

# **SARS-CoV-2 Antigen RAPID TEST Kit**

(Fluorescence Immunochromatography)

## **Instruction for Use**

**CE**

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

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## 1. INTENDED USE

The novel coronaviruses belong to the  $\beta$  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-anti-gen 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 (4mL)
- 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

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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.

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## 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.

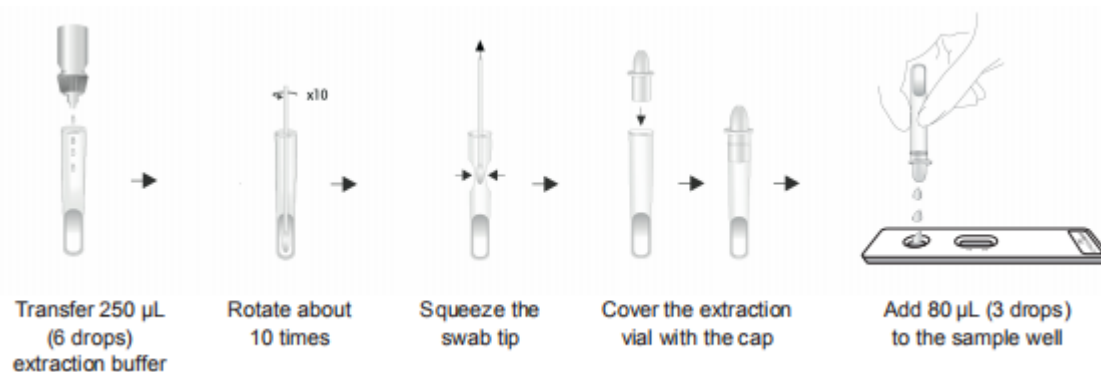
## 10. TEST PROCEDURE FOR SWAB SAMPLES

Step 1: Transfer 250  $\mu$ L (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 flashlight. Interpret the result within 10 seconds under the illumination of the fluorescent flashlight. Long time exposure under the UV light will cause a diminishing of the fluorescent signal and may affect the interpretation of the result.



## 11. TEST PROCEDURES FOR SERUM SAMPLES

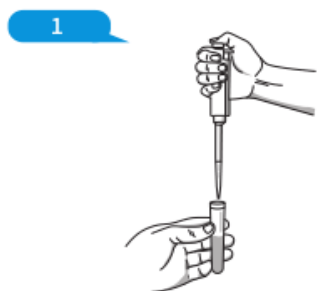
Step 1: Take out the test cassette and sample to be tested and let it reach room temperature.

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

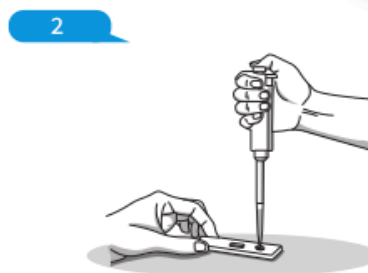
Step 3: Write the sample number on the test cassette.

Step 4: Pipette 80µl (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.

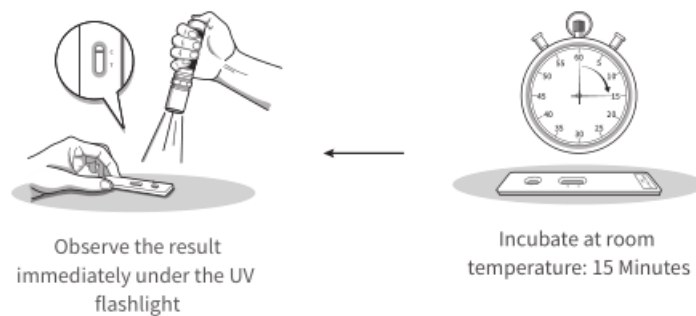
Step 5: After 15 minutes have elapsed observe the test results by illuminating the interpretation window with the fluorescent flashlight. Interpret the result within 10 seconds under the illumination of the fluorescent flashlight. Long time exposure under the UV light will cause a diminishing of the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



