WHO Emergency Use Assessment SARS-CoV-2 IVDs
PUBLIC REPORT

Product: RADI COVID-19 Detection Kit
Manufacturer: KH Medical Co., Ltd.
EUL Number: EUL 0538-214-00
Outcome: Accepted.

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety, and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents.
- Product Dossier Review: assessment of the documentary evidence of safety and performance. This evaluation of limited scope is to verify critical analytical and performance characteristics.

RADI COVID-19 Detection Kit with product code RV008, CE-mark regulatory version manufactured by KH Medical Co., Ltd, located at 201, Jinwiseo-ro, Jinwi-myeon, Pyeongtaek-si, Gyeonggi-do, 17712, Republic of Korea, was listed as eligible for WHO procurement on 30 September 2021.

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the table below.

Version	Summary of amendment	Date of report amendment
2.0	1. Change of facility location from location from #C-1402, 947, Hanam-daero, Hanam-si, Gyeonggi[1]do, 12982, Republic of Korea to 201, Jinwiseo-ro, Jinwi-myeon, Pyeongtaek-si, Gyeonggi-do, 17712, Republic of Korea.	23 June 2022

	2. Replacement of "COVID-19" with SARS-CoV-2 on product component labels and change of address on the IFU to reflect the new location.	

Intended use

According to the claim of intended use from KH Medical Co., Ltd, *"RADI COVID-19 Detection Kit is an in vitro diagnostic medical device, based on Real-time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA). It is intended for the qualitative detection of RNA (S gene and RdRp gene) of SARS-CoV-2 from nasopharyngeal and oropharyngeal swab specimens to aid in the diagnosis of SARS-CoV-2 infection. Positive results are indicative of SARS-CoV- 2 RNA detection, but may not represent the presence of transmissible virus. Negative results do not preclude SARS-CoV- 2 infection and should not be used as the sole basis for patient management decisions. Results must be combined with clinical observations, patient history, and epidemiological information."*

Specimen type(s) that were validated

Nasopharyngeal and oropharyngeal swab specimens.

Assay description

According to the claim of assay description from KH Medical Co., Ltd, "*SARS-CoV-2 which is the causative agent for an outbreak (COVID-19) first reported on 31 Dec 2019 in China, is a new beta coronavirus. Infection with SARS-CoV-2 causes respiratory symptoms, fever, fatigue and in severe cases, pneumonia, severe acute respiratory syndrome, organ failure and even death. The test is designed to detect two RNA targets of the SARS-CoV-2 (S gene and RdRP gene) and also to detect human RNase P as an Internal Positive Control (IPC) in a clinical sample."*

Test kit contents

Component	**100 tests (product code RV008)**
SARS-CoV-2 Primer & Probe Mixture	500 µl x 1 vial
3X RT Master Mix	1 000 µl x 1 vial
SARS-CoV-2 Positive Control	300 µl x 1 vial
RNase free Water	1 000 µl x 1 vial
SARS-CoV-2 Extraction Control	1 000 µl x 1 vial

Items required but not provided

- Micropipette
- Sterilized pipette tips with filter barriers
- Centrifuge
- Disposable powder-free gloves
- Real Time PCR machine: CFX96 Real-Time PCR Detection System, Bio-Rad (Cat.no: 1845097 / S/W ver.: 1.6)
- Consumables relating the RT-PCR
    - 96-Well PCR Plate
    - 1.5 mL microcentrifuge tubes (Dnase/Rnase free)
    - PCR Plate Sealing film (adhesive, optical)
    - 0.1 mL flat PCR tube 8-cap strips (optical)
- Nucleic acid extraction kit: QIAamp Viral RNA Mini Kit (Qiagen, Cat.no: 52906)
- Viral Transport Medium: Clinical Virus Transport Medium (Noble Biosciences, Cat.no: UTNFS-3B-2) (Swabs included)
- Cold block
- Vortex mixer
- PCR Tube rack

Storage
The test kit should be stored at -25°C to -15°C.

Shelf-life upon manufacture
8 months

Warnings/limitations
See attached instructions for use.

Product dossier assessment

KH Medical Co., Ltd submitted a product dossier for RADI COVID-19 Detection Kit as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and an external assessor appointed by WHO.

Post listing Commitments for EUL:

As a requirement to listing, the manufacturer is required to:

1. Estimate the limit of detection with the WHO international standard by 31 December 2021. This commitment is under review.

2. Implement new labelling approved under EUL assessment by 31 December 2021. This commitment was fulfilled. Issue closed.

Risk benefit assessment conclusion: Acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, KH Medical Co., Ltd was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff and external technical experts (assessors), it was established that sufficient information was provided by KH Medical Co., Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_ 347 "*.

Quality management documentation assessment conclusion: Acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-prequalification activities are required to maintain the prequalification status:

1. Notification to WHO of any planned changes to a prequalified product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9).

KH Medical Co., Ltd is also required to report complaints related to the product. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO

guidance "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"*[1]

Scope and duration of procurement eligibility

The RADI COVID-19 Detection Kit with product code RV008 manufactured by KH Medical Co., Ltd is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, KH Medical Co., Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. KH Medical Co., Ltd is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days, and any changes made to the product.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

[1] https://www.who.int/publications/i/item/9789240015319

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

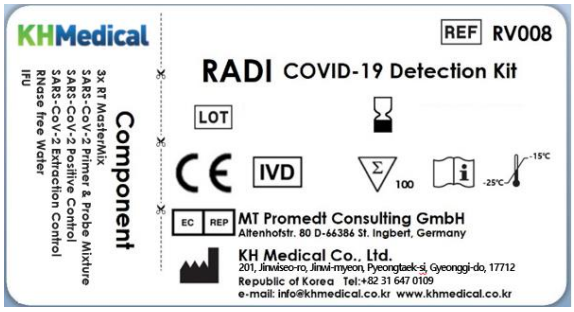
RADI COVID-19 Detection Kit (RV008)

