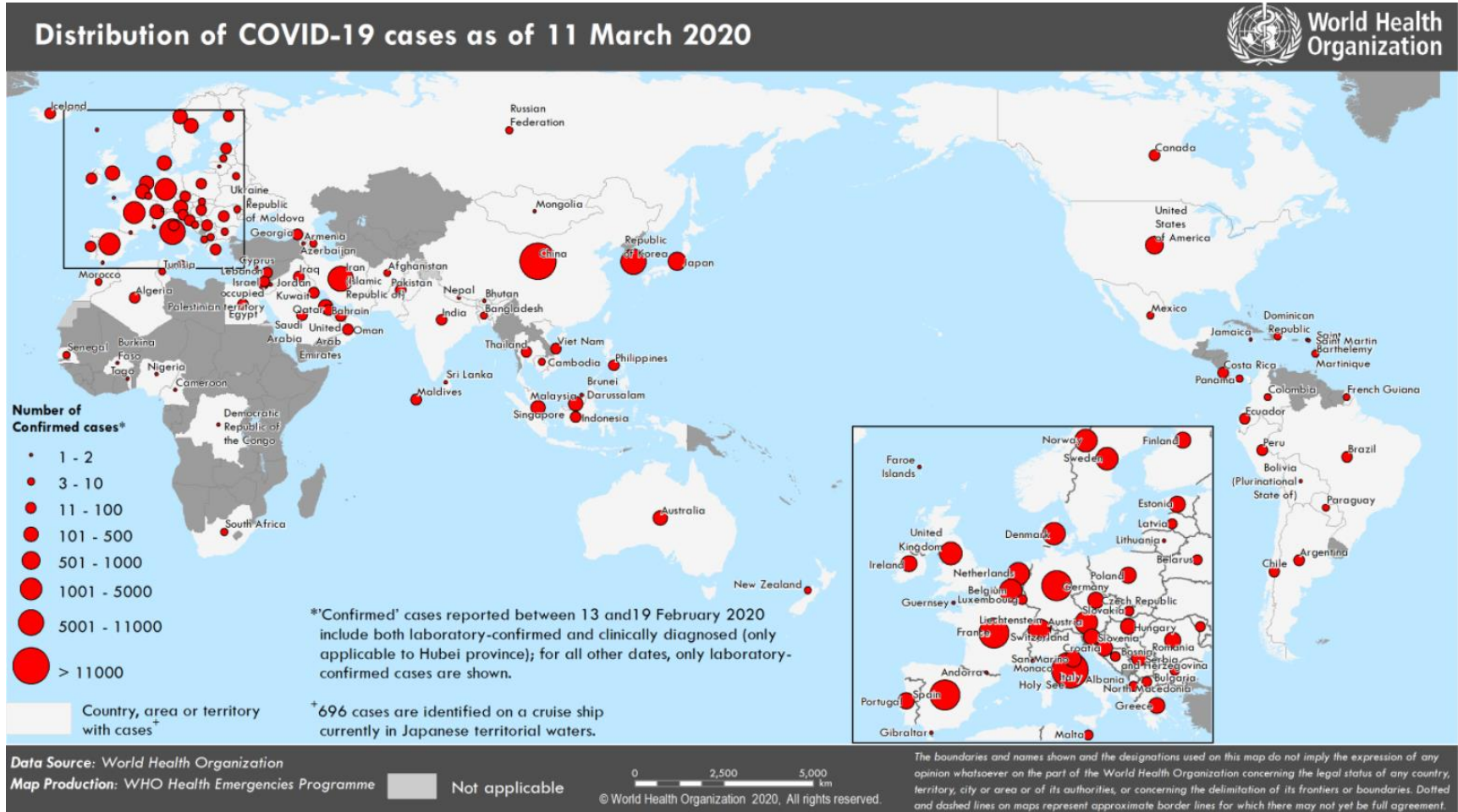
Reference: Korea Disease Control Division Infectious Disease Analysis Center

- HCoV Alpha (229E, NL63) 39-40% of homology
- HCoV Beta (OC43, HKU1) 42~43% of homology
- MERS-CoV 50% of homology
- **SARS-CoV 77.5% of homology**
- SARS-CoV-2 has strong genetic similarity to **bat SARS-like CoV (85.1% homology)**, from which it likely originated, although an intermediate reservoir such as a pangolin is thought to be involved.

# Global Outbreak of COVID-19

**118,322 confirmed / 2,853 deaths**

11 March 2020, WHO COVID-19 Situation Report - 51





## II. SARS-CoV-2 Specimen

# Types of Specimen

**Both samples should be collected from lower and upper respiratory tract**

| No. | Types of specimen       |   | Container   |
|-----|-------------------------|---|---|
| 1   | Lower respiratory tract | <ul style="list-style-type: none"> <li>Sputum<br/>(not mandatory)</li> </ul>                      | <ul style="list-style-type: none"> <li><b>Container</b> Sterilized tube (50ml)</li> <li><b>Volume</b> &gt;3ml</li> </ul>  |
| 2   | Upper respiratory tract | <ul style="list-style-type: none"> <li>Nasopharyngeal swab</li> <li>Oropharyngeal swab</li> </ul> | <ul style="list-style-type: none"> <li><b>Container</b> Both NP and OP swabs should be collected to the same medium containing viral transport media (VTM)</li> </ul> |

Table. Types of sample for COVID-19



# How to Collect Specimen (1)

## Lower respiratory | Sputum

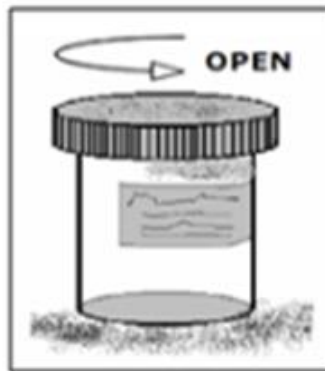
- **Sputum** can be extracted by coughing into the sterilized container (make sure not to be contaminated by saliva)

\* Avoid any contamination or leakage during delivery by sealing completely.

Figure. How to collect sputum



1. Wash your mouth out



2. Use a sterilized container



3. Cough until sputum comes out



4. Seal it completely (Keep in 4°C)

## How to Collect Specimen (2)

### Upper respiratory | Nasopharyngeal swab + Oropharyngeal swab

- **Nasopharyngeal swab** can be collected by scratching the nasopharynx through the nostril.
- **Oropharyngeal swab** can be collected by scratching the pharynx having your tongue down.

