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IVD AND POCT PRODUCTS MANUFACTURER

SARS-CoV-2 antigen IVD kit SALIVA

Analytical Performance Evaluation

Contents

The analytical performance evaluation document of SARS-CoV-2 antigen IVD kit SALIVA (Immuno-colloidal Gold) (hereinafter referred to as the "kit") mainly includes the analytical performance evaluation of three batches of trial-produced kits, including the analytical performance evaluation of accuracy, specificity, LOD, and repeatability of three batches of trial-produced kits. The analytical performance evaluation of specificity includes the interference test evaluation of common interfering substances, and the performance evaluation of the kit test specificity by using other pathogens.

IReagent materials

1.The kit

Name: SARS-CoV-2 antigen IVD kit SALIVA

Specification: 1/20 tests/kit ; trial-produced kits lot No.: 202008001, 202008002 and 202008003.

2.References

Enterprise references (20200629).

Table1.Enterprise reference plate information reference

References	type	concentration	References	type	concentration
N1	H1N1(2009)	10^6Copies/mL	P1	SARS-CoV-2	5.5×10^6 Copies/mL
N2	H3N2	10^6 Copies/mL	P2	SARS-CoV-2	2×10^6 Copies/mL
N3	H1N1	10^6 Copies/mL	P3	SARS-CoV-2	5.8×10^5 Copies/mL
N4	Influenza B virus	10^6 Copies/mL	P4	SARS-CoV-2	5×10^6 Copies/mL
N5	mycoplasma pneumonia	10^6CFU/mL	P5	SARS-CoV-2	2×10^6 Copies/mL
N6	Respiratory Syncytial Virus	10^6 Copies/mL	P6	SARS-CoV-2	8×10^5 Copies/mL
N7	Enterovirus 68	10^6 Copies/mL	L1	SARS-CoV-2	1.2×10^6 Copies/mL
N8	Chlamydia pneumonia	10^6CFU/mL	L2	SARS-CoV-2	6×10^5 Copies/mL
N9	healthy volunteer	/	L3	SARS-CoV-2	3×10^5 Copies/mL
R1	SARS-CoV-2	5×10^7 Copies/mL	L4	SARS-CoV-2	1.2×10^6 Copies/mL
R2	SARS-CoV-2	5×10^6Copies/mL	L5	SARS-CoV-2	6×10^5 Copies/mL
			L6	SARS-CoV-2	3×10^5 Copies/mL

IIEvaluation contents and methods

1.Evaluation of kit test accuracy

1.1. Coincidence rate of enterprise positive references

The enterprise positive references P1~P6 are tested with three batches of trial-produced kits, and the test results shall all be positive for the novel coronavirus antigen. That is, the coincidence rate of enterprise positive references is 100%.

1.2Evaluation of samples from different regions

1.2.1. Repeatability

Ten samples infected with novel coronavirus from different regions at different times are selected and diluted for 100 times with the negative sample. The diluted samples are subject to PCR calibration, and the original concentrations of samples are calculated and determined. The original samples are tested with three batches of trial-produced kits, and each sample is tested in decuplicate to evaluate the kit test accuracy.

1.2.2. Lowest LOD

Ten samples infected with novel coronavirus from different regions at different times are gradiently diluted to 3 series with the negative sample. Each series is tested in triplicate to determine the maximum test dilution of the kit. The dilution of 100% detection is selected as the estimated LOD. Ten samples from different regions are diluted to the estimated LOD concentration, which are continuously diluted to 2 times and 5 times of dilution. Each dilution sample is tested in 20 replicates. The lowest LOD of 10 samples tested with three batches of trial-produced kits is evaluated.

2.Kit specificity analysis

The kit specificity analysis includes the coincidence rate of enterprise negative references tested with the kit, and the analytical performance evaluation of the anti-interference ability of common interfering substances and anti-crossing reaction of other pathogens.

2.1.Coincidence rate of enterprise negative references

The enterprise negative references N1~N9 are tested with three batches of trial-produced kits, and the test results are all negative for the novel coronavirus antigen. That is, the coincidence rate of enterprise negative references is 100%.

2.2.Cross reaction

2.2.1.Pathogen crossing

Pathogens of human coronavirus (HCoV-OC43, HCoV-229E, HCoV-HKU1 and HCoV-NL63); novel influenza A (H1N1) virus (2009); seasonal H1N1, H3N2, H5N1, and H7N9 influenza virus; influenza B virus (Yamagata and Victoria); respiratory syncytial virus types A and B; parainfluenza virus types 1, 2, 3; rhinoviruses A, B, and C; adenoviruses 1, 2, 3, 4, 5, 7, and 55; enteroviruses A, B, C, and D; EB virus; measles virus; human cytomegalovirus; rotavirus; norovirus; mumps virus; varicella-zoster virus; and mycoplasma pneumoniae are selected for the analytical performance evaluation of the kit cross specificity.

The concentrations of all pathogens are shown in the table below:

S/N	Name of pathogen	Minimum concentration requirement	Positive or negative	Species
1	HCoV-HKU1	＞10^6copies/mL	Novel coronavirus negative	Coronavirus
2	HCoV-OC43	＞10^6copies/mL	Novel coronavirus negative	Coronavirus
3	HCoV-NL63	＞10^6copies/mL	Novel coronavirus negative	Coronavirus
4	HCoV-229E	＞10^6copies/mL	Novel coronavirus negative	Coronavirus
5	Novel Influenza A (H1N1) Virus (2009)	＞10^6copies/mL	Novel coronavirus negative	Influenza A virus
6	Seasonal H1N1 influenza virus	＞10^6copies/mL	Novel coronavirus negative	Influenza A virus
7	Influenza A virus (H3N2)	＞10^6copies/mL	Novel coronavirus negative	Influenza A virus
8	Influenza A virus (H5N1)	＞10^6copies/mL	Novel coronavirus negative	Influenza A virus
9	Influenza A virus (H7N9)	＞10^6copies/mL	Novel coronavirus negative	Influenza A virus
10	Influenza B virus (Yamagata)	＞10^6copies/mL	Novel coronavirus negative	Influenza B virus
11	Influenza B virus (Victoria)	＞10^6copies/mL	Novel coronavirus negative	Influenza B virus
12	Respiratory syncytial virus type A	＞10^6copies/mL	Novel coronavirus negative	Pneumonia virus
13	Respiratory syncytial virus type B	＞10^6TCID_{50}/ mL	Novel coronavirus negative	Pneumonia virus
14	Parainfluenza virus type 1	＞10^6copies/mL	Novel coronavirus	Respiratory virus