Packaging

1. BOX

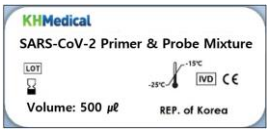
2. Label (REF : RV008)

2.1 Box label(REF : RV008)



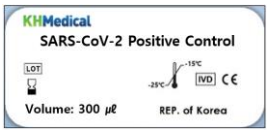
2.2 Tube labels(REF : RV008)

<SARS-COV-2 Primer & Probe Mixture>



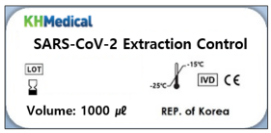
<3X RT MasterMix>

<SARS-CoV-2 Positive Control>



<RNase free water>

<SARS-CoV-2 Extraction Control>



2. Instructions for Use[2]

[2] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

RADI COVID-19 Detection Kit IVD -25°C~-15°C Σ100 REF RV008

This instruction must be read carefully prior to use. Reliability of assay results cannot be guaranteed if there is any deviation from the instructions.

1. Intended use

The RADI COVID-19 Detection Kit is an in vitro diagnostic medical device, based on Real-time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA).

It is intended for the qualitative detection of RNA (S gene and RdRp gene) of SARS-CoV-2 from nasopharyngeal and oropharyngeal swab specimens to aid in the diagnosis of SARS-CoV-2 infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Results must be combined with clinical observations, patient history, and epidemiological information[1].

2. Summary and Explanation

The SARS-CoV-2 which is the causative agent for an outbreak (COVID-19) first reported on 31 Dec 2019 in China, is a new beta coronavirus. Infection with SARS-CoV-2 causes respiratory symptoms, fever, fatigue and in severe cases, pneumonia, severe acute respiratory syndrome, organ failure and even death[2].

The test is designed to detect two RNA targets of the SARS-CoV-2 (S gene and RdRP gene) and also to detect human RNase P as an Internal Positive Control (IPC) in a clinical sample.

3. Precautions and Warnings

1) For in vitro diagnostic use (IVD) only.
2) This assay needs to be carried out by trained personnel.
3) Please wear a mask and disposable gloves when handling.
4) Prior to commencing an IVD test, make sure all the reagents are melted well on the operating room temperature.
5) The acceptable operating temperature range is 4°C to 36 °C. Testing out of the operating temperature range may lead to false results.
6) When the control value is out of the expected range (see "**10. Quality Control**"), it is indicative of instability or deterioration of the kit.
7) To prevent contamination, prepare reagents in a PCR workstation or equivalent amplicon-free area.
8) To avoid contamination from the positive control (PC), pipette the PC last.
9) Do not use the kit after its expiration date.
10) Always use sterile pipette tips with filters and use a new tip every time a volume is dispensed.
11) Avoid eyes, skin and clothing contact with reagents. In case of any contact, flush with flowing water.
12) Follow standard precautions for infectious waste management. All patient specimens and positive controls should be considered to be potentially infectious and handled with precautions.
13) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
14) Handle all specimens considering as infectious and follow the safe laboratory procedures[3].
15) Specimen processing should be performed in accordance with national biological safety regulations.
16) RNA should be maintained on cold block or on ice during preparation to ensure stability.
17) Dispose of used kit reagents and human specimens according to local, state, and federal regulations.
18) Do not reuse disposable materials after use.
19) Do not pool reagents from different lots or from different kits of the same lot.

4. Kit Components

Materials Provided (100 tests/kit)

Lid Color of Tube	Component	Volume (μℓ)
Brown	SARS-CoV-2 Primer & Probe Mixture	500
Yellow	3X RT MasterMix	1,000
Red	SARS-CoV-2 Positive Control	300
Blue	RNase free Water	1,000
Red (Yellow sticker on top)	SARS-CoV-2 Extraction Control*	1,000

*** Note for SARS-CoV-2 Extraction Control**

The Extraction Control is a recombinant plasmid DNA containing RNase P as IPC. Prior to extraction, the extraction control can be added to specimens to serve as a total process control.

Use of SARS-CoV-2 Extraction control is optional and supplemental procedure to make sure the test result validity and total quality control from the extraction process.

Materials Required BUT NOT PROVIDED

- Micropipette
- Sterilized pipette tips with filter barriers
- Centrifuge
- Disposable powder-free gloves
- Real Time PCR machine
  - ✓ CFX96 Real-Time PCR Detection System, Bio-rad (Cat.no: 1845097 / S/W ver.: 1.6)
- Consumables relating the RT-PCR
  - ✓ 96-Well PCR Plate
  - ✓ 1.5 mL microcentrifuge tubes (Dnase/Rnase free)
  - ✓ PCR Plate Sealing film (adhesive, optical)
  - ✓ 0.1 mL flat PCR tube 8-cap strips (optical)
- Nucleic acid extraction kit
  - ✓ QIAamp® Viral RNA Mini Kit (Qiagen, Cat.no: 52906)
- Viral Transport Medium
  - ✓ Clinical Virus Transport Medium (Noble Biosciences, Cat.no: UTNFS-3B-2) (Swabs included)
- Cold block
- Vortex mixer
- PCR Tube rack

5. Storage

- All components should be stored at -25°C to -15°C upon arrival.
- Exposure time at Room temperature (25°C) should not exceed 30 minutes after the component being mixed.
- Freeze-thaw cycles should not exceed 5 times.

