CE IVD

COVID-19 Antigen/FLU A+B Antigen Combo Rapid Test Cassette (Colloidal Gold Method)

【Product Name】

COVID-19 Antigen/FLU A+B Antigen Combo Rapid Test Cassette (Colloidal Gold Method)

【Package specification】

1 Test/Bag, 1/10/20/50/100 Tests/Box

【Intended Use 】

This kit is a rapid test for the qualitative detection of antigens to SARS-CoV-2 and Flu A+B in human nasopharyngeal/oropharyngeal swab specimens or saliva/sputum specimens.

This kit is for professional in vitro diagnosis only.

【Principle】

This kit applies the double antibody sandwich assay technology to test the SARS-CoV-2 antigens*. After dropping a suitable amount of sample onto the sample collection hole, it will move forward alongside the test card. If there is SARS-CoV-2 antigens in the specimen, the antigens will bind to colloidal gold labeled SARS-CoV-2 antibodies and be captured by the other SARS-CoV-2 antibodies in the test line (T line), showing a red reaction line, indicating a positive in SARS-CoV-2 antigens; otherwise, it is negative. The quality control area (C line) should be red in any circumstances, to indicate that the test is valid, otherwise it is necessary to test the sample again.

This kit applies the double antibody sandwich assay technology to test influenza A+B antigens. After dropping a suitable amount of sample onto the sample collection hole, it will move forward alongside the test card. If there is influenza A/B or A+B antigens in the specimen, the antigens will bind to colloidal gold labeled influenza A/B or A+B antibodies, and be captured by the other influenza A、B antibodies in the test line (A/B line), showing red reaction lines, indicating a positive in influenza A/B or A+B antigens; otherwise, it is negative. The quality control area (C line) should be red in any circumstances, to indicate that the test is valid, otherwise it is necessary to test the sample again.

*According to our investigation, several site mutations have occurred in the spike protein at the position of N501Y, E484K, K417N in UK and of N501Y, P681H, 69-70 in SA. Since the recognition site of the raw materials used in our antigen test is the nucleocapsid protein (also known as nucleoprotein or protein N) antigens, which is different from the mutation sites, we expect our products are theoretically able to detect variants including those in the UK and SA.

【Components】

COVID-19 Antigen/FLU A+B Antigen Combo Rapid Test Cassette: Individually packaged in aluminum foil bags per person. The kit consists of a sample pad, a gold-labeled pad labeled with a gold-labeled mouse anti-human SARS-COV-2 monoclonal antibody I, a nitrocellulose coated with a mouse anti- human SARS-COV-2 monoclonal antibody II, and a goat anti-mouse IgG antibody. The kit consists of a sample pad, a gold-labeled pad labeled with a gold-labeled mouse anti-human influenza A and influenza B monoclonal antibody I, a nitrocellulose coated with a mouse anti-human influenza A and influenza B monoclonal antibody II, and a goat anti-mouse IgG antibody. It consists of plain film, absorbent paper, plastic backing and plastic template.

- Test Cassettes: each cassette with desiccant in individual foil pouch
- Sterilized Swabs: single use swab for specimen collection
- Saliva/Sputum Collector: saliva/sputum specimen collection
- Extraction Tubes: ampoule containing 0.3 ml of extraction reagent
- Package Insert

Materials required but not provided

- Timer

【Storage & Expiry】

The unopened kit should be stored at 2-30℃, and the period of validity is tentatively 12 months. When open the foil pouch, the test cassette is suggested to be used within one hour.

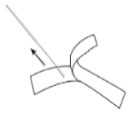
Production date and expiry date are shown on the label.

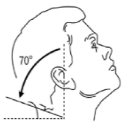
【Specimen】

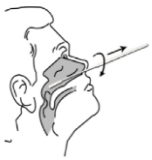
Acceptable specimen type for testing is a direct swab specimen or a swab in viral transport media (VTM) without denaturing agents.

Prepare the extraction tube according to the Test Procedure and use the sterile swab provided in the kit for specimen collection.