Pipette the serum: 80µl

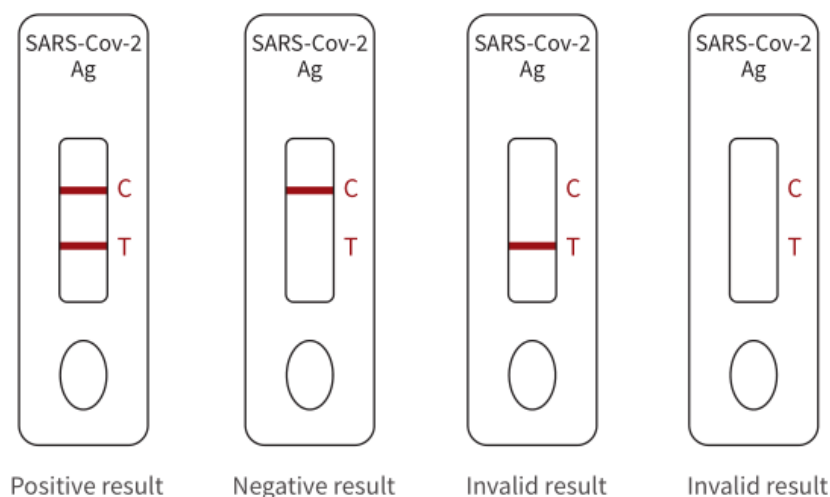


3 drops (≅ 80µl)



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



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

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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 collection 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 ( $2.0 \times 10^4$  TCID<sub>50</sub>/ml) (IVCAS 6.7512), that has been inactivated at 56°C for 30 minutes has been diluted to 200 TCID<sub>50</sub>/ml, 100 TCID<sub>50</sub>/ml, 40 TCID<sub>50</sub>/ml, 20 TCID<sub>50</sub>/ ml, 10 TCID<sub>50</sub>/ml, 5 TCID<sub>50</sub>/ml samples. Each sample was tested 3 times.

| Test Concentration (TCID <sub>50</sub> /ml) | Test cycles | 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<sub>50</sub> /ml and the limit of detection for swab samples was determined at 40 TCID<sub>50</sub> /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                         | $1.0 \times 10^6$ CFU/ml                      | Negative                 |
| Hepatitis C Virus (HCV)                  | $1.2 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Hepatitis B Virus (HBV)                  | $2.2 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Influenza B                              | $1.0 \times 10^{6.67}$ TCID <sub>50</sub> /ml | Negative                 |
| Influenza A                              | $1.0 \times 10^{5.67}$ TCID <sub>50</sub> /ml | Negative                 |
| Herpes Simplex Virus-1 (HSV-1)           | $1.6 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Herpes Simplex Virus-2 (HSV-2)           | $2.1 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Human Immunodeficiency Virus – 1 (HIV-1) | $3.2 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Enterovirus                              | $3.6 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Staphylococcus epidermidis               | $1.0 \times 10^6$ CFU/ml                      | Negative                 |
| Legionella pneumophila                   | $3.5 \times 10^6$ CFU/ml                      | Negative                 |
| Chlamydia pneumoniae                     | $1.7 \times 10^6$ CFU/ml                      | Negative                 |
| Mycoplasma pneumoniae                    | $1.5 \times 10^6$ CFU/ml                      | Negative                 |
| Parainfluenza virus                      | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Respiratory syncytial virus              | $2.1 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Adenovirus                               | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Cytomegalovirus (CMV)                    | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Epstein-Barr Virus (EBV)                 | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Varicella Zoster Virus (VZV)             | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Parvovirus B19                           | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Streptococcus pneumoniae                 | $1.0 \times 10^6$ CFU/ml                      | Negative                 |
| Streptococcus pyogenes                   | $1.6 \times 10^6$ CFU/ml                      | Negative                 |
| Staphylococcus aureus                    | $1.2 \times 10^6$ CFU/ml                      | Negative                 |
| Human coronavirus 229E                   | $1.3 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Human coronavirus OC43                   | $1.5 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| Human coronavirus (NL63)                 | $1.0 \times 10^5$ TCID <sub>50</sub> /ml      | Negative                 |
| MERS                                     | $1.0 \times 10^5$ TCID <sub>50</sub> /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/ml     |
| Hemoglobin            | 1 mg/ml        |
| $\alpha$ - interferon | 2000 IU/ml     |
| Zanamivir             | 142ng/ml       |
| Ribavirin             | 6 $\mu$ g/ml   |
| Oseltamivir           | 40 $\mu$ g/ml  |
| Levofloxacin          | 40 mg/ml       |
| Ceftriaxone           | 156 $\mu$ g/ml |
| Meropenem             | 0.2 mg/ml      |
| Tobramycin            | 4 $\mu$ g/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                         | $1.0 \times 10^6$ CFU/ml                      | Positive             |
| Hepatitis C Virus (HCV)                  | $1.2 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Hepatitis B Virus (HBV)                  | $2.2 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Influenza B                              | $1.0 \times 10^{6.67}$ TCID <sub>50</sub> /ml | Positive             |
| Influenza A                              | $1.0 \times 10^{5.67}$ TCID <sub>50</sub> /ml | Positive             |
| Herpes Simplex Virus-1 (HSV-1)           | $1.6 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Herpes Simplex Virus-2 (HSV-2)           | $2.1 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Human Immunodeficiency Virus – 1 (HIV-1) | $3.2 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Enterovirus                              | $3.6 \times 10^5$ TCID <sub>50</sub> /ml      | Positive             |
| Staphylococcus epidermidis               | $1.0 \times 10^6$ CFU/ml                      | Positive             |
| Legionella pneumophila                   | $3.5 \times 10^6$ CFU/ml                      | Positive             |
| Chlamydia pneumoniae                     | $1.7 \times 10^6$ CFU/ml                      | Positive             |
| Mycoplasma pneumoniae                    | $1.5 \times 10^6$ CFU/ml                      | Positive             |

