Novel Coronavirus（SARS-CoV-2）

Antigen Detection Kit

(Beijing Savant Biotechnology Co.,Ltd).

2020-03

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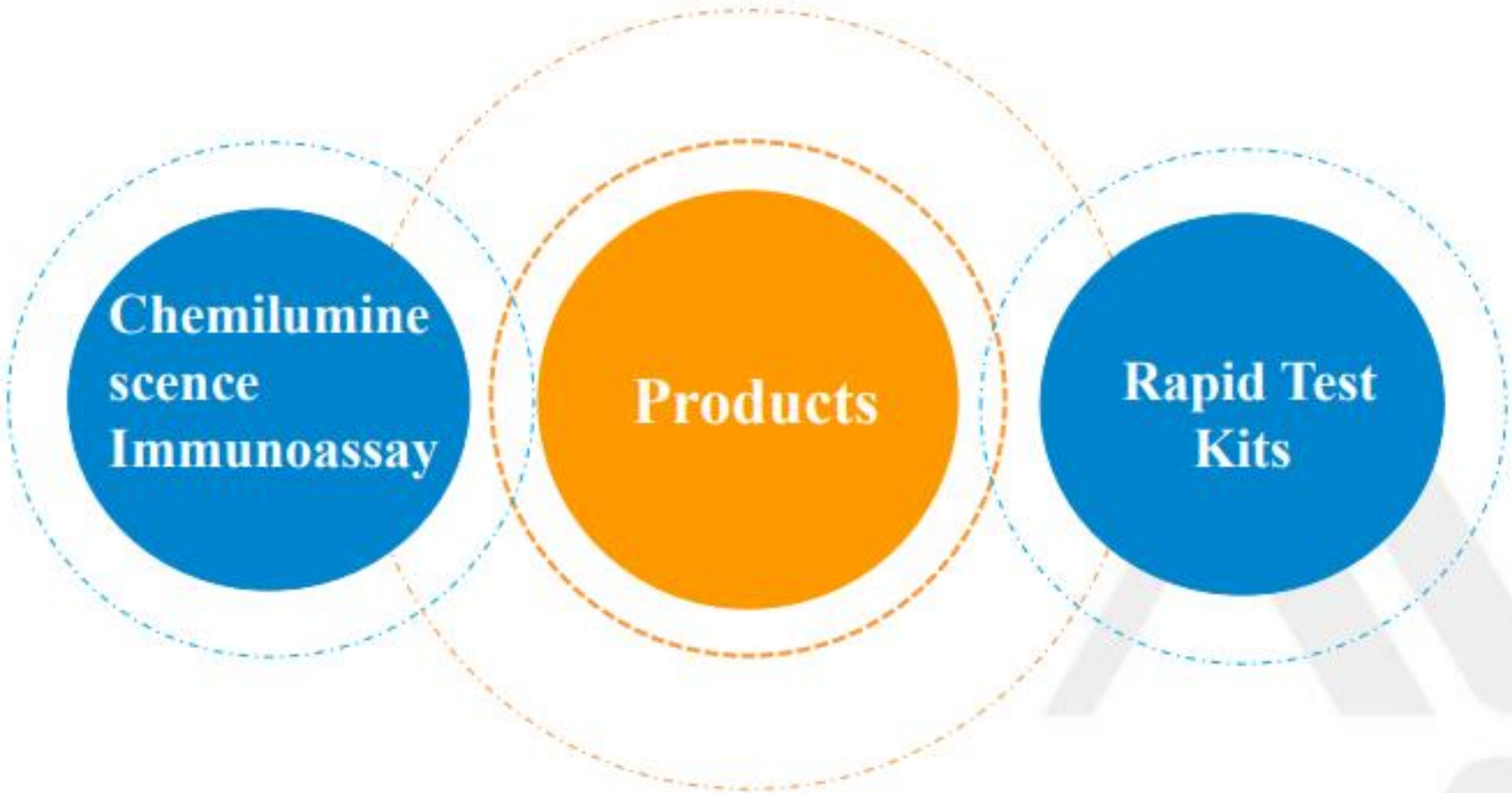

Focus on Field of Quantitative Immunoassay | Own Core Technologies

At present, the company has applied for 65 patents, among which 30 patents have been authorized.

Proprietary Name	Application Number or Patent Number	Status
The preparation and application of surface activated fluorescent microspheres	ZL201610804733.X	Authorized
A rapid detection immunochromatography kit for a novel coronavirus N protein, preparation and application	202010136117.8	First trial
A rapid detection immunochromatography kit for novel coronavirus N protein and application method	202010136067.3	First trial
A novel coronavirus N protein detection kit and application	202010136153.4	First trial