1. Nasopharyngeal Swab Specimen Collection







1. Remove the swab from the package.

2. Tilt patient's head back about 70°.

3. Insert the swab through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient, indicating contact with the nasopharynx. (Swab should reach depth equal to distance from nostrils to outer opening of the ear.) Gently rub and roll the swab. Leave swab in place for several seconds to absorb secretions.

4. Slowly remove swab while rotating it.

Specimens can be collected from both sides using the same swab, but it is not necessary to collect specimens from both sides if the minitip is saturated with fluid from the first collection. If a deviated septum or blockage creates difficulty in obtaining the specimen from nostril, use the same swab to obtain the specimen from the other nostril.

2. Anterio nasal Swab Specimen Collection (nose front)

1. Carefully insert the swab into the patient's nostril, up to 2.5 cm deep from the edge of the nostril.
2. Sweep along the mucous membrane in the nostril to ensure that both mucus and cells are collected. Rotate the swab several times.

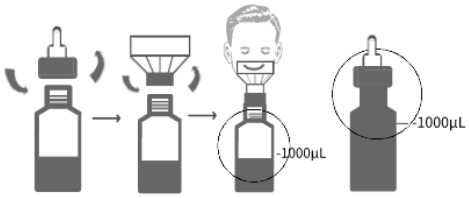
3. Pull the swab out of the nasal cavity.

3. Oropharyngeal Swab Specimen Collection

Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx and avoid touching the tongue, teeth, and gums.

4. Saliva/Sputum (Recommended) Specimen Collection

1. Take out the buffer tube, open the dripper cap.
2. Gently turn the saliva/sputum collector and tighten it onto the buffer tube.
3. Spit saliva or sputum into the oval mouth of the saliva/sputum collector, make the saliva/sputum flow into the tube completely until it meets the 1000 µl marker line.

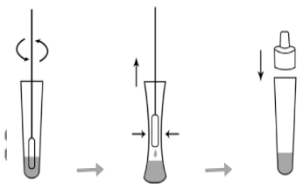


【Test Procedure】

Note: Allow the test devices, reagents and specimens to equilibrate to room temperature (15-30℃ or 59-86℉) prior to testing.

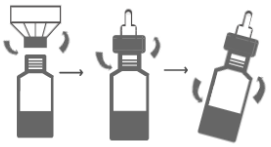
Specimen Handling (except for Saliva/Sputum):

1. Insert the swab specimen into the extraction tube which contains extraction reagent. Roll the swab at least 5 times while pressing the head against the bottom and side of the extraction tube. Leave the swab in the extraction tube for one minute.
2. Remove the swab while squeezing the sides of the tube to extract the liquid from the swab. The extracted solution will be used as test sample.
3. Cover the extraction tube with a dropper tip tightly.

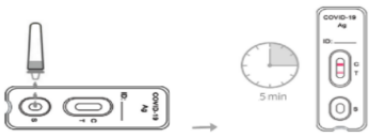


Handling the Saliva/Sputum Specimen:

1. Remove the saliva/sputum collector.
2. Close the dripper cup and tighten it.
3. Shake the tube at least 10 times, make sure the saliva or sputum mix well with sample buffer.



4. Remove the test cassette from the sealed pouch.
5. Reverse the specimen extraction tube, holding the tube upright, transfer 3 drops (approximately 100µl) slowly to the specimen well (S) of the test cassette, then start the timer.
6. Wait for colored lines to appear. Interpret the test results at 10 minutes. Do not read results after 20 minutes.



【Specimen Transport and Storage】

Freshly collected specimens should be processed as soon as possible, but no later than one hour after specimen collection. Specimen collected may be stored at 2-8℃ for no more than 24 hours; Store at -70℃ for a long time but avoid repeated freeze-thaw cycles. If transport of samples with viral transport medium (VTM) is required, minimal dilution of the sample is recommended, as dilution may result in decreased test sensitivity. Whenever possible, 1 milliliter or less is best to avoid excessive dilution of the patient sample. While holding the swab, remove the cap from the tube.

