COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit

(Colloidal Gold)

REF:CG02Ag-01
REF:CG02Ag-25
REF:CG02Ag-50

Instruction for Use

【Product Name】

COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit (Colloidal Gold)

【Packing Specification】

1 Test/ Kit, 25 Tests/Kit, 50 Tests/Kit

【Intended Use】

The AmonMed™ COVID-19/Influenza A/Influenza B Virus Antigen Test Kit (Colloidal Gold) is used for in vitro qualitative detection of COVID-19, influenza A and influenza B virus antigens in human throat swabs, nasal swabs and Virus Transport Medium(VTM) specimens. This test is for professional in-vitro diagnostic use and preliminary screening use only.

【Test Principle】

This kit uses immunochromatographic technology for the qualitative detection of COVID-19/influenza A (type A)/influenza B (type B) virus antigen in human throat swabs and nasal swabs by double antibody sandwich detection principle. If the sample contains COVID-19, type A, type B influenza virus antigen and antigen concentration higher than LoD, COVID-19, type A, type B influenza virus antigen will combine with colloidal gold labeled COVID-19, type A, type B influenza virus antibodies and form colloidal gold antibody-antigen complexes. Colloidal gold antibody-antigen complexes move forward along the nitrocellulose membrane under chromatography to the detection area, and bind to another Covid-19, type A and type B influenza virus antibody which were fixed on the detection areas on nitrocellulose membrane respectively to form purple detection lines, and the detection results are positive. Conversely, if the sample contains no Covid-19, type A, or type B influenza virus antigens, or if the antigen concentrations are below the minimum detected amount, no purplish red bands are present in the detection areas, and the results are negative. No matter whether there is COVID-19, type A or type B influenza virus antigen in the sample or not, the purplish red quality control line (C) in the test card should be present. The purplish red band in the quality control area is the standard to determine whether there are enough samples and whether the chromatography process is normal, and also serves as the internal control standard of the reagent.

【Main Components】

Number	Components		Specification		
1	Test card	Nitrocellulose membranes were coated with COVID-19 antibody, influenza A virus antibody, influenza B virus antibody, and Goat anti-mouse antibody. The conjugate pad of colloidal gold-labeled COVID-19 antibody, influenza A virus antibody, influenza B virus antibody.	1 test/kit	25 tests/kit	50 tests/kit
2	Extraction solution	TritonX-100, Nacl, Sodium Deoxycholate, SDS, Tris-Hcl buffer	1mL×1 bottle	5mL×2 bottles	5mL×4 bottles
3	Extraction tube/cap		1×1 set	1×25 sets	1×50 sets
4	Sample collection swab		1×1 piece	1×25 pieces	1×50 pieces
5	Instruction for use		1×1 piece	1×1 piece	1×1 piece

【Materials Required but Not Provided】

1. Timer
2. Any necessary personal protective equipment
3. Biohazard or sharps container
4. Micropipette

【Storage & Stability】

1. Store at 2℃~30℃, and it is valid for 18 months. Do not freeze the kit or its components.
2. After the aluminum foil pouch is unsealed, the test card should be used immediately.

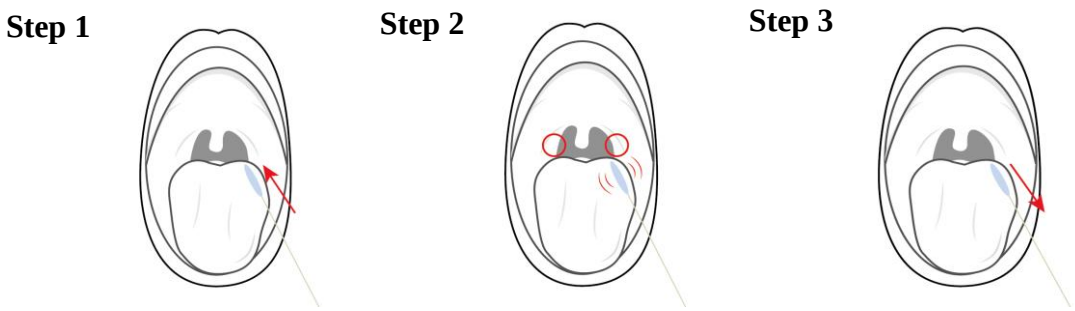
【Specimen Collection】

1. Throat Swab:

Step 1: Let the inspector's head tilt slightly, mouth open, and make "ah" sounds. Expose the pharyngeal tonsils on both sides.