6. Specimen collection, transportation and storage

- RADI COVID-19 Detection Kit is optimized and verified for viral RNA extracted from upper respiratory specimens such as nasopharyngeal or oropharyngeal swabs specimens from symptomatic individuals suspected of COVID-19.
- Specimens types of nasopharyngeal swab, oropharyngeal swab are stable up to 48 hours at 2℃ to 8℃.
- Collect nasopharyngeal swab, oropharyngeal swab according to CDC guidelines and manufacturer's protocol for sample collection. Follow specimen collection devices manufacturer instructions for proper collection method.
- For the specimen collection and preparation, users are recommended referring to CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html)

7. Extraction (including Extraction control usage)

When SARS-CoV-2 Extraction control is used with nucleic acid extraction kit, please take the following steps.

a) Freshly collected specimens should be used to collect RNA to ensure suitable RNA quality and quantity.
b) Users should prepare the positive control (PC) and no template control (*NTC), simultaneously alongside the specimen.
***NTC**: RNase free water is supplied as a kit component.
c) Add 10 μℓ of SARS-CoV-2 Extraction Control to each specimen to be extracted.
d) Make sure that the total volume of Specimen sample (including 10 μℓ of SARS-CoV-2 Extraction Control) to be extracted should be matched with the manufacturer's instruction for the volume of specimen required for extraction.
e) QIAamp® Viral RNA Mini Kit (Qiagen) is recommended to extract the RNA from the freshly collected specimen and must follow the manufacturer's instructions.
f) The extracted RNA should be used immediately or stored at -70℃ for use later.
g) Precautions should be taken while handling the positive control to avoid cross-contamination of other samples in the test run.
h) False positive results may appear due to the failure to take proper precautions while handling the positive control.

Note:

- The extraction protocol or extraction kit's quality may affect real-time PCR results
- The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology.

8. PCR Mixture Protocol

<u>All reagent should be mixed in the preparation area (PCR workstation or equivalent amplicon-free area).</u>

a) As the kit (reagents) is stored in a frozen condition, take kit out and thaw thoroughly at ambient temperature.
b) Vortex and centrifuge briefly. the reagents should be kept on ice at all times of the experiments.
c) Calculate the number of reactions (n) that will be included in the test.
d) The number of the reactions should include NTC and Positive control (1 tube each) and each specimen.
e) Calculate the concentration of "3X RT MasterMix" and "SARS-CoV-2 Primer & Probe Mixture" needed for desired number of reactions and aliquot them into a 1.5 ml tube and vortex to mix well.

<u>Reagent and ingredient concentration for PCR Mixture preparation</u>:

Component		Volume (μℓ) 1 reaction	Volume (μℓ) n reaction
PCR Mixture	3X RT MasterMix	10	10 x number of reaction (n)
	SARS CoV-2 Primer & Probe Mixture	5	5 x number of reaction (n)
Extracted RNA, PC, NTC		15	15 x number of reaction (n)
Total Volume		30	30 x number of reaction (n)

f) Prepare 96-well plates or tube strips for real-time RT-PCR based on the estimated number of reactions (n).
g) Aliquot 15 μℓ of PCR-Mix into each well.
h) Add 15 μℓ of extracted RNA (specimen) into each well and note the specimen ID and well position (table above).
i) For negative well, add 15 μℓ of RNase free water (table above).
j) For positive well, add 15 μℓ of Positive Control (table above).
k) Cover the plate or tubes, vortex and spin down then transfer them into PCR area for PCR run.
l) The remaining Reaction Mix and reagents must be stored at -20℃.

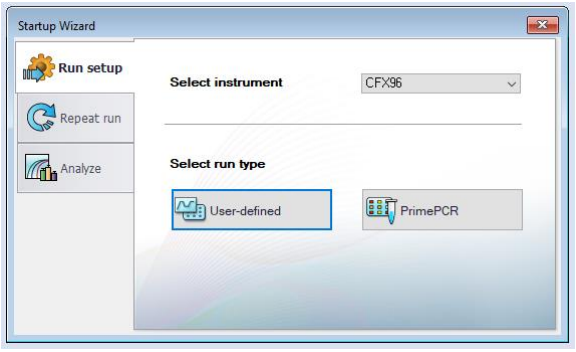
Note.

- Considering the pipette loss, users may add one more rection PCR mixture to the calculated quantity.
- To avoid contamination from the PC, pipette the positive control last.
- Must not change the volumes for reagent preparation specified in table above or the volume of the sample addition in the process below. Such changes could cause false results.
- Considering the pipette loss, users may add one more rection PCR mixture to the calculated quantity.

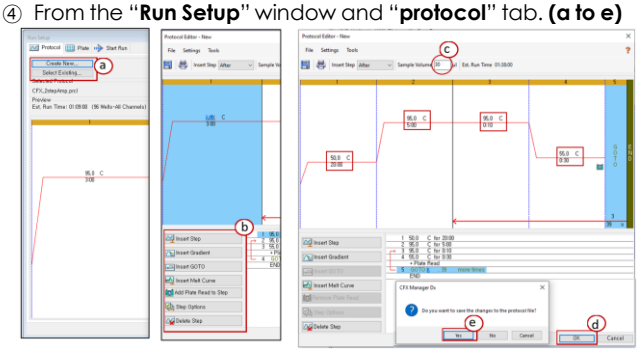
9. PCR amplification Protocol

① Access the Bio-Rad CFX software to program the PCR protocol.
② Double-click the icon to open the "**Bio-Rad CFX Dx**" software.

③ Click "**User-defined**" from the pop-up window when CFX software is open.



④ From the "**Run Setup**" window and "**protocol**" tab. **(a to e)**



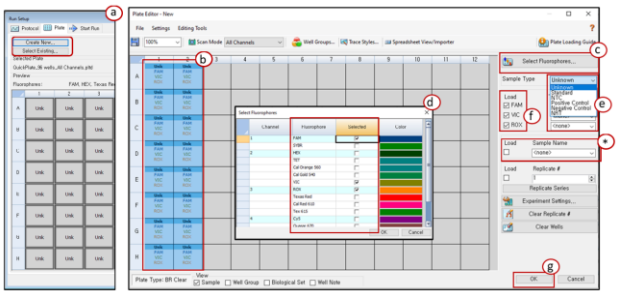
a) Click the "**Create New**" button to create a new protocol from "**Protocol Editor**" window or the "**Select Existing**" to open a predefined protocol.
b) Click "**Insert Step, Delete Setup**" or other buttons to create a specific protocol.
c) Adjust the sample volume to 30 µℓ.
d) After creating specific protocol click "**OK**" button that will open another window.
e) In the new saving window click "**Yes**" to save the protocol.

