In vitro Diganostic medical device

Asan qPCR Test® COVID-19/RSV/Flu/AdV

1. LOT number and Expiry date
Refer to external labelling

2. Intended use
The Asan qPCR Test® COVID-19/RSV/Flu/AdV is an in vitro diagnostic medical device that carefully diagnoses SARS-CoV-2 genes (RdRp gene, N gene), Influenza A (M gene), Influenza B (NS1 gene), Respiratory Synthetic Virus A (L gene), RSV B (N gene), and Adenovirus (Hexon gene) in real-time reverse transcription polymerase chain reaction (RT-qPCR).

3. How to use
(1) Product description
Asan qPCR Test® Covid-19/RSV/Flu/AdV is a One-step RT-PCR product using a real-time reverse transcription polymerase chain reaction (RT-PCR), and One-step RT-PCR uses a mixture of materials required for a reverse transcription reaction and materials required for a PCR reaction in one tube. Using this, cDNA is synthesized from RNA extracted from the sample in the same tube, and PCR for target genes is performed from the synthesized cDNA.
The RT-qPCR method uses Primer & Probe, where the amplification product amplified by Primer binds to Probe, Polymerase breaks down Probe, and the fluorescence signal of the generated Reporter eye is measured in real time. In this process, the fluorescence signal increases according to the amount of the amplification product. The RNA extraction process and whether the PCR reaction inhibitor in the extracted RNA is mixed can be confirmed by the Human RNase P gene, which is an internal control (IC), and whether a sample is properly collected and extracted.
This product consists of RdRp gene, N gene targeting the novel coronavirus (SARS-CoV-2), gene L gene targeting the Respiratory Synthesis Virus (RSV) type A, gene N gene targeting the RSVB type simultaneously amplifying and detecting Tube 1, gene M gene targeting the Influenza A type virus, gene NS1 gene targeting the Influenza B type virus, and tube 2 that simultaneously amplifies and detects the gene Hexon gene targeting the AdV (Adenovirus). Through this, the fluorescence signal of each tube can be measured and qualitatively detected.

Primer/Probe Mix 1

Detection target	Reporter
RdRp gene	Cal Red 610
N gene	FAM
RSV (L gene, N gene)	Cy5-5
Internal Control (IC)	CY5

Primer/Probe Mix 2

Detection target	Reporter
Flu A	HEX
Flu B	FAM
Adenovirus	Cal Red 610
Internal Control (IC)	CY5

(2) Kit Components

	Components	Volume	Quantity
1	Onestep qPCR Master Mix	1.1 mL	1 tube
2	Primer/Probe Mix 1	400 µL	1 tube
3	Primer/Probe Mix 2	400 µL	1 tube
4	Positive Control 1	250 µL	1 tube
5	Positive Control 2	400 µL	1 tube
6	Negative Control	250 µL	1 tube

※ Material and devices required but not provided
CFX 96™ Real-Time PCR Dectection system(Bio-Rad)
- Nucleic acid extraction system or kit
- Benchtop centrifuge with a rotor for 1.5/2.0 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Appropriate reaction plate or tube
- Pipettes
- Pipette tips with filters
- Disposible latex gloves

(3) Specimen preparation and storage
1) The specimen that can be used is a human nasopharyngeal swab.
2) Specimens stored in transport medium are stable for up to 72 hours in refrigerated storage (2~8℃), and must be stored frozen under -70℃ for long-term storage.
3) Test sensitivity may decrease when samples are stored for a long period of time or are repeatedly frozen and thawed.
※ All clinical specimens are handled as potentially infectious.

4. Procedure
(1) Nucleic acid extraction
It is recommended to use the QIAamp DSP RNA Viral Mini Kit as a nucleic acid extraction reagent, and follow the manufacturer's instructions.

(2) How to test
[Preparation of reagent]
1) Open the box, and thaw each components
2) Lightly voltex each completely melted component and spin-down.