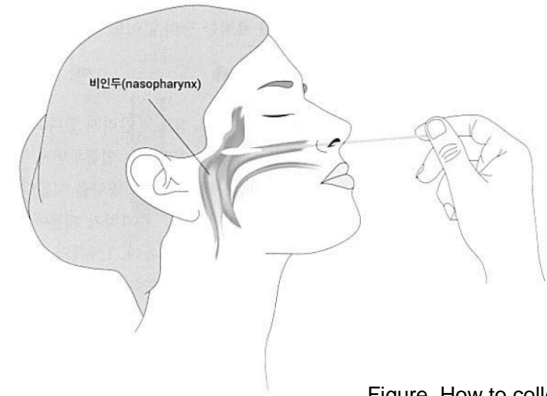


Figure. How to collect the NP swab

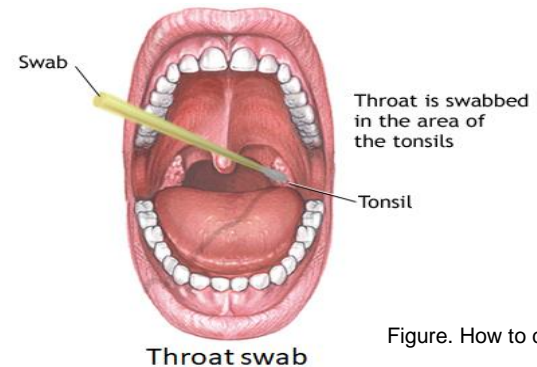


Figure. How to collect the OP swab

✓ **Collect the NP and OP swabs in one VTM.**

\* When collect specimen, it should be wearing the individual protection such as mask, gloves, gown and goggle

# Storage of Specimen

**Table 1. Specimens to be collected from symptomatic patients**

Guidance on specimen collection (adapted from reference 5)

| Specimen type   | Collection materials                                       | Transport to laboratory | Storage till testing                 | Comment  |
|---|--|-------------------------|--------------------------------------|--|
| Nasopharyngeal and oropharyngeal swab   | Dacron or polyester flocked swabs*                         | 4 °C                    | ≤5 days: 4 °C<br>>5 days: -70 °C     | The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.   |
| Bronchoalveolar lavage  | sterile container *  | 4 °C                    | ≤48 hours: 4 °C<br>>48 hours: -70 °C | There may be some dilution of pathogen, but still a worthwhile specimen  |
| (Endo)tracheal aspirate, nasopharyngeal aspirate or nasal wash                | sterile container *  | 4 °C                    | ≤48 hours: 4 °C<br>>48 hours: -70 °C |  |
| Sputum  | sterile container  | 4 °C                    | ≤48 hours: 4 °C<br>>48 hours: -70 °C | Ensure the material is from the lower respiratory tract  |
| Tissue from biopsy or autopsy including from lung                             | sterile container with saline                              | 4 °C                    | ≤24 hours: 4 °C<br>>24 hours: -70 °C |  |
| Serum (2 samples acute and convalescent possibly 2-4 weeks after acute phase) | Serum separator tubes (adults: collect 3-5 ml whole blood) | 4 °C                    | ≤5 days: 4 °C<br>>5 days: -70 °C     | Collect paired samples: <ul style="list-style-type: none"> <li>• acute – first week of illness</li> <li>• convalescent – 2 to 3 weeks later</li> </ul> |
| Whole blood   | collection tube  | 4 °C                    | ≤5 days: 4 °C<br>>5 days: -70 °C     | For antigen detection particularly in the first week of illness  |
| Urine   | urine collection container                                 | 4 °C                    | ≤5 days: 4 °C<br>>5 days: -70 °C     |  |

Reference: WHO/2019-nCoV

# Prior to Real-Time PCR

## 1. Specimen pretreatment

- **NP and OP swabs**

extract the nucleic acids by re-suspending from the VTM or PBS

- **Sputum**

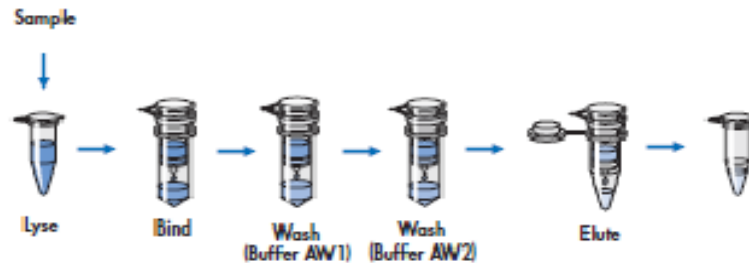
Add the same amount of 2X sputum lysis buffer and shake the incubation for 30 minutes

## 2. Extraction of nucleic acids

Commercially available viral RNA extract reagents or automation instruments can be used. Follow the instructions given by the manufacturer.

Recommended extraction kit: **QIAamp® Viral RNA mini Kit (Cat. no. 52904)**

### QIAamp Viral RNA Mini Spin Procedure



\* **Setting values might be different depending on sites (hospitals)**



# III. STANDARD M nCoV Real-Time Detection kit

# Advantages of STANDARD M nCoV

- ✓ Designed according to **WHO / KCDC** recommendations
- ✓ Easy to use **1 Tube** reaction
- ✓ Separately configured **Internal Control (IC)** to verify the whole process
- ✓ **Simple interpretation of results** (2 Target (RdRp / E gene) + 1 IC)
- ✓ Outstanding analytical sensitivity **5 copies/reaction**
- ✓ A wide range of instrument compatibility **CFX96 / AB7500 / LC480**
- ✓ **Fast TAT** - **Within 90 minutes** after extraction, **12 months of stability**
- ✓ **Obvious patient sample test results**
- ✓ **CE-IVD, KEUA** (Korean Emergency Use Authorization)

# WHO Recommendations on COVID-19 Testing

Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases.

Interim guidance  
2 March 2020



**Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases**  
<https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117>

## 4. Laboratory testing for COVID-19 virus

Laboratories undertaking testing for COVID-19 virus should adhere strictly to appropriate biosafety practices.

### **Nucleic acid amplification tests (NAAT) for COVID-19 virus**

Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT such as real-time reverse-transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary. The viral genes targeted so far include the N, E, S and RdRP genes. Examples of protocols used may be found [here](#). RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility. Heat treatment of samples prior to RNA extraction is not recommended.