Parameters to create a PCR protocol:

Step	Temperature	Time	Cycle
1. cDNA synthesis	50 ℃	20 min	1
2. Pre-Denaturation	95 ℃	5 min	1
3. Denaturation	95 ℃	10 sec	45
4. Annealing & Extension	**55*** ℃	30 sec	

* Fluorogenic data is collected during this step through the FAM, VIC and ROX channels.

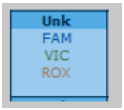
⑤ **Selecting wells and fluorophore setup (a to g):**



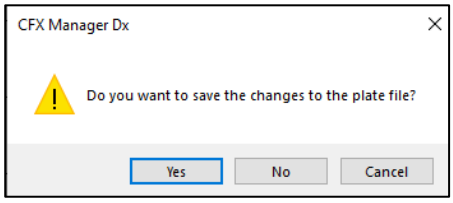
a) Under "**Plate**" tab, click "**Create New**" button to create a new plate or click "**Select Existing**" button to invoke the existing plate file.
b) Select the wells where the samples will be placed for PCR run.
c) Click the "**Select Fluorophores**" tab to select the desired fluorophores for PCR.
d) Select the dye "**FAM**", "**VIC**" and "**ROX**" and click "**OK**".

Target	Reporter
S gene	FAM
RdRP gene	VIC
IPC (Internal Control)	ROX

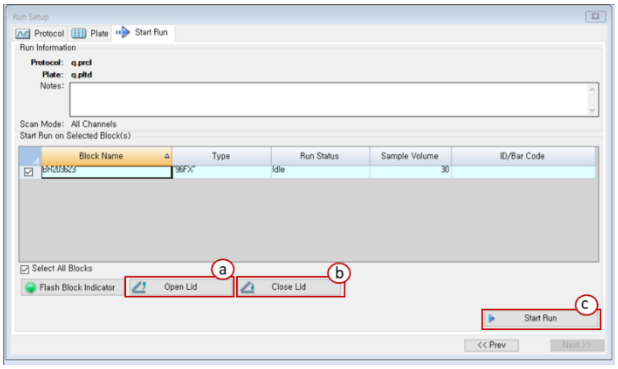
e) Click the drop-down menu from the "**Sample Type**" tab and select desired sample types such as Unknown, NTC etc. one by one.
f) Select "**FAM**", "**VIC**" and "**ROX**" for samples. After finishing the steps from (a) to (f) the wells will look like as below.



Optional: the user can add a sample name for each well from the "Sample Name" field as necessary.
g) Click ok and click "**Yes**" from the Pop-up window to save the plate setup.



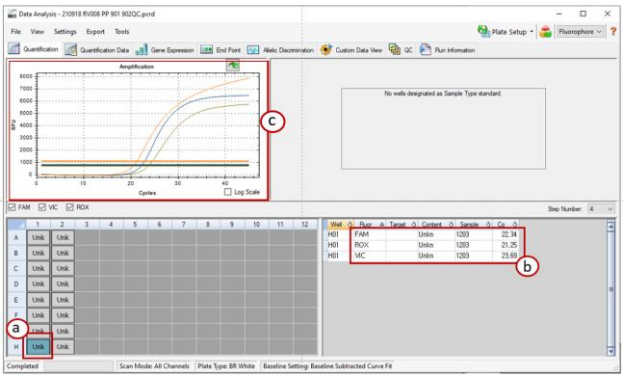
⑥ After finishing plate setup click "**Start Run**" tab to add sample plate or tube strips.



a) From the "**Start Run**" tab, click "**Open Lid**" to open the device and add the plate/tube strips into the sample rack.
b) After adding plate/tube strips click "**Close Lid**" to close the device lid.
c) When lid is fully closed and device is ready for run click "**Start Run**" which will open a window asking to save the run. Save the run with desired name to a desired folder.

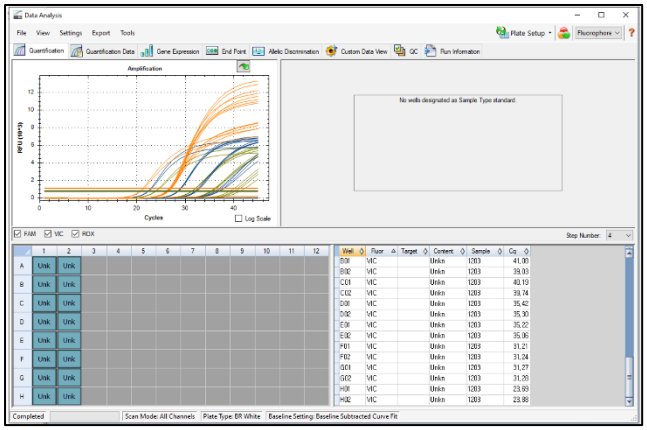
The experiment run will begin automatically after the run is saved properly.

⑦ **PCR Result Analysis (a to c)**: After the run is completed and the threshold is adjusted follow the following steps to analysis the results.



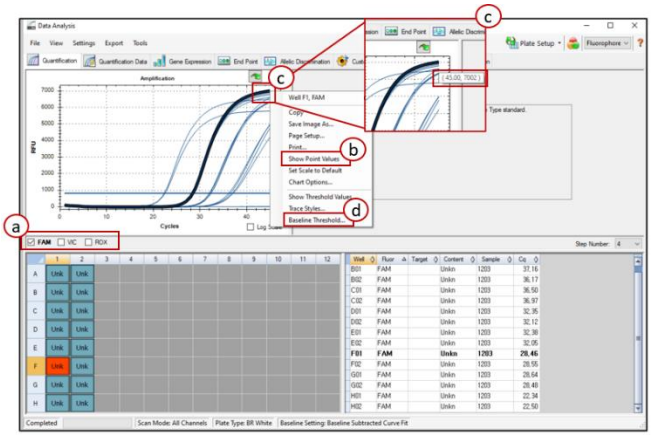
a) Click the desired well to read the result (Ct values).
b) At the lower right side of the window the Ct values of different dyes will be read.
c) The amplification curve will be shown on the upper left.