Insert the swab into the tube until the breakpoint is level with the tube opening. Bend the swab shaft at a 180 degrees angle to break it off at the breaking point. You may need to gently rotate the swab shaft to complete the breakage. Based on data generated with influenza virus, or nasopharyngeal swabs in VTM are stable for up to 72 hours at 2° to 8°C.

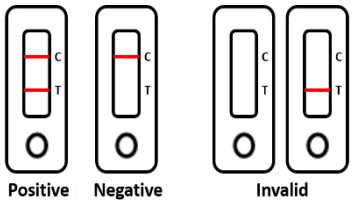
Note: When using viral transport medium (VTM), it is important to ensure that the VTM containing the sample is warmed to room temperature. Cold samples will not flow correctly and can lead to erroneous or invalid results. Several minutes will be required to bring a cold sample to room temperature.

【Explanation of test results】

COVID-19 Antigen

As shown in Figures, the test results are as follows:

1. **Positive:** two red lines, both test line (T line) and control line (C line) appear in color.
2. **Negative:** only a clear red line appears in the C line.
3. **Invalid result:** no signal appears in C line, indicating that this test is invalid, and should be done again.



FLU A+B Antigen

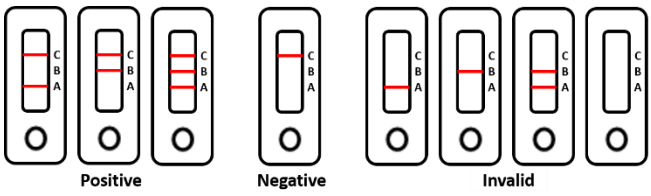
As shown in Figures, the test results are as follows:

1. **Influenza A Positive:** One red band appears in the control region (C), and another red band in the A region (A).
2. **Influenza B Positive:** One red band appears in the control region (C), and another red band in the B region (B).
3. **Influenza A+B Positive:** One red band appears in the control region (C), and two other red bands appear in both A region (A) and B region (B).

Note: *Co-infection with influenza A and B is very rare. A clinical specimen that generates positive results for both A and B should be considered an invalid result, and another test should be performed. If the test is again positive for both influenza A and B, the specimen should be re-tested by another method prior to reporting of results.*

4. Negative: Only one red band appears in the control region (C), and no band appears either in the A region (A) or B region (B).

5. Invalid result: no signal appears in C line, indicating that this test is invalid, and should be done again.



【Limitations】

1. The test results are qualitative, and only used as in vitro assisted diagnosis.

2. Based on the limitation of antigen test assays, the minimum detection limit (sensitivity analysis) is generally lower than nucleic acid methods, so the researchers should focus more on the negative results. They should make comprehensive judgement based on other test results as well. It is suggested to do nucleic acid testing or virus isolation and culture to assist the judgement.

3. Inappropriate sampling, transportation, treatment and low-level of virus in the sample can all lead to false-negative result.

4. The test results are only used as an assisted clinical diagnosis. They are not the only reference for clinical diagnosis. The final diagnosis of the disease should be after the comprehensive judgement of every clinical and laboratory results.

【Performance Indicators】

COVID-19 Antigen

1. Positive reference rate: eight positive reference specimens (P1-P8) were detected, all results should be positive.

2. Minimum detection limit: the study used cultured SARS-CoV-2 virus, which is β-propiolactone and heat inactivated and spiked into nasopharyngeal swab specimen. The Limit of Detection (LoD) is $5\times10^{2.55}$ $TCID_{50}$/ml.

3. Reproducibility: use 10 tests with the same batch number, test the reproducibility reference specimens (R), the result should all be positive, and the signal in T line should be uniform.

4. Cross Reactivity: no cross-reactivity was observed with recombinant MERS-CoV NP protein when tested at the concentration of 50 μg/ml. No cross-reactivity was observed with the following viruses when tested at the concentration of 1.0×10^6 PFU/ml: Influenza A (H1N1), Influenza A (H3N2), Influenza B (Yamagata), Influenza B (Victoria), Adenovirus (type 3), Human metapneumovirus, Parainfluenza virus (type 2), Respiratory syncytial virus, Enterovirus, Rhinovirus, Human coronavirus 229E, Human coronavirus OC43, Human coronavirus NL63. No cross-reactivity was observed with the following bacteria when tested at the concentration of 1.0×10^7 CFU/ml: Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella pneumophila, Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus.