			negative	
15	Parainfluenza virus type 2	＞10^6copies/mL	Novel coronavirus negative	Mumps virus
16	Parainfluenza virus type 3	＞10^6copies/mL	Novel coronavirus negative	Respiratory virus
17	Rhinovirus A	＞10^6copies/mL	Novel coronavirus negative	Rhinovirus
18	Rhinovirus B	＞10^6copies/mL	Novel coronavirus negative	Rhinovirus
19	Rhinovirus C	＞10^6copies/mL	Novel coronavirus negative	Rhinovirus
20	Adenovirus type 1	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
21	Adenovirus type 2	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
22	Adenovirus type 3	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
23	Adenovirus type 4	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
24	Adenovirus type 5	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
25	Adenovirus type 7	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
26	Adenovirus type 55	＞10^6copies/mL	Novel coronavirus negative	Adenovirus
27	Human metapneumovirus	＞10^6copies/mL	Novel coronavirus negative	Metapneumovirus
28	Enterovirus A	＞10^6copies/mL	Novel coronavirus negative	Enterovirus
29	Enterovirus B	＞10^6copies/mL	Novel coronavirus negative	Enterovirus
30	Enterovirus C	＞10^6copies/mL	Novel coronavirus negative	Enterovirus
31	Enterovirus D	＞10^6copies/mL	Novel coronavirus negative	Enterovirus
32	EB virus	＞10^6copies/mL	Novel coronavirus negative	Lymphophilic viruses
33	Measles virus	＞10^6copies/mL	Novel coronavirus negative	Measles virus
34	Human cytomegalovirus	＞10^6copies/mL	Novel coronavirus negative	Cytomegalovirus
35	Rotavirus	＞10^6copies/mL	Novel coronavirus negative	Rotavirus
36	Norovirus	＞10^6copies/mL	Novel coronavirus negative	Norovirus
37	Mumps virus	＞10^6copies/mL	Novel coronavirus negative	Mumps virus
38	Herpes zoster virus	＞10^6copies/mL	Novel coronavirus negative	herpes virus
39	Mycoplasma pneumoniae	＞10^6CFU/mL	Novel coronavirus negative	Mycoplasma

2.2.2.Evaluation of samples from healthy persons

Twenty saliva samples from healthy persons are tested with three batches of trial-produced kits to verify the crossover with healthy persons.

2.3.Analysis of interfering substances

The sample types tested with this kit are saliva samples. The common interfering substances are mainly endogenous interfering substances and drug interfering substances, such as mucin, 20% (v/v) human blood, nasal spray or nose drop (phenylephrine, oxymetazoline, sodium chloride (with preservative)), nasal dermal steroid (beclomethasone, dexamethasone, flunisolide, triamcinolone acetonide, budesonide, mometasone, and

fluticasone), antiviral drugs (interferon-α, zanamivir, ribavirin, oseltamivir, peramivir, lopinavir, ritonavir, and arbidol), antibiotics (levofloxacin, azithromycin, ceftriaxone and meropenem), antibacterial drug (tobramycin), and allergic symptom reliever (histamine dihydrochloride). In this study, the anti-interference analysis performance of the kit is evaluated with these interfering substances.

Basic samples: Four saliva samples (3 positive samples and 1 negative sample) are selected as the basic samples.

2.3.1.Endogenous interfering substances

2.3.1.1. Mucin

The interfering substance: 0.5g mucin is dissolved to the concentration of 120mg/dL with 1×PBS buffer solution.

The interference samples: The 200 mL mucin with the concentration of 120 mg/dL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the mucin concentration in the interference sample is 60 mg/dL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.1.2. 20%(v/v) human blood

The interference samples: The 60 mL human blood is added to the 240 mL basic samples, which is mixed upside down as the interference samples. At the moment, the concentration of the blood interfering substance in the sample is 20%.

The contrast sample: The 60mL 1×PBS buffer solution is added to the 240 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.Exogenous interfering substances

2.3.2.1. Phenylephrine

The interfering substance: The phenylephrine is dissolved to the concentration of 4mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL phenylephrine with the concentration of

4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the phenylephrine concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.2. Oxymetazoline

The interfering substance: The oxymetazoline is dissolved to the concentration of 4mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL oxymetazoline with the concentration of 4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the oxymetazoline concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.3. Sodium chloride (with preservative)

The interfering substance: The 2g sodium chloride is dissolved to the concentration of 40 mg/mL with pure water. The preservative is added to the dissolved sodium chloride solution with the concentration of 0.2%.

The interference samples: The 200 mL sodium chloride with the concentration of 40mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the sodium chloride concentration in the interference sample is 20mg/mL, with 0.1% of preservative.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.4. Beclomethasone

The interfering substance: The beclomethasone is dissolved to the concentration of 40mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL beclomethasone with the concentration of 40mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the beclomethasone concentration in the interference sample is 20mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.5. Dexamethasone

The interfering substance: The dexamethasone is dissolved to the concentration of 40mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL dexamethasone with the concentration of 40mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the dexamethasone concentration in the interference sample is 20mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.6. Flunisolide

The interference samples: The 200 mL flunisolide with the concentration of 40μg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the flunisolide concentration in the interference sample is 20μg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.7. Triamcinolone acetonide

The interfering substance: The triamcinolone acetonide is dissolved to the concentration of 4mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL triamcinolone acetonide with the concentration of 4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the triamcinolone acetonide concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The interfering basic control sample and the interference sample are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.8. Budesonide

The interfering substance: The budesonide is dissolved to the concentration of 4mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL budesonide with the concentration of 4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the budesonide concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.9. Mometasone

The interference samples: The 200 mL mometasone with the concentration of 4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the mometasone concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.10. Fluticasone

The interference samples: The 200 mL fluticasone with the concentration of 4mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the fluticasone concentration in the interference sample is 2mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.11. Zanamivir

The interference samples: The 200 mL zanamivir with the concentration of 40mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the zanamivir concentration in the interference sample is 20mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.12. Peramivir

The interference samples: The 200 mL peramivir with the concentration of 2mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the peramivir concentration in the interference sample is 1mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.13. Interferon-α

The interfering substance: The interferon-α is dissolved to the concentration of 1600IU/mL with 1×PBS buffer solution.

