In vitro diagnostic medical devices

Asan qPCR Test® Flu/RSV/AdV

1. Manufacturing number and expiration date
Refer to External (Packaging Box) Markings (LOT, EXP)

2. Purpose of use
Asan qPCR Test® Flu/RSV/AdV is an in vitro diagnostic medical device that helps diagnose the virus infection of Influenza A virus (M gene), Influenza B virus (NS1 gene), Respiratory Syntial Virus (RSV) A (L gene), RSV B (N gene), and Adenovirus(AdV) (Hexon gene) in real-time reverse transcription polymerase chain reaction (Real-time RT-PCR).

3. How to use it
(1) inspection principle
Asan qPCR Test® Flu/RSV/AdV is a One-step RT-PCR product using real-time reverse transcription polymerase chain reaction (Real-time 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 real-time PCR 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 can simultaneously amplify the gene Mene targeting the Influenza A type virus, NS1 gene targeting the Influenza B type virus, Lene targeting the Respiratory Synthesis Virus (RSV) type A, and Hexon gene targeting the RSVB type and AdV (AdV) type and qualitatively detect the fluorescence signal by measuring it.

(2) Components

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

(3) Preparation and storage methods of specimens
1) Available specimens are human nasopharyngeal smear specimens.
2) Samples stored in the transport medium should be recognized for up to 72 hours when refrigerated (2~8℃), and frozen under -70℃ for long-term storage.
3) Specimens can be stored for a long period of time, or test sensitivity can be reduced when freezing and thawing are repeated.
※ All clinical specimens are treated as likely to carry infectious pathogens.

(4) Nucleic acid extraction
The use of the QIAamp DSP RNA Viral Mini Kit (Seoul In vitro Receipt No. 14-250) is recommended for nucleic acid extraction reagents, and the manufacturer's manual is followed.

(5) Inspection Method
[Ready for the reagent]
1) Unlock the product box and defrost each component.
2) Lightly vortex each completely melted component and spin-down.

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

	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) Each PCR tube is dispensed with 5 μl of the extracted Viral RNA sample, and each is dispensed with 5 μL using Positive Control (PC) and Negative Control (NC). (To minimize contamination, it is dispensed in the order of NC > Sample > PC.)

[Real-time PCR amplification]
1) After dispensing the PCR tube is lightly spin-down and placed in a PCR well.
2) Drive the equipment according to the relevant equipment manual.
3) Refer to the RT-PCR equipment settings in the following table.
<CFX96™ Dx System Fluorescence Settings>

Target	Flu A	Flu B	RSV	AdV	IC
Fluorophore	HEX	FAM	Cy5-5	CalRed610	Cy5

<Real-time PCR Condition setting>

	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	

4. Determination of the result
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.

Target	Ct Value	Result
Flu A	Ct ≤ 35	Influenza A virus positive
Flu B	Ct ≤ 36	Influenza B virus positive
RSV	Ct ≤ 36	Respiratory Syncytial Virus positive
AdV	Ct ≤ 36	Adenovirus positive
IC	Ct ≤ 33	Internal control positive

Case	Flu A	Flu B	RSV	AdV	IC	Interpretation
1	+	-	-	-	+/-	Flu A positive
2	-	+	-	-	+/-	Flu B positive
3	-	-	+	-	+/-	RSV positive
4	-	-	-	+	+/-	AdV positive
5	+	+	-	-	+/-	Flu A positive, Flu B positive
6	+	-	+	-	+/-	Flu A positive, RSV positive
7	+	-	-	+	+/-	Flu A positive, AdV positive
8	-	+	+	-	+/-	Flu B positive, RSV positive
9	-	+	-	+	+/-	Flu B positive, AdV positive
10	-	-	+	+	+/-	RSV positive, AdV positive
11	+	+	+	-	+/-	Flu A positive, Flu B positive, RSV positive
12	+	+	-	+	+/-	Flu A positive, Flu B positive, AdV positive
13	+	-	+	+	+/-	Flu A positive, RSV positive, AdV positive
14	-	+	+	+	+/-	Flu B positive, RSV positive, AdV positive
15	+	+	+	+	+/-	Flu A positive, Flu B positive, RSV positive, AdV positive
16	-	-	-	-	-	Negative
17	-	-	-	-	-	Invalid/Re-test

- Even if the internal control material (IC) comes out negative, it may 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.
- Invalid/Re-test : The inspection is invalidated, and the re-inspection is carried out.