⑧ **Threshold Adjustment (a to f):**
Open the file from the folder where the data was saved after completion of the PCR run and follow the following steps to adjust the baseline threshold.



a) Select only one dye and deselect the others

b) Place the mouse arrow (cursor) anywhere of the amplification curve window view (upper left) and right click to open the pop-up menu and select "Show Point Values".



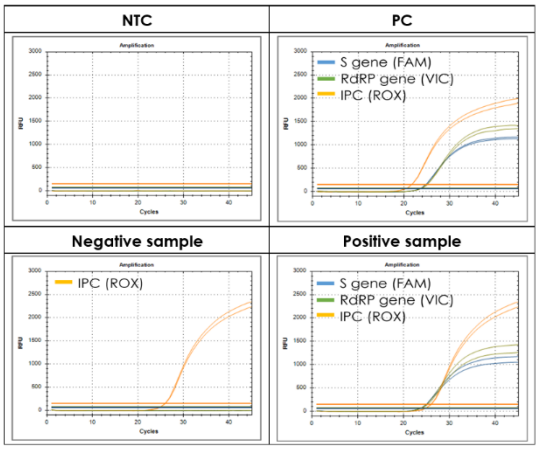
c) Place the mouse arrow at the highest Ct curve to view the highest RFU (delta Rn) value and note the value. The threshold is 1/20 of maximum delta Rn.

Threshold = delta Rn/20 (here delta Rn for **FAM**= 7002)
[**Threshold** = 7002/20 = **350.10** (please note the threshold)]

d) Place the mouse arrow (cursor) anywhere of the amplification curve window view and right click to open the pop-up menu and select "**Baseline Threshold**" to open the "**Baseline Threshold**" pop-up.
e) Select "**User Defined**" and input the threshold calculated (Here it is **350.10** for **FAM**).
f) Click "**OK**" to set the baseline threshold for specific dye.

Note:
- Threshold for each dye is different. So, adjusting baseline threshold for multiple dye cannot be done at the same time.
- Threshold for each dye should be done separately.
- Threshold can be different depending on the device used.

Reference Images after Threshold adjusted amplification curve graphs from CFX96



10. Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results are also invalid. A negative control and a positive control should be set for each batch.

Control	Targets		
	S gene (FAM)	RdRP gene (VIC)	IPC (ROX)
NTC	No Ct or Ct>40	No Ct or Ct>40	No Ct or Ct>40
PC	20≤Ct≤30	20≤Ct≤30	20≤Ct≤30
*Extraction Control	-	-	Ct≤40

*If Extraction Control is used without specimen as extraction control, Ct value of S gene and RdRP gene do not appear.

11. Test Interpretation

Test interpretation of RADI COVID-19 Detection Kit is described as below.

Ct value of Target genes	Result on Clinical specimens
≤40	**Detected (+)**
No Ct or > 40	**Not detected (ND) (-)**

Cases	Target			Result
	S (FAM)	RdRP (VIC)	IPC* (ROX)	
1	≤40	≤40	≤40	Positive
2	≤40	≤40	> 40 or No Ct	Positive**
3	≤40	No Ct	≤40	Positive
4	≤40	No Ct	> 40 or No Ct	Positive**
5	No Ct	≤40	≤40	Positive
6	No Ct	≤40	> 40 or No Ct	Positive**
7	> 40 or No Ct	> 40 or No Ct	≤40	Negative
8	> 40 or No Ct	> 40 or No Ct	> 40 or No Ct	Invalid*** (Re-testing)

* The valid IPC Ct range is ≤40. In positive cases, IPC may not be detected due to competitive reaction with target genes.

** If the S and/or RdRP gene are detected but IPC may not be detected due to high concentration of target genes that may lead suppression of IPC signal due to competitive reaction. this result can still be valid and should be interpreted as positive.

*** Results are invalid. Re-extraction and retest are needed. During retest, if any of SARS-CoV-2 target has a Ct≤40 and internal control has a Ct of ≤ 40 or no Ct then it is judged as positive. If the two targets have no Ct (or Ct > 40) and internal control has a Ct of ≤ 40, then it is judged as negative.

If all targets and internal control are >40 or no Ct, then judge the results according to No. 8

Since the internal control is used for monitoring the complete process of sampling, nucleic acid extraction and amplification, it is not possible to determine where the error lies when there are abnormal internal control results, so it is necessary to do the complete process again.

If results are still abnormal after this, test results need to be reported as "**Specimen inhibition**". Another specimen should be collected following appropriate specimen collection, transportation and storage protocols.

12. Test Limitations

1) Performance of the kit has been established in nasopharyngeal swab and oropharyngeal swab specimens from symptomatic individuals suspected of COVID-19.
2) This kit is a qualitative test and does not provide the quantitative value.
3) All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
4) Negative results do not preclude SARS-CoV-2 virus and should not be used as the sole basis for treatment or other patient management decisions.
5) Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
6) False positive results may happen from cross-contamination between patient samples, specimen mix-up and/or RNA contamination during product handling.
7) False-negative results may arise from:
   - Improper sample collection
   - A sample at concentrations near or below the limit of detection of the test.
   - Degradation of the viral RNA during specimen transport and/or storage.
   - Mutation in the SARS-CoV-2 virus.
   - Failure to follow the Instructions for Use provided

13. Performance characteristics

Analytical sensitivity (Limit of Detection)

In order to determine limit of detection (LoD) of RADI COVID-19 Detection Kit, a tentative LoD was determined with several diluted concentrations using synthesized RNA by in vitro transcription in 5 replicates. After fixing a tentative LoD, the claimed LoD was determined with 20 replicates of diluted concentrations spanning tentative LoD.

LoD is 0.66 copies/μℓ.