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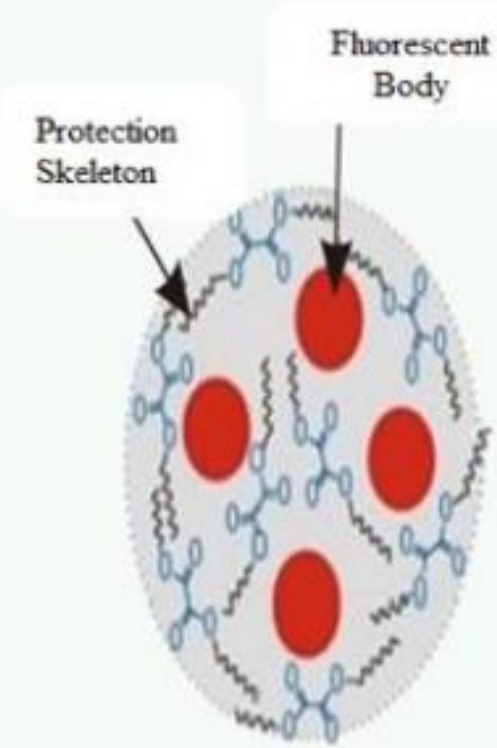
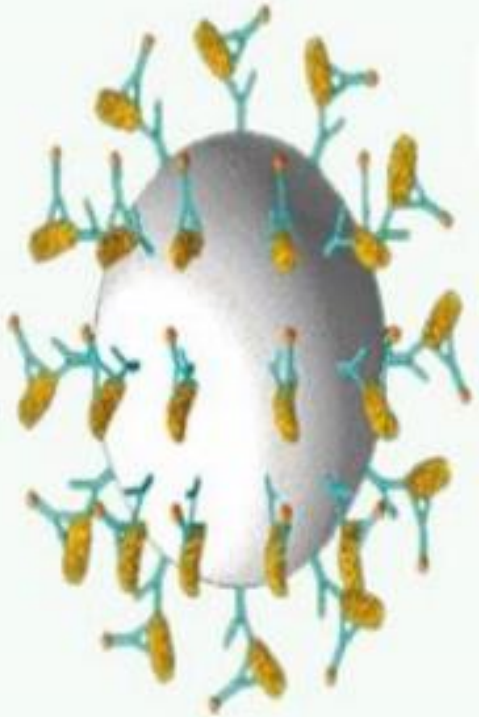
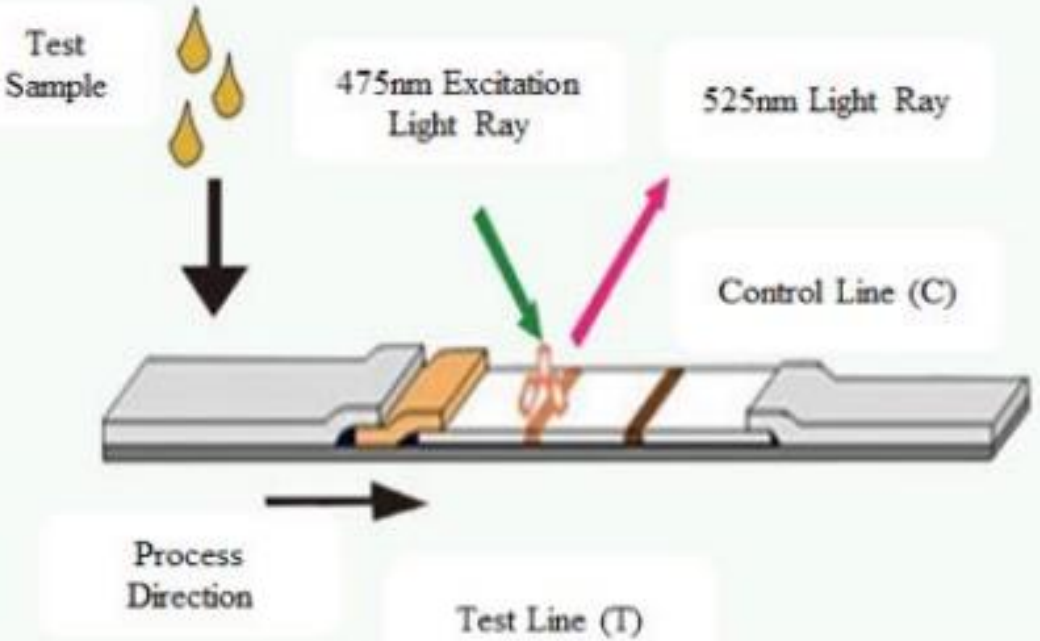