The interference samples: The 200 mL interferon-α with the concentration of 1600IU/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the interferon-α concentration in the interference sample is 800IU/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.14. Ribavirin

The interfering substance: The ribavirin is dissolved to the concentration of 20mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL ribavirin with the concentration of 20mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the ribavirin concentration in the interference sample is 10mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.15. Oseltamivir

The interfering substance: The oseltamivir is dissolved to the concentration of 120ng/mL with 1×PBS buffer solution.

The interference samples: The 200 mL oseltamivir with the concentration of 120ng/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the oseltamivir concentration in the interference sample is 60ng/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.16. Levofloxacin

The interfering substance: The levofloxacin is dissolved to the concentration of 20μg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL levofloxacin with the concentration of 20μg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the levofloxacin concentration in the interference sample is 10μg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.17. Azithromycin

The interfering substance: The azithromycin is dissolved to the concentration of 2mg/L with 1×PBS buffer solution.

The interference samples: The 200 mL azithromycin with the concentration of 2mg/L is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the azithromycin concentration in the interference sample is 1mg/L.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.18. Tobramycin

The interfering substance: The tobramycin is dissolved to the concentration of 1.2mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL tobramycin with the concentration of 1.2mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the tobramycin concentration in the interference sample is 0.6mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.19. Histamine dihydrochloride

The interference samples: The 200 mL histamine dihydrochloride with the concentration of 10mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the mometasone concentration in the interference sample is 5mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.20. Lopinavir

The interference samples: The 200 mL lopinavir with the concentration of 1000mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the lopinavir concentration in the interference sample is 500mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.21. Ritonavir

The interference samples: The 200 mL ritonavir with the concentration of 120mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the ritonavir concentration in the interference sample is 60mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.22. Arbidol

The interfering substance: The arbidol is dissolved to the concentration of 1400ng/mL with 1×PBS buffer solution.

The interference samples: The 200 mL arbidol with the concentration of 1400ng/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the arbidol concentration in the interference sample is 700ng/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.23. Ceftriaxone

The interfering substance: The ceftriaxone is dissolved to the concentration of 80μg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL ceftriaxone with the concentration of 80μg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference

samples. At the moment, the ceftriaxone concentration in the interference sample is 40μg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

2.3.2.24.Meropenem

The interfering substance: The meropenem is dissolved to the concentration of 400mg/mL with 1×PBS buffer solution.

The interference samples: The 200 mL meropenem with the concentration of 400mg/mL is added to the 200 mL basic samples, which is mixed upside down as the interference samples. At the moment, the meropenem concentration in the interference sample is 200mg/mL.

The contrast sample: The 200mL 1×PBS buffer solution is added to the 200 mL basic samples, which is mixed upside down as the interfering basic sample.

The contrast sample and the interference samples are tested as per IFU of the kit, and the consistency between the test results is compared.

3.Kit repeatability evaluation

3.1.Finished kit repeatability evaluation

3.1.1. Enterprise repeatability reference evaluation

The enterprise repeatability references R1 and R2 are tested with three batches of trial-produced kits in decuplicate, respectively and the test results are all positive for the novel coronavirus antigen.

3.2.Intra-lot/inter-lot and intra-day/inter-day precision

Two operators used three batches of trial production kits to detect strong positive samples in the morning and afternoon on the same day, the medially positive sample, the critically positive sample, and the negative sample in 20 replicates, respectively; and the intra-lot, inter-lot, and intra-day repeatability of the kit is evaluated. Three batches of kits are used by the same operator to test the strongly positive sample, the medially positive

sample, the critically positive sample, and the naluated.

3.3.Inter-operator and inter-location precision

The same batch of kits are used by 2 operators in the same day in 2 locations to test the strongly positive sample, the medially positive sample, the critically positive sample, and the negative sample in 20 replicates, respectively; and the inter-operator and inter-location kit test repeatability is evaluated.

3.4.Evaluation indicator

3.4.1. Strongly positive sample:

The enterprise repeatability reference R1 is selected as the strongly positive sample, and the test results shall be all positive for the novel coronavirus antigen.

3.4.2. Medially positive sample:

The enterprise repeatability reference R2 is selected as the medially positive sample, and the test results shall be all positive for the novel coronavirus antigen.

3.4.3. Critically positive sample:

Select 1 case of culture and use the new coronavirus negative saliva sample to dilute to 5×10^4Copies/mL, as the test sample, the test result should be the new coronavirus antigen positive detection rate of 90%~100%.

3.4.4. Negative sample

One novel coronavirus antigen negative sample is selected as the negative sample for repeatability evaluation, and the test results shall all be negative for the novel coronavirus antigen.

4.LOD

4.1.Verification of enterprise LOD references

Enterprise LOD references L1~L6 are tested with three batches of trial-produced kits. The following shall be met: Test results of L1, L2, L4, and L5 are positive for the novel coronavirus antigen, and the test results of L3 and L6 may be positive or negative for the novel coronavirus antigen.

4.2.Validation and verification of lowest LOD

4.2.1.Lowest LOD of virus culture

4.2.1.1.Determination of estimated LOD

Three samples in the novel coronavirus culture are gradiently diluted with the sample extraction solution in kit and the negative saliva sample based on the PCR quantitative results to obtain the diluted samples C01~C24 with the concentration of 10^6, 10^5, 10^4 and 10^3Copies/mL. Each dilution series is tested with three batches of trial-produced kits in triplicate, and the minimum dilution ratio with positive test results for all 3 replicates is selected as the estimated LOD.