- **Tentative LoD** : 0.66 copies/μℓ

Target	copies/μℓ	Mean Ct	Result in agreement	Percent agreement
S gene	6600	21.57	5/5	100 %
	660	26.17	5/5	100 %
	66	29.98	5/5	100 %
	6.6	33.50	5/5	100 %
	0.66	**37.92**	**5/5**	**100 %**
	0.06	39.81	1/5	20 %
RdRP gene	6600	22.32	5/5	100 %
	660	25.95	5/5	100 %
	66	29.48	5/5	100 %
	6.6	33.09	5/5	100 %
	0.66	**37.32**	**5/5**	**100 %**
	0.06	*ND	0/5	0%

* ND: Not detected

- **Claimed LoD**: 0.66 copies/μℓ

Target	copies/μℓ	Mean Ct	Result in agreement	Percent agreement
S gene	2.33	37.29	20/20	100%
	2.00	37.35	20/20	100%
	0.66	**38.24**	**20/20**	**100%**
	0.5	38.27	17/20	85%
RdRP gene	2.33	36.93	20/20	100%
	2.00	37.01	20/20	100%
	0.66	**38.16**	**20/20**	**100%**
	0.5	38.49	16/20	80%

Cut off Value

Limit of detection was decided as the cut off value. A Ct value of 40 was set as the cut off of RADI COVID-19 Detection Kit.

Interfering test

RADI COVID-19 Detection Kit does not have any interference with following interfering substances.

No.	Interfering Substances	Concentrations	Interference
1	Whole blood	10 %V/V	No interference
2	DNA extracted	303.5 μg/ml	No interference
3	Nasal spray	10 % V/V	No interference
4	Nasal ointment	5 % V/V	No interference
5	Triamcinolone	10 % V/V	No interference
6	salbutamol	2 mg/mL	No interference
7	Benzocaine	2 μmol/L	No interference
8	Levofloxacin	1 mg/mL	No interference

No.	Interfering Substances	Concentrations	Interference
9	Tobramycin	10 μg/mL	No interference
10	Nasal gel	1 mg/mL	No interference
11	Oseltamivir	2 mg/mL	No interference
12	Mucin	100 μg/mL	No interference
13	Biotin	220 μg/mL	No interference
14	Human Anti-mouse Antibody (HAMA)	25 μg/mL	No interference

Cross Reactivity – Wet testing

Wet-testing for 63 microorganisms (including pooled human nasal wash) was evaluated at the concentrations listed in the table below. Each microorganism was tested with RADI COVID-19 Detection Kit in triplicate. RADI COVID-19 Detection Kit showed no cross-reaction with any tested pathogens. Additionally, in case of pooled human nasal wash, the microbial interference study was evaluated (spiking with 3X LoD SARS-CoV-2) in triplicate. There was no observed microbial interference in the pooled human nasal wash in the presence of SARS-CoV-2 virus.

Source	No.	Cross Reactivity pathogens	Concentration
NCCP	14547	*Salmonella Enteritidis*	$9.9x10^8$copies/ml
NCCP	16207	*Salmonella Typhimurium*	$9.2x10^8$copies/ml
NCCP	15758	*Salmonella enterica*	$9.7x10^8$copies/ml
NCCP	14641	*Salmonella Typhi*	$9.6x10^8$copies/ml
NCCP	13713	*Vibrio parahaemolyticus*	$9.2x10^8$copies/ml
NCCP	12843	*Vibrio cholera*	$1.1x10^9$copies/ml
NCCP	16296	*Bacillus cereus*	$8x10^8$copies/ml
NCCP	16297	*Bacillus subtilis*	$1.1x10^9$copies/ml
NCCP	15938	*Bacillus infantis*	$9.4x10^8$copies/ml
NCCP	15871	*Staphylococcus aureus*	$1.5x10^9$copies/ml
NCCP	14669	*Staphylococcus hominis*	$1.9x10^9$copies/ml
NCCP	11192	*Campylobacter jejuni*	$2.8x10^9$copies/ml
NCCP	14714	*Listeria monocytogenes*	$1.5x10^9$copies/ml
NCCP	15661	*Escherichia coli*	$8.8x10^8$copies/ml
NCCP	15911	*Clostridium perfringens*	$1.3x10^9$copies/ml
NCCP	12713	*Yersinia enterocolitica*	$9.6x10^8$copies/ml
NCCP	16366	*Clostridium tertium*	$1.1x10^9$copies/ml
NCCP	11820	*Clostridium difficile*	$1.0x10^9$copies/ml
NCCP	16330	*Salmonella Stanley*	$9.7x10^8$copies/ml
NCCP	14708	*Haemophilus parainfluenzae*	$2.2x10^9$copies/ml
NCCP	14675	*Haemophilus haemolyticus*	$2.3x10^9$copies/ml
NCCP	14785	*Haemophilus influenzae*	2.3×10^9copies/ml
NCCP	13753	*Neisseria meningitidis*	$2.0x10^9$copies/ml
NCCP	15882	*Streptococcus pneumoniae*	2.2×10^9copies/ml
NCCP	43193	Adenovirus	$9.0x10^{10}$ copies/ml
NCCP	43248	Dengue virus	$5.8x10^{11}$copies/ml
NCCP	43280	Zika virus	$5.9x10^{11}$copies/ml
NCCP	43132	Chikungunya virus	$5.4x10^{11}$copies/ml
NCCP	41308	Japanese Encephalitis virus	$5.9x10^{11}$copies/ml
NCCP	43165	Enterovirus	$8.7x10^{11}$ copies/ml
NIBSC	06/202	HPV 16 DNA	$4.1x10^{11}$copies/ml
NIBSC	06/206	HPV 18 DNA	$4.1x10^{11}$copies/ml
NCCP	40204	Measles virus	$5.8x10^{11}$copies/ml
NCCP	41205	Coxsackievirus	$1.2x10^{12}$copies/ml
NCCP	43221	Coxsackievirus	$1.2x10^{12}$copies/ml
NCCP	43225	Rhinovirus	$1.2x10^{12}$ copies/ml
NCCP	43230	Influenza A virus (H3N2)	$6.8x10^{11}$ copies/ml
NCCP	43231	Influenza A virus(H1N1)	$6.8x10^{11}$ copies/ml
NCCP	43232	Influenza B virus	$6.3x10^{11}$ copies/ml
NCCP	43238	Respiratory Syncytial virus A	$6x10^{11}$ copies/ml
NCCP	43239	Respiratory Syncytial virus B	$6x10^{11}$ copies/ml
NCCP	43281	Vaccinia virus	$4.7x10^{10}$ copies/ml
NCCP	43214	human Coronavirus NL63	$3.3x10^{11}$ copies/ml
NCCP	72002	*Legionella pneumophila*	$9.5x10^8$ copies/ml
NCCP	72026	*Bordetella pertussis*	$4.5x10^8$ copies/ml
NCCP	72006	*Pseudomonas aeruginosa*	$7x10^8$ copies/ml
NCCP	72077	*Mycobacterium tuberculosis*	$3.1x10^8$ copies/ml
NCCP	72059	*Staphylococcus epidermidis*	$7.3x10^8$ copies/ml
NCCP	43261	SFTS virus	$1x10^{12}$ copies/ml
NCCP	43108	Herpes Simplex virus 2	$4.1x10^{10}$ copies/ml
NCCP	43110	Herpes Simplex virus 1	$4.2x10^{10}$ copies/ml
NCCP	41003	Echovirus	$8.6x10^{11}$ copies/ml
KBPV	VR-9D	Human coronavirus 229E	$1.7x10^{13}$ copies/ml
KBPV	VR-8D	Human coronavirus OC43	$6.8x10^{12}$ copies/ml
KBPV	VR-86D	Human Metapneumovirus	$3.3x10^{13}$ copies/ml
KBPV	VR-64D	Parainfluenza virus 1	$5.5x10^{13}$ copies/ml
KBPV	VR-45D	Parainfluenza virus 2	$5.6x10^{12}$ copies/ml
KBPV	VR-46D	Parainfluenza virus 3	$7.3x10^{12}$ copies/ml
KBPV	VR-69D	Parainfluenza virus 4	$3.5x10^{13}$ copies/ml
ATCC	700294DQ	Streptococcus pyrogenes	$4.9x10^8$ copies/ml
ATCC	29342DQ	Mycoplasma pneumoniae	$4.6x10^8$ copies/ml
EVA	004N-02005	SARS-coronavirus	$1x10^7$ copies/ml
Medicore	-	Pooled human nasal wash*	100%

