COVID-19 Antigen Test kit (Colloidal Gold)

Analysis of Performance Evaluation

Product Name:	COVID-19 Antigen Test Kit (Colloidal Gold)
Applicant	SINGUWAY BIOTECH INC.
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In accordance with the relevant requirements of the "Guiding Principles for Analytical Performance Evaluation of In Vitro Diagnostic Reagents" issued by the State Food and Drug Administration, and the Clinical & Laboratory Standards Institute (CLSI) guidelines, the Singuway Biotech Inc. conducts a series of analytical performance evaluations on the product new coronavirus antigen detection kit (colloidal gold method) Related research and verification work.

The summary is as follows:

Content

A. MAIN EXPERIMENTAL MATERIALS AND INSTRUMENTS

Product Name: COVID-19 Antigen Test Kit (Colloidal Gold)

Packing Size: 25 tests/box

Lot Number: 20200601, 20200602, 20200603

B. PERFORMANCE ASSESSMENT INFORMATION

1. Determination of analytical sensitivity - Limit of Detection (LoD)

1.1 Synthetic antigen test

(1). Experimental methods

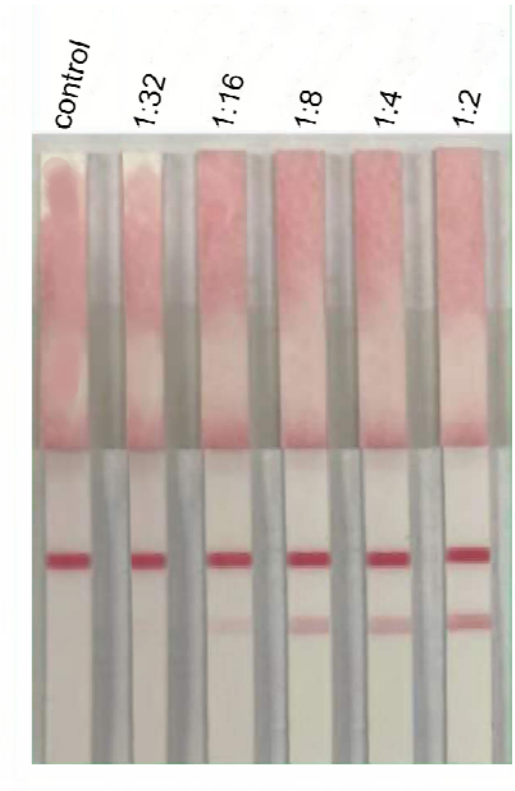
The Limit of Detection for direct swab was established using synthesized SARS-CoV-2 nucleocapsid protein. Presumed negative natural nasal swab specimens were eluted in Phosphate-buffered saline (PBS). Swab eluates were combined and mixed thoroughly to create a clinical matrix pool to be used as the diluent. Inactivated SARS-CoV-2 isolate was diluted in this natural nasal swab matrix pool to generate virus dilutions for testing.

The synthesized SARS-CoV-2 nucleocapsid protein (0.4ng/ml) was mixed and diluted with the negative diluent with the dilution ratios of 1:1, 1:2, 1:4, 1:8, 1:16, and 1:32.

Three batches of COVID-19 Antigen Test Kits (Colloidal Gold) were used to test the diluted samples. Each sample was tested in parallel 20 times in each batch, and the detection rate was calculated.

The LoD was determined as the lowest virus concentration that was detected ⩾ 95% of the time.

(2). Experimental results



Control: Negative sample

The experimental results are shown in Table 1.

Table 1: Determination of experimental results by the lowest detectable quantity

Lot number / Dilution	20200601	20200602	20200603	Positive rate (%)
Original	20/20+	20/20+	20/20+	100
1:1	20/20+	20/20+	20/20+	100
1:2	20/20+	20/20+	20/20+	100
1:4	20/20+	20/20+	20/20+	100
1:8	20/20+	20/20+	20/20+	100
1:16	19/20+	19/20+	20/20+	98.7
1:32	11/20+	15/20+	12/20+	63.3

The result showed that at the dilution ratio of 1:16, the detection rate of reagent positive samples reaches more than 95%. The Limit of Detection is 25 pg/ml.