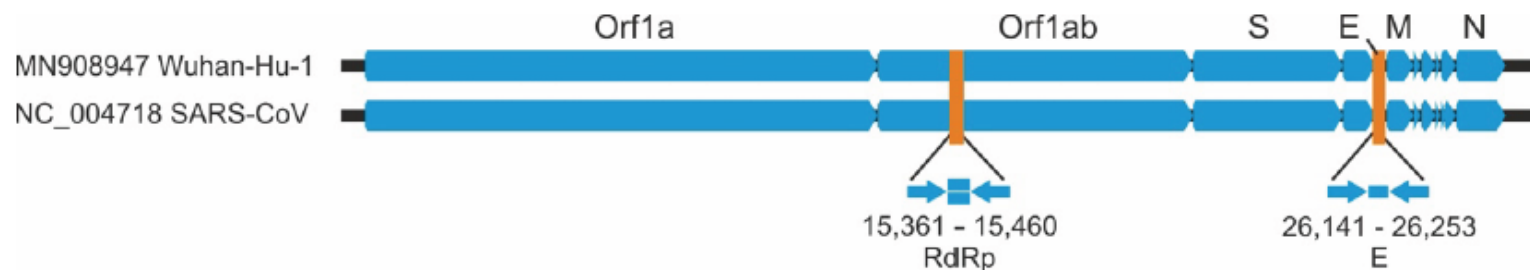
✓ **WHO recommended rRT-PCR for SARS-CoV-2 Laboratory testing.**

# WHO Recommendations for Primer/Probe

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for 2019-nCoV E gene assay is available via EVAg. Synthetic control for 2019-nCoV RdRp is expected to be available via EVAg from Jan 21st onward.

**First line screening assay: E gene assay**

**Confirmatory assay: RdRp gene assay**



[Diagnostic detection of Wuhan coronavirus 2019 by real-time RT-PCR – Charité, Berlin Germany \(17 January 2020\)](#)

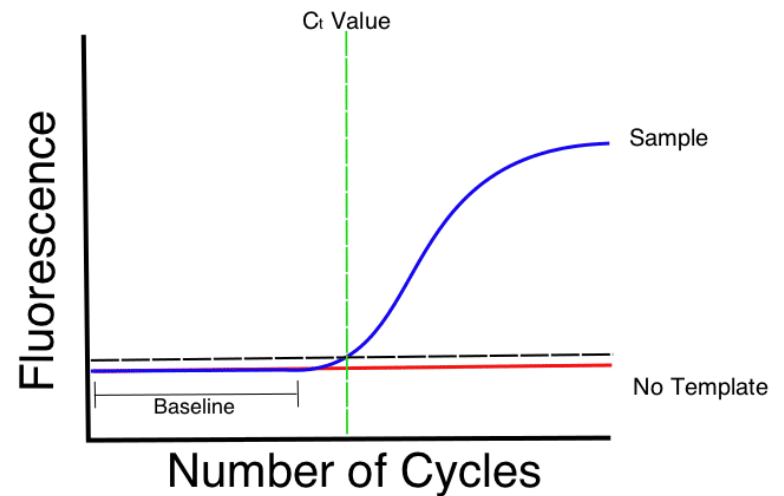
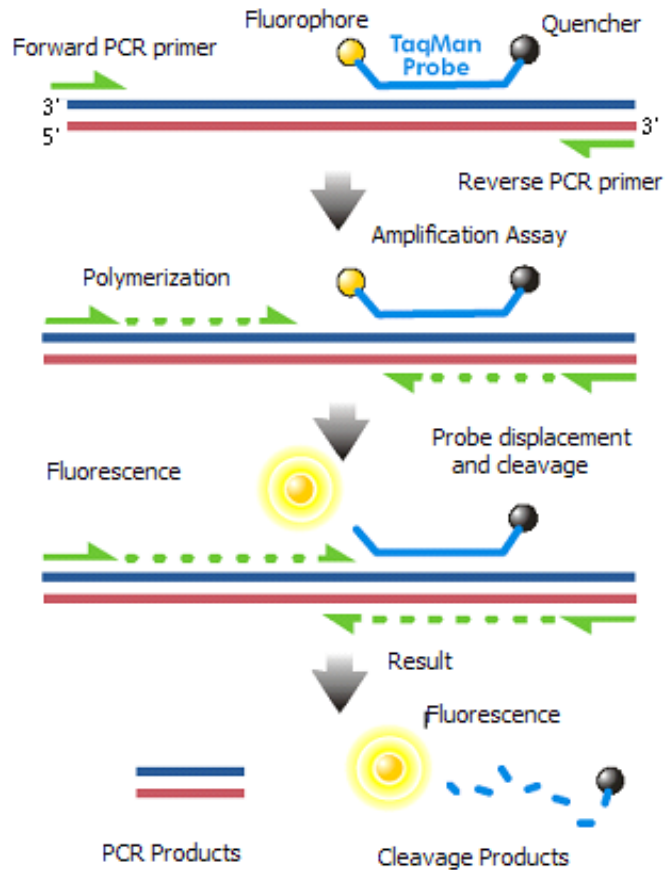
- ✓ **WHO (Berlin) recommended target gene: ORF1ab (RdRp) gene, E gene = **KCDC****
- ✓ **WHO recommend Protocols:**  
Select 1 out of 6 protocols (from China, Germany, Hong Kong, Thailand, Japan or USA)



# 1-1. WHO Recommended Testing Method

## rRT-PCR | Real-time reverse transcription polymerase chain reaction

- TaqMan probe system



## 1-2. WHO Recommended Primer/Probe

### Protocol of KCDC (Korea Centers for Disease Control & Prevention)




Modified WHO (Berlin) protocol

| Target gene              | Primer/probe             | Sequence                   | Note                      |
|--------------------------|--------------------------|----------------------------|---------------------------|
| ORF1ab<br>(RdRp)<br>gene | ORF1 gene-forward primer | GTGARATGGTCATGTGTGGCGG     | RdRp_SARsR-F2<br>(German) |
|                          | ORF1 gene-revers primer  | CARATGTTAAASACACTATTAGCATA | RdRp_SARsR-R1<br>(German) |
|                          | ORF1 gene-probe (FAM)    | CAGGTGGAACCTCATCAGGAGATGC  | RdRp_SARsR-P2<br>(German) |
| E gene                   | E gene-forward primer    | ACAGGTACGTTAATAGTTAATAGCGT | E_Sabeco-F1 (German)      |
|                          | E gene-reverse primer    | ATATTGCAGCAGTACGCACACA     | E_Sabeco-R2 (German)      |
|                          | E gene-probe (JOE)       | ACACTAGCCATCCTTACTGCGCTTCG | E_Sabeco-P1 (German)      |