4.2.1.2.Validation of Lowest LOD

Use the kit sample extract and negative saliva samples to dilute to 4 series concentrations near the estimated detection limit, labeled as C25-C48, and each sample concentration is tested in 20 replicates, and the concentration with 90% ~ 100% of detection rate is selected as the lowest LOD.

4.2.1.3.Verification of lowest LOD

Select 3 culture samples, and according to their PCR quantitative results, use the kit sample extract and negative saliva samples to be diluted to the detection limit concentration, labeled C49-C54. The concentration of each sample is tested with three batches of trial-produced kits in 20 replicates, the detection rate should be 90%~100%.

4.2.2.Lowest LOD of antigen

4.2.2.1.Determination of estimated LOD of antigen concentration

N protein is gradiently diluted with the sample extraction solution in kit and the negative saliva sample to obtain the samples NP01~NP08 with the concentration of 1ng/mL、100pg/mL、10pg/mL and 1pg/mL. Each dilution series is tested with three batches of trial-produced kits in triplicate, and the minimum dilution ratio with positive test results for all 3 replicates is selected as the estimated LOD.

4.2.2.2.Validation of Lowest LOD of antigen concentration

Use the kit sample extract and negative saliva samples to be diluted to 4 series concentrations near the estimated detection limit, labeled as NP09-NP16, and each sample concentration is tested in 20 replicates, and the concentration with 90% ~ 100% of detection rate is selected as the lowest LOD.

4.2.2.3.Verification of Lowest LOD of antigen concentration

N protein is diluted with the sample extraction solution in kit to the concentration of the lowest LOD, mark as NP17-NP18,which is tested with three batches of trial-produced kits in 20 replicates.

4.2.3.Lowest LOD of virus titer

4.2.3.1. Determination of estimated LOD

The collected samples were separated and cultured using the Vero E6 cell line. After 2 days in a 37°C CO2 incubator, the lesions of the cells were observed and diluted and tested with nucleic acid. Cell cultures with positive nucleic acid test results were selected for labeling to obtain the new coronavirus Cultures. Dilute the virus stock solution (10-1, 10-2...10-10, etc.) with the incubation medium 10 times the culture, inoculate the culture plate, incubate in a 37℃ CO2 incubator for 1 hour, take out the culture plate and aspirate the virus liquid (Drawing from low concentration to high concentration can avoid channeling), add 200 μl of maintenance solution and continue to incubate in 37℃ CO2 incubator for 2 days. Take out the culture plate and observe the cell pathology under the microscope. Find out the virus dilution factor that can cause half of the cell bottle or tube infection, and calculate the TCID50 of the virus solution to obtain 3 culture samples.

Use the supporting sample extract and negative saliva samples to perform serial dilutions on the 3 culture samples, to obtain the samples S01~S24 with the titers of 5000$TCID_{50}$/mL, 500 $TCID_{50}$/mL, 50 $TCID_{50}$/mL and 5 $TCID_{50}$/mL. Each dilution series is tested with the kits in triplicate, and the minimum dilution ratio with positive test results for all 3 replicates is selected as the estimated LOD.

4.2.3.2.Validation of Lowest LOD

Use the kit sample extract and negative saliva samples to be diluted to 4 series concentrations near the estimated detection limit, labeled as S25-S48, and each sample concentration is tested in 20 replicates, and the concentration with 90% ~ 100% of detection rate is selected as the lowest LOD.

4.2.3.3.Verification of lowest LOD

Three samples in the culture are diluted with the sample extraction solution in kit and the negative saliva sample to the titer of LOD, labeled as S49-S54. The concentration of each sample is verified with three batches of trial-produced kits in 20 replicates.

5.Hook effect

Three cases of novel coronal culture and one case of recombinant N protein were selected for gradient dilution using negative samples, respectively. The concentrations of 3 samples were 2×10^8、5×10^7、5×10^5、5×10^4Copies/mL；The concentrations of N protein were 1000ng/mL、100ng/mL、10ng/mL、1ng/mL. The kit HooK effect is evaluated.

IIIResults

1.Kit accuracy test

1.1.Coincidence rate of enterprise positive references

The enterprise positive references are tested with three batches of trial-produced kits, and the test results are shown in Table 1.

Table 1: Statistics of Results of Enterprise Positive References Tested with Three Batches of Kits (+ for Positive)

Number	Test requirement	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
P1	+	+	+	+
P2	+	+	+	+
P3	+	+	+	+
P4	+	+	+	+
P5	+	+	+	+
P6	+	+	+	+

The test results show that the coincidence rate of enterprise positive references tested with three batches of kits is 100%.

1.2.Evaluation of samples from different regions

1.2.1.Repeatability

Table 2: Statistics of Results of 10 Samples from Different Regions Tested in Decuplicate(+/+)

Number	Source	Acquisition time	The original concentration Copies/mL	Measured concentration Copies/mL	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
1	Shenzhen	2020.10.10	1×10^8	1×10^8	10/10	10/10	10/10

2	Tianjin	2020.02.09	1×10^7	1×10^7	10/10	10/10	10/10
3	Zhengzhou	2020.02.01	5×10^8	5×10^8	10/10	10/10	10/10
4	Zhengzhou	2020.08.16	1×10^7	1×10^7	10/10	10/10	10/10
5	Wuhan	2020.02.12	1×10^7	1×10^7	10/10	10/10	10/10
6	Shenzhen	2020.09.16	5×10^7	5×10^7	10/10	10/10	10/10
7	Tianjin	2020.02.17	1×10^7	1×10^7	10/10	10/10	10/10
8	Zhengzhou	2020.02.09	1×10^7	1×10^7	10/10	10/10	10/10
9	Zhengzhou	2020.09.11	1×10^8	1×10^8	10/10	10/10	10/10
10	Wuhan	2020.01.31	6×10^6	6×10^6	10/10	10/10	10/10

The test results show that 10 samples from patients infected with novel coronavirus from different regions are positive for the novel coronavirus antigen when tested in decuplicate, and the kit can detect the novel coronavirus antigen in saliva samples from patients with novel coronavirus infection.

