SARS-CoV-2 Antigen RAPID TEST KIT

【Instruction for Use】

CE

For in vitro diagnostic use only
Store at 2°C -30°C

(Fluorescence Immunochromatography)

CONTENT

1. INTENDED USE

The novel coronaviruses belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection: asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The genome of coronavirus encodes spike protein, envelope protein, membrane protein and nucleocapsid. In the process of viral assembly, N protein binds to viral RNA and leads to the formation of spiral nucleocapsid. N protein is a highly immunogenic phosphoprotein, which is related to viral genome replication and cell signalling. Because of the conserved sequence of N protein, detection of SARS-CoV-2 N protein is of great clinical significance.

This rapid kit is used for the qualitative detection of SARS-CoV-2 nucleocapsid protein antigen (hereinafter referred to as SARS-CoV-2 N-antigen) in human serum and nasopharyngeal swab and/or oropharyngeal swab samples.

2. TEST PRINCIPLE

This rapid kit uses a fluorescence immunochromatography method to detect SARS-CoV-2 N antigen. The sample to be tested is applied to the sample window of the test cassette.

The SARS-CoV-2 N-antigen in the sample forms a complex with the antibody labeled with fluorescent microspheres. This complex migrates along the membrane and reaches the test region (T-line) on which a second antibody against the SARS-CoV-2 N-antigen is applied. Unbound fluorescent microspheres migrate along the membrane to the control region (C-line) and are bound by the control region anti-body. The test result in the test window is made visible with a UV lamp with a wavelength of 365 nm. If both the T-line and the C-line fluoresce, the test result is SARS-CoV-2 N-antigen positive; if only the C-line fluoresces and no T-line becomes visible, the test result is SARS-CoV-2 N-antigen negative. If no C-line becomes visible the test result is invalid and the sample must be retested with a new test cassette.

3. KIT COMPONENTS

25 Test Cassettes

25 Pipettes

1 Instruction For Use

25 Swabs

2 Extraction Buffer (4 mL)

25 Extraction Vials/Caps

4. WARNINGS AND PRECAUTIONS

4.1. For in vitro diagnostic use only. Do not use after expiration date.

4.2. Samples should be considered as potentially infectious. Operators should wear protective clothing, masks, gloves and are advised to take other appropriate safety precautions to avoid or reduce the risk of infection.

4.3. This test should be performed at 18-30°C. The test and samples must be brought to room temperature before the test is performed.

4.4. Follow the instructions for use carefully. The accuracy of the assay results cannot be guaranteed if there is any deviation from the instructions For Use.

4.5. Operators must handle the potentially contaminated materials safely according to local requirements.

4.6. Wipe and wash away any sample spills with highly effective disinfectant. Avoid splashing and the formation of aerosols.

4.7. Use a new clean disposable pipette/extraction vial for each sample to avoid cross contamination.

4.8. Do not look into the UV light directly.

4.9. Dispose of all samples and potentially contaminated materials as if they were infectious waste in a biohazard waste container.

4.10. Once the test cassette is removed from the pouch, perform the test as soon as possible to avoid being humidified. The test cassette is sensitive to humidity as well as to heat.

4.11. Do not use the test cassette if the pouch is damaged or if the seal is broken.

4.12. The test cassette cannot be reused.

5. STORAGE CONDITIONS AND SHELF LIFE

The test can be stored at 2°C-30°C for 12 months from the date of manufacture. The test cassette inside the foil bag shall be used within 1 hour after opening.

6. APPLICABLE INSTRUMENTS

UV light with a wavelength of 365nm.

7. SAMPLE REQUIREMENTS

7.1 Applicable to human serum and to nasopharyngeal swab and/or oropharyngeal swab samples.

7.2. It is recommended that the samples are tested at the time of sample collection.

7.3. If the swab samples are not tested immediately, they should be stored in a dry and clean tube tightly sealed (place tip of swab into a tube and snap/cut off the applicator stick).

The swabs can be stored at 2–8°C for up to 24 hours.

7.4. Serum samples can be stored for 5 days at 2–8°C. For long-term storage the sample should be stored at –20°C. Avoid repeated freezing and thawing of samples. The samples can be subjected to a maximum of 3 freezing/thawing cycles.