✓ **Multiplex:** detecting 2 type of genes in a tube by using different fluorophore for each probe

## 2. Easy to Use 1 Tube reaction

**1-Tube Reaction** | Higher Throughput! User hands-on time ↓, Error ↓

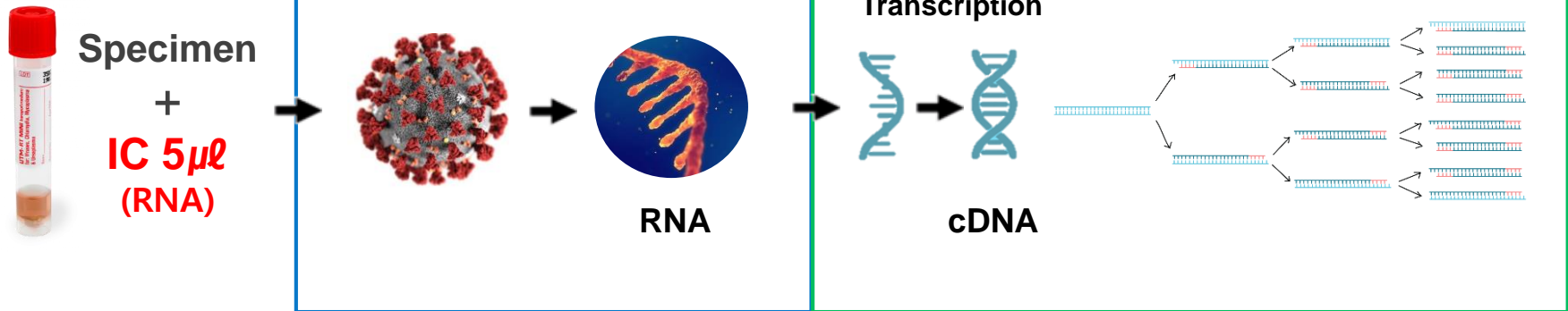
|   | 1 Tube Reaction<br>STANDARD M nCoV Real-Time Detection kit  | 2 Tube Reaction<br>Competitors  |
|---|---|---|
| <b>Protocol</b>                                       | <p>[ Simple procedure ]</p>  <p>RT-PCR Mix (RdRP/E gene)<br/>+<br/>RNA</p>                 |  <p>RT-PCR Mix (RdRP gene)<br/>+<br/>RNA</p>  <p>RT-PCR Mix (E gene)<br/>+<br/>RNA</p>  |
| <b>Interpretation</b>                                 | <p>[ Clear interpretation for results ]</p> <ul style="list-style-type: none"> <li>• IC (Internal control) Valid – Confirm result</li> <li>• IC Invalid – Retest</li> </ul> | <ul style="list-style-type: none"> <li>• 1 tube IC Valid / 2 tube IC Valid – Confirm result</li> <li>• <b>1 tube IC Invalid</b> / 2 tube IC Valid – <b>Retest</b></li> <li>• 1 tube IC Valid / <b>2 tube IC Invalid</b> – <b>Retest</b></li> <li>• <b>1 tube IC Invalid</b> / <b>2 tube IC Invalid</b> – <b>Retest</b></li> </ul> |
| <b>User Hands-on time</b>                             | <p>[ Minimized user error ]</p> <p>RT-PCR Mix 1 time / Dispense NA 1 time</p>   | <p>1 tube: RT-PCR Mix 1time / Dispense NA 1 time<br/>2 tube: RT-PCR Mix 1time / Dispense NA 1 time</p> <p><b>Hands-on time X 2 / User errors could be X 2</b></p>   |
| <b>Test per run</b><br>(1 Instrument, 1 PC, 1 NC/run) | <p><b>94 tests</b> [ Throughput X2 ]</p>  | <p>46 tests</p>   |
| <b>Test per day</b><br>(5 run/day)                    | <p><b>470 tests</b></p>   | <p><b>230 tests</b></p>   |