5. Interference: the following potential interference substances were evaluated with the COVID-19 Antigen Rapid Test Cassette at the concentrations listed below and were found not to affect test performance.

Substance	Concentration	Substance	Concentration
3 OTC nasal sprays	10%	Guaiacol glycerol ether	20 mg/ml
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Mupirocin	250 μg/ml
4-acetamidophenol	10 mg/ml	Oxymetazoline	10 mg/ml
Acetylsalicylic acid	20 mg/ml	Phenylephrine	10 mg/ml
Albuterol	20 mg/ml	Phenylpropanolamine	20 mg/ml
Chlorpheniramine	5 mg/ml	Relenza ® (zanamivir)	20 mg/ml
Dexamethasone	5 mg/ml	Rimantadine	500 ng/ml
Dextromethorphan	10 mg/ml	Tamiflu ® (oseltamivir)	100 mg/ml
Diphenhydramine	5 mg/ml	Tobramycin	40 mg/ml
Doxylamine succinate	1 mg/ml	Triamcinolone	14 mg/ml
Flunisolide	3 mg/ml	Nasopharyngeal swab containing respiratory syncytial virus	1.0×10^6 PFU/ml
Oropharyngeal swab containing respiratory syncytial virus	1.0×10^6 PFU/ml	Nasopharyngeal swab containing influenza virus	1.0×10^6 PFU/ml
Oropharyngeal swab containing influenza virus	1.0×10^6 PFU/ml		

6. Clinical Performance

Different samples from 436 patients were detected by COVID-19 Antigen Rapid Test and the RT-PCR.

A. Nasopharyngeal and Oropharyngeal Swab Specimen

The clinical performance of COVID-19 Antigen Rapid Test Cassette was established in prospective studies with Nasopharyngeal and Oropharyngeal Swab collected from 436 individual symptomatic patients (within 7 days of onset) and asymptomatic patients who were suspected of COVID-19.

COVID-19 antigen		RT-PCR		Total
		Positive	Negative	
NDH testing kit	Positive	103	2	105
	Negative	2	329	331
Total		105	331	436

Sensitivity (PPA): 98.10% (103/105), (95% CI: 93.32%, 99.48%)

Specificity (NPA): 99.40% (329/331), (95% CI: 97.82%, 99.83%)

B. Anterior nasal Swab Specimen (nose front)

The clinical performance of COVID-19 Antigen Rapid Test Cassette was established in prospective studies with nasal

swabs collected from 436 individual symptomatic patients (within 7 days of onset) and asymptomatic patients who were suspected of COVID-19.

COVID-19 antigen		RT-PCR		Total
		Positive	Negative	
NDH testing kit	Positive	97	3	100
	Negative	8	328	336
Total		105	331	436

Sensitivity (PPA): 92.38% (97/105), （95% CI: 85.68%，96.09%）
Specificity (NPA): 99.09% (328/331), （95% CI: 97.37%，99.69%）

C. Saliva/Sputum Specimen

The clinical performance of COVID-19 Antigen Rapid Test Cassette was established in prospective studies with Saliva/Sputum specimen collected from 436 individual symptomatic patients (within 7 days of onset) and asymptomatic patients who were suspected of COVID-19.

COVID-19 antigen		RT-PCR		Total
		Positive	Negative	
NDH testing kit	Positive	93	2	95
	Negative	12	329	341
Total		105	331	436

Sensitivity (PPA): 88.57% (93/105), （95% CI: 81.08%，93.34%）
Specificity (NPA): 99.40% (329/331), （95% CI: 97.82%，99.83%）

7. High-dose Hook Effect: The COVID-19 Antigen Rapid Test was tested up to $1.0 \times 10^{6.01}$ $TCID_{50}$/ml of inactivated SARS-CoV-2 and no high-dose hook effect was observed.