- Let the serum reach room temperature and mix well before testing. If there are visible particles in the serum, it should be centrifuged in order to remove the precipitate.
- If there is a lot of lipid (Triglyceride concentration over 37 mmol/L), hemolysis or turbidity in the serum, please do not use the sample to avoid affecting the result interpretation.

8. MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- UV light with a wavelength of 365nm
- Sample vortex mixer

9. COLLECTION OF SWAB SAMPLES

9.1. The test can be performed according to the standard nasopharyngeal swab or oropharyngeal swab sample collection procedure.

9.2. Nasopharyngeal swab sample collection: Tilt back the head of the patient 70 degrees. Insert swab into nostril (swab should reach depth equal to the distance from nostrils to outer opening of the ear). Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.

9.3. Oropharyngeal swab sample collection: Insert swab into the posterior pharynx and tonsillar areas. Rub swab over both tonsils and posterior oropharynx and avoid touching the tongue, teeth and gums.

9.4. It is recommended that the sample is tested at the time of sample collection.

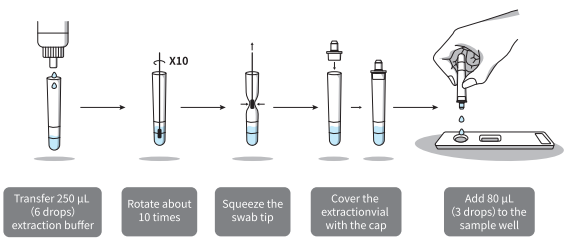
10. TEST PROCEDURE FOR SWAB SAMPLES

Step 1: Transfer 250 µL(6 drops) extraction buffer to the sample extraction vial.

Step 2: Insert the swab which has collected secretions into the extraction buffer and rotate about 10 times to dissolve the sample in the buffer as much as possible.

Step 3: Squeeze out the swab tip by pressing the side of the extraction tube to keep as much liquid as possible in the tube.

Step 4: Cover the vial with the cap.

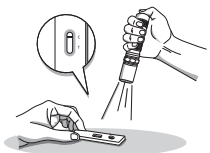


Step 5: Tear open the aluminum foil bag, take out the test cassette and place it on a horizontal surface.

Step 6: Write the sample number on the test cassette.

Step 7: Apply 80 µL (3 drops) of the sample extract into the sample hole of the test cassette. Ensure that there is no bubble during the operation.

Step 8: After 15 minutes have elapsed observe the test results by illuminating the interpretation window with the fluorescent flash light. Interpret the result within 10 seconds under the illumination of the fluorescent flash light. Long time exposure under the UV light will cause a diminishing of the fluorescent signal and may affect the interpretation of the result.



Observe the result
immediately under the UV flashlight

11. TEST PROCEDURES FOR SERUM SAMPLES

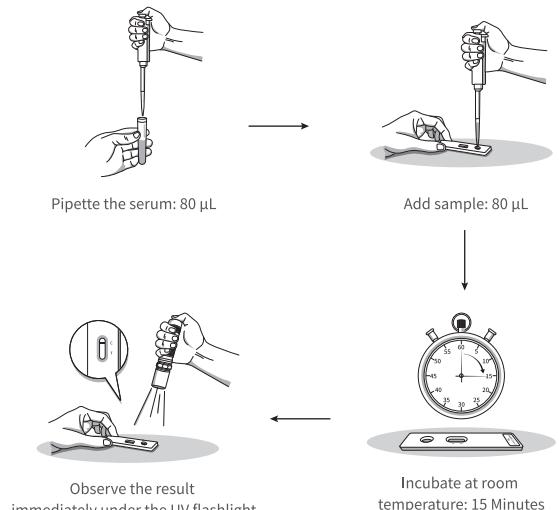
Step 1: Take out the test cassette and sample to be tested and let it reach room temperature.

Step 2: Tear open the aluminium foil bag, take out the test cassette and place it on a horizontal surface.

Step 3: Write the sample number on the test cassette.

Step 4: Pipette 80 µL (3 drops with the included pipette) of the sample to be tested and apply it into the sample hole on the test cassette. Ensure that there is no bubble during the operation.