Pic 1: Fluorescent Microsphere

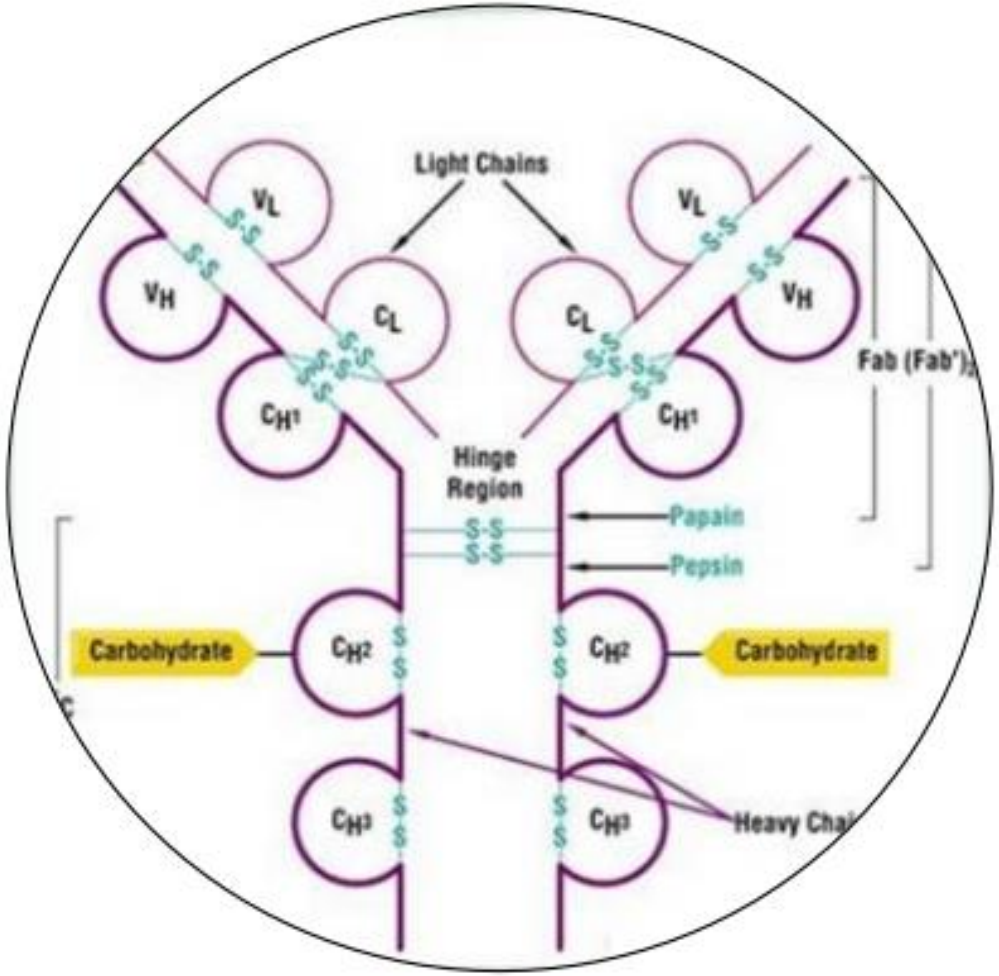


Pic 2: Fluorescent Microsphere-labeled Antibody



Pic 3: Fluorescent Test Strip

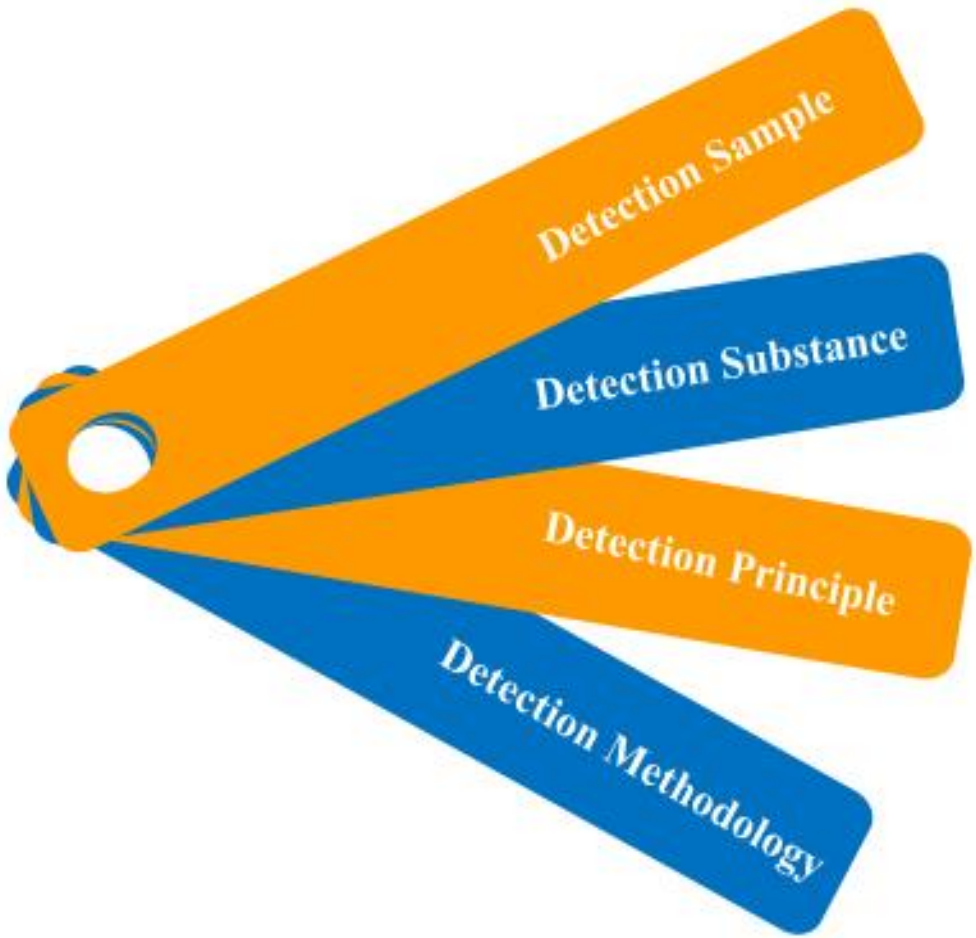
Fluorescence Microsphere Dry Quantification Technology