1.2 Inactivated Isolate

(1). Experimental methods

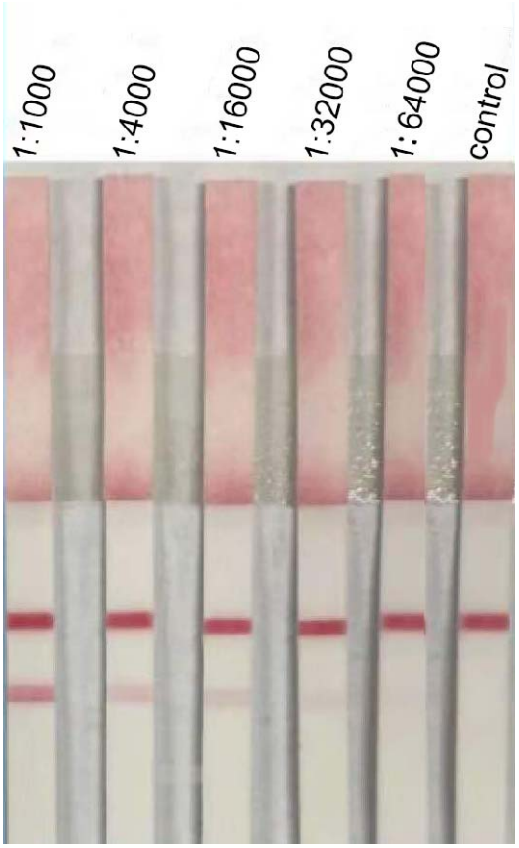
The heat-inactivated SARS-CoV-2 isolate（$1\times10^7 TCID_{50}$/ml）was diluted the negative diluent at the dilution ratios of 1:1000, 1:4000, 1:8000, 1:16000, 1:32000, and 1:64000.

The COVID-19 antigen detection kit (colloidal gold method) detects the diluted samples and determines the Limit of Detection.

The lot number 20200601of COVID-19 Antigen Test Kits (Colloidal Gold) was used to test the diluted samples. Each sample was tested in parallel 5 times, and the detection rate was calculated.

The LoD was determined as the lowest virus concentration that was detected ⩾95% of the time.

(2). Experimental results



The experimental results are shown in Table 2.

Table 2: Determination of experimental results by the lowest detectable quantity

Dilution	Concentration（$TCID_{50}$/ml）	Test results	Positive rate (%)
Original	1×10^{7}	5/5+	100
1:1000	1×10^{4}	5/5+	100
1:4000	2×10^{3}	5/5+	100
1:16000	600	5/5+	100
1:32000	300	5/5+	100
1:64000	150	5/3+	60

The result showed that at the dilution ratio of 1:32000 (300 $TCID_{50}$/ml), the detection rate of reagent positive samples reaches more than 95%.

(3). Confirmation test

Three batches of COVID-19 Antigen Test Kits (Colloidal Gold) were used to test the diluted samples at the dilution ratio of 1:32000 (300 $TCID_{50}$/ml). Each sample was tested in parallel 20 times in each batch, and the detection rate was calculated. Table 3

Lot number	20200601	20200602	20200603	Positive rate (%)
	20/20+	20/20+	19/20+	98.3

The Limit of Detection of COVID-19 antigen detection kit (colloidal gold method) is 300 $TCID_{50}$/ml.