FLU A Antigen and FLU B Antigen

1. Positive reference rate: eight positive reference specimens (P1-P8) were detected, all results should be positive.

2. Minimum detection limit:

the study used cultured Influenza A/B virus, which is β-propiolactone and heat inactivated and spiked into nasopharyngeal swab specimen.

Influenza A(H1N1): 1.0×10^3 $TCID_{50}$/ml

Influenza A (H3N2)：1.0×10^3 $TCID_{50}$/ml

Influenza A (H1N1pdm09)：5.0×10^3 $TCID_{50}$/ml

Influenza B (Yamagata)：1.0×10^4 $TCID_{50}$/ml

Influenza B (Victoria)：1.0×10^3 $TCID_{50}$/ml

3. Reproducibility: use 10 tests with the same batch number, test the reproducibility reference specimens (R), the result should all be positive, and the signal in T line should be uniform.

4. Cross Reactivity:

To determine the analytical specificity of the Influenza A/B Test, 69 commensal or pathogenic microorganisms (24 viruses, 45 bacteria) that may be present in the upper respiratory tract were tested.

Positive and negative specimens were spiked with these microbes. Bacterial or yeast isolates were evaluated at a concentration of 10^7-10^8 org/ml. Viral isolates were inoculated at a concentration of 10^4-10^8 $TCID_{50}$/ml. Adenovirus 18 and Parainfluenza virus 3 were tested at 10^2 $TCID_{50}$/ml. None of the microorganisms tested yielded a positive result with the influenza-negative samples or interfered with detection of the influenza A or B positive samples. Both the negative and positive respiratory specimens were positive when spiked with influenza A strain A2/Aichi/2/68(H3N2) or influenza B strain Hong Kong 5/72.

Virus other than Influenza A/B viruses

Human adenovirus B, C	Adenovirus type 10, 18
Human coronavirus OC43	Coxsackie virus A9, B5
Human herpesvirus 2, 5	Echovirus 2, 3, 6
Herpes simplex virus 1	Human rhinovirus 2, 14, 16
Measles	Sendai virus
Parainfluenza virus 2,3	Respiratory syncytial virus
Varicella-Zoster	

Bacteria

Acinetobacter calcoaceticus	Bacteroides fragilis	Bordetella pertussis
Bacillus cereus	Bacillus subtilis	Bordetella parapertussis
Branhamella catarrhalis	Chlamydia pneumoniae	Corynebacterium diphtheria
Citrobacter freundii	Enterobacter cloacae	Enterococcus faecalis
Escherichia coli	Gardnerella vaginails	Haemophilus influenzae
Klebsiellaoxytoca	Klebsiella pneumoniae	Lactobacillus casei
Lactobacillus plantarum	Legionella pneumophila	Listeria monocytogenes
Moraxella catarrhalis	Mycobacterium avium	Mycobacterium intracellulare
Mycobacterium tuberculosis	Mycoplasma pneumoniae	Neisseria meningitides
Neisseria sicca	Neisseria subflava	Nocardia asteroids
Proteus vulgaris	Pseudomonas aeruginosa	Serratia liquifaciens
Staphylococcus aureus	Staphylococcus epidermidis	Streptococcus Groups A, B, C, F,
Streptococcus mutants	Streptococcus pneumoniae	Streptococcus salivaris
Streptococcus sanguis	Yersinia enterocolitica	

5. Interference: the following potential interference substances were evaluated with the Influenza A/B Rapid Test at the concentrations listed below and were found not to affect test performance.