* There was no microbial interference in the pooled human nasal wash.

In-silico analysis for inclusivity (Including Variant)

In silico analysis for Inclusivity was conducted by comparing RADI COVID-19 Detection Kit primers and probes to an alignment of SARS-CoV-2 sequences (n=8850) and 3 kinds of SARS-CoV-2 variants, Alpha (Lineage B.1.1.7) sequences (n=9121), Beta (Lineage B.1.351) sequence (n=641), and Gamma (Lineage P.1) sequences (n=913) in GISAID EpiCoV™ database.

The MUSCLE alignment was generated by Multiple sequence alignment using ClustalW and viewed in Jalview program (version 2.11.1.3) and Geneious prime® 2020.2.1 program.

The coverage of all primers and probes was 100%. The table below shows the results of this coverage analysis.

<Coverage (%) of novel SARS-CoV-2>

Target	Primer/Probe	Coverage (%)
S gene	S Forward primer	8850/8850 (100%)
	S Forward Probe	8850/8850 (100%)
	S Reverse Primer	8850/8850 (100%)
RdRP gene	Orf1 Forward Primer	8850/8850 (100%)
	Orf1 Forward Probe1	8850/8850 (100%)
	Orf1 Reverse Probe2	8850/8850 (100%)
	Orf1 Reverse Primer	8850/8850 (100%)

<Coverage (%) of 3 kinds of SARS-CoV-2 variants>

Target	Primer/Probe	Coverage (%)		
		Alpha (Lineage B.1.1.7)	Beta (Lineage B.1.351)	Gamma (Lineage P.1)
S gene	S Forward primer	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
	S Forward Probe	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
	S Reverse Primer	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
RdRP gene	Orf1 Forward Primer	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
	Orf1 Forward Probe1	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
	Orf1 Reverse Probe2	9121/9121 (100%)	641/641 (100%)	913/913 (100%)
	Orf1 Reverse Primer	9121/9121 (100%)	641/641 (100%)	913/913 (100%)

In-silico analysis for exclusivity

The in-silico analysis for possible cross-reaction with all the organisms listed in table was conducted by mapping primers in RADI COVID-19 Detection Kit individually to the sequences downloaded from NCBI database.

As a result of in-silico analysis, in-silico analysis showed <80% homology between the cross-reacting microorganisms and the test primers/probes.

No.	Pathogen	No.	Pathogen
1	Human coronavirus 229E	16	Respiratory syncytial virus B
2	Human coronavirus OC43	17	Rhinovirus
3	Human coronavirus HKU1	18	Chlamydia pneumoniae
4	Human coronavirus NL63	19	Haemophilus influenzae
5	SARS-coronavirus	20	Legionella pneumophila
6	MERS-coronavirus	21	Mycobacterium tuberculosis
7	Adenovirus (e.g. C1 Ad. 71)	22	Streptococcus pneumoniae
8	Human Metapneumovirus (hMPV)	23	Streptococcus pyogenes
9	Parainfluenza virus 1	24	Bordetella pertussis
10	Parainfluenza virus 2	25	Mycoplasma pneumoniae
11	Parainfluenza virus 3	26	Pneumocystis jirovecii (PJP)
12	Parainfluenza virus 4	27	Candida albicans
13	Influenza A & B	28	Pseudomonas aeruginosa
14	Enterovirus (e.g. EV68)	29	Staphylococcus epidermis
15	Respiratory syncytial virus A	30	Streptococcus salivarius

Note.