1.2.2.Lowest LOD

The test results with 10 samples from different regions diluted to the estimated LOD of are shown in the table below.

Table 3: Statistics of Maximum dilution Test Results of Different Samples Gradiently Diluted

Number	Source	Concentration (Copies/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
1	Shenzhen	1×10^6	3/3	3/3	3/3
		1×10^5	3/3	3/3	3/3
		1×10^4	0/3	0/3	0/3
2	Tianjin	1×10^5	3/3	3/3	3/3
		1×10^4	1/3	1/3	3/3
		1×10^3	0/3	0/3	0/3
3	Zhengzhou	5×10^6	3/3	3/3	3/3
		5×10^5	3/3	3/3	3/3
		5×10^4	0/3	0/3	0/3
4	Zhengzhou	1×10^5	3/3	3/3	3/3
		1×10^4	1/3	0/3	1/3

		1×10^3	0/3	0/3	0/3
5	Wuhan	1×10^5	3/3	3/3	3/3
		1×10^4	0/3	1/3	0/3
		1×10^3	0/3	0/3	0/3
6	Shenzhen	5×10^5	3/3	3/3	3/3
		5×10^4	3/3	3/3	3/3
		5×10^3	0/3	0/3	0/3
7	Tianjin	1×10^5	3/3	3/3	3/3
		1×10^4	1/3	1/3	0/3
		1×10^3	0/3	0/3	0/3
8	Zhengzhou	1×10^5	3/3	3/3	3/3
		1×10^4	1/3	0/3	0/3
		1×10^3	0/3	0/3	0/3
9	Zhengzhou	1×10^6	3/3	3/3	3/3
		1×10^5	3/3	3/3	3/3
		1×10^4	0/3	1/3	0/3
10	Wuhan	5×10^4	3/3	3/3	3/3
		5×10^3	0/3	0/3	0/3
		5×10^2	0/3	0/3	0/3

Table 4: Statistics of LOD of Samples from Different Regions

Number	Source	Concentration (Copies/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
1	Shenzhen	1×10^5	20/20	20/20	20/20
		5×10^4	19/20	20/20	20/20
		2×10^4	5/20	8/20	6/20
2	Tianjin	1×10^5	20/20	20/20	20/20
		5×10^4	20/20	19/20	20/20
		2×10^4	7/20	6/20	5/20
3	Zhengzhou	5×10^4	20/20	20/20	20/20
		2.5×10^4	11/20	10/20	12/20
		1×10^4	1/20	3/20	2/20
4	Zhengzhou	1×10^5	20/20	20/20	20/20
		5×10^4	19/20	20/20	20/20
		2×10^4	5/20	6/20	10/20
5	Wuhan	1×10^5	20/20	20/20	20/20
		5×10^4	20/20	20/20	20/20
		2×10^4	11/20	9/20	8/20
6	Shenzhen	5×10^4	20/20	20/20	20/20
		2.5×10^4	16/20	15/20	14/20
		1×10^4	3/20	5/20	2/20
7	Tianjin	1×10^5	20/20	20/20	20/20

		5×10^4	19/20	20/20	19/20
		2×10^4	7/20	8/20	6/20
8	Zhengzhou	1×10^5	20/20	20/20	20/20
		5×10^4	19/20	19/20	20/20
		2×10^4	9/20	6/20	9/20
9	Zhengzhou	1×10^5	20/20	20/20	20/20
		5×10^4	20/20	20/20	20/20
		2×10^4	4/20	5/20	5/20
10	Wuhan	5×10^4	20/20	20/20	20/20
		2.5×10^4	10/20	13/20	13/20
		1×10^4	5/20	7/20	5/20

The test results show that the detection rate of the lowest LOD of 10 samples from different regions tested with three batches of kits are consistent.

2.Kit specificity test

2.1. Coincidence rate of enterprise negative references

The enterprise negative references are tested with three batches of trial-produced kits, and the test results are shown in the table.

Table 5: Statistics of Results of Enterprise Negative References Tested with Three Batches of Kits (- for Negative)

Number	Test requirement	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
N1	-	-	-	-
N2	-	-	-	-
N3	-	-	-	-
N4	-	-	-	-
N5	-	-	-	-
N6	-	-	-	-
N7	-	-	-	-
N8	-	-	-	-
N9	-	-	-	-

The test results show that the coincidence rate of enterprise negative references tested with three batches of kits is 100%.

2.2. Cross reaction

2.2.1. Pathogen crossing

Test results of other pathogens tested with three batches of kits are shown in the

following table.

Table 6: Statistics of Test Results of Other Pathogens Tested with Three Batches of Kits (- for Negative)