Substance	Concentration	Substance	Concentration
3 OTC nasal sprays	10%	Guaiacol glycerol ether	20 mg/ml
3 OTC mouthwashes	10%	Mucin	1%
3 OTC throat drops	10%	Mupirocin	250 μg/ml
4-acetamidophenol	10 mg/ml	Oxymetazoline	10 mg/ml
Acetylsalicylic acid	20 mg/ml	Phenylephrine	10 mg/ml
Albuterol	20 mg/ml	Phenylpropanolamine	20 mg/ml
Chlorpheniramine	5 mg/ml	Relenza ® (zanamivir)	20 mg/ml
Dexamethasone	5 mg/ml	Rimantadine	500 ng/ml
Dextromethorphan	10 mg/ml	Tamiflu ® (oseltamivir)	100 mg/ml
Diphenhydramine	5 mg/ml	Tobramycin	40 mg/ml
Doxylamine succinate	1 mg/ml	Triamcinolone	14 mg/ml
Flunisolide	3 mg/ml		

6. Clinical Performance

Different samples from 436 patients were detected by the Influenza A/B Rapid Test and the cell culture.

A. Nasopharyngeal and Oropharyngeal Swab Specimen

FLU A		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	146	3	149
	Negative	5	282	287
Total		151	285	436

Sensitivity (PPA): 96.69% (146/151), （95% CI: 92.48%，98.58%）

Specificity (NPA): 98.95% (282/285), （95% CI: 96.95%，99.64%）

FLU B		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	160	3	163
	Negative	7	266	273
Total		167	269	436

Sensitivity (PPA): 95.81% (160/167), （95% CI: 91.60%，97.96%）

Specificity (NPA): 98.88% (266/269), （95% CI: 96.77%，99.62%）

B. Anterior nasal Swab Specimen (nose front)

FLU A		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	141	5	146
	Negative	10	280	290
Total		151	285	436

Sensitivity (PPA): 93.38% (141/151), （95% CI: 88.24%，96.36%）

Specificity (NPA): 98.25% (280/285), （95% CI: 95.96%，99.25%）

FLU B		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	152	4	156
	Negative	15	265	280
Total		167	269	436

Sensitivity (PPA): 91.02% (152/167), （95% CI: 85.71%，94.48%）

Specificity (NPA): 98.51% (265/269), （95% CI: 96.24%，99.42%）

C. Saliva/Sputum Specimen

FLU A		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	131	4	135
	Negative	20	281	301
Total		151	285	436

Sensitivity (PPA): 86.75% (131/151), （95% CI: 80.43%，91.26%）

Specificity (NPA): 98.60% (281/285), （95% CI: 96.45%，99.45%）

FLU B		CELL CULTURE		Total
		Positive	Negative	
NDH testing kit	Positive	138	3	141
	Negative	29	266	295
Total		167	269	436

Sensitivity (PPA): 82.63% (138/167), （95% CI: 76.17%，87.63%）

Specificity (NPA): 98.88% (266/269), （95% CI: 96.77%，99.62%）

【Precautions】

1. This kit is for science research use and in vitro detection only, read the instruction careful before the test, and strictly follow the instruction.
2. The collection, storage and testing of the sample should strictly obey *the guide of the laboratory testing technologies of pneumonia caused by the novel coronavirus* and the *guide of biosafety in novel coronavirus laboratory*.
3. This kit is for disposable use. Do not re-use it or use it after expiry.
4. Avoid high temperature during test. The test card and pretreatment reagents shall be restored to room temperature before use to avoid excessive humidity.
5. After test, the used test card and reagents shall be treated as biomedical waste.
6. The desiccant in the aluminum foil pouch, which shall not be taken internally.
7. The specimen shall be regarded as infectious product, and the operation shall be in accordance with the operation specifications of infectious disease laboratory.
8. If you have any questions or suggestions during the use of this kit, please contact the manufacturer.

【Manufacturer】

Manufacturer: Wuhan NanoDiagnosis for Health Biotechnology Co., Ltd
Address: Fl. 1-5, Building B4, Biolake, 666 Gaoxin Avenue, Donghu New Technology Development Zone, Wuhan City, Hubei Province, 430075, P.R.China
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Email: sales@ndh-biotech.com
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EC REP

European Authorized Representative:
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Tel: +31644168999

【Manual Approved/Amended Date】

Approval Date: April 2021

【Definition of Symbols】