In terms of Microbial interference with above microorganisms, the in-silico analysis revealed less than 80% homology or low possibility of interaction and/or interference between the microorganisms and the test primers/probe(s). The microbial interference study is covered by in-silico analysis for Exclusivity.

Repeatability

Ten replicates of three concentrations test were performed. CV showed less than 5% for each concentrations of Negative sample (0.0 copies/㎕), 3X LoD (2.00 copies/㎕), 5X LoD (3.33 copies/㎕). Under above test condition the CV values of test results were within acceptable criteria.

Target	S gene			RdRP gene			IPC		
Conc.	Negative	3X LoD	5X LoD	Negative	3X LoD	5X LoD	Negative	3X LoD	5X LoD
Mean Ct	*ND	36.17	36.01	*ND	35.93	35.03	32.31	32.66	32.81
SD	-	0.74	0.72	-	0.37	0.27	0.16	0.10	0.14
CV (%)	-	2.03	1.99	-	1.02	0.77	0.50	0.30	0.42

*ND: Not detected

Reproducibility

To test the reproducibility of RADI COVID-19 Detection Kit, a synthesized RNA by in vitro transcription was diluted into three concentrations (Negative sample (0.0 copies/㎕), 3X LoD (2.00 copies/㎕), 5X LoD (3.33 copies/㎕).

Test runs of three concentrations were performed in four replicates (each day) for 15 days using 3 different lots (total 180 tests) by two different operators of the RADI COVID-19 Detection Kits.

Mean Ct value and CV (%) of Between Lot, Between Day and Between Run is calculated.

All CV values does not exceed 5%.

	Target	Concentration	Mean Ct	SD	CV (%)
Between Lot	S gene	Negative	ND*	-	-
		3X LoD	36.33	0.52	1.43
		5X LoD	35.79	0.44	1.23
	RdRP gene	Negative	*ND	-	-
		3X LoD	35.67	0.48	1.34
		5X LoD	35.10	0.42	1.21
	IPC	Negative	32.24	0.18	0.54
		3X LoD	32.23	0.10	0.32
		5X LoD	32.16	0.08	0.25

* ND: Not detected

	Target	Concentration	Mean Ct	SD	CV (%)
Between Day	S gene	Negative	*ND	-	-
		3X LoD	36.44	0.49	1.35
		5X LoD	35.68	0.54	1.51
	RdRP gene	Negative	*ND	-	-
		3X LoD	35.74	0.42	1.17
		5X LoD	34.98	0.52	1.49
	IPC	Negative	32.37	0.16	0.49
		3X LoD	32.74	0.16	0.47
		5X LoD	32.74	0.16	0.48

* ND: Not detected

	Target	Concentration	Mean Ct	SD	CV (%)
Between Run	S gene	Negative	*ND	-	-
		3X LoD	36.31	0.61	1.69
		5X LoD	35.86	0.47	1.32
	RdRP gene	Negative	*ND	-	-
		3X LoD	35.82	0.53	1.48
		5X LoD	35.14	0.54	1.55
	IPC	Negative	32.47	0.16	0.48
		3X LoD	32.70	0.06	0.17
		5X LoD	32.71	0.16	0.49

* ND : Not detected

	Target	Concentration	Mean Ct	SD	CV (%)
Between operator	S gene	Negative	*ND	-	-
		3X LoD	36.23	0.78	2.15
		5X LoD	35.56	0.35	0.98
	RdRP gene	Negative	*ND	-	-
		3X LoD	35.33	0.47	1.33
		5X LoD	34.80	0.43	1.23
	IPC	Negative	32.41	0.07	0.23
		3X LoD	32.91	0.16	0.48
		5X LoD	32.88	0.15	0.46

*ND: Not detected

Clinical Performance

The clinical performance of RADI COVID-19 Detection Kit has been independently evaluated in different countries from different continents such as FIND (Switzerland, Europe), PCR-DX, LLC (USA, north America), Center for Disease Prevention and Control (Saudi Arabia, Asia), RIVM (Netherland, Europe), GHESKIO lab (Haiti, Latin America), MOH (Uganda, Africa), Clinical laboratory of Albert Einstein Israelite hospital (Brazil, Latin America), and Green Cross Lab. (South Korea, Asia). A total number of 764 samples have been retrospectively collected and evaluated, and 366 of them were SARS-CoV-2 positive and the rest were negative samples.

The specimen type of these evaluations was nasopharyngeal swab and/or oropharyngeal swab sample.

As shown in the table below, the comprehensive sensitivity and specificity of the RADI COVID-19 Detection Kit was found

to be 98.9% and 100% respectively.

No.	Site	Result for Sensitivity (n= samples tested)	Result for Specificity (n= samples tested)
1	FIND in Switzerland [6,7]	100% (n=50)	100% (n=100)
2	Clinical validation from US Laboratory (PCR-DX, LLC)	100% (n=111)	100% (n=96)
3	Study from Saudi CDC[4]	100% (n=31)	100% (n=24)
4	MOH in Uganda	94% (n=31)	100% (n=45)
5	Clinical laboratory of Albert Einstein Israelite hospital, Brazil	100% (n=14)	100% (n=8)
6	Dutch National Institute for Public Health (RIVM) in Netherland[5]	92% (n=13)	100% (n=11)
7	GHESKIO in Haiti	100% (n=6)	100% (n=5)
8	Study from Korea (Green Cross laboratories)	99.09% (n=110)	100% (n=109)
Comprehensive Sensitivity%		**98.9% (362/366)** (95CI% : 97.2 – 99.6)	
Comprehensive Specificity%		**100.0% (398/398)** (95CI% : 99.0 – 100.0)	

n= number of samples evaluated.

14. Troubleshooting

Please contact to info@khmedical.co.kr for Troubleshooting guide.

15. Bibliography

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16. Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number		Consult instruction for use
LOT	Lot number	-25℃ -15℃	Storage at -25℃ to -15℃
	Use by date		Manufacturer
Σ	Contains sufficient for tests	CE	CE mark
IVD	In vitro diagnostic Medical Device	EC REP	Authorized representative in the European community

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CE IVD