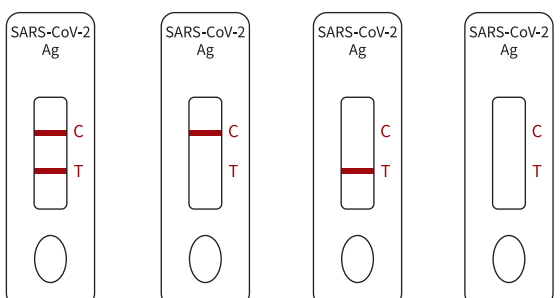
Step 5: After 15 minutes have elapsed observe the test results by illuminating the interpretation window with the fluorescent flash light. Interpret the result within 10 seconds under the illumination of the fluorescent flash light. Long time exposure under the UV light will cause a diminishing of the fluorescent signal and may affect the interpretation of the result.



12. INTERPRETATION OF THE RESULTS

12.1. Under the UV flashlight, if a visible red fluorescent band appears in the detection area(T) and the control region (C) at the same time, the test is SARS-CoV-2-N-protein positive; if a red fluorescent band becomes visible in the control region(C),

and no visible red fluorescent band in the detection area(T), the test is SARS-CoV-2-N-proteinis negative;if there is no visible red fluorescent band in the control region(C), regardless of whether there is a red fluorescent band visible to in the detection area(T), the test result is invalid and the sample needs to be tested again with a new test cassette.



Positive result　Negative result　Invalid result　Invalid result

12.2. Due to the complex structure of bioactive substances in samples and the difference of antigen antibody specificity, the possibility of false positive results cannot be completely ruled out when using this kit. If the test results are inconsistent with the clinical indications, other appropriate test methods should be used for confirmation.

12.3. If the SARS-CoV-2 N-protein is positive, it is an indicator SARS-CoV-2 infection. A negative result of SARS-CoV-2 N-antigen cannot completely rule out a SARS-CoV-2 infection. A negative result can be caused if the sample is below the detection limit or if the anti-N-antigen antibodies have been produced and are present in the serum which decreases the N-antigen.

12.4. The test results of this kit are only used as the basis of auxiliary diagnosis. Clinical diagnosis should be combined with clinical symptoms and other diagnostic methods.

13. LIMITATION OF THE PROCEDURES

13.1. Hyperlipidemia, hemolysis samples, samples contaminated with microorganisms, repeated freezing and thawing more than 3 times or serum samples after heat inactivation may affect the accuracy of the detection and may lead to erroneous results.

13.2. Serum samples with severe jaundice or serious contamination may lead to false results.

13.3. The accuracy of the test depends on the sample collec-

tion process. Improper sample collection, improper sample storage or repeated freezing and thawing of the sample may affect the test result.

14. PERFORMANCE CHARACTERISTICS

14.1. Detection Limit

The detection limit (LoD) for serum samples was determined with negative serum samples added with recombinant N-antigen; the test was repeated 60 times, 3.5 pg/mL has been determined as the LoD. The LoD for negative swab samples was determined with swab samples added with recombinant N-antigen; the test was repeated 60 times, 7.0 pg/mL has been determined as the LoD.

14.2. Virus Detection Limit

Novel coronavirus stock solution (2.0×10^4 $TCID_{50}$/mL) (IVCAS 6.7512), that has been inactivated at 56°C for 30 minutes has been diluted to 200 $TCID_{50}$/mL, 100 $TCID_{50}$/mL, 40 $TCID_{50}$/mL, 20 $TCID_{50}$/ mL, 10 $TCID_{50}$/mL, 5 $TCID_{50}$/mL samples. Each sample was tested 3 times.

Test Concentration ($TCID_{50}$/mL)	Test times	Test Result Serum Samples	Test Result Swab Specimen
20000	3	3/3 Positive	3/3 Positive
200	3	3/3 Positive	3/3 Positive
100	3	3/3 Positive	3/3 Positive
40	3	3/3 Positive	3/3 Positive
20	3	3/3 Positive	1/3 Positive
10	3	1/3 Positive	0/3 Positive
5	3	0/3 Positive	0/3 Positive

The limit of detection for serum samples is determined at 20 $TCID_{50}$/mL and the limit of detection for swab samples was determined at 40 $TCID_{50}$/mL.

