REF: 208.05.25.01

SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography)

Instructions for Use

CE

For Professional Use Only
For in vitro diagnostic use only
Store at 2℃ - 30℃

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, plasma (heparin, dipotassium EDTA, and sodium citrate) and fingertip blood.

2. TEST PRINCIPLE

The test analyte in the sample will form a complex with the fluorescent microsphere-labelled antibody on the conjugate pad under chromatography, and the complex will continue to be chromatographed on the nitrocellulose membrane until reaching test line (T line), then captured by the test line antibody. The unbound microspheres are chromatographed to the quality control line (C line) and captured by the antibody on the quality control line. The fluorescence signal intensity can be detected and analyzed by BOH-180 Fluorescent Immunoassay Analyzer. The analyzer converts the fluorescence signals into corresponding concentrations.

3. KIT COMPONENTS

1 Instructions for Use
1 Extraction Buffer (4 ml)
25 Test Cassettes
25 Droppers

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 15-30℃. The test and samples must be brought to room temperature before testing.

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. Use new clean disposable pipette, tip and tube for each sample to avoid cross contamination.

4.7. Although the test kit uses detergents in the extraction buffer which neutralize SARS-CoV-2,

dispose of all samples and materials as if they were infectious waste in a biohazard waste container.

4.8. 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.

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

4.10. The test cassette cannot be reused.

5. STORAGE CONDITIONS AND SHELF LIFE

The test can be stored at 2℃-30℃ 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

BOH-180 Fluorescent Immunoassay Analyzer produced by Biohit Healthcare (Hefei) Co., Ltd.

7. SAMPLE REQUIREMENTS

7.1 Applicable to human serum, plasma (heparin, dipotassium (K_2)-EDTA, and fingertip blood .

7.2 For serum and plasma (heparin, K_2-EDTA, and sodium citrate) samples, the samples should be tested immediately after collection. Serum and plasma (heparin, K_2-EDTA, and sodium citrate) samples can be stored for 5 days at 2-8℃. If long-term storage is required, it should be stored at -20℃ (It has been confirmed that the sample can be stored for 3 months at -20℃). Serum or plasma specimens can be subjected to a maximum of 3 freezing/ thawing cycles.

7.3 For fingertip blood, a safety lancet is recommended to make a finger prick. After puncturing the skin, use clean gauze to wipe away the first drop of blood to avoid specimen dilution with interstitial fluid. With the patient’s hand pointing downward, firmly grasp the finger towards the base with the thumb placed along the length of the patient's finger. Gently massage along the length of the finger towards the tip, using a light squeeze-and-release motion to allow large droplets of blood to form and encourage continuous blood flow. If using a capillary tube or pipette, allow a large drop of blood to form, position the device horizontally, and lightly touch the drop of blood (avoid touching the skin); allow the blood drop to be drawn into the collection vessel by capillary action (avoid air bubbles).

7.4 Let the samples reach room temperature and mix well before testing. When there are visible particles, the sample should be centrifuged before the test to remove the particles.

8. MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Quality control
- Sample Vortex Mixer
- 10-100μL Pipette and Tips
- Lancet
- Test Tubes

9. TEST PROCEDURE

Step 1: Take out the sample to be tested and let it reach room temperature. Mix the sample well before testing.

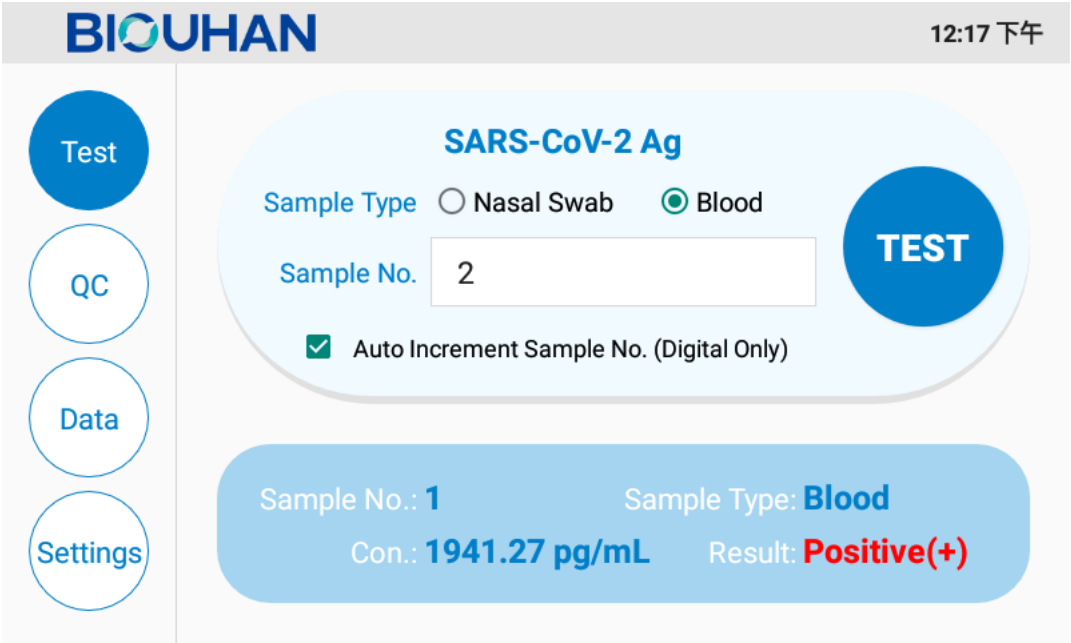
Step 2: Tear the aluminium foil bag to open, take out the test cassette and place it on the horizontal surface.