### 3. Separately configured Internal Control

**Internal Control** | verify the whole process NA extraction to RT-PCR

NA extraction

NA amplification



# 4. Simple Interpretation of Results

|   | SD BIOSENSOR<br>M nCoV Real-Time Detection kit |            |               |                      | 2 Tube Reaction<br>Competitor A |            |               |                      | 3 Gene Detection<br>Competitor B |                |            |               |                      |            |            |               |               |
|---|--|------------|---------------|----------------------|---------------------------------|------------|---------------|----------------------|----------------------------------|----------------|------------|---------------|----------------------|------------|------------|---------------|---------------|
| <b>COVID-19 Positive</b>                          | <b>RdRp (R) +</b>                              | <b>E +</b> | <b>IC +/-</b> | <b>nCoV Positive</b> | 1.                              | <b>R +</b> | <b>IC +/-</b> | <b>nCoV Positive</b> | <b>R +</b>                       | <b>E +</b>     | <b>N +</b> | <b>IC +/-</b> | <b>nCoV Positive</b> |            |            |               |               |
|   |  |            |               |                      | 2.                              | <b>E +</b> | <b>IC +/-</b> |                      |                                  |                |            |               |                      |            |            |               |               |
| <b>Inconclusive (RdRp/N Positive, E Negative)</b> | <b>R +</b>                                     | <b>E -</b> | <b>IC +/-</b> | <b>Re-test</b>       | 1.                              | <b>R +</b> | <b>IC +/-</b> | <b>Re-test</b>       | <b>R +</b>                       | <b>E +</b>     | <b>N -</b> | <b>IC +/-</b> | <b>Re-test</b>       |            |            |               |               |
|   |  |            |               |                      |                                 |            |               |                      | <b>R +</b>                       | <b>E -</b>     | <b>N +</b> | <b>IC +/-</b> | <b>Re-test</b>       |            |            |               |               |
|   |  |            |               |                      | <b>R +</b>                      | <b>E -</b> | <b>N -</b>    |                      | <b>IC +/-</b>                    | <b>Re-test</b> |            |               |                      |            |            |               |               |
|   |  |            |               |                      | 2.                              | <b>E -</b> | <b>IC +/-</b> |                      | <b>R -</b>                       | <b>E +</b>     | <b>N +</b> | <b>IC +/-</b> | <b>Re-test</b>       |            |            |               |               |
|   |  |            |               |                      |                                 |            |               |                      | <b>R -</b>                       | <b>E -</b>     | <b>N +</b> | <b>IC +/-</b> | <b>Re-test</b>       |            |            |               |               |
| <b>Near-source Coronavirus Positive</b>           | <b>R -</b>                                     | <b>E +</b> | <b>IC +/-</b> | <b>CoV Positive</b>  | 1.                              | <b>R -</b> | <b>IC +</b>   | <b>CoV Positive</b>  | <b>R -</b>                       | <b>E +</b>     | <b>N -</b> | <b>IC +/-</b> | <b>CoV Positive</b>  |            |            |               |               |
|   |  |            |               |                      | 2.                              | <b>E +</b> | <b>IC +/-</b> |                      |                                  |                |            |               |                      |            |            |               |               |
|   |  |            |               |                      | 1.                              | <b>R -</b> | <b>IC -</b>   | <b>Re-test</b>       |                                  |                |            |               |                      | <b>R -</b> | <b>E +</b> | <b>N -</b>    | <b>IC +/-</b> |
|   |  |            |               |                      |                                 |            |               |                      |                                  |                |            |               |                      | 2.         | <b>E +</b> | <b>IC +/-</b> |               |
| <b>Negative</b>                                   | <b>R -</b>                                     | <b>E -</b> | <b>IC +</b>   | <b>Negative</b>      | 1.                              | <b>R -</b> | <b>IC +</b>   | <b>Negative</b>      | <b>R -</b>                       | <b>E -</b>     | <b>N -</b> | <b>IC +</b>   | <b>Negative</b>      |            |            |               |               |
|   |  |            |               |                      | 2.                              | <b>E -</b> | <b>IC +</b>   |                      |                                  |                |            |               |                      |            |            |               |               |
| <b>Re-test (IC Invalid)</b>                       | <b>R -</b>                                     | <b>E -</b> | <b>IC -</b>   | <b>Re-test</b>       | 1.                              | <b>R -</b> | <b>IC -</b>   | <b>Re-test</b>       | <b>R -</b>                       | <b>E -</b>     | <b>N -</b> | <b>IC -</b>   | <b>Re-test</b>       |            |            |               |               |
|   |  |            |               |                      | 2.                              | <b>E -</b> | <b>IC +</b>   |                      |                                  |                |            |               |                      |            |            |               |               |
|   |  |            |               |                      | 1.                              | <b>R -</b> | <b>IC +</b>   | <b>Re-test</b>       |                                  |                |            |               |                      | <b>R -</b> | <b>E -</b> | <b>N -</b>    | <b>IC -</b>   |
|   |  |            |               |                      |                                 |            |               |                      |                                  |                |            |               |                      | 2.         | <b>E -</b> | <b>IC -</b>   |               |

## 5. Outstanding Analytical Sensitivity / Cross-reactivity

- **Sensitivity : 5 copies/reaction (10µl reaction) = 5x10<sup>2</sup> copies/ml**

| Concentration(copies/mL)      | ORF 1ab gene | E gene      |
|-------------------------------|--------------|-------------|
| 5x10 <sup>3</sup> copies / ml | 100%(20/20)  | 100%(20/20) |
| 1x10 <sup>3</sup> copies / ml | 100%(20/20)  | 100%(20/20) |
| 5x10 <sup>2</sup> copies / ml | 95%(19/20)   | 95%(19/20)  |
| 1x10 <sup>2</sup> copies / ml | 65%(13/20)   | 70%(14/20)  |

- **Cross Reactivity : There is no cross reactivity to the 21 substances below.**

| No. | Category                             | Cross-reactivity substance  | ID | ORF 1ab gene | E gene | Result   |
|-----|--------------------------------------|-----------------------------|----|--------------|--------|----------|
| 1   | Non 2019-nCoV coronavirus infections | MERSr-CoV                   | 1  | No Ct        | No Ct  | negative |
|     |                                      | HCoV-229E                   | 2  | No Ct        | No Ct  | negative |
|     |                                      | HCoV-HKU1                   | 3  | No Ct        | No Ct  | negative |
|     |                                      | HCoV-NL63                   | 4  | No Ct        | No Ct  | negative |
|     |                                      | HCoV-OC43                   | 5  | No Ct        | No Ct  | negative |
| 2   | Non 2019-nCoV Viral infections       | Influenza A virus           | 6  | No Ct        | No Ct  | negative |
|     |                                      | Influenza B virus           | 7  | No Ct        | No Ct  | negative |
|     |                                      | Respiratory syncytial virus | 8  | No Ct        | No Ct  | negative |
|     |                                      | Rhinovirus                  | 9  | No Ct        | No Ct  | negative |
|     |                                      | Parainfluenza virus         | 10 | No Ct        | No Ct  | negative |
|     |                                      | Adenovirus                  | 11 | No Ct        | No Ct  | negative |
| 3   | Bacteria                             | Legionella pneumophila      | 12 | No Ct        | No Ct  | negative |
|     |                                      | Chlamydia pneumoniae        | 13 | No Ct        | No Ct  | negative |
|     |                                      | Mycoplasma pneumoniae       | 14 | No Ct        | No Ct  | negative |
|     |                                      | Haemophilus influenzae      | 15 | No Ct        | No Ct  | negative |
|     |                                      | Moraxella catarrhalis       | 16 | No Ct        | No Ct  | negative |
|     |                                      | Streptococcus pyogenes      | 17 | No Ct        | No Ct  | negative |
|     |                                      | Bowman Animal bacterium     | 18 | No Ct        | No Ct  | negative |
|     |                                      | Klebsiella pneumoniae       | 19 | No Ct        | No Ct  | negative |
|     |                                      | Pseudomonas aeruginosa      | 20 | No Ct        | No Ct  | negative |
|     |                                      | Streptococcus pneumonia     | 21 | No Ct        | No Ct  | negative |