Step 2: Hold the swab and wipe the pharyngeal tonsils on both sides of the inspector with a little hard back and forth at least 3 times.

Step 3: Place the swab specimen in the extraction tube with the extraction solution added in advance, rotate the swab for about 10 seconds, and press the swab head against the wall to release the antigen in the swab.

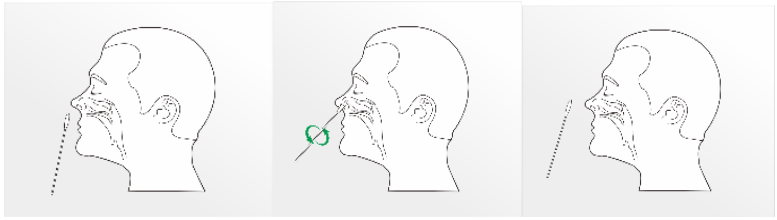


2. Nasal Swab:

Step 1: Insert the swab into one nostril of the inspector. The swab tip should be inserted up to 2.5cm (1 inch) from the edge of the nostril.

Step 2: Roll the swab 5 times along the mucosa inside the nostril to ensure that both mucus and cells are collected.

Step 3: Using the same swab, repeat this process for the other nostril to ensure that an adequate sample is collected from both nasal cavities. Withdraw the swab from the nasal cavity.

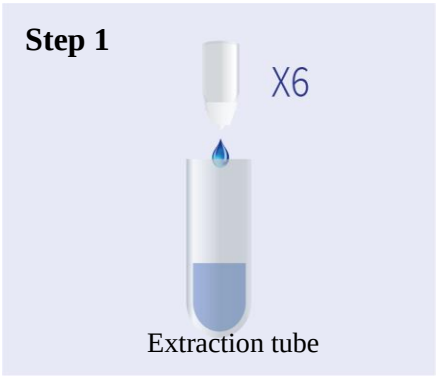


Step 1 Step 2 Step 3

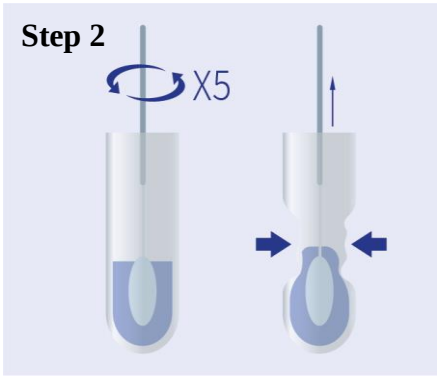
【Test Procedures】

For Swab Specimens

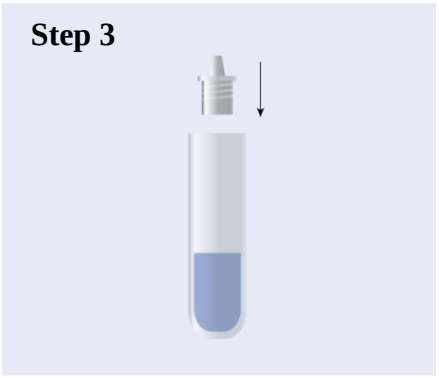
1. Open the package and take out the test card to balance to room temperature (15~30°C) prior to testing. Place the extraction tube on the workbench. The extraction solution bottle is pressed vertically downward to allow the extraction solution to drip freely into the extraction tube without touching the edge of the tube. Add 6 drops (about 300μL) of extraction solution to the extraction tube.
2. Insert the swab into the extraction tube. While squeezing the extraction tube, stir the swab more than 5 times. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab. Dispose of swabs according to biohazard waste disposal method.
3. Press the cap tightly onto the extraction tube.
4. Apply 2 drops (about 80μL) of extracted specimen to the specimen well of the test card, and then start the timer.
5. Read the results at 15 minutes, and the results after 20 minutes are no longer valid.



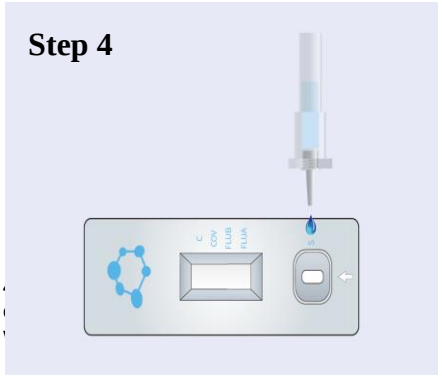
1. Add 6 drops (about 300μL) of extraction solution to the extraction tube.