Directional Marking Technology

Guarantee the Integrity of binding sites

Detection Principle



Throat Swab

Novel Coronavirus Nucleocapsid (N) Antigen

Double Antibody Sandwich Method

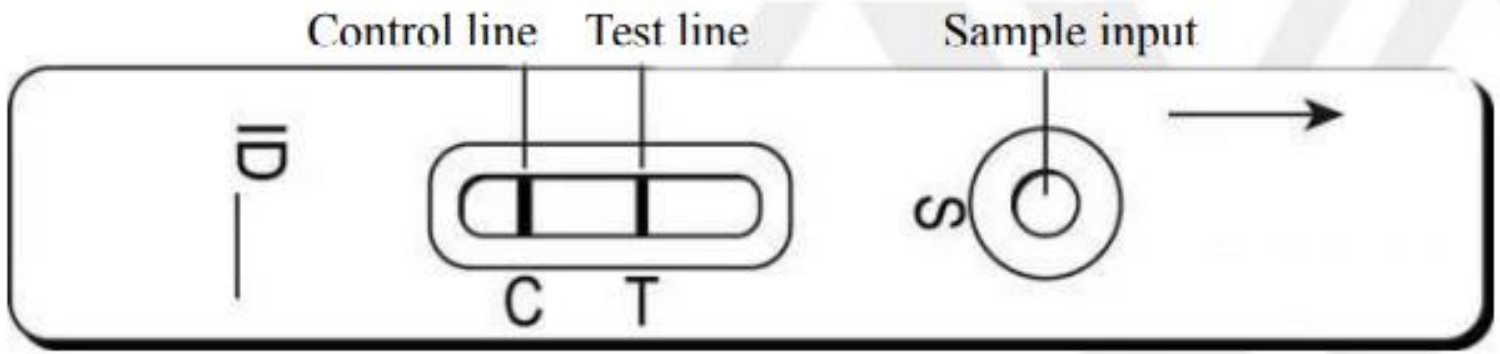
Fluorescence Immunochromatography Test

Kit Components

Components	Specification	Per package
Detection Card	Novel Coronavirus （SARS-CoV-2） Antigen Detection Card	50 tests /100 tests
Virus Preservation Solution	25 ml/bottle	1 bottle/2 bottles

Notes:

The amount of virus preservation solution：**500ul/test**



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Comparing Different Detection Method

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	Nucleic acids detection	Antigens detection	Antibodies detection
Detection Period	At least 1h	15min	15min
Specificity and Sensitivity	Good Specificity and High Sensitivity	Good Specificity, High Sensitivity	Low specificity, Low sensitivity
Automation Degree	Medium	Low	Low
Operate Difficulty Level	Difficulty and Complex	Easy	Easy
Appear (Detectable) Time	Early	Early	Late
Detection Sample	Throat Swab		Serum

Specificity：Response false positive rate, good specificity means low false positive rate.

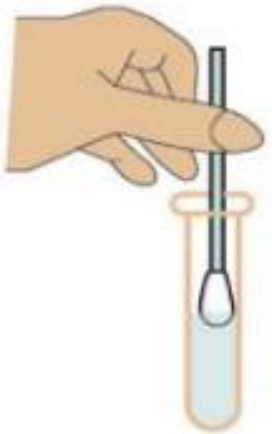
Sensitivity：Response False negative rate, high Sensitivity means low false negative rate.

Kit Performance Advantage

- Quick and Easy
- Good Specificity
- High Sensitivity
- Short Window Period

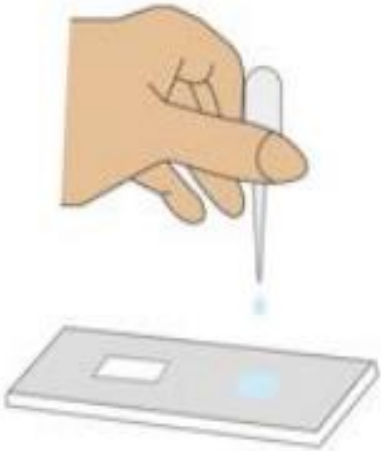
Applying to the requirement of all levels Disease Control Department (Such as Standard laboratory, Mobile laboratory and On-site testing)

1 Quick and Easy



Step 1: Sample Collection and Handling

Put throat swab sample into virus preservation solution and extraction (Sample collection should conform to standard) . After extraction, the virus preservation solution is test sample. If there are sticky samples or suspended matter in samples. These samples should be centrifugation.



Step 2: Sample Input

Add 60μL test sample into detection card

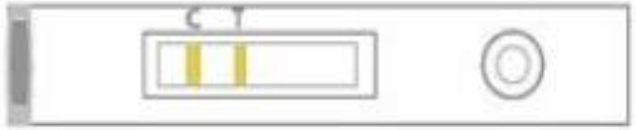
Step 3: Detection

Let the detection card standing about 15 minutes in room temperature. Using UV light to obtain Final Result.

Negative

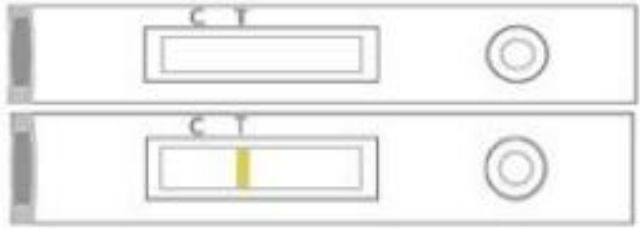
Negative Result: Only Control Line (C) appear.