Step 3: Pipette 10μL from the tube for serum and plasma samples, draw 1 drop (20μL) using the dropper for fingertip blood sample to the sample well of test cassette. Apply 2 drops of extraction buffer to the sample well.

Step 4: After 15 minutes, insert the test cassette into the cassette slot of BOH-180. Press Test, and BOH-180 will automatically run test and generate test results.

10. INTERPRETATION OF THE RESULTS

10.1. The analyzer converts the fluorescence signals into corresponding concentrations. Concentration higher than or equal to 8.92 pg/mL indicates SARS-CoV-2 antigen positive while concentration lower than 8.92 pg/mL indicates SARS-CoV-2 antigen negative. If the chromatography is not successful and the analyzer gives "Result: Failure", it indicates that the test fails and the sample needs to be retested with a new test cassette.



10.2. 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.

11. LIMITATION OF THE PROCEDURES

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

11.2. Samples with severe jaundice or serious pollution will lead to wrong results.

11.3. The presence of sodium azide in the sample will affect the experimental results. Sodium azide cannot be used as a sample preservative.

11.4. If the time interval of sample adding is too long, it may cause the deviation of test results.

12. PERFORMANCE CHARACTERISTICS

Limit of Blank

The LoB is confirmed as 5.88 pg/mL.

Limit of Detection

The LoD is confirmed as 8.92 pg/mL.

Linear Range

In the concentration range of 9.60-1545.30 pg/mL, the correlation coefficient (r) is not less than 0.990.

Accuracy

Recovery test is used to evaluate the accuracy of the kit. The recovery rate is in the range of 85.0% ~ 115.0%.

Precision

CVs of intra-assay, inter-assay, intra-day, inter-day, different operators and different labs variation study are not higher than 15%.

Cross-reactivity

The cross-reactivity was evaluated by testing a panel of common organisms that could potentially cross-react with SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography) in serum samples. Results did not show any cross reactivity with the potentially cross-reactive organisms at the concentrations listed below.

No.	Potential Cross-Reactant	Test Concentration	Cross Reactivity (Yes/No)
1	Escherichia coli	1.0×10^{6} CFU/mL	No
2	Hepatitis C Virus (HCV)	1.2×10^{5} $TCID_{50}$/mL	No
3	Hepatitis B Virus (HBV)	2.2×10^{5} $TCID_{50}$/mL	No
4	Influenza B	$1.0\times10^{6.67}$$TCID_{50}$/mL	No
5	Influenza A	$1.0\times10^{5.67}$$TCID_{50}$/mL	No
6	Herpes Simplex -1 (HSV-1)	1.6×10^{5} $TCID_{50}$/mL	No
7	Herpes Simplex Virus-2 (HSV-2)	2.1×10^{5} $TCID_{50}$/mL	No

8	Human Immunodeficiency Virus -1 (HIV-1)	3.2×10^5 $TCID_{50}$/mL	No
9	Enterovirus	3.6×10^5 $TCID_{50}$/mL	No
10	Staphylococcus epidermidis	1.0×10^6 CFU/mL	No
11	Legionella pneumophila	3.5×10^6 CFU/mL	No
12	Chlamydia pneumoniae	1.7×10^6 CFU/mL	No
13	Mycoplasma pneumoniae	1.5×10^6 CFU/mL	No
14	Parainfluenza virus	1.0×10^5 $TCID_{50}$/mL	No
15	Respiratory syncytial virus	2.1×10^5 $TCID_{50}$/mL	No
16	Adenovirus	1.0×10^5 $TCID_{50}$/mL	No
17	Cytomegalovirus (CMV)	1.0×10^5 $TCID_{50}$/mL	No
18	Epstein-Barr Virus (EBV)	1.0×10^5 $TCID_{50}$/mL	No
19	Varicella Zoster Virus (VZV)	1.0×10^5 $TCID_{50}$/mL	No
20	Parvovirus B19	1.0×10^5 $TCID_{50}$/mL	No
21	Streptococcus pneumoniae	1.0×10^6 CFU/mL	No
22	Streptococcus pyogenes	1.6×10^6 CFU/mL	No
23	Staphylococcus aureus	1.2×10^6 CFU/mL	No
24	Human coronavirus 229E	1.3×10^5 $TCID_{50}$/mL	No
25	Human coronavirus OC43	1.5×10^5 $TCID_{50}$/mL	No
26	Human coronavirus (NL63)	1.0×10^5 $TCID_{50}$/mL	No

Microbial Interference Studies

Microbial interference of SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography) was evaluated by testing a panel of high prevalence respiratory pathogens that could potentially interfere with SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography). Results did not show any interference with the microbial substances at the concentrations listed below.