Name of pathogen	Concentration	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
HCoV-HKU1	1.5×10^6copies/mL	-	-	-
HCoV-OC43	1.1×10^6copies/mL	-	-	-
HCoV-NL63	1.0×10^6copies/mL	-	-	-
HCoV-229E	3.8×10^6copies/mL	-	-	-
Novel Influenza A (H1N1) Virus (2009)	1.8×10^6copies/mL	-	-	-
Seasonal H1N1 influenza virus	8×10^6copies/mL	-	-	-
Influenza A virus (H3N2)	1.2×10^6copies/mL	-	-	-
Influenza A virus (H5N1)	1.5×0^6copies/mL	-	-	-
Influenza A virus (H7N9)	1.5×10^6copies/mL	-	-	-
Influenza B virus (Yamagata)	1.5×10^6copies/mL	-	-	-
Influenza B virus (Victoria)	1.5×10^6copies/mL	-	-	-
Respiratory syncytial virus type A	1.5×10^6copies/mL	-	-	-
Respiratory syncytial virus type B	$1.5\times10^6TCID_{50}$/ mL	-	-	-
Parainfluenza virus type 1	1.5×10^6copies/mL	-	-	-
Parainfluenza virus type 2	1.5×10^6copies/mL	-	-	-
Parainfluenza virus type 3	1.5×10^6copies/mL	-	-	-
Rhinovirus A	1.5×10^6copies/mL	-	-	-
Rhinovirus B	1.5×10^6copies/mL	-	-	-
Rhinovirus C	1.5×10^6copies/mL	-	-	-
Adenovirus type 1	1.5×10^6copies/mL	-	-	-
Adenovirus type 2	1.5×10^6copies/mL	-	-	-
Adenovirus type 3	1.5×10^6copies/mL	-	-	-
Adenovirus type 4	1.5×10^6copies/mL	-	-	-
Adenovirus type 5	1.5×10^6copies/mL	-	-	-
Adenovirus type 7	1.5×10^6copies/mL	-	-	-
Adenovirus type 55	1.5×10^6copies/mL	-	-	-
Human metapneumovirus	1.5×10^6copies/mL	-	-	-
Enterovirus A	1.5×10^6copies/mL	-	-	-
Enterovirus B	1.5×10^6copies/mL	-	-	-
Enterovirus C	1.5×10^6copies/mL	-	-	-
Enterovirus D	1.5×10^6copies/mL	-	-	-
EB virus	1.5×10^6copies/mL	-	-	-
Measles virus	1.5×10^6copies/mL	-	-	-
Cytomegalovirus	1.5×10^6copies/mL	-	-	-

Rotavirus	1.5×10^6copies/mL	-	-	-
Norovirus	1.5×10^6copies/mL	-	-	-
Mumps virus	1.5×10^6copies/mL	-	-	-
Varicella-zoster virus	1.5×10^6copies/mL	-	-	-
Mycoplasma pneumoniae	1.5×10^6CFU/mL	-	-	-

The test results show that the test results are all negative when the common cross substances are tested with three batches of kits, indicating that the above-mentioned pathogens have no effect on the test results of the kits.

2.2.2.Verification of samples from healthy persons

Twenty saliva samples from healthy persons are tested with three batches of kits, and the test results are shown in the table.

Table 7: Statistics of Results of saliva Samples from Healthy Persons Tested with Three Batches of Kits (- for Negative)

Sample type	Sample No.	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Sample 1	-	-	-
	Sample 2	-	-	-
	Sample 3	-	-	-
	Sample 4	-	-	-
	Sample 5	-	-	-
	Sample 6	-	-	-
	Sample 7	-	-	-
	Sample 8	-	-	-
	Sample 9	-	-	-
	Sample 10	-	-	-
	Sample 11	-	-	-
	Sample 12	-	-	-
	Sample 13	-	-	-
	Sample 14	-	-	-
	Sample 15	-	-	-
	Sample 16	-	-	-
	Sample 17	-	-	-
	Sample 18	-	-	-
	Sample 19	-	-	-
	Sample 20	-	-	-

The test results show that 20 saliva samples from healthy persons are tested with three batches of kits, and the test results are all negative.

2.3. Analysis of interfering substances

2.3.1. Endogenous interfering substances

2.3.2.1. Mucin

Table 8: Statistics of Mucin Interference Results (+ for Positive, and - for Negative)

Sample type	Mucin (60mg/dL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic samples and the interference samples with mucin tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of mucin in the samples is not higher than 60 mg/dL.

2.3.2.2. 20%(v/v) human blood

Table 9: Statistics of 20%(v/v) Human Blood Interference Results (+ for Positive, and - for Negative)

Sample type	20%(v/v) human blood	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+

	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic samples and the 20% (v/v) human blood interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of 20% (v/v) human blood in the samples is not higher than 60 mg/dL.

2.3.2. Exogenous interfering substances

2.3.2.1. Phenylephrine

Table 10: Statistics of Interference Test Results of Phenylephrine

Sample type	Phenylephrine (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the phenylephrine interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of phenylephrine in the samples is not higher than 2mg/dL.

2.3.2.2. Oxymetazoline

Table 11: Statistics of Interference Test Results of oxymetazoline (+ for Positive, and - for Negative)

Sample type	Oxymetazoline (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the oxymetazoline interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of oxymetazoline in the samples is not higher than 2mg/dL.

2.3.2.3. Sodium chloride (with preservative)

Table 12: Statistics of Interference Test Results of Sodium Chloride (with Preservative) (+ for Positive, and - for Negative)

Sample type	Sodium chloride (20mg/mL), with 0.1% of preservative	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive	+	+	+

	sample-3			
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the sodium chloride (with preservative) interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of sodium chloride (with preservative) in the samples is not higher than 20mg/mL (0.1% of preservative).

2.3.2.4. Beclomethasone

Table 13: Statistics of Interference Test Results of Beclomethasone (+ for Positive, and - for Negative)

Sample type	Beclomethasone (20mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the beclomethasone interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of beclomethasone in the samples is not higher than 20mg/mL.

2.3.2.5. Dexamethasone

Table 14: Statistics of Interference Test Results of Dexamethasone (+ for Positive, and - for Negative)

Sample type	Dexamethasone (20mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the dexamethasone interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of dexamethasone in the samples is not higher than 20mg/mL.

2.3.2.6. Flunisolide

Table 15: Statistics of Interference Test Results of Flunisolide (+ for Positive, and - for Negative)

Sample type	Flunisolide (20μg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the flunisolide interference samples tested with three batches of kits are consistent, indicating

that the test results of the kits are not affected when the concentration of flunisolide in the samples is not higher than 20μg/mL.

2.3.2.7. Triamcinolone acetonide

Table 16: Statistics of Interference Test Results of Triamcinolone Acetonide (+ for Positive, and - for Negative)

Sample type	Triamcinolone acetonide (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the triamcinolone acetonide interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of triamcinolone acetonide in the samples is not higher than 2mg/mL.

2.3.2.8. Budesonide

Table 17: Statistics of Interference Test Results of Budesonide (+ for Positive, and - for Negative)

Sample type	Budesonide (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference	+	+	+

	sample -2			
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the budesonide interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of budesonide in the samples is not higher than 2mg/mL.