5. Quality control

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

Target	Flu A (HEX)	Flu B (FAM)	RSV (Cy5-5)	Adenovirus (CalRed610)	IC (Cy5)
PC	Ct≤35	Ct≤36	Ct≤36	Ct≤36	Ct≤33
NC	N/A	N/A	N/A	N/A	N/A

6. Performance

(1) Analytical sensitivity-Minimum Detection Limit

Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus, and Adenovirus standards were diluted step by step and tested twice a day twice a day for three days in five repetitions in one test with two lots to confirm the lowest concentration detected more than 95%.

Detection gene	Minimum detection limit
	Detection concentration(Copies/μL)
Flu A-H1N1	1.14 Copies/μL
Flu A-H3N2	1.13 Copies/μL
Flu B	2.18 Copies/μL
RSV A	2.0 Copies/μL
RSV B	0.82 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

In order to confirm the competitive interference between the analytes of Influenza A Virus, Influenza B Virus, Respiratory Synthetic Virus, and Adenovirus, 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 it was suitable within the determination criteria, and it was confirmed that another target at a high concentration (100xLoD) did not affect the analysis sensitivity of the corresponding target at each low concentration (2xLoD).

standard substance	combination			
Influenza A Virus	Low Concentration(2xLoD)	High Concentration	High Concentration	High Concentration
Influenza B Virus	High Concentration	Low Concentration(2xLoD)	High Concentration	High Concentration
Respiratory Syncytial Virus	High Concentration	High Concentration	Low Concentration(2xLoD)	High Concentration
Adenovirus	High Concentration	High Concentration	High Concentration	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.

number	cross-material	concentration	detection status
1	Coronavirus OC43	$1.58\times10^{5}TCID_{50}/mL$	Not detected
2	Coronavirus NL63	$1\times10^{5}TCID_{50}/mL$	Not detected
3	Coronavirus 229E	$1\times10^{5}TCID_{50}/mL$	Not detected
4	Human metapenumovirus-3 type B1	$1.25\times10^{5.34}TCID_{50}/mL$	Not detected
5	Parainfluenza virus type 1	$1.14\times10^{8}TCID_{50}/mL$	Not detected
6	Parainfluenza virus type 2	$1\times10^{5}TCID_{50}/mL$	Not detected
7	Parainfluenza virus type 3	$3.21\times10^{7}TCID_{50}/mL$	Not detected
8	Parainfluenza virus type 4A	$1.25\times10^{5.58}TCID_{50}/mL$	Not detected
9	Adenovirus type 1	$3.21\times10^{7}TCID_{50}/mL$	Not detected
10	Adenovirus type 3	$4.75\times10^{5}TCID_{50}/mL$	Not detected
11	Adenovirus type 5	$1.25\times10^{6.53}TCID_{50}/mL$	Not detected
12	Adenovirus type 7a	$1\times10^{5}TCID_{50}/mL$	Not detected
13	Adenovirus type 8	$1\times10^{5}TCID_{50}/mL$	Not detected
14	Adenovirus type 11	$1.25\times10^{6.29}TCID_{50}/mL$	Not detected
15	Influenza A H1N1 Virus	$4.17x10^{5}TCID_{50}/ml$	Not detected
16	Influenza A H1N1pdm09 Virus	13.3ng/μL	Not detected
17	Influenza A H3N2 Virus	16.4ng/μL	Not detected
18	Influenza B Virus	12.7ng/μL	Not detected
19	Influenza B Virus [1A Clade]	13.3ng/μL	Not detected
20	Respiratory syncytial virus type A	130.1ng/μL	Not detected
21	Respiratory syncytial virus type B	102.7ng/μL	Not detected
22	MERS-Coronavirus (Florida/USA-2_Saudi Arabia_2014)	$1\times10^{5}TCID_{50}/mL$	Not detected
23	Human coronavirus HKU1	$5.5x10^{5}$copies/μL	Not detected
24	Human SARS Coronavirus	$1.8x10^{4}$copies/μL	Not detected
25	Human bocavirus	$6.5x10^{5}$copies/μL	Not detected
26	Rhinovirus B42	$1.31\times10^{5}TCID_{50}/mL$	Not detected
27	Enterovirus B111	$0.7\times10^{4}TCID_{50}/mL$	Not detected
28	Bordetella pertussis	$1.227x10^{7}$copies/μL	Not detected
29	Candida albicans	$4.519x10^{7}$copies/μL	Not detected
30	Streptococcus pneumoniae	$2.318x10^{7}$copies/μL	Not detected
31	Globicatella sanguinis	$2.368x10^{7}$copies/μL	Not detected
32	Klebsiella pneumoniae	$7.891x10^{6}$copies/μL	Not detected
33	Mycobacterium goodii	$8.053x10^{6}$copies/μL	Not detected
34	Mycobacterium fortuitum	$6.017x10^{6}$copies/μL	Not detected
35	Streptococcus pyogenes	$2.317x10^{7}$copies/μL	Not detected
36	Staphylococcus aureus	$1.542x10^{7}$copies/μL	Not detected
37	Staphylococcus epidermidis	$1.653x10^{7}$copies/μL	Not detected
38	Bacillus licheniformis	$1.302x10^{7}$copies/μL	Not detected
39	Paenibacillus timonensis	$1.195x10^{7}$copies/μL	Not detected
40	Lactobacillus johnsonii	$2.816x10^{7}$copies/μL	Not detected
41	Streptococcus salivarius	$2.383x10^{7}$copies/μL	Not detected
42	Haemophilus influenzae	$2.825x10^{7}$copies/μL	Not detected
43	Haemophilus parainfluenzae	$2.294x10^{7}$copies/μL	Not detected
44	Legionella pneumophila	$1.366x10^{7}$copies/μL	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.