2. Insert the swab into an extraction tube. While squeezing the extraction tube, stir the swab more than 5 times. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab.



3. Press the cap tightly onto the extraction tube.



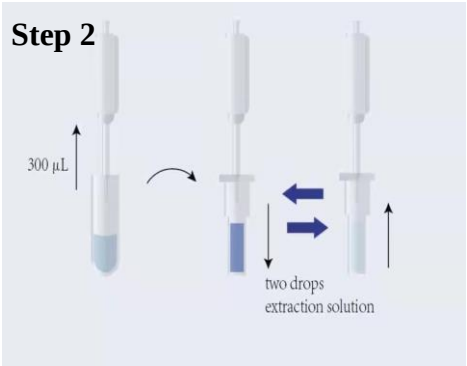
4. Apply 2 drops of extracted specimen to the specimen well of the test card.

5. Read the test result at 15-20 minutes.

For Transport Medium Specimens

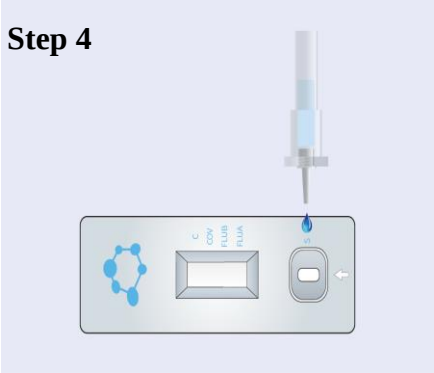
1. Open the package and take out the test card to balance to room temperature (15~30°C) prior to testing. Place the extraction tube on the workbench. The bottle of extraction solution is pressed vertically downward to allow the extraction solution to drip freely into the extraction tube without touching the edge of the tube. Add 2 drops of extraction solution to the extraction tube.
2. Use a micropipette to collect 300μL of specimen from the collection cup or VTM. Mix the specimen with extraction solution.
3. Press the cap tightly onto the extraction tube.
4. Apply 2 drops (about 80μL) of extracted specimen to the specimen well of the test card, and then start the timer.
5. Read the results at 15 minutes, and the results after 20 minutes are no longer valid.

1. Add 2 drops (about 100μL) of extraction solution to the extraction tube.



2. Use a micropipette to remove 300μL specimen into extraction tube with two drops (about 100μL) extraction solution.

3. Press the cap tightly onto the extraction tube.



4. Apply 2 drops of extracted specimen to the specimen well of the test card.

5. Read the test result at 15-20 minutes.

【Interpretation of Test Results】

1. Negative Result

A reddish purple Control line (C position) only, with no Test line at the FluA, FluB, CoV positions, indicates that Influenza A, B viral antigen or SARS-CoV-2 antigen has not been detected. A negative result does not exclude influenza viral or SARS-CoV-2 viral infection.

NOTE: Determination of negative results should not be made before 15 minutes.

2. Positive Result

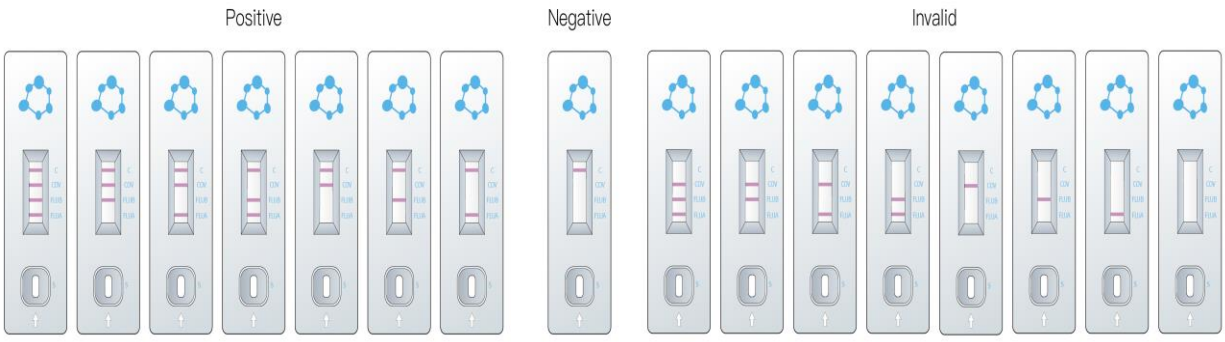
Determination of a positive result is made at fifteen (15) minutes. A reddish purple Control line (C position) and a reddish purple Test line (FluA, FluB or CoV position) indicate that Influenza A, B and/or SARS-CoV-2 antigen has been detected. Lines at the FluA and C positions indicate the presence of Influenza type A viral antigen, lines at the FluB and C positions indicate the presence of Influenza type B viral antigen, and lines at the COV and C positions indicate the presence of COVID-19 antigen in the specimen. A positive result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.