No.	Microbial Substance	Test Concentration	Microbial Interference (Yes/No)
1	Escherichia coli	1.0×10^6 CFU/mL	No
2	Hepatitis C Virus (HCV)	1.2×10^5 $TCID_{50}$/mL	No
3	Hepatitis B Virus (HBV)	2.2×10^5 $TCID_{50}$/mL	No
4	Influenza B	$1.0\times10^{6.67}$$TCID_{50}$/mL	No
5	Influenza A	$1.0\times10^{5.67}$$TCID_{50}$/mL	No
6	Herpes Simplex -1 (HSV-1)	1.6×10^5 $TCID_{50}$/mL	No
7	Herpes Simplex Virus-2 (HSV-2)	2.1×10^5 $TCID_{50}$/mL	No
8	Human Immunodeficiency Virus -1 (HIV-1)	3.2×10^5 $TCID_{50}$/mL	No
9	Enterovirus	3.6×10^5 $TCID_{50}$/mL	No
10	Staphylococcus epidermidis	1.0×10^6 CFU/mL	No
11	Legionella pneumophila	3.5×10^6 CFU/mL	No
12	Chlamydia pneumoniae	1.7×10^6 CFU/mL	No
13	Mycoplasma pneumoniae	1.5×10^6 CFU/mL	No
14	Parainluenza virus	1.0×10^5 $TCID_{50}$/mL	No

15	Respiratory syncytial virus	2.1×10^5 $TCID_{50}$/mL	No
16	Adenovirus	1.0×10^5 $TCID_{50}$/mL	No
17	Cytomegalovirus (CMV)	1.0×10^5 $TCID_{50}$/mL	No
18	Epstein-Barr Virus (EBV)	1.0×10^5 $TCID_{50}$/mL	No
19	Varicella Zoster Virus (VZV)	1.0×10^5 $TCID_{50}$/mL	No
20	Parvovirus B19	1.0×10^5 $TCID_{50}$/mL	No
21	Streptococcus pneumoniae	1.0×10^6 CFU/mL	No
22	Streptococcus pyogenes	1.6×10^6 CFU/mL	No
23	Staphylococcus aureus	1.2×10^6 CFU/mL	No
24	Human coronavirus 229E	1.3×10^5 $TCID_{50}$/mL	No
25	Human coronavirus OC43	1.5×10^5 $TCID_{50}$/mL	No
26	Human coronavirus (NL63)	1.0×10^5 $TCID_{50}$/mL	No

Endogenous Interference

The endogenous Interference was evaluated by testing a panel of potentially interfering substances in negative and 3xLoD samples containing recombinant SARS-CoV-2 N protein. The endogenous interference substances listed below did not interfere with the test results of the SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography).

No.	Interfering Substance	Concentration	Endogenous **Interference (Yes/No)**
1	Bilirubin	0.3mg/mL	No
2	Triglyceride	37 mmol/L	No
3	Hemoglobin	10mg/mL	No
4	α - interferon	2ng/mL	No
5	Zanamivir	142ng/mL	No
6	Ribavirin	6μg/mL	No
7	Oseltamivir	1μg/mL	No
8	Levofloxacin	2 mg/mL	No
9	Ceftriaxone	156μg/mL	No
10	Meropenem	0.2 mg/mL	No
11	Tobramycin	4μg/mL	No

High-dose Hook Effect

No high-dose hook effect was observed when tested with up to a concentration of 100 ng/ml of recombinant SARS-CoV-2 N protein with the SARS-CoV-2 Antigen Rapid Test Kit.

Clinical Evaluation

The sensitivity of the test was determined with 178 PCR confirmed positive serum samples. The specificity was determined with 357 PCR confirmed negative serum samples. The sensitivity and specificity of the test was compared to a commercial PCR test. A sensitivity of 96.63% and a specificity of 99.72% were determined for the SARS-CoV-2 Antigen Rapid Test Kit.

		PCR results	
		Positive	Negative
SARS-CoV-2 Antigen RAPID TEST Kit	Positive	172	1
	Negative	6	356
	Total	178	357
Sensitivity		96.63% (95CI:92.81%-98.75%)	
Specificity		99.72% (95CI: 98.45%-99.99%）	

13. PROCEDURAL NOTES

13.1. Read the Instructions for Use carefully before performing the test.

13.2. Testing needs to be performed under proper testing conditions.

13.3. Protect the test cassette from moisture.

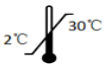
13.4. All reagents and samples should reach room temperature before use.

13.5. Do not use turbid or contaminated samples.

13.6 False negative results will be caused when the antigen titer in the sample is lower than the detection limit.

14. 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

	Temperature limit at 2 ℃~30 ℃.
	Contents sufficient for 25 tests.
	Do not re-use
	Caution
	Keep dry

15. GENERAL INFORMATION

Manufacturer

Name: Biohit Healthcare (Hefei) Co., Ltd.

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Authorized representative in the European Union

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16. DATE OF ISSUE

SARS-CoV-2 Antigen Rapid Test Kit (Fluorescence Immunochromatography) insert.

Version 01, June 07, 2021.