Positive



Positive Result: Both Control Line (C) & Test Line (T) appear.

Note: If the color of test Line is light. It also should be considered as POSITIVE.



Invalid Result: Discard the test if there is no visible Control Line (C),

or only Test Line (T) available. Repeat the test with new Detection Kit .

Interpretation Under UV Light , No need for complicated instruments

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2 Good Specificity

Specification Analysis

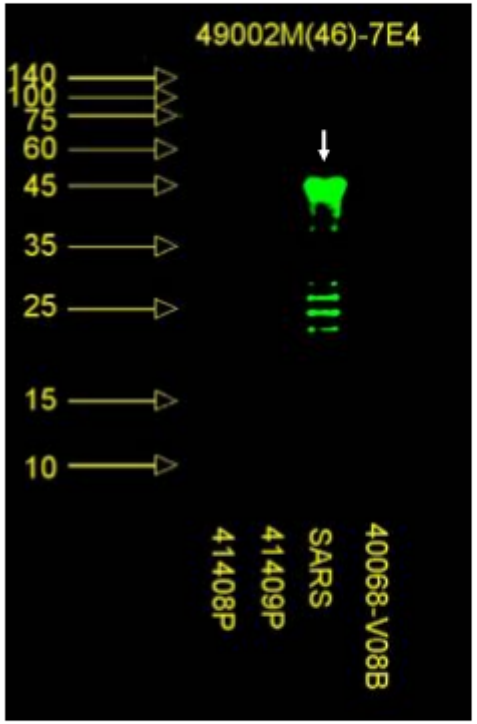
Cross validation with recombinant proteins:

It has been proved that there is no obvious cross-reaction between N protein of common human infection coronavirus (HKV1, OC43, 229E, NL63) and bat source coronavirus (HKV8, HKV10).

There was a little cross-reaction with the N protein of sars-cov.

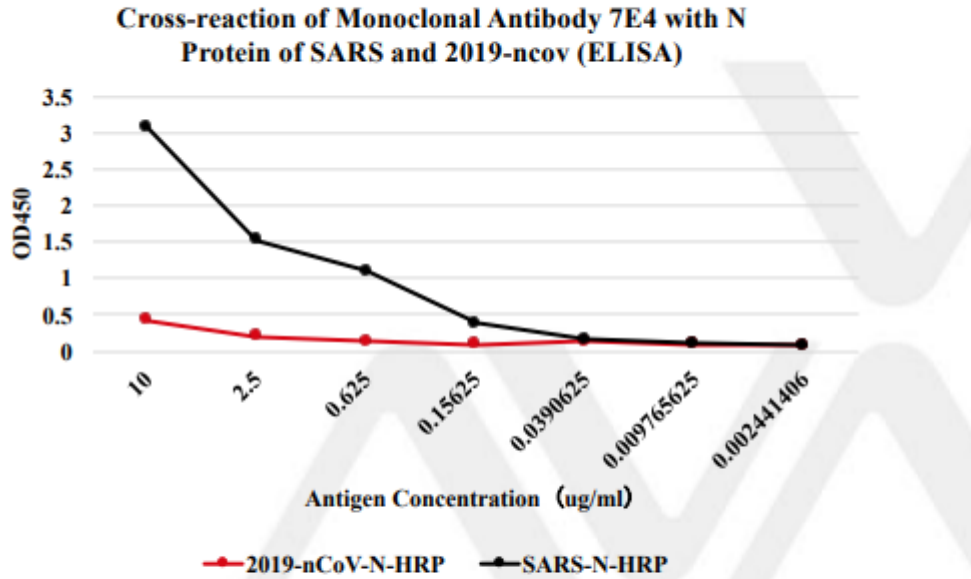
Number	Viral NP Source	Sample Volume	Test Value	Results
1	HKV1 (common human infection coronavirus)	60μl	0.003	Negative
2	OC43(common human infection coronavirus)	60μl	0.004	Negative
3	229E(common human infection coronavirus)	60μl	0.003	Negative
4	NL63(common human infection coronavirus)	60μl	0.001	Negative
5	HKV8(bat source coronavirus)	60μl	0.005	Negative
6	HKV10(bat source coronavirus)	60μl	0.003	Negative

Specific Against Sars-cov N Protein Antibody



1: 41408P: 2019-nCoV-N protein; 2: 41409P: 2019-nCoV-N protein(C end)

3: SARS: SARS-N protein; 4: 40068-V08B：MERS-N protein



Monoclonal antibody 7E4 and sars-cov N protein-specific antibody may be used for background clearance or virus typing in new crown infected samples