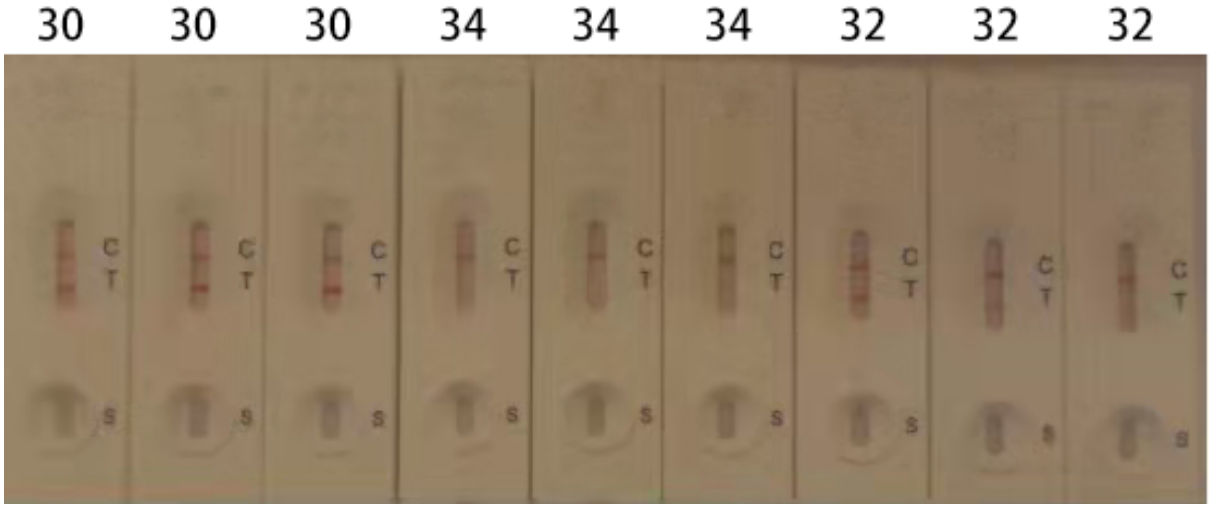
1.3 Inactivated isolate

(1). Experimental methods

One inactivated isolate sample was diluted to Ct values of 26, 28, 30, 32, and 34.

The COVID-19 antigen detection kit (colloidal gold method) detects the diluted samples and determines the Limit of Detection.

(2). Experimental results



The experimental results are shown in the Table 4.

Table 4: Determination of experimental results by the lowest detectable quantity

Ct	20200601	20200602	20200603	Positive rate (%)
20(stock solution)	+	+	+	100
26	+	+	+	100
28	+	+	+	100
30	+	+	+	100
32	+	+	+	100
34	-	-	-	0
NC	-	-	-	0

The result showed that at the Ct of 32, the detection rate of reagent positive samples reaches more than 95%. The Limit of Detection is Ct of 32.

1.4 Summary

The Limit of Detection of the COVID-19 Antigen detection kit is 300 $TCID_{50}$/mL(25 pg/mL, Ct=32 (2000-5000copies/mL).

2. Analysis of specificity assessment data

To evaluate the effects of these factors on the reagents, the clinical samples of patients with these diseases were examined to determine whether they had cross-reactions with the reagents.

2.1 Cross-reaction

(1). Sample source

The specimens used for cross-reaction are as follows:

Less infectious samples, such as respiratory syncytial virus, rhinovirus, adenovirus, enterovirus, EB Virus, Measles virus, human cytomegalovirus, Rotavirus, Norovirus, Mumps virus, varicella-zoster virus, parainfluenza virus type II, Mycoplasma pneumoniae, H1N1; H3N2; fluB and other respiratory related samples. For example, the corresponding pathogen from the Fourth Hospital of Shenzhen is positive by fluorescence PCR.

The purchased and cultivated standard inactivated virus strain is purchased from Beina Biotech and the interference concentration was selected as 50μg/ml.

Those extremely infectious samples cannot be obtained for testing and can directly choose the proteins sold on the market: Endemic human coronavirus (HKU1, OC43, NL63, 229E), SARS, MERS; purchased from Beijing Biodragon.

In order to prevent antigens for the cross-reaction caused by the difference, the virus surface protein of each epitope is selected for experiment. The interference concentration of protein is 100ng/mL.