NOTE: The Test line (reddish purple line) may vary in shade and intensity (light or dark, weak or strong) depending on the concentration of antigen detected. The intensity of the Control line should not be compared to that of the Test line for the interpretation of the test result. Even a light or faint Test line must be interpreted as a positive result.

3. Invalid Result

A reddish purple line should always appear at the Control line position (C position). If a line does not form at the Control line position in 15 minutes, the test result is invalid and the test should be repeated with a new test card.

NOTE: Co-infection with Influenza A, B and/or COVID-19 is rare. If results are positive for more than one antigen, i.e., Flu A, B and/or COVID-19, the inspector specimens should be re-tested.



【Performance Characteristics】

1. Limit of Detection (LoD)

Limit of detection of this test kit for different subtypes and strains of COVID-19 / Influenza A/ Influenza B virus are as follows:

Virus Strain	Limit of Detection	Virus Strain	Limit of Detection
FluA/141609 (Influenza A virus) (A/14160(H1N1)	8.0×10^3TCID$_{50}$/mL	FluB/1715	3.0×10^3TCID$_{50}$/mL

FluA/phylum30 (Influenza A virus)(H1N1)	5.1×10^5TCID$_{50}$/mL	Swine influenza A virus/4/09(H1N1)	7.9×10^3TCID$_{50}$/mL
Seasonal influenza A/ HongKong/403946/09(H1N1)	2.0×10^4TCID$_{50}$/mL	FluB/1704	6.3×10^3TCID$_{50}$/mL
FluA/44045(H3N2)	4.8×10^3TCID$_{50}$/mL	FluB/179	2.1×10^3TCID$_{50}$/mL
FluA924(H3N2)	4.8×10^3TCID$_{50}$/mL	FluB/668	1.4×10^4TCID$_{50}$/mL
FluA/302/54(H5N1)	7.2×10^3TCID$_{50}$/mL	COVID-19(swab)	5.0×10^2TCID$_{50}$/mL
FluA/pig/2/01(H1N1)	1.7×10^3TCID$_{50}$/mL	COVID-19(VTM)	4.0×10^3TCID$_{50}$/mL
Swine influenza A virus/ 415742/09(H1N1)	2.0×10^3TCID$_{50}$/mL		

2. Analytical Specificity

2.1 Cross Reactivity

2.1.1 COVID-19, influenza A virus and influenza B virus does not cross reactivity.

2.1.2 The test result is lower than the corresponding concentration of the substance in the table below, which has no effect on the negative and positive test results of the reagent, and there is no cross-reaction.

Potential Cross-Reactant		**Test Concentration**
Virus	Adenovirus	1.0×10^6TCID$_{50}$/mL
	Human metapneumovirus (hMPV)	1.0×10^6TCID$_{50}$/mL
	Rhinovirus	1.0×10^6PFU/mL
	Enterovirus/Coxsackievirus B4	1.0×10^6TCID$_{50}$/mL
	Human coronavirus OC$_{43}$	1.0×10^6TCID$_{50}$/mL
	Human coronavirus 229E	1.0×10^6TCID$_{50}$/mL
	Human coronavirus NL63	1.0×10^6TCID$_{50}$/mL
	Human parainfluenza virus1	1.0×10^6TCID$_{50}$/mL
	Human parainfluenza virus2	1.0×10^6TCID$_{50}$/mL
	Human parainfluenza virus3	1.0×10^6TCID$_{50}$/mL
	Human parainfluenza virus4	1.0×10^6TCID$_{50}$/mL
	Influenza A	1.0×10^6 TCID$_{50}$/mL
	Influenza B	1.0×10^6TCID$_{50}$/mL
	Measles Virus	1.0×10^6TCID$_{50}$/mL
	Respiratory Syncytial Virus A	1.0×10^6PFU/mL
	Human rhinovirus1A	1.0×10^6TCID$_{50}$/mL
	Mumps Virus	1.0×10^6TCID$_{50}$/mL
Bacteria	Bordetella pertussis	1.0×10^6 cells/mL
	Chlamydia pneumoniae	1.0×10^6 PFU/mL
	Haemophilus influenzae	1.0×10^6 cells/mL
	Legionella pneumophila	1.0×10^6cells/mL
	Mycoplasma pneumoniae	1.0×10^6 U/mL