2.3.2.9. Mometasone

Table 18: Statistics of Interference Test Results of Mometasone (+ for Positive, and - for Negative)

Sample type	Mometasone (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the mometasone interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of mometasone in the samples is not higher than 2mg/mL.

2.3.2.10. Fluticasone

Table 19: Statistics of Interference Test Results of Fluticasone (+ for Positive, and - for Negative)

Sample type	Fluticasone (2mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the fluticasone interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of fluticasone in the samples is not higher than 2mg/mL.

2.3.2.11. Zanamivir

Table 20: Statistics of Interference Test Results of Zanamivir (+ for Positive, and - for Negative)

Sample type	Zanamivir (20mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample	-	-	-

	-4			

The test results show that the results of the interference basic control samples and the zanamivir interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of zanamivir in the samples is not higher than 20mg/mL.

2.3.2.12. Peramivir

Table 21: Statistics of Interference Test Results of Peramivir (+ for Positive, and - for Negative)

Sample type	Peramivir 1mg/mL	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the peramivir interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of peramivir in the samples is not higher than 1mg/mL.

2.3.2.13. Lopinavir

Table 22: Statistics of Interference Test Results of Lopinavir (+ for Positive, and - for Negative)

Sample type	Lopinavir (500mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+

	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the lopinavir interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of lopinavir in the samples is not higher than 500mg/mL.

2.3.2.14. Ritonavir

Table 23: Statistics of Interference Test Results of Ritonavir (+ for Positive, and - for Negative)

Sample type	Ritonavir (60mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the ritonavir interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of ritonavir in the samples is not higher than 60mg/mL.

2.3.2.15. Interferon-α

Table24: Statistics of Interference Test Results of Interferon-α (+ for Positive, and - for Negative)

Sample type	Interferon-α (800IU/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the interferon-α interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of interferon-α in the samples is not higher than 800IU/mL.

2.3.2.16. Ribavirin

Table 25: Statistics of Interference Test Results of Ribavirin (+ for Positive, and - for Negative)

Sample type	Ribavirin (10mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-

	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the ribavirin interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of ribavirin in the samples is not higher than 10mg/mL.

2.3.2.17. Oseltamivir

Table 26: Statistics of Interference Test Results of Oseltamivir (+ for Positive, and - for Negative)

Sample type	Oseltamivir (60ng/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the oseltamivir interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of oseltamivir in the samples is not higher than 60ng/mL.

2.3.2.18. Arbidol

Table 27: Statistics of Interference Test Results of Arbidol (+ for Positive, and - for Negative)

Sample type	Arbidol (700ng/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+

	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the arbidol interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of arbidol in the samples is not higher than 700ng/mL.

2.3.2.19. Levofloxacin

Table 28: Statistics of Interference Test Results of Levofloxacin (+ for Positive, and - for Negative)

Sample type	Levofloxacin (10μg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the levofloxacin interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of levofloxacin in the

samples is not higher than 20μg/mL.

2.3.2.20. Azithromycin

Table 29: Statistics of Interference Test Results of Azithromycin (+ for Positive, and - for Negative)

Sample type	Azithromycin (1mg/L)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the azithromycin interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of azithromycin in the samples is not higher than 1mg/L.

2.3.2.21. Ceftriaxone

Table 30: Statistics of Interference Test Results of Ceftriaxone (+ for Positive, and - for Negative)

Sample type	Ceftriaxone (40μg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample	+	+	+

	-3			
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the ceftriaxone interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of ceftriaxone in the samples is not higher than 40μg/mL.

2.3.2.22. Meropenem

Table 31: Statistics of Interference Test Results of Meropenem (+ for Positive, and - for Negative)

Sample type	Meropenem (200mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the meropenem interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of meropenem in the samples is not higher than 200mg/mL.

2.3.2.23. Tobramycin

Table 32: Statistics of Interference Test Results of Tobramycin (+ for Positive, and - for Negative)

Sample type	Tobramycin (0.6mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive	+	+	+

	sample-1			
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the tobramycin interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of tobramycin in the samples is not higher than 0.6mg/mL.

2.3.2.24. Histamine dihydrochloride

Table 33: Statistics of Interference Test Results of Histamine Hydrochloride (+ for Positive, and - for Negative)

Sample type	Histamine dihydrochloride (5mg/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
saliva	Interference positive sample-1	+	+	+
	Interference sample -1	+	+	+
	Interference positive sample-2	+	+	+
	Interference sample -2	+	+	+
	Interference positive sample-3	+	+	+
	Interference sample -3	+	+	+
	Interference negative sample-4	-	-	-
	Interference sample -4	-	-	-

The test results show that the results of the interference basic control samples and the histamine dihydrochloride interference samples tested with three batches of kits are consistent, indicating that the test results of the kits are not affected when the concentration of histamine dihydrochloride in the samples is not higher than 5mg/mL.

3.**Kit repeatability evaluation**

3.1.Finished kit repeatability evaluation

3.1.1.Enterprise repeatability reference evaluation

The enterprise repeatability references are tested with three batches of trial-produced kits in decuplicate, and the test results are shown in the table.

Table 34: Statistics of Results of Enterprise Repeatability References Tested with Three Batches of Kits (+ for Positive)

Number	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
R1（+/+）	10/10	10/10	10/10
R2（+/+）	10/10	10/10	10/10

The test results show that the enterprise repeatability references tested with three batches are all positive for novel coronavirus.