Specification Analysis

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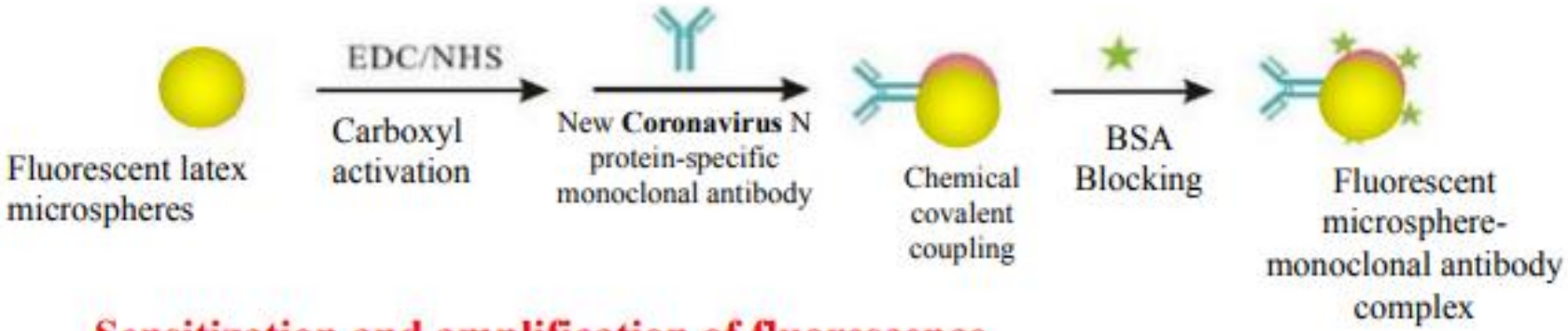
Cross validation with pathogen:

There was no cross-reaction between the kit and influenza a/b virus, measles virus, mumps virus, rubella virus, varicella zoster virus, staphylococcus aureus, pseudomonas aeruginosa, etc.

Reference article number	Types and types of pathogens	Test results（T/C）		Judgment
		（T/C）		
PC01	B/Victoria	0.003		Negative
PC02	B/Victoria	0.008		Negative
PC03	B/yamagata	0.006		Negative
PC04	B/yamagata	0.007		Negative
PC05	Type A H1N1	0.009		Negative
PC06	Type A H1N1	0.006		Negative
PC07	Seasonal H1N1	0.004		Negative
PC08	Seasonal H3N2	0.009		Negative
PC09	Seasonal H3N2	0.012		Negative
PC10	Seasonal H7N9	0.003		Negative
NC01	Measles virus	0.009		Negative
NC02	Mumps virus	0.010		Negative
NC03	Rubella virus	0.005		Negative
NC04	Varicella-zoster virus	0.002		Negative
NC05	Staphylococcus aureus	0.005		Negative
NC06	Staphylococcus aureus	0.003		Negative

3 High Sensitivity

Sensitivity Improvement Strategy



Sensitization and amplification of fluorescence immunolabeled signals

Sampling with **Throat Swab (Synchronized with nucleic acid sampling process)**

PBS preservation solution

After 15 minutes immune chromatography reaction process,get antigen qualitative test results

The method conditions were optimized to select the best virus protein preservation solution.

The detection sensitivity was further improved by blocking and inhibiting common coronavirus.

Comparison Experimental Results Between Collaurum and Fluorescent Microspheres

0ng/ml 50ng/ml 100ng/ml **300ng/ml** 1ug/ml

0ng/ml 50ng/ml **100ng/ml** 300ng/ml

0ng/ml **50ng/ml** 100ng/ml 300ng/ml

Collaurum marking (visual)

300ng/ml visible to the naked eye

50ng/ml、100ng/ml invisible to the naked eye

Fluorescent microsphere marking (visual)

100ng/ml Yellow inspection lines are visible to the naked eye

Fluorescent color

50ng/ml、100ng/ml Fluorescence color is more obvious

试用情况说明

北京华科泰生物技术股份有限公司提供一台 Savant-100 及相应 2019-nCoV 检测试剂盒供中国计量科学研究院前沿计量科学中心试用，我中心采用 GBW(E)091097 新型冠状病毒核衣壳蛋白溶液标准物质配制系列溶液，用该仪器及配套试剂测定，结果如表 1 所示：

表 1　系列标准溶液测定值

浓度值	T/C	报告结果
9 pg/mL	0.029	阴性
43 pg/mL	0.023	阴性
87 pg/mL	0.025	阴性
430 pg/mL	0.033	阴性
859 pg/mL	0.029	阴性
8.6 ng/mL	0.026	阴性
43 ng/mL	0.055	阳性
85 ng/mL	0.111	阳性