(2). cross activity

Use the Interrupt pathogens blow as samples to test, the result is as follow Table 5

Interrupt pathogens	concentration	1	2	3
SARS-CoV	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
MERS-CoV	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
HCOV-HKU1	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
HCOV-NL63	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
HCOV-OC43	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
HCoV-229E	100ng/ml（about 1×10^7TCID50）	Neg	Neg	Neg
EB Virus	1×10^8copies/ml	Neg	Neg	Neg

CMV	1×10^{8}copies/ml	Neg	Neg	Neg
VZV	1×10^{6}copies/ml	Neg	Neg	Neg
Parvovius B19	1×10^{6}copies/ml	Neg	Neg	Neg
HIV-1	2×10^{7}IU/ml	Neg	Neg	Neg
HIV-2	4×10^{6}IU/ml	Neg	Neg	Neg
HBV	5×10^{7}IU/ml	Neg	Neg	Neg
HCV	2×10^{7}IU/ml	Neg	Neg	Neg
HSV-1	1×10^{6}copies/ml	Neg	Neg	Neg
HSV-2	7×10^{5}copies/ml	Neg	Neg	Neg
E.coil	1×10^{6}CFU/ml	Neg	Neg	Neg
Streptococcus Pneumoniae	1×10^{6}CFU/ml	Neg	Neg	Neg
Streptococcus Pyogenes	1×10^{6}CFU/ml	Neg	Neg	Neg
S. aureus	1×10^{6}CFU/ml	Neg	Neg	Neg
Staphylococcus epidermidis	1×10^{6}CFU/ml	Neg	Neg	Neg
M.Pneumoniae	5×10^{6}CFU/ml	Neg	Neg	Neg
C. Pneumoniae	5×10^{6}CFU/ml	Neg	Neg	Neg
H1N1	1×10^{5}TCID50/ml	Neg	Neg	Neg
fluB	1×10^{5}TCID50/ml	Neg	Neg	Neg
H3N2	1×10^{5}TCID50/ml	Neg	Neg	Neg
respiratory syncytial virus	1×10^{5}TCID50/ml	Neg	Neg	Neg
rhinovirus	1×10^{5}TCID50/ml	Neg	Neg	Neg
Adenovirus（Ad3）	1×10^{5}PFU/ml	Neg	Neg	Neg
Adenovirus（Ad7）	8×10^{4}PFU/ml	Neg	Neg	Neg

enterovirus	1×10^5TCID50/ml	Neg	Neg	Neg

From the above experimental results, it can be concluded that the test results of the kit are all negative, indicating that the above pathogens do not affect the test results of the kit.

(3). interference experience

Select low positive sample (3×LoD),add all pathogen and repeat 3 times marked as L1, L2,L3; in addition, select negative samples (marked as N1, N2) and blank swabs (marked as B); add the above interference substances to make the final concentration reach the interference concentration.

(4). Experimental results

The results of the cross reaction test of the Coronavirus Antigen Detection kit (Colloidal Gold) were as follows: Table 6

Interrupt pathogens	**concentration**	**L1**	**L2**	**L3**	**N**	**B**	**CR%**
SARS-CoV	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
MERS-CoV	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
HCOV-HKU1	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
HCOV-NL63	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
HCOV-OC43	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
HCoV-229E	100ng/ml（about 1×10^7TCID50）	Pos	Pos	Pos	Neg	Neg	0%
FluA(H1N1)	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%
fluB	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%
FluA(H3N2)	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%
respiratory syncytial virus	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%
rhinovirus	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%
Adenovirus（Ad3）	5×10^4PFU/ml	Pos	Pos	Pos	Neg	Neg	0%
Adenovirus（Ad7）	4×10^4PFU/ml	Pos	Pos	Pos	Neg	Neg	0%
enterovirus	5×10^4TCID50/ml	Pos	Pos	Pos	Neg	Neg	0%