|                              |                                            |          |
|------------------------------|--------------------------------------------|----------|
| Parainfluenza virus          | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Respiratory syncytial virus  | 2.1×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Adenovirus                   | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| HAMA                         | 600 ng/ml                                  | Positive |
| Cytomegalovirus (CMV)        | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Epstein-Barr Virus (EBV)     | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Varicella Zoster Virus (VZV) | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Parvovirus B19               | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Streptococcus pneumoniae     | 1.0×10 <sup>6</sup> CFU/ml                 | Positive |
| Streptococcus pyogenes       | 1.6×10 <sup>6</sup> CFU/ml                 | Positive |
| Staphylococcus aureus        | 1.2×10 <sup>6</sup> CFU/ml                 | Positive |
| Human coronavirus 229E       | 1.3×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Human coronavirus OC43       | 1.5×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| Human coronavirus (NL63)     | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |
| MERS                         | 1.0×10 <sup>5</sup> TCID <sub>50</sub> /ml | Positive |

#### 14.5 Hook Effect

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

#### 14.6 Clinical Evaluation

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%)  |          |

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 | PPA                              |
|-----------------------------|------------------|--------------|----------------------------------|
| 0-3                         | 27               | 29           | 93.10%<br>(95CI:77.23%~99.15%)   |
| 4-7                         | 33               | 33           | 100.00%<br>(95CI:89.42%~100.00%) |
| ≤7                          | 60               | 62           | 96.77%<br>(95CI:88.83%~99.61%)   |

The specificity of the test using 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%<br>(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

|                                                                                     |                                    |
|-------------------------------------------------------------------------------------|------------------------------------|
|  | In vitro diagnostic medical device |
|  | Catalogue Number                   |
|  | Batch Code                         |
|  | Manufacturer                       |

|                                                                                   |                                  |
|-----------------------------------------------------------------------------------|----------------------------------|
|  | Date of Manufacture              |
|  | Use by date                      |
|  | Do Not Use if Package is Damaged |
|  | Consult Instruction for Use      |
|  | Temperature Limit at 2°C~30°C.   |
|  | Contents Sufficient for 25 Tests |
|  | Do Not Re-use                    |
|  | Caution                          |
|  | Keep Dry                         |

## 17.GENERAL INFORMATION

### Applicant/ Manufacturer

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

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