[Mixing components]
1) Considering the type of specimen to be tested and the number of specimens N (number of specimens to be tested + 1 positive control + 1 negative control + 1 extra), prepare a reaction solution 15 *µℓ* x N as shown in the table below using Onestep qPCR Master Mix and Primer/Probe Mix 1, 2.

	Components	1 Test Volume	N Test Volume
1	Onestep qPCR Master Mix	11 µL	11 µL × N
2	Primer/Probe Mix	4 µL	4 µL × N
3	Viral RNA Sample	5 µL	5 µL × N
	Total	20 µL	20 µL × N

2) The prepared reaction solution is lightly vortexed to prevent bubble from being generated, followed by spin-down.
3) Dispense 15 µl of the reaction solution prepared in the PCR

tube.

4) In each PCR tube, 5 μl of the extracted Viral RNA sample is dispensed, and the positive control group is dispensed with positive control (PC) 1 for Primer/Probe Mix 1, positive control (PC) 2 for Pirmer/Probe Mix 2, and the negative control group is dispensed with 5 μℓ each using Negative Control (NC). (To minimize contamination, it is dispensed in the order of NC > Sample > PC.)

[Amplication of Real-time PCR]

1) After dispensing, spin down the PCR tube and place it into the PCR well.
2) Operate the equipment following the user manual instructions.
3) Refer to the RT-PCR equipment settings in the following table.

Primer/Probe Mix 1

Target	SARS-CoV-2 RdRp gene	SARS-CoV-2 N gene	RSV	IC
Fluorophore	CalRed610	FAM	Cy5-5	Cy5

Primer/Probe Mix 2

Target	Flu A	Flu B	Adenovirus	IC
Fluorophore	HEX	FAM	Cal Red 610	Cy5

<Setting of Real-time PCR condition>

	Stage	Temperature	Time	Cycle
1	cDNA synthesis	50℃	5min	1
2	RTase Inactivation	95℃	3min	1
3	Pre-Denaturation	95℃	5s	5
4	Pre-Annealing/ Extension	60℃	40s	
5	Denaturation	95℃	1s	40
6	Annealing / Extension	60℃	5s	

5. Result analysis

When the inspection is completed, open the appropriate experimental data of the analysis program and analyze the results, check the Cut-off (Ct Value) value for the target gene, and set the threshold to 300 for FluA and 500 for IC.

Primer/Probe Mix 1

Target	Ct Value	Result
COVID-19 (RdRp gene)	Ct ≤ 36	SARS-CoV-2 RdRp gene positive
COVID-19 (N gene)	Ct ≤ 36	SARS-CoV-2 N gene positive
RSV	Ct ≤ 36	Respiratory Syncytial Virus positive
IC	Ct ≤ 33	Internal control positive

Primer/Probe Mix 2

Target	Ct Value	Result
Flu A	Ct ≤ 35	Influenza A positive
Flu B	Ct ≤ 36	Influenza B positive
Adenovirus	Ct ≤ 36	Adenovirus positive
IC	Ct ≤ 33	Internal control positive

primer/Probe Mix 1

Case	SARS-CoV-2 RdRp gene	SARS-CoV-2 N gene	RSV	IC	Interpretation
1	+	+	-	+/-	SARS-CoV-2 positive
2	+	-	-	+/-	SARS-CoV-2 Inconclusive
3	-	+	-	+/-	SARS-CoV-2 Inconclusive
4	-	-	+	+/-	RSV positive
5	+	+	+	+/-	SARS-CoV-2, RSV positive
6	-	-	-	+	Negative
7	-	-	-	-	Invalid

Primer/Probe Mix 2

Case	Flu A	Flu B	AdV	IC	Interpretation
1	+	-	-	+/-	Flu A positive
2	-	+	-	+/-	Flu B positive
3	-	-	+	+/-	AdV positive
4	+	+	-	+/-	FluA, Flu B positive
5	+	-	+	+/-	Flu A, AdV positive
6	-	+	+	+/-	FluB, AdV positive
7	+	+	+	+/-	Flu A, Flu B, AdV positive
8	-	-	-	+	Negative
9	-	-	-	-	Invalid