## 6. A Wide Range of Instrument Compatibility

**Instrument Compatibility** | instruments mainly used in laboratories



CFX 96  
**Bio-Rad**



ABI 7500/7500 Fast  
**Applied Biosystems**



LightCycler 480  
**Roche**



STANDARD M nCoV Real-Time Detection kit

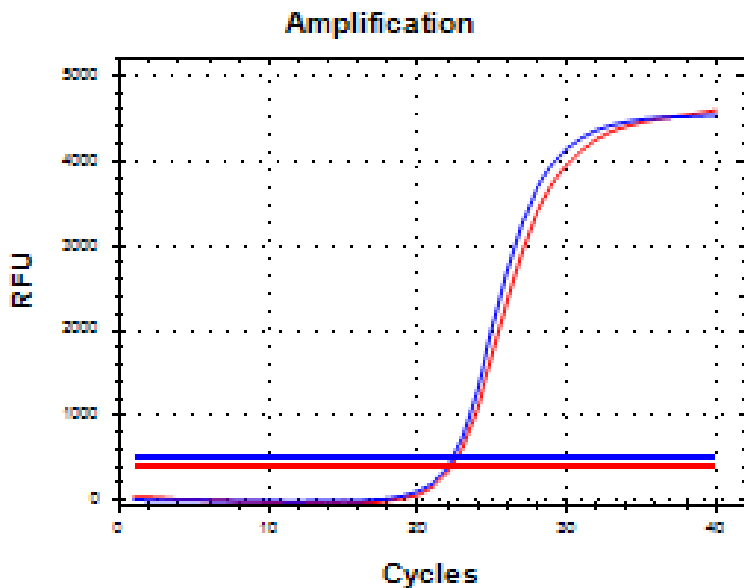


Competitors

# 7. Obvious Patient Sample Test Result

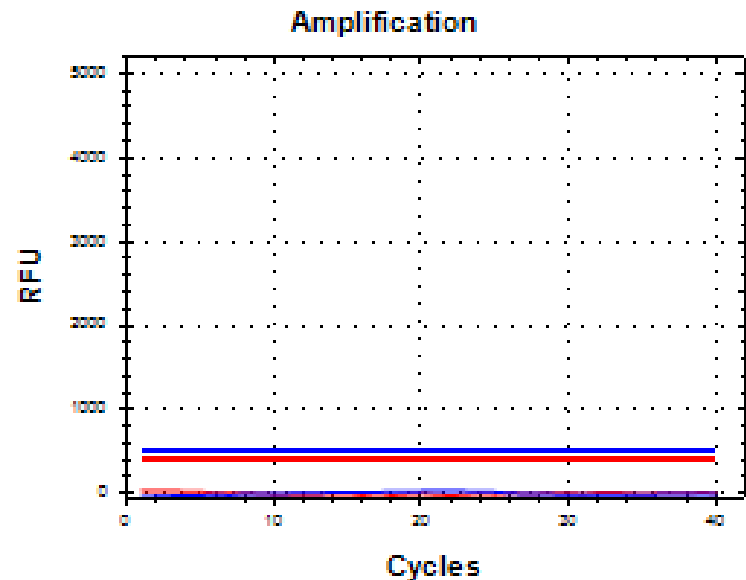
**Positive – Sensitive result** | 10 $\mu$ l RNA, Low LoD: 5 copies/rxn

**Negative- No abnormal signal** | Flat amplification curve



| Well | Fluor | Target | Content | Cq    |
|------|-------|--------|---------|-------|
| A01  | FAM   | RdRP   | Unkn    | 22,29 |
| A01  | HEX   | E      | Unkn    | 22,28 |

**Example of positive result:**  
Similar Ct value for RdRp gene & E gene



| Well | Fluor | Target | Content | Cq  |
|------|-------|--------|---------|-----|
| B01  | FAM   | RdRP   | Unkn    | N/A |
| B01  | HEX   | E      | Unkn    | N/A |

**Example of negative result:**  
No abnormal signal for negative sample  
(Abnormal signal was observed with other kit)



# Comparison Test Results

**Date:** 13 March, 2020

**Site:** A Hospital (Korea)

**Specimen:** 1~8 Hospitalized positive patient sample (sample type random)  
 9~22 Negative sample (sample type random)

|    | A (Cut-off: 35 Ct) |          |              |          | SD BIOSENSOR (36 Ct) |              |          | Result   |
|----|--------------------|----------|--------------|----------|----------------------|--------------|----------|----------|
|    | RdRp (FAM)         | IC (JOE) | E (FAM)      | IC (JOE) | RdRp (FAM)           | E (JOE)      | IC (CY5) |          |
| 1  | 29.72              | 18.23    | 28.35        | 18.37    | 26.57                | 26.92        | 23.21    | Positive |
| 2  | 30.16              | 18.46    | 28.45        | 18.37    | 27.52                | 27.92        | 24.34    | Positive |
| 3  | 31.32              | 18.76    | 30.26        | 18.40    | 28.80                | 29.14        | 23.53    | Positive |
| 4  | 32.06              | 18.34    | 32.01        | 18.45    | 30.33                | 30.16        | 24.28    | Positive |
| 5  | 32.33              | 18.12    | 31.11        | 18.26    | 29.13                | 29.38        | 23.30    | Positive |
| 6  | 32.68              | 18.19    | 32.21        | 18.32    | 30.23                | 30.66        | 23.24    | Positive |
| 7  | 37.25              | 18.44    | 34.32        | 18.29    | 28.49                | 29.11        | 23.93    | Positive |
| 8  | 38.34              | 18.18    | Undetermined | 18.46    | 34.44                | 34.90        | 24.04    | Positive |
| 9  | Undetermined       | 18.46    | Undetermined | 18.59    | Undetermined         | Undetermined | 23.52    | Negative |
| 10 | Undetermined       | 18.22    | Undetermined | 18.46    | Undetermined         | Undetermined | 23.29    | Negative |
| 11 | Undetermined       | 18.25    | Undetermined | 18.21    | Undetermined         | Undetermined | 24.00    | Negative |
| 12 | Undetermined       | 18.36    | Undetermined | 18.41    | Undetermined         | Undetermined | 25.72    | Negative |
| 13 | Undetermined       | 18.41    | Undetermined | 18.59    | Undetermined         | Undetermined | 23.29    | Negative |
| 14 | Undetermined       | 18.25    | Undetermined | 18.36    | Undetermined         | Undetermined | 23.10    | Negative |
| 15 | Undetermined       | 19.29    | Undetermined | 18.29    | Undetermined         | Undetermined | 23.69    | Negative |
| 16 | Undetermined       | 18.32    | Undetermined | 18.38    | Undetermined         | Undetermined | 23.61    | Negative |
| 17 | Undetermined       | 18.32    | Undetermined | 18.40    | Undetermined         | Undetermined | 24.19    | Negative |
| 18 | Undetermined       | 18.21    | Undetermined | 18.38    | Undetermined         | Undetermined | 24.17    | Negative |
| 19 | Undetermined       | 18.32    | 37.49        | 18.39    | Undetermined         | Undetermined | 25.09    | Negative |
| 20 | Undetermined       | 18.31    | Undetermined | 18.52    | Undetermined         | Undetermined | 25.73    | Negative |
| 21 | Undetermined       | 18.30    | Undetermined | 18.36    | Undetermined         | Undetermined | 23.36    | Negative |
| 22 | Undetermined       | 18.46    | 38.01        | 18.79    | Undetermined         | Undetermined | 24.10    | Negative |