HPIVs-1	1×10^6Copies/ml	Pos	Pos	Pos	Neg	Neg	0%
HPIVs-2	1×10^6Copies/ml	Pos	Pos	Pos	Neg	Neg	0%
HPIVs-3	1×10^6Copies/ml	Pos	Pos	Pos	Neg	Neg	0%
HPIVs-4	1×10^6Copies/ml	Pos	Pos	Pos	Neg	Neg	0%
Haemophilus influenzae	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
Streptococcus Pneumoniae	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
Streptococcus Pyogenes	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
candida albicans	7×10^6CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
bordetella pertussis	1×10^6CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
M.Pneumoniae	5×10^6CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
C. Pneumoniae	5×10^6CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
Legionella Pneumoniae	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
S. aureus	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
Staphylococcus epidermidis	5×10^5CFU/ml	Pos	Pos	Pos	Neg	Neg	0%
MTB	3×10^4 bacteria/ml	Pos	Pos	Pos	Neg	Neg	0%
PJP（protein）	5ng/ml	Pos	Pos	Pos	Neg	Neg	0%
Pooled human nasal wash	100%(V/V; as a solution medium)	Pos	Pos	Pos	Neg	Neg	0%

From the above experimental results, it can be concluded that the test results of the kit are also positive, indicating that the above pathogens do not affect the test results of the kit.

(5). Conclusion

None of the above samples and this reagent produce cross reaction.

2.2 Interference test

2.2.1 Endogenous interference

(1). Experimental Methods

In order to investigate the influence of possible endogenous substances in the sample on the test

results, this experiment was conducted under the condition of each interfering substance close to the maximum potential clinical concentration. The test subjects included 3 cases of blood, 3 cases of saliva, nasal secretions and 100 cases of negative nasal swabs. Use the qualified Coronavirus Antigen Detection kit (Colloidal Gold) to test the above samples, record the number of negative and positive detections, screen for possible endogenous interfering substances, and determine the acceptable limit of interfering substances concentration.

(2). Experimental results

The results of the Coronavirus Antigen Test kit (Colloidal Gold)(Table 7):

Table7 Effects of Endogenous Interference Substances on the Detection Results

Interference	Con	Sample type	Results	
			Pos	Neg
Whole Blood	4%	Positive	3/3	0/3
		Negative	0/3	3/3
Mucin	0.5%	Positive	3/3	0/3
		Negative	0/3	3/3
Normal nasal swab	/	Positive	100/100	0/100
		Negative	0/100	100/100

(3). Conclusion

The experimental results showed that the above interfering substances were positive without false negative, negative without false positive, and no interference with kit detection.

2.2.2 Drug interference

(1). Experimental methods

In order to investigate the influence of the possible drugs in the sample on the test results, common drugs were tested. Use the qualified COVID-19 antigen detection kit (colloidal gold method) to test the above samples, record the number of negative and positive detections, screen for possible endogenous interfering substances, and determine the acceptable limit of interfering substances concentration.

(2). Experimental results

The results of the Coronavirus Antigen Detection kit (Colloidal Gold)(Table8):

Table 8 Effects of Endogenous Interference Substances on Detection Results

Interference	Concert	Strong positive	Medium positive	Weak positive	Negative
Ribavirin	10mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Jin Ying	5mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Mint	5mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Interferon	10U/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Amoxicillin	10mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Aspirin	5mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
chloraseptine	1.5mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Naso GEL	5%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
CVS nasal drop	15%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
CVS Nasal Spray	15%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Zicam	5%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Afrin	15%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Homeopathic（ALkalol ）	1：10	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Sore throat Phenol Spray	15%（V/V）	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Tobramycin	4μg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Mupirocin	10mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Fluticasone propionate	5%(V/V)	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg
Tamiflu	5mg/ml	3/3 Pos	3/3 Pos	3/3 Pos	3/3 Neg

(3). Conclusion

The experimental results show that the above-mentioned interfering substances have no false negatives when they are positive and no false positives when they are negative. They have no interference effect on the detection of the kit.