- Even if the internal control material (IC) comes out negative, it can be determined as positive. When each detection gene is strong positive, the IC result value may be inhibited due to the high concentration of the detection gene, so even if the IC is negative, it is determined as positive.
- SARS-CoV-2 Inconclusive: If all genes are not positive, retest is conducted.
- Invalid/Re-test : invalidation of the test, retest is conducted.

6. Quality control and validity of results

Each test shall be tested together with Positive Control and Negative Control to ensure that the following Ct value criteria are met.

Primer/Probe Mix 1

Target	RdRp gene (CalRed 610)	N gene (FAM)	RSV (Cy5-5)	IC (Cy5)
PC	Ct≤36	Ct≤36	Ct≤36	Ct≤33
NC	N/A	N/A	N/A	N/A

Primer/Probe Mix 2

Target	Flu A (HEX)	Flu B (FAM)	Adenovirus (Cal Red 610)	IC (Cy5)
PC	Ct≤36	Ct≤36	Ct≤36	Ct≤33
NC	N/A	N/A	N/A	N/A

7. Performance

(1) Analytical sensitivity – Limit of detection (LoD)

SARS-CoV-2 (SARS-CoV-2 RNA Control, Vircell), RSVA, RSV B, Influenza A Virus, Influenza B Virus (Invitro transcribed RNA), and Adenovirus standards were diluted step by step in two lots, and the lowest concentration detected more than 95% was confirmed by repeated testing twice a day twice a day for three days in five repetitions.

Detection gene	Limit of detection (LoD)
	Detection concentration(Copies/μL)
RdRp gene	0.8 Copies/μL
N gene	0.8 Copies/μL
RSV A	2.0 Copies/μL
RSV B	0.82 Copies/μL
Flu A-H1N1	1.14 Copies/μL
Flu A-H3N2	1.13 Copies/μL
Flu B	2.18 Copies/μL
Adenovirus	0.17 Copies/μL

(2) Precision - Repeatability

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were tested twice a day in one lot for 5 days by the same experimenter and three times per test. As a result of the test, it was confirmed that the detected Ct result value within the test, between the test, and between the test date was 5% CV 〈, showing that it was valid for repeatability.

(3) Precision – Reproducibility

Three samples of high concentration (100xLoD), medium concentration (10xLoD), and low concentration (2xLoD) were tested for reproducibility between places, between testers, and between lots in 1 set. Two testers in two laboratories were tested twice a day for 5 days with two lot products, and when tested repeatedly three times for each test, it was confirmed that the negative and positive results were 100% consistent and the detected Ct result value was 5% CV 〈, showing that it was valid for reproducibility.

(4) Competitive interference

To confirm the competitive interference between Tube 1: SARS-CoV-2, RSV and Tube 2: Influenza A, Influenza B, and Adenovirus analytes, the test was repeated 20 times with one lot product for each concentration under the following conditions.

As a result of the test, it was confirmed that both tubes were suitable within the judgment criteria, and it was confirmed that other targets at high concentrations (100xLoD) did not affect the analysis sensitivity of the corresponding targets at low concentrations (2xLoD).

Primer/Probe Mix 1

Standard substance	Combination	
SARS-CoV-2	Low concentration (2xLoD)	High concentration
Respiratory Syncytial Virus	High concentration	Low concentration (2xLoD)

Primer/Probe Mix 2

Standard substance	Combination		
Influenza A Virus	Low concentration (2xLoD)	High concentration (100xLoD)	High concentration (100xLoD)
Influenza B Virus	High concentration (100xLoD)	Low concentration (2xLoD)	High concentration (100xLoD)
Adenovirus	High concentration (100xLoD)	High concentration (100xLoD)	Low concentration (2xLoD)

(5) Analytical specificity - cross-reaction

As a result of selecting 44 cross-materials of various microorganisms and viruses related to respiratory diseases and present in samples and repeated testing three times per sample, it was confirmed that 44 pathogens did not cross-react.