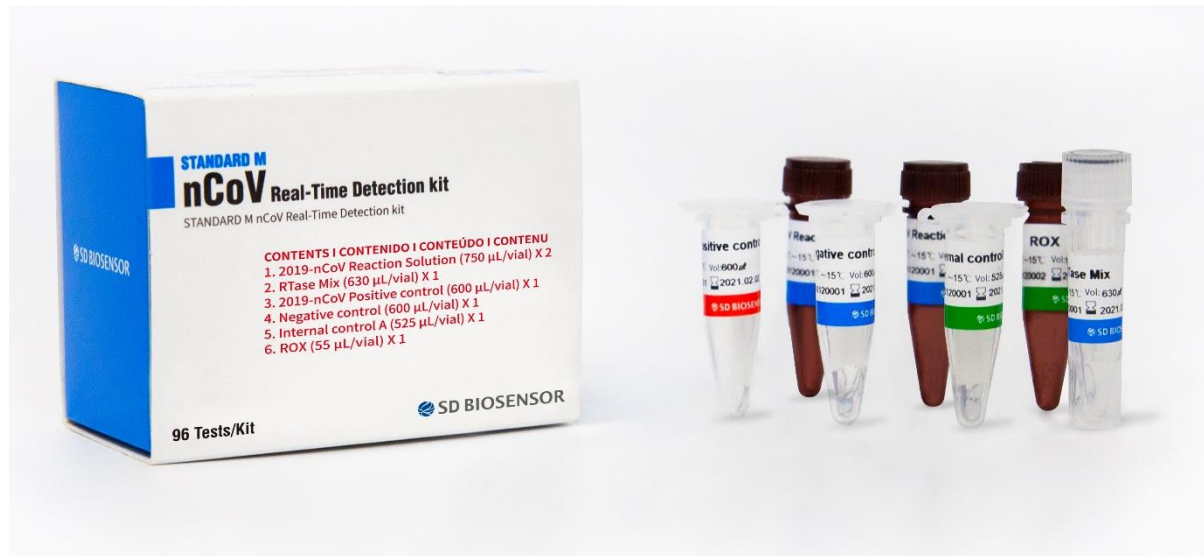
More sensitive results  
 (previously collected specimen was positive with A kit.)

No abnormal signal for negative results



# IV. STANDARD M nCoV Procedure

# Contents



|   | Contents (96T/kit)                 | Quantity             |
|---|------------------------------------|----------------------|
| 1 | <b>2019-nCoV Reaction Solution</b> | 750 $\mu$ l/vial x 2 |
| 2 | <b>RTase Mix</b>                   | 630 $\mu$ l/vial x 1 |
| 3 | <b>2019-nCoV Positive control</b>  | 600 $\mu$ l/vial x 1 |
| 4 | <b>Negative control</b>            | 600 $\mu$ l/vial x 1 |
| 5 | <b>Internal control A</b>          | 525 $\mu$ l/vial x 1 |
| 6 | <b>ROX*</b>                        | 55 $\mu$ l/vial x 1  |
| 7 | <b>Instructions for Use</b>        | 1                    |

- **Cat. no.: 11NCO10**
- **96 Tests/Kit**
- **Storage: -15°C ~ -25°C**
- **Stability: 12 months**
- **ROX: Reference dye (ABI 7500)**

# Protocol

## Target |

| Detection target   | Reporter           |
|--------------------|--------------------|
| ORF1ab (RdRp) gene | <b>FAM</b>         |
| E gene             | <b>JOE/VIC/HEX</b> |
| Internal control   | <b>CY5</b>         |

## PCR Mixture + RNA **10µl** |

1) CFX96 (Bio-Rad) / LC480 (Roche)

|                         | Reagent  | Dosage/<br>reaction |
|-------------------------|--|---------------------|
| 1                       | 2019-nCoV Reaction Solution  | <b>14µl</b>         |
| 2                       | RTase Mix  | <b>6µl</b>          |
| 3                       | Internal control A<br>(amplification directly)   | <b>0.5µl</b>        |
| Total<br>vol. /<br>well | <b>20.5µl</b> (IC from NA amplification step)<br><i>20µl (5µl of IC from NA extraction step. Add<br/>0.5µl of Internal control A into the wells<br/>assigned as PC and NC)</i> |                     |

## Cycle Condition |

| Reaction              | Temp(°C)           | Time  | Cycle |
|-----------------------|--------------------|-------|-------|
| Reverse transcription | 50°C               | 15:00 | 1     |
| Initial denaturation  | 95°C               | 3:00  | 1     |
| Pre-amplification     | 95°C               | 0:05  | 5     |
|                       | 60°C               | 0:40  |       |
| Amplification         | 95°C               | 0:05  | 40    |
|                       | 60°C               | 0:40  |       |
|                       | Scan (FAM/JOE/CY5) |       |       |

2) ABI7500/7500 Fast (Applied Biosystems)

|                         | Reagent  | Dosage/<br>reaction |
|-------------------------|--|---------------------|
| 1                       | 2019-nCoV Reaction Solution  | <b>14µl</b>         |
| 2                       | RTase Mix  | <b>6µl</b>          |
| 3                       | ROX (Passive Reference)  | <b>0.5µl</b>        |
| 4                       | Internal control A<br>(amplification directly)   | <b>0.5µl</b>        |
| Total<br>vol. /<br>well | <b>21µl</b> (IC from NA amplification step)<br><i>20.5µl (5µl of IC from NA extraction step. Add<br/>0.5µl of Internal control A into the wells<br/>assigned as PC and NC)</i> |                     |