	Streptococcus pneumoniae	1.0×10^6 cells/mL
	Streptococcus pyogenes (group A)	1.0×10^6 cells/mL
	Mycobacterium tuberculosis	1.0×10^6 cells/mL
	Staphylococcus aureus	1.0×10^6 org/mL
	Staphylococcus epidermidis	1.0×10^6 org/mL
	E.coli	1.0×10^6 cells/mL
	Mycobacterium tuberculosis Lehmann and Neumann	1.0×10^6 cells/mL
	Neisseria meningitidis	1.0×10^6 cells/mL
	Pseudomonas aeruginosa	1.0×10^6 cells/mL
	Neisseria gonorrheae	1.0×10^6 cells/mL
	Staphylococcus pyogenes	1.0×10^6 cells/mL
Yeast	Candida albicans	1.0×10^6 cells/mL

Note: 1 $TCID_{50}$ /mL≈0.7 CFU/mL

2.2 Endogenous Interfering Substances

To assess substances with the potential to interfere with the performance of the AmonMed™ COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit (Colloidal Gold), positive and negative samples were tested with the addition of potentially interfering substances. All samples tested produced expected results, demonstrating that the COVID-19/Influenza A/Influenza B Virus performance was not affected by any of the 22 potentially interfering substances listed in the table below at the concentrations tested.

Substance	**Concentration**	**Substance**	**Concentration**
Mucin	1 mg/mL	Fluticasone Propionate	0.5 mg/mL
Whole Blood	1%	Phenol	1 mg/mL
Phenylephrine	10 mg/mL	Tamiflu (Oseltamivir Phosphate)	0.5%
Sodium Chloride (i.e. NeilMed)	20%	Mupirocin	1 mg/mL
Cromolyn	15%	Tobramycin	1 mg/mL
Oxymetazoline	10 mg/mL	Beclomethasone	1 mg/mL
Fluconazole	5%	Dexamethasone	1 mg/mL
Benzocaine, Menthol	1 mg/mL	Flunisolide	1 mg/mL
Galphimia glauca, Sabadilla	20%	Mometasone	1 mg/mL
Zincum gluconium (i.e. Zicam)	1%	Sulphur	1%
Nasal washes	10%	Zanamivir	1 mg/m

3. Clinical Performance

This clinical trial was conducted only on influenza A virus (H1N1), seasonal (H3N2) subtype and COVID-19. The test results of this reagent were compared with 452 clinical samples of similar products. The results are as follows.

Influenza A Virus Antigen		Result of Comparison Test		Subtotal
		Positive	Negative	
COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit	Positive	98	9	107
	Negative	7	338	345
Subtotal		105	347	452
Relative Sensitivity:	98/105	93.33% (95% CI*: 86.87%～96.73%)		
Relative Specificity:	338/347	97.41% (95% CI*: 95.14%～98.63%)		
Accuracy:	436/452	96.46% (95% CI*: 94.33%～97.81%)		

Influenza B Virus Antigen		Result of Comparison Test		Subtotal
		Positive	Negative	
COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit	Positive	112	11	123
	Negative	8	321	329
Subtotal		120	332	452
Relative Sensitivity:	112/120	93.33% (95% CI*: 87.39%～96.58%)		
Relative Specificity:	321/332	96.69% (95% CI*: 94.17%～98.14%)		
Accuracy:	433/452	95.80% (95% CI*: 93.53%～97.29%)		

COVID-19 Antigen		Result of Comparison Test		Subtotal
		Positive	Negative	
COVID-19/Influenza A/Influenza B Virus Antigen Assay Kit	Positive	89	5	94
	Negative	6	352	358
Subtotal		95	357	452
Relative Sensitivity:	89/95	93.68% (95% CI*: 86.90%～97.07%)		
Relative Specificity:	352/357	98.60% (95% CI*: 96.76%～99.40%)		
Accuracy:	441/452	97.57% (95% CI*: 95.70%～98.64%)		

*** 95% Confidence Interval**

4. High Dose Hook Effect

The validated results of the maximum concentration value without hook effect are shown in the table below.