No	Pathogen name	Concentration	Dection or not
1	Coronavirus OC43	$1.58\times10^{5}TCID_{50}$/mL	N.D
2	Coronavirus NL63	$1\times10^{5}TCID_{50}$/mL	N.D
3	Coronavirus 229E	$1\times10^{5}TCID_{50}$/mL	N.D
4	Human metapenumovirus-3 type B1	$1.25\times10^{5.34}TCID_{50}$/mL	N.D
5	Parainfluenza virus type 1	$1.14\times10^{8}TCID_{50}$/mL	N.D
6	Parainfluenza virus type 2	$1\times10^{5}TCID_{50}$/mL	N.D
7	Parainfluenza virus type 3	$3.21\times10^{7}TCID_{50}$/mL	N.D
8	Parainfluenza virus type 4A	$1.25\times10^{5.58}TCID_{50}$/mL	N.D
9	Adenovirus type 1	$3.21\times10^{7}TCID_{50}$/mL	N.D
10	Adenovirus type 3	$4.75\times10^{5}TCID_{50}$/mL	N.D
11	Adenovirus type 5	$1.25\times10^{6.53}TCID_{50}$/mL	N.D
12	Adenovirus type 7a	$1\times10^{5}TCID_{50}$/mL	N.D
13	Adenovirus type 8	$1\times10^{5}TCID_{50}$/mL	N.D
14	Adenovirus type 11	$1.25\times10^{6.29}TCID_{50}$/mL	N.D
15	Influenza A H1N1 Virus	$4.17x10^{5}TCID_{50}$/ml	N.D
16	Influenza A H1N1pdm09 Virus	13.3ng/μL	N.D
17	Influenza A H3N2 Virus	16.4ng/μL	N.D
18	Influenza B Virus	12.7ng/μL	N.D
19	Influenza B Virus [1A Clade]	13.3ng/μL	N.D
20	Respiratory syncytial virus type A	130.1ng/μL	N.D
21	Respiratory syncytial virus type B	102.7ng/μL	N.D
22	MERS-Coronavirus (Florida/USA-2_Saudi Arabia 2014)	$1\times10^{5}TCID_{50}$/mL	N.D
23	Human coronavirus HKU1	$5.5x10^{5}$copies/μL	N.D
24	Human SARS Coronavirus	$1.8x10^{4}$copies/μL	N.D
25	Human bocavirus	$6.5x10^{5}$copies/μL	N.D
26	Rhinovirus B42	$1.31\times10^{5}TCID_{50}$/mL	N.D
27	Enterovirus B111	$0.7\times10^{4}TCID_{50}$/mL	N.D
28	Bordetella pertussis	$1.227x10^{7}$copies/μL	N.D
29	Candida albicans	$4.519x10^{7}$copies/μL	N.D
30	Streptococcus pneumoniae	$2.318x10^{7}$copies/μL	N.D
31	Globicatella sanguinis	$2.368x10^{7}$copies/μL	N.D
32	Klebsiella pneumoniae	$7.891x10^{6}$copies/μL	N.D
33	Mycobacterium goodii	$8.053x10^{6}$copies/μL	N.D

34	Mycobacterium fortuitum	$6.017x10^6$copies/μL	N.D
35	Streptococcus pyogenes	$2.317x10^7$copies/μL	N.D
36	Staphylococcus aureus	$1.542x10^7$copies/μL	N.D
37	Staphylococcus epidermidis	$1.653x10^7$copies/μL	N.D
38	Bacillus licheniformis	$1.302x10^7$copies/μL	N.D
39	Paenibacillus timonensis	$1.195x10^7$copies/μL	N.D
40	Lactobacillus johnsonii	$2.816x10^7$copies/μL	N.D
41	Streptococcus salivarius	$2.383x10^7$copies/μL	N.D
42	Haemophilus influenzae	$2.825x10^7$copies/μL	N.D
43	Haemophilus parainfluenzae	$2.294x10^7$copies/μL	N.D
44	Legionella pneumophila	$1.366x10^7$copies/μL	N.D