# Interpretation of Results

**Ct Cut-off** | the same criteria for Sample/PC/NC

| Target                   | Ct Value       | 결과                                    |
|--------------------------|----------------|---------------------------------------|
| ORF1ab (RdRp) gene (FAM) | <b>Ct ≤ 36</b> | 2019-nCov ORF1ab (RdRp) gene positive |
| E gene (JOE/VIC/HEX)     | <b>Ct ≤ 36</b> | E gene positive                       |
| Internal Control (CY5)   | <b>Ct ≤ 32</b> | Internal control positive             |

**Interpretation of result for each sample |**

| ORF1ab (RdRp) gene FAM | E gene JOE/VIC/HEX | Internal Control CY5      | Result                           |
|------------------------|--------------------|---------------------------|----------------------------------|
| <b>Positive</b>        | <b>Positive</b>    | <b>Positive/Negative*</b> | 2019-nCoV positive               |
| <b>Positive</b>        | <b>Negative</b>    | <b>Positive/Negative*</b> | Inconclusive*                    |
| <b>Negative</b>        | <b>Positive</b>    | <b>Positive/Negative*</b> | Near-source Coronavirus positive |
| <b>Negative</b>        | <b>Negative</b>    | <b>Positive</b>           | 2019-nCoV negative               |
| <b>Negative</b>        | <b>Negative</b>    | <b>Negative</b>           | Invalid / Re-test                |

\* In case FAM/JOE\* signal is strong, Cy5 (IC) could be undetermined. If the retest result is still the same, interpret the result except for the CY5 (IC) result.

\*\* RdRp positive/ E negative (**Inconclusive**): If the repeated result is still the same, confirm the result using other methods such as viral sequencing.

# Comparison Chart (1)

|                                 | SD BIOSENSOR<br>M nCoV Real-Time Detection kit                | Kogene<br>PowerChek 2019-nCoV Real-time<br>PCR kit                | Seegene<br>Allplex 2019-nCoV Assay  |
|---------------------------------|---|---|---|
| <b>Test/Kit</b>                 | <b>96</b>   | 50  | 100   |
| <b>Tube/Test</b>                | <b>1 Tube (3-plex)</b>  | <b>2 Tube (2-plex)</b>  | 1 Tube (4-plex)   |
| <b>Target gene</b>              | <b>RdRp gene</b> - FAM<br><b>E gene</b> - JOE/VIC/HEX         | 1 tube: RdRp gene - FAM<br>2 tube: E gene - FAM                   | RdRp gene – Cal Red 610<br>N gene- Quasar 670<br>E gene - FAM   |
| <b>Sample type</b>              | Sputum<br>Nasopharyngeal swab<br>Oropharyngeal swab           | Sputum,<br>Nasopharyngeal swab<br>Oropharyngeal swab              | Sputum, Nasopharyngeal swab<br>Oropharyngeal swab<br>nasopharyngeal aspirate, Bronchial<br>cleaning fluid |
| <b>Applicable analyzer</b>      | CFX96, ABI 7500/7500 Fast,<br>LC480                           | CFX96, ABI7500, Gentier96   | <b>CFX96</b>  |
| <b>Sample volume</b>            | 10 $\mu$ l  | 10 $\mu$ l<br>(1 tube: 5 $\mu$ l / 2 tube: 5 $\mu$ l)             | 8 $\mu$ l   |
| <b>Limit of Detection (LoD)</b> | RdRp: <b>5</b> copies/reaction<br>E: <b>5</b> copies/reaction | RdRp: <b>38</b> copies/reaction<br>E: <b>28.5</b> copies/reaction | RdRp: <b>100</b> copies/reaction<br>E: <b>100</b> copies/reaction<br>N: <b>100</b> copies/reaction        |
| <b>Testing Time (on CFX96)</b>  | <b>90 Mins</b>  | <b>120 Mins</b>   | 110 Mins  |
| <b>Target Cut-off</b>           | 36Ct (40 cycle)   | 35Ct (40 cycle)   | 40Ct (45 cycle)   |
| <b>Storage temp.</b>            | -25~-15°C   | -25~-15°C   | Below -20°C   |
| <b>Expiration date</b>          | <b>12 Months</b>  | <b>6 Months</b>   | 8 Months  |

# Comparison Chart (2)

|                                   | <b>SD BIOSENSOR</b><br>M nCoV Real-Time Detection kit                                       | <b>Solgent</b><br>DiaPlexQ Novel Coronavirus<br>(2019-nCoV) Detection Kit | <b>Biosewom</b><br>Real-Q 2019-nCOV Detection Kit               |
|-----------------------------------|---|---|---|
| <b>Test/Kit</b>                   | <b>96</b>   | 20 / 100  | -   |
| <b>Tube/Test</b>                  | <b>1 Tube (3-plex)</b>  | 1 Tube (3-plex)   | -   |
| <b>Target gene</b>                | <b>RdRp gene</b> - FAM<br><b>E gene</b> - JOE/VIC/HEX                                       | <b>RdRp gene</b> - FAM<br><b>E gene</b> - JOE/VIC                         | <b>RdRp gene</b><br><b>E gene</b>                               |
| <b>Sample type</b>                | Sputum<br>Nasopharyngeal swab<br>Oropharyngeal swab   | Sputum<br>Nasopharyngeal swab<br>Oropharyngeal swab                       | Sputum<br>Nasopharyngeal swab<br>Oropharyngeal swab             |
| <b>Applicable analyzer</b>        | CFX96, ABI 7500/7500 Fast,<br>LC480   | CFX96, ABI 7500/7500 Fast   | CFX96, ABI 7500/7500 Fast                                       |
| <b>Sample volume</b>              | 10 $\mu$ l  | -   | -   |
| <b>Limit of Detection (LoD)</b>   | RdRp: 5 copies/reaction<br>E: 5 copies/reaction<br>(= <b>0.5 copies/<math>\mu</math>l</b> ) | RdRp: 2 copies/ $\mu$ l<br>E: 2 copies/ $\mu$ l                           | RdRp: 6.51~6.87 copies/ $\mu$ l<br>E: 7.60~7.99 copies/ $\mu$ l |
| <b>Testing Time</b><br>(on CFX96) | <b>90 Mins</b>  | <b>120 Mins</b>   | -   |
| <b>Target Cut-off</b>             | 36Ct (40 cycle)   | -   | -   |
| <b>Storage temp.</b>              | -25~-15 $^{\circ}$ C  | -   | -   |
| <b>Expiration date</b>            | <b>12 Months</b>  | -   | -   |

# Thank You

SD BIOSENSOR, Ltd.