14.3 Cross-reactivity Studies

The cross-reactivity was evaluated by testing a panel of microbials that could potentially cross-react with the SARS-CoV-2 Antigen rapid test in serum and swab samples. The results do not show any cross reactivity with the below listed microbial substances:

Microbial Substance	Test Concentration	Cross-reactivity Results
Escherichia coli	1.0×10^{6} CFU/mL	Negative
Hepatitis C Virus (HCV)	1.2×10^{5} $TCID_{50}$/mL	Negative
Hepatitis B Virus (HBV)	2.2×10^{5} $TCID_{50}$/mL	Negative
Influenza B	$1.0\times10^{6.67}$ $TCID_{50}$/mL	Negative
Influenza A	$1.0\times10^{5.67}$ $TCID_{50}$/mL	Negative
Herpes Simplex Virus-1 (HSV-1)	1.6×10^{5} $TCID_{50}$/mL	Negative
Herpes Simplex Virus-2 (HSV-2)	2.1×10^{5} $TCID_{50}$/mL	Negative
Human Immunodeficiency Virus – 1 (HIV-1)	3.2×10^{5} $TCID_{50}$/mL	Negative
Enterovirus	3.6×10^{5} $TCID_{50}$/mL	Negative
Staphylococcus epidermidis	1.0×10^{6} CFU/mL	Negative
Legionella pneumophila	3.5×10^{6} CFU/mL	Negative
Chlamydia pneumoniae	1.7×10^{6} CFU/mL	Negative
Mycoplasma pneumoniae	1.5×10^{6} CFU/mL	Negative
Parainfluenza virus	1.0×10^{5} $TCID_{50}$/mL	Negative
Respiratory syncytial virus	2.1×10^{5} $TCID_{50}$/mL	Negative
Adenovirus	1.0×10^{5} $TCID_{50}$/mL	Negative
Cytomegalovirus (CMV)	1.0×10^{5} $TCID_{50}$/mL	Negative
Epstein-Barr Virus (EBV)	1.0×10^{5} $TCID_{50}$/mL	Negative
Varicella Zoster Virus (VZV)	1.0×10^{5} $TCID_{50}$/mL	Negative
Parvovirus B19	1.0×10^{5} $TCID_{50}$/mL	Negative
Streptococcus pneumoniae	1.0×10^{6} CFU/mL	Negative
Streptococcus pyogenes	1.6×10^{6} CFU/mL	Negative
Staphylococcus aureus	1.2×10^{6} CFU/mL	Negative
Human coronavirus 229E	1.3×10^{5} $TCID_{50}$/mL	Negative
Human coronavirus OC43	1.5×10^{5} $TCID_{50}$/mL	Negative
Human coronavirus (NL63)	1.0×10^{5} $TCID_{50}$/mL	Negative
MERS	1.0×10^{5} $TCID_{50}$/mL	Negative

14.4. Interference Studies

14.4.1. Endogenous Interference Substances Studies

The endogenous interference substances listed below do not interfere with the test results of the SARS -CoV- 2 antigen rapid test:

Interfering Substance	Concentration
Bilirubin	0.3mg/mL
Triglyceride	37 mmol/L
Hemoglobin	1 mg/mL

α - interferon	2000 IU/mL
Zanamivir	142 ng/mL
Ribavirin	6 μg/mL
Oseltamivir	40 μg/mL
Levofloxacin	40 mg/mL
Ceftriaxone	156 μg/mL
Meropenem	0.2 mg/mL
Tobramycin	4 μg/mL
HAMA	600 ng/mL

14.4.2. Microbial Interference Studies:

The following pathogens had no influence on the test results on SARS-CoV-2 N-antigen positive samples in the tested concentration:

Microbial Interfering Substance	Test Concentration	Interference Results
Escherichia coli	1.0×10^{6} CFU/mL	Positive
Hepatitis C Virus (HCV)	1.2×10^{5} $TCID_{50}$/mL	Positive
Hepatitis B Virus (HBV)	2.2×10^{5} $TCID_{50}$/mL	Positive
Influenza B	$1.0\times10^{6.67}$ $TCID_{50}$/mL	Positive
Influenza A	$1.0\times10^{5.67}$ $TCID_{50}$/mL	Positive
Herpes Simplex Virus-1 (HSV-1)	1.6×10^{5} $TCID_{50}$/mL	Positive
Herpes Simplex Virus-2 (HSV-2)	2.1×10^{5} $TCID_{50}$/mL	Positive
Human Immunodeficiency Virus – 1 (HIV-1)	3.2×10^{5} $TCID_{50}$/mL	Positive
Enterovirus	3.6×10^{5} $TCID_{50}$/mL	Positive
Staphylococcus epidermidis	1.0×10^{6} CFU/mL	Positive
Legionella pneumophila	3.5×10^{6} CFU/mL	Positive
Chlamydia pneumoniae	1.7×10^{6} CFU/mL	Positive
Mycoplasma pneumoniae	1.5×10^{6} CFU/mL	Positive
Parainfluenza virus	1.0×10^{5} $TCID_{50}$/mL	Positive
Respiratory syncytial virus	2.1×10^{5} $TCID_{50}$/mL	Positive
Adenovirus	1.0×10^{5} $TCID_{50}$/mL	Positive
HAMA	600 ng/mL	Positive
Cytomegalovirus (CMV)	1.0×10^{5} $TCID_{50}$/mL	Positive
Epstein-Barr Virus (EBV)	1.0×10^{5} $TCID_{50}$/mL	Positive
Varicella Zoster Virus (VZV)	1.0×10^{5} $TCID_{50}$/mL	Positive
Parvovirus B19	1.0×10^{5} $TCID_{50}$/mL	Positive
Streptococcus pneumoniae	1.0×10^{6} CFU/mL	Positive
Streptococcus pyogenes	1.6×10^{6} CFU/mL	Positive

Human coronavirus 229E	1.3×10^5 $TCID_{50}$/mL	Positive
Human coronavirus OC43	1.5×10^5 $TCID_{50}$/mL	Positive
Human coronavirus (NL63)	1.0×10^5 $TCID_{50}$/mL	Positive
MERS	1.0×10^5 $TCID_{50}$/mL	Positive

14.5. Hook Effect

200 ng/mL recombinant N protein has been prepared with negative serum-and negative swab samples. No Hook effect was observed.

14.6. Clinical Evaluation

Site 1 (Germany): The sensitivity of the test using swab samples was determined with 85 PCR confirmed positive swab samples with a Ct value ⩽29. The specificity was determined with 250 PCR confirmed negative swab samples. The sensitivity and specificity of the test was compared to a commercialize PCR test. A sensitivity of 97.6% and a specificity of 99.6% were determined for the SARS-CoV-2 Antigen RAPID TEST KIT.

		PCR	
		Positive	Negative
SARS-CoV-2 Antigen RAPID TEST KIT	Positive	83	1
	Negative	2	249
	Total	85	250
Sensitivity		97.6% (93.17%-98.7%)	
Specificity		99.6% (97.3%-99.9%)	

Site 2 (China): The sensitivity of the test with serum samples was determined in a retrospective study with 62 by PCR confirmed COVID-19 patients. The serum samples were collected on the same day as the swab samples. The test results were as follows:

Days from onset of symptoms	Antigen positive	Total number	Sensitivity
0-3	27	29	93.10% (95CI: 77.23%~99.15%)
4-7	33	33	100.00% (95CI: 89.42%～100.00%)
⩽7	60	62	96.77% (95CI: 88.83%~99.61%)

The specificity of the test with serum samples was determined in a retrospective study with 188 samples that were confirmed negative by PCR. The test results were as follows:

Antigen negative	Total number	Specificity
186	188	98.9% (95CI: 96.21%~99.87%)

15. PROCEDURAL NOTES

15.1. Read this manual carefully before performing the test.

15.2. Testing needs to be performed under proper testing conditions. All samples and materials shall be handled according to the local requirements for infectious diseases laboratory.

15.3. Protect the test cassette from moisture.

15.4. All reagents and samples should reach room temperature before use.

15.5. Do not use lipemic samples.

15.6. Do not use hemolytic samples.

15.7. Do not use turbid or contaminated samples.

15.8. Do not store this kit in a frozen condition.

15.9. The interpretation of the test results must be carried out in strict accordance with this manual.

16. EXPLANATION OF THE SYMBOLS USED

IVD	In vitro diagnostic medical device
REF	Catalogue Number
LOT	Batch Code
	Manufacturer
	Date of Manufacture
	Use by date
	Do Not Use if Package is Damaged
	Consult Instruction for Use
2°C 30°C	Temperature Limit at 2°C~30°C.
Σ 25	Contents Sufficient for 25 Tests
	Do Not Re-use
	Caution
	Keep Dry

17. GENERAL INFORMATION

Applicant/ Manufacturer

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