Virus Name	Maximum Concentration Value
Influenza A Virus	$5.1\times10^{8}TCID_{50}/mL$
Influenza B Virus	$5.6\times10^{6}TCID_{50}/mL$
COVID-19	$1.6\times10^{6}TCID_{50}/mL$

【Limitations】

1. This kit is only used for qualitative detection of nucleoprotein antigen of COVID-19 /Influenza A virus / Influenza B virus in samples, and cannot determine the concentration of nucleoprotein antigen of COVID-19 / influenza A virus / influenza B virus in samples.
2. If the test result is negative, but the inspector has clinical symptoms, it is recommended to repeat sampling or other test methods for testing. The negative result can not completely rule out the possibility of infection with COVID-19 and influenza A and B virus.
3. The test results of this kit are only for clinicians' reference and should not be used as the only basis for clinical diagnosis.

The clinical management of inspectors should be considered in combination with their symptoms or signs, medical history, other laboratory tests and treatment reactions.
4. If the test result is positive, it is suggested that further tests should be conducted to confirm the subtype of the virus, and the public health and prevention institutions should consult and negotiate with each other.
5. Possibility analysis of false negative results:
5.1 Improper sample collection, transportation and treatment, too low concentration of the tested substance in the sample, not fresh sample or repeated freezing and thawing of the sample may lead to false negative results.
5.2 The variation of virus gene may lead to the change of antigenic determinants, which may lead to false negative results. The use of monoclonal antibody reagents is more prone to this kind of situation.
5.3 The most suitable sample type and the best sampling time after infection may not be confirmed for the new type of COVID-19, Influenza A virus, or Influenza B virus. Therefore, the possibility of false negative results will be reduced if the samples are collected in different parts of the same inspector.
6. The sensitivity of COVID-19, Influenza A virus and Influenza B virus cannot be completely ruled out by negative results. The test results are negative but clinical symptoms exist, and combined with other test results should be comprehensively judged. It is recommended that the negative results should be checked by nucleic acid detection or virus isolation and culture identification.
7. The variation of virus gene may lead to the change of antigenic determinants, which may lead to false negative results. The use of monoclonal antibody reagents is easier to cause this kind of situation.
8. This test kit is a colloidal gold test kit, which is only used for preliminary screening, and can not be used as a diagnostic result.

【Warnings and Precautions】
1. This kit is only used for in vitro diagnosis. Do not use expired products.
2. Do not use if the test card package is damaged.
3. Test results are meant to be visually determined.
4. Do not eat, drink, or smoke in the area where the specimens and kit contents are handled.
5. Do not swallow the reagent or if the extract comes into contact with skin, eyes and mucous membranes. Once in contact, flush the contaminated area with plenty of water.
6. Appropriate protective measures shall be taken during the collection, disposal, storage, mixing and testing of samples. After testing, all clinical samples, non-reusable reagents and waste shall be disposed of as infectious agents.
7. Please use the swab and extraction solution provided in this kit. Do not mix different batches of reagent and sample extract.
8. The operator should pay attention to safety measures, such as wearing protective clothing, wearing gloves, etc., used swab, test card, extraction tube, etc., before the removal of pollution, high pressure steam disinfection is recommended.
9. For detection of influenza A viruses or subtypes, small changes in epitopes due to small mutations in nucleic acid sequences may result in negative results or reduced analytical sensitivity of the reagent.
10. Inadequate or inappropriate sample collection, storage and transport may result in incorrect results.

【Approval Date and Revision Date of the Instruction for Use】
Approval Date: 2021.01.20
Revision Date: 2021.03.10
Date of Issue: 2021.03.10
Version: V2.0

【Index of Symbols】

IVD	The product is used in vitro, please don't swallow it.		Please don't reuse it
	Expiry date		Please read the instruction book carefully before using
	Warning, please refer to the instruction in the annex		Manufacturer
30°C 2°C	Temperature scope within which the product is reserved	LOT	Batch number
EC REP	European union authorization representative		Keep dry
	Avoid overexposure to the sun		Don't use the product when the package is damaged
	Date of manufacture	REF	Catalog Number
CE	The product meets the basic requirements of European in vitro diagnostic medical devices directive 98/79/EC		

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EC REP

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