选择 200 ng/mL 溶液考察检测系统重复性，6 次重复分析结果见表 2。

表 2 重复性试验结果

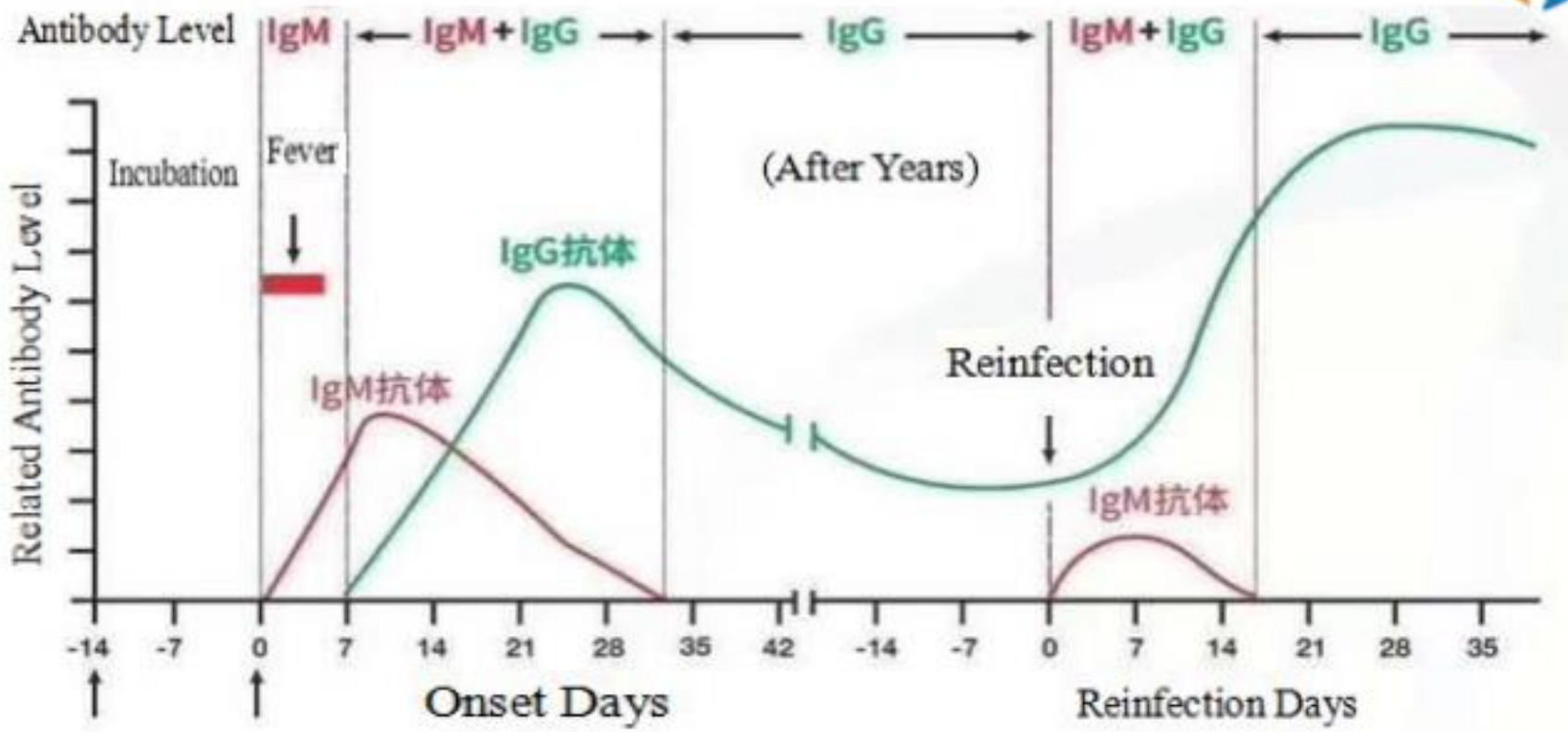
200 ng/mL 溶液	1	2	3	4	5	6	mean	SD	CV（%）
T/C	0.157	0.121	0.123	0.144	0.133	0.164	0.140	0.018	13

前沿计量科学中心

2020 年 3 月 3 日

4 Short Window Period

Short Window Period



1. Antigen detection is direct evidence of viral infection.

2. New crown pneumonia is also contagious during the incubation period. If new crown potential patients gets the antigen detection, it could have implications for potential patients.

3. There is a window period for antibody detection. Detection antigen has a high probability to obtain positive results at that time. The results could provide strong evidence for the disease evaluation.

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Discussion on Nucleic Acid Detection and Antigen Detection

Nucleic Acid Detection can detect viral RNA. Antigen detection can specifically detect novel coronavirus N protein. The results are significant, because both methods can be detected in the early stages.

Nucleic Acid Detection has the advantage of sensitivity, specificity, accuracy that is widely used clinically. It is also a "gold standard" in various disease detections.

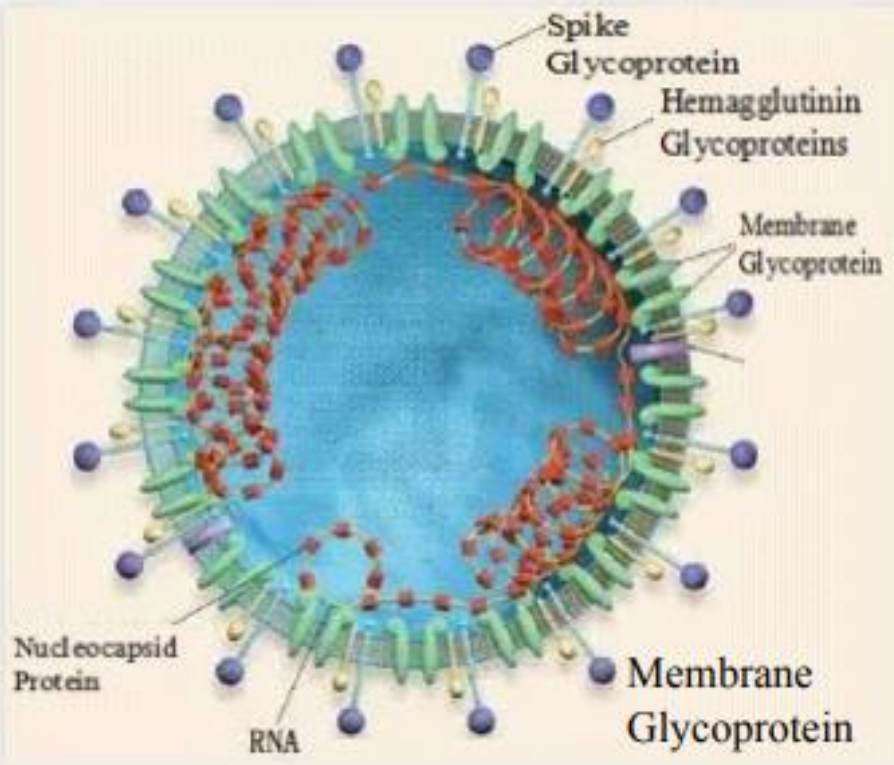
But, the results of Nucleic Acid Detection have a lot of uncertainty in New Coronavirus.