number	interfering substance	concentration	Presence of interference
1	Saline Nasal spray	10%(V/V)	none
2	Zanamivir	3mg/ml	none
3	Bilirubin, Conjugated	0.05mg/ml	none
4	Albumin	0.24g/ml	none
5	Haemoglobin	2mg/ml	none
6	Biotin	300μg/ml	none
7	Tobramycine	24μg/ml	none
8	Mucin	0.1mg/ml	none
9	Human genomic DNA	10μg/ml	none

7. Storage method and duration of use

Store in an airtight container at –20℃ and the period of use is 12 months from the date of manufacture and 1 month after opening, and the number of times of cooling/thawing shall be limited to 4 times.

8. Precautions for Use

[General precautions]

(1) This product is for in vitro diagnostics only.

(2) This test is for professional use only.

(3) Before the start of the inspection, thoroughly understand the manual and inspect it according to the method of use.

(4) All specimens taken from humans should be considered potentially infectious. Compliance with Good Laboratory Practice (GLP) standards can help to maintain personal safety.

(5) Do not allow reagents to touch the skin, eyes, or mucous membranes of this product, and if it does, wash it immediately with a large amount of water.

(6) If cross-contamination of specimens is not properly controlled during specimen handling and processing, false-positive results can be obtained.

[Storage precautions]

(1) When storing the product, it should be stored below -20℃.

(2) Do not use products that have expired.

[Precautions for inspection]

(1) Before using, check each component of the product with the naked eye for leakage. If there is a leak, do not use it.

(2) When using the product, do not mix it with other Lot's products.

(3) Be careful because repeated freezing and defrosting of the product may affect the test results.

(4) This product is optimized for the recommended real-time gene amplification device, and incorrect result values may be output when used in other equipment.

(5) Be careful not to contaminate the components of the product by nucleic acid extracts, PCR products, or Positive Control. To prevent contamination of the product, it is recommended to use filter tips.

(6) If the components of the product leak into the equipment, clean according to the equipment manual and remove contamination of the equipment surface.

(7) All specimens used in the test shall be considered to be

potentially infectious, use appropriate protective equipment, and be tested in facilities such as biosafety level 2 or higher.

(8) If the specimen is improperly collected, transported, or handled, it may affect the test results and should be handled with care.

[Precautions for determining results]

(1) Only the test results obtained using this product are not used for the purpose of diagnosing diseases. The diagnosis should be made by a doctor based on the results of this test, clinical findings, and other clinical results.

(2) If the concentration of Flu A, Flu B, RSV, 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 Flu A, Flu B, RSV, and Adenovirus.

(3) If there is a mutation in the site where the included primer and probe of the product bind, the sensitivity may be lowered.

(4) The present test cannot exclude diseases caused by other bacteria or viral pathogens.

[Precautions for product disposal]

(1) Dispose of products and samples in accordance with the standards and methods of the Waste Management Act after use.

9. Date of preparation and revision

Date of preparation : 2024.07

[Item name] genetic testing reagent for high-risk infections
[Classification number (class)] K05030.01(3)
[Model name] Asan qPCR Test® Flu/RSV/AdV

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