N.D: Not detected

(6) Analytical specificity - interference response

As a result of evaluating the presence or absence of interference by adding 9 types of interference substances that can be present in the sample, no interference was observed up to that concentration.

No	Interference material	Concentration	Interference or not
1	Saline Nasal spray	10%(V/V)	N.A
2	Zanamivir	3mg/ml	N.A
3	Bilirubin, Conjugated	0.05mg/ml	N.A
4	Albumin	0.24g/ml	N.A
5	Haemoglobin	2mg/ml	N.A
6	Biotin	300μg/ml	N.A
7	Tobramycine	24μg/ml	N.A
8	Mucin	0.1mg/ml	N.A
9	Human genomic DNA	10μg/ml	N.A

N.A: Not available

8. Storage method and period of use

(1) Store the kit in its original sealed packaging at -20℃. or below, away from direct sunlight. Kit contents are stable until the expiration date printed on the outer box.

(2) The number of freeze/thaw cycles is limited to four

9. Precautions

[General precautions]

(1) This product is for in vitro diagnostic use only.

(2) This test should only be conducted by trained professionals.

(3) Before starting the assay, thoroughly read the user manual and follow the instructions for use.

(4) All specimens collected from humans should be considered potentially infectious. Follow Good Laboratory Practice (GLP) when handling samples to prevent contamination.

(5) Avoid contact of this product with skin, eyes, or mucous membranes. If contact occurs, rinse immediately with plenty of water.

(6) Improper control of sample cross-contamination during handling and processing may lead to false positive results.

[Precautions for storage]

(1) Store the product below -20℃.

(2) Do not use products beyond their expiration date.

[Precautions when test]

(1) Before use, visually inspect each component of the product for leaks. Do not use if a leak is detected.

(2) Do not mix this product with products from other lots during use.

(3) Avoid repeated freezing and thawing of the product, as it may affect test results.

(4) This product is optimized for use with the recommended real-time gene amplification device. Use with other equipment may result in incorrect results.

(5) Take care to prevent contamination of product components by nucleic acid extracts, PCR products, or positive controls. We recommend using filter tips to prevent contamination.

(6) In case of spills onto equipment, clean according to the equipment manual and remove contamination from surfaces.

(7) All samples used in testing are potentially infectious. Use appropriate protective equipment and conduct testing in facilities such as biosafety cabinets of Biosafety Level 2 or higher.

(8) Improper collection, transport, or handling of samples may affect test results. Handle samples with caution.

[Precautions for determining results]

(1) Do not utilize test results obtained with this product for disease diagnosis. Diagnosis should be based on test results, clinical findings, and other clinical data evaluated by a healthcare professional.

(2) If the concentration of SARS-CoV-2, RSV, Flu A, B and Adenovirus in the sample is below the detection limit, or if an amplification inhibitor in the sample is included, a false negative result may come out, so the negative result alone cannot exclude the infection of SARS-CoV-2, RSV, Flu A, B and Adenovirus

(3) Sensitivity may decrease if there are mutations in the primer and probe binding regions included in the product.

(4) This test does not exclude diseases caused by other bacterial or viral pathogens.

[Precautions for disposal]

(1) Dispose of products and samples following guidelines and methods outlined in the Waste Management Act.

10. Creation and revision date

Date of preparation : 2024.09

[Product name] High-risk infectious agent genetic testing reagent
[Classification number (grade)] K05030.01(3)
[Model name] Asan qPCR Test® Covid-19/RSV/Flu/AdV