There were reports that the positive rate of Nucleic Acid Detection was “only” about 30%-50% !

Why to Carry On Antigen Detection?

Nucleocapsid protein (N protein) is guide synthesized by viral RNA. N protein can induce the body produce strong immune responses in New Coronavirus infection. It is also used as a target for the diagnosis of coronavirus infection.

New Coronavirus is a single - stranded sense RNA virus with large molecular weight and easy mutation. The virus might have a variation of nucleotide sequence during propagation. If the mutation occurs in the primer-binding region of nucleic acid amplification, false negative will occur. But bind N protein is relatively stable and it can be detected even if the RNA mutates.



The Result Analysis of Nucleic Acid Detection and Antigen Detection

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The result analysis of Nucleic Acid Detection and Antigen detection (Same sampling time, Total number of confirmed cases is 35)						
Consistent Results				Inconsistent Results		
Nucleic Acid Detection Positive and Antigen Detection positive	Nucleic Acid Detection Negative and Antigen Detection Negative			Nucleic Acid Detection Negative and Antigen Detection Positive		Nucleic Acid Detection Suspected Antigen Detection Negative
3	Inpatient	**10**		Inpatient	**13**	1
	Discharge	**6**		Discharge	**2**	

Results of the two methods were inconsistent in 16 samples. Antigen detection has 15 cases that fit with the clinical diagnosis.

The company has test **70** employees with **1** false positive case.

Beijing CDC has test **60** cases with **1** false positive case.

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In-patients Case：Nucleic Acid Detection negative, Antigen detection positive

Female, 60 years old

Background Information on Preliminary Diagnostic ：Novel Coronavirus Pneumonia (Serious illness)

Transferred to the hospital after diagnosed in the outer court

Sampling Time：2020.2.21

Sample Collection：Throat Swab

Nucleic Acid Detection Result：Double Negative + Negative

Antigen Detection Result：Positive

In **23** hospitalized cases, Antigen detection has **15** cases that fit with the clinical diagnosis.

Clinical Case 2

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Discharge Case：Nucleic Acid Detection Negative, Antigen detection Negative

Male, 51 years old

Background Information on Preliminary Diagnostic ：Novel Coronavirus Pneumonia

Transferred to the hospital after diagnosed in the outer court

Sampling Time：2020.2.21

Discharge Time：2020.2.22

Sample Collection：Throat Swab

Nucleic Acid Detection Result：Double Negative + Negative

Antigen Detection Result：Negative

In **8** hospitalized cases, Antigen detection has **6** cases that fit with the clinical diagnosis.

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Discharge Case：Nucleic Acid Detection Negative, Antigen detection Positive

Male, 71 years old

Background Information on Preliminary Diagnostic ：Novel Coronavirus Pneumonia

Sampling Time：2020.02.21

Discharge Time：2020.2.22

Sample Collection：Throat Swab

Nucleic Acid Detection Result：Double Negative + Negative

Antigen Detection Result：Positive

In **8** hospitalized cases, there were **2** similar cases that should be worthy of attention

Clinical Case 4

In-patients Case：Inconsistent results of Nucleic Acid Detection, diagnosed by sequencing
But Antigen detection was positive consistently.

Male, 39 years old

Background Information on Preliminary Diagnostic ：Novel Coronavirus Pneumonia + hypertension

Sample Collection：Throat Swab

Detection Method	Sampling Time	Results	Detection Method	Sampling Time	Results
Nucleic Acid	2.20	Double Negative	Antigen	2.21	Weak Positive
	2.23	Single Positive		2.23	Weak Positive
	2.25	Negative		2.29	Positive
	2.27	Positive(Sequencing)		03.02	Positive

Clinical Case 5

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In-patients Case：Inconsistent results of Nucleic Acid Detection, Antigen was tested from negative to positive

Male, 67 years old

Background Information on Preliminary Diagnostic ：Novel Coronavirus Pneumonia

Sample Collection：Throat Swab

Detection Method	Sampling Time	Results	Detection Method	Sampling Time	Results
Nucleic Acid	2.20	Double Negative	Antigen	2.21	Negative
	2.22	Double Negative			
	2.23	Single Positive		2.23	Positive
	2.27	Negative		2.28	Positive
	2.29	Negative		3.02	Weak Positive

There were **15** cases by clinical follow-up observation since 2.21.
11 cases were similar as shown above.

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Production Environment

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Industrialization

Products : 65

The annual capacity of rapid teat kits

20million pieces

The annual capacity of Chemiluminescence

60million pieces