

Beijing Savant Biotechnology Co., Ltd.

New Coronavirus (SARS-CoV-2) N Protein Detection Kit (Fluorescence Immunochromatography)

Emergency Use Authorization

April

Cross-reactivity (Analytical Specificity) test

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1. PURPOSE

Verify the analytical specificity of the test kit.

2. DEVICE USED IN THIS STUDY

Savant-100 Fluorescence immunochromatographic analyzer and test kit was tested in this study.

3. METHOD

This study is divided into three parts according to the method of obtaining test samples.

3.1 test using positive pathogenic samples stored in pathogen monitoring

The first part was performed at the Tianjin Center for Disease Control and Prevention in China.

Use of positive pathogenic samples stored in routine pathogenic surveillance.

20 healthy human oropharyngeal swab samples and 24 samples corresponding to 23 viruses and 1 pathogen was tested using our test kit.

The 24 samples used include:

- (1) 10 strains,
- (2) 9 swabs
- (3) 2 countries issued inactivated viruses Strains
- (4) 2 stool suspensions and
- (5) 1 serum sample

Among all the 24 samples listed above, 17 samples were tested once with the test kit as positive stock solution , and 7 samples were tested once with the test kit after dilution.

3.2 test using positive and negative reference materials from the China CDC of China.

This section is performed in the laboratory.

The reference materials used in this test include 1 virus and 2 microorganisms from China National Institute of Food and Drug Control.

Dissolve the reference material in 500 μ L of deionized water, and each reference material was tested once with a test kit.

3.3 test using genetically engineered virus recombinant N proteins

This section is performed in the laboratory.

Genetically engineered virus recombinant N proteins was tested, include 4 species from Nanjing Keltai Biotechnology Technology Co., Ltd.

Dilute the recombinant N protein to 1 $\mu\text{g}/\text{mL}$ with the sample preservation solution, and test each virus recombinant N protein with the test kit once.

4. **CRITIERIA**

4.1 When the test result of the sample is negative, the pathogen or microorganism in the sample is considered to have no cross-reaction at this concentration;

4.2 When the test result of the sample is positive, continue to dilute the sample and find the lowest concentration that produces a positive measurement result.

5. RESULTS AND ANALYSIS

5.1 healthy human oropharyngeal swab samples result

All the 20 healthy human oropharyngeal swab samples tested in the section 3.1 of this file were negative.

5.2 pathogen samples result

Please see result listed below:

Table1 Results of different samples

NO.	Substance	Source	Type	Dilution multiple	Test Kit Result
1	Human coronavirus HCoV-HKU1/NL63	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 3.368	Negative
2	Human coronavirus HCoV- OC43	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 35.582	Negative
3	Human coronavirus HCoV-NL63	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff:28.736	Negative
4	Human coronavirus HCoV-229E	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff:27.908	Negative
5	Mycoplasma pneumoniae	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 30.783	Negative
6	Novel influenza A H1N1 virus (2009)	pathogenic surveillance	Strain	Titer: 1:64	Negative
7	H3N2	pathogenic surveillance	strain	Titer: 1:64	Negative
8	H5N1	China CDC	Inactivated virus	stock solution	Negative
9	H7N9	China CDC	Inactivated virus	stock solution	Negative
10	Influenza B Yamagata	pathogenic surveillance	strain	Titer: 1:64	Negative
11	Influenza B Victoria	pathogenic surveillance	strain	Cutoff: 28.24	Negative
12	Respiratory syncytial virus	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 20.32	Negative
13	Rhinovirus A	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 25.096	Negative
14	Adenovirus type7	pathogenic surveillance	strain	Cutoff: 15.75	Negative
15	Adenovirus type 55	pathogenic surveillance	strain	Sequencing	Negative
16	Enterovirus A	pathogenic surveillance	strain	stock solution	Negative