2.3 HOOK effects

2.3.1 Experimental methods

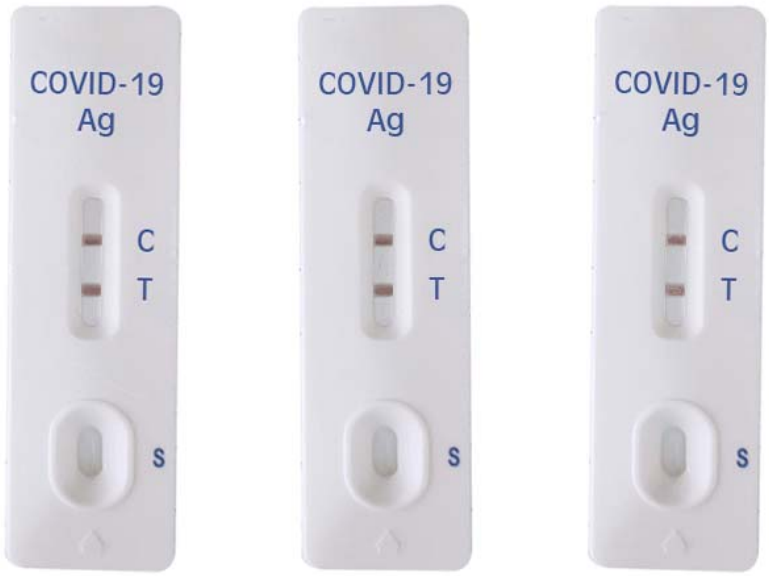
The test was performed with 10x, 100x, 1000x the LOD （3000TCID50/ml、30000 TCID50/ml、300000 TCID50/ml） of heat-inactivated SARSCoV-2 strainand repeated three times to verify whether there is a hook effect.

2.3.2 Assessment standard

Three times should be strongly positive

2.3.3 Experimental results Table 9

Repeat	1	2	3
10×LoD	Strong positive	Strong positive	Strong positive
100×LoD	Strong positive	Strong positive	Strong positive
1000×LoD	Strong positive	Strong positive	Strong positive



The results showed that samples below 300000 TCID50/ml did not induce HOOK effects.

2.4 Repeatability

2.4.1 Experimental Methods

Positive protein and negative samples were measured for 5 days and 4 times a day.

2.4.2 Assessment standard

Positive conformity rate not 18/20

2.4.3 Experimental results

Table10 Results of Precision Testing Experiments

Reagent / days		Positive protein		Negative samples	
		+	-	+	-
1	20200601	4	0	0	4
	20200602	4	0	0	4
	20200603	4	0	0	4
2	20200601	4	0	0	4
	20200602	4	0	0	4
	20200603	4	0	0	4
3	20200601	4	0	0	4
	20200602	4	0	0	4
	2020603	4	0	0	4
4	20200601	4	0	0	4
	20200602	4	0	0	4
	20200603	4	0	0	4
5	20200601	4	0	0	4
	20200602	4	0	0	4
	20200603	4	0	0	4

2.4.4 Conclusion

Use 3 batches of Coronavirus Antigen Test kits (Colloidal Gold) to perform 20 consecutive repeated tests on the specimens. The test results show that the positive rate of positive samples is 100%, and the negative rate of negative samples is 100%, indicating that the repeatability of kit is good.

3. Summary

3.1 Sensitivity

The minimum detectable quantity of the kit was 25pg /ml recombinant protein,300TCID50/ml or ct32positive samples;

3.2 Specificity

(2019-nCoV) New coronavirus Antigen Test show no cross reaction with followed positive samples: Endemic human coronavirus (HKU1), OC43, NL63, 229E), influenza A virus, influenza B virus, respiratory syncytial virus, rhinovirus, adenovirus, enterovirus, EB Virus, Measles virus, human cytomegalovirus,Rotavirus,Norovirus,Mumps virus,varicella-zoster virus,parainfluenza virus type II,Mycoplasma pneumoniae,and not less than 100health swab specimens.

3.3 HOOK effect

Test the 1000LoD COVID-19antigen sample, the result should be strongly positive.

3.4 Repeatability and Reproducibility

Tests showed positive results with all positive samples and showed negative results with negative samples. There was no significant difference observed to the same sample when repeatedly testing 10tests in the same batch. No appreciable intra and inter lot variation were observed among different tests for each lot, different lots, different operators at different test sites in different time for the same sample.

The results demonstrated that the repeatability and reproducibility of (2019-nCoV) New coronavirus Antigen Test are findings satisfaction.