				Cutoff: 23.28	
17	Enterovirus B	pathogenic surveillance	strain	stock solution Cutoff: 23.23	Negative
18	Epstein-Barr virus	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 32.6	Negative
19	Measles virus	pathogenic surveillance	strain	stock solution Cutoff: 34	Negative
20	Human cytomegalovirus	pathogenic surveillance	serum	stock solution Cutoff: 33.46	Negative
21	Rotavirus	pathogenic surveillance	Stool suspension	Cutoff: 12	Negative
22	Norovirus	pathogenic surveillance	Stool suspension	Cutoff: 21	Negative
23	Mumps virus	pathogenic surveillance	strain	Cutoff: 20.32	Negative
24	Varicella-zoster virus	pathogenic surveillance	oropharyngeal swab	stock solution Cutoff: 18	Negative
25	Seasonal H1N1	national reference materials	Lyophilizer	500µL/piece	Negative
26	Staphylococcus aureus	national reference materials	Lyophilizer	500µL/piece	Negative
27	Pseudomonas aeruginosa	national reference materials	Lyophilizer	500µL/piece	Negative
28	HKU8 type N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	1µg/mL	Negative
29	HKU10 type N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	1µg/mL	Negative
30	MERS-CoV N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	1µg/mL	Negative
31	SARS-CoV N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	1µg/mL	Positive

Note:

a. Results of No.1~24 is from 3.1 in Method section, No.25~27 is from 3.2, and No.28~31 is from 3.3.

b. cross-reaction sample screening was referred to "2019 New Coronavirus Antigen Antibody Detection Reagent Registration Technical Review Essentials (Trial)"

As Table 1 shows, the SARS-CoV N protein produced a positive response, after test with the diluted sample in 1 µg / mL, 500 ng / mL, 200 ng / mL, 100 ng / mL and 50 ng / mL, we determine that 50 ng/mL was the lowest concentration at which a positive reaction occurred. The results are shown in Table 2.

Table 2 Results of SARS-CoV N protein

Item	SARS-CoV N protein	Test result
1	1 µg/mL	Positive
2	500 ng/mL	Positive
3	200 ng/mL	Positive
4	100 ng/mL	Positive
5	50 ng/mL	Negative

6. CONCLUSION

2.1 30 substances in the test concentration will not cause cross-reactions to the test kit.

2.2 The recombinant virus SARS-CoV N protein will not produce cross-reactions to the test card at <50 ng / mL,

Please see Table 3 as reference.

Table 3

NO.	Substance	Source	Type	Concentration
1	Human coronavirus HCoV-HKU1/NL63	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
2	Human coronavirus HCoV- OC43	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
3	Human coronavirus HCoV-NL63	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
4	Human coronavirus HCoV-229E	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
5	Mycoplasma pneumoniae	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
6	Novel influenza A H1N1 virus (2009)	pathogenic surveillance	strain	positive sample stock solution
7	H3N2	pathogenic surveillance	strain	positive sample stock solution
8	H5N1	China CDC	Inactivated virus	positive sample stock solution
9	H7N9	China CDC	Inactivated virus	positive sample stock solution
10	Influenza B Yamagata	pathogenic surveillance	strain	positive sample stock solution dilute 17.7times
11	Influenza B Victoria	pathogenic surveillance	strain	positive sample stock solution dilute 17.7times
12	Respiratory syncytial virus	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
13	Rhinovirus A	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
14	Adenovirus type 7	pathogenic	strain	positive sample stock

		surveillance		solution dilute 17.7 times
15	Adenovirus type 55	pathogenic surveillance	strain	positive sample stock solution dilute 17.7 times
16	Enterovirus A	pathogenic surveillance	strain	positive sample stock solution
17	Enterovirus B	pathogenic surveillance	strain	positive sample stock solution
18	Epstein-Barr virus	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
19	Measles virus	pathogenic surveillance	strain	positive sample stock solution
20	Human cytomegalovirus	pathogenic surveillance	serum	positive sample stock solution
21	Rotavirus	pathogenic surveillance	Stool suspension	positive sample stock solution 17.7 times
22	Norovirus	pathogenic surveillance	Stool suspension	positive sample stock solution 17.7 times
23	Mumps virus	pathogenic surveillance	strain	positive sample stock solution 51 times
24	Varicella-zoster virus	pathogenic surveillance	oropharyngeal swab	positive sample stock solution
25	Seasonal H1N1	national reference materials	Lyophilizer	500µL/piece
26	Staphylococcus aureus	national reference materials	Lyophilizer	500µL/piece
27	Pseudomonas aeruginosa	national reference materials	Lyophilizer	500µL/piece
28	HKU8 type N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	≤1µg/mL
29	HKU10 type N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	≤1µg/mL
30	MERS-CoV N protein	Genetically engineered virus recombinant N protein	Viral recombinant N protein	≤1µg/mL
31	SARS-CoV N protein	Genetically engineered virus recombinant N	Viral recombinant N protein	<50ng/mL

		protein		
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