3.2.Intra-lot/inter-lot and intra-day/inter-day precision

Table 35: Statistics of Intra-lot, Inter-lot and Intra-day Evaluation Test Results

Time	Test indicator	Test requirement	Operator 1			Operator 2		
			Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
Morning	Strongly positive sample(n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）
	Medially positive sample(n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）
	Critically positive sample(n=20)	Detection rate (90~100%)	19/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	19/20（+/+）	20/20（+/+）
	Negative sample(n=20)	Coincidence rate (100%)	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）

Afternoon	Strongly positive sample(n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）
	Medially positive sample(n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）
	Critically positive sample(n=20)	Detection rate (90~100%)	20/20（+/+）	19/20（+/+）	20/20（+/+）	19/20（+/+）	20/20（+/+）	20/20（+/+）
	Negative sample(n=20)	Coincidence rate (100%)	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）

The test results show that the strongly positive sample, the medially positive sample, and the critically positive sample are all positive for the novel coronavirus antigen when tested with three batches of kits by 2 operators on the same day. The negative coincidence rate of the negative sample is 100%, indicating that the kits have good intra-lot, inter-lot and intra-day test repeatability.

Table 36: Statistics of Inter-day Evaluation Test Results

Detection Indicator	Detection Requirements	Time	202008001batch of test results	202008002batch of test results	202008003batch of test results
Strongly positive sample (n=60)	Coincidence rate（100%）	1day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		2day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		3day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		4day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		5day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		6day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		7day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		8day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		9day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		10day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		11day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		12day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		13day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		14day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		15day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		16day	3/3（+/+）	3/3（+/+）	3/3（+/+）

		17day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		18day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		19day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		20day	3/3（+/+）	3/3（+/+）	3/3（+/+）
Medially positive sample(n=60)	Coincidence rate（100%）	1day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		2day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		3day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		4day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		5day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		6day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		7day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		8day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		9day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		10day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		11day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		12day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		13day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		14day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		15day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		16day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		17day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		18day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		19day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		20day	3/3（+/+）	3/3（+/+）	3/3（+/+）
Critically positive sample（n=60）	Coincidence rate（90%~100%）	1day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		2day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		3day	2/3（-/+）	3/3（+/+）	3/3（+/+）
		4day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		5day	3/3（+/+）	2/3（-/+）	3/3（+/+）
		6day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		7day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		8day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		9day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		10day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		11day	3/3（+/+）	3/3（+/+）	2/3（-/+）
		12day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		13day	2/3（-/+）	3/3（+/+）	3/3（+/+）
		14day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		15day	3/3（+/+）	3/3（+/+）	3/3（+/+）

		16day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		17day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		18day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		19day	3/3（+/+）	3/3（+/+）	3/3（+/+）
		20day	3/3（+/+）	3/3（+/+）	3/3（+/+）
Negative sample（n=60）	Coincidence rate（100%）	1day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		2day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		3day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		4day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		5day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		6day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		7day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		8day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		9day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		10day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		11day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		12day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		13day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		14day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		15day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		16day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		17day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		18day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		19day	3/3（-/-）	3/3（-/-）	3/3（-/-）
		20day	3/3（-/-）	3/3（-/-）	3/3（-/-）

The test results show that the strongly positive sample, the medially positive sample, and the critically positive sample are all positive for the novel coronavirus antigen when tested with three batches of kits. The negative coincidence rate of the negative sample is 100%, indicating that the kits have good inter-day test repeatability.

3.3.Inter-operator and inter-location precision

Table 37: Statistics of Inter-operator and Inter-location Repeatability Evaluation Test Results of Kits

Test indicator		Operator 1	Operator 2	Operator 1	Operator 2
		Laboratory 1	Laboratory 2	Laboratory 2	Laboratory 1
Strongly positive sample (n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）

Medially positive sample (n=20)	Detection rate (100%)	20/20（+/+）	20/20（+/+）	20/20（+/+）	20/20（+/+）
Critically positive sample (n=20)	Detection rate (90%~100%)	20/20（+/+）	19/20（+/+）	19/20（+/+）	20/20（+/+）
Negative sample (n=20)	Coincidence rate (100%)	20/20（-/-）	20/20（-/-）	20/20（-/-）	20/20（-/-）

The test results show that the strongly positive sample, the medially positive sample, and the critically positive sample are all positive for the novel coronavirus antigen when tested with one batch of kits by 2 operators in 2 locations. The negative coincidence rate of the negative sample is 100%, indicating that the kits have good inter-operator and inter-location test repeatability.

4.Lowest LOD

4.1. Verification of enterprise LOD references

Table 38: Statistics of Results of Enterprise LOD References L1~L6 Tested with Three Batches of Kits (+ for Positive, - for Negative, and/for Positive or Negative)

Number	Test requirement	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
L1	+	+	+	+
L2	+	+	+	+
L3	/	+	+	+
L4	+	+	+	+
L5	+	+	+	+
L6	/	-	+	+

The test results show that the test results of enterprise LOD references tested with three batches of kits meets the test requirements.

4.2. Determination of Lowest LOD

4.2.1.Lowest LOD of Virus culture

4.2.1.1. Determination of estimated LOD

Table 39: Table of original concentration of each culture

No.	RNA Copies/mL	$TICID_{50}$/mL
Culture viral1	2.03×10^{8}	8.12×10^{5}

Culture viral2	2.10×10^8	8.40×10^5
Culture viral3	2.06×10^8	8.24×10^5

Table 40: Validation of estimated LOD

Sample	Matrix	Concentration (Copies/mL)	Positives detected (+/+)	Positive rate detected
C1	Sample extraction solution	10^6	3/3	100%
C2		10^5	3/3	100%
C3		10^4	3/3	100%
C4		10^3	0/3	0%
C5		10^6	3/3	100%
C6		10^5	3/3	100%
C7		10^4	2/3	66.7%
C8		10^3	0/3	0%
C9		10^6	3/3	100%
C10		10^5	3/3	100%
C11		10^4	2/3	66.7%
C12		10^3	1/3	33.3%
C13	saliva	10^6	3/3	100%
C14		10^5	3/3	100%
C15		10^4	2/3	66.7%
C16		10^3	0/3	0%
C17		10^6	3/3	100%
C18		10^5	3/3	100%
C19		10^4	2/3	66.7%
C20		10^3	0/3	0%
C21		10^6	3/3	100%

C22		10^5	3/3	100%
C23		10^4	2/3	66.7%
C24		10^3	0/3	0%

The results show that the minimum dilution ratios of different positive samples are different. The dilution of 10^5Copies/mL with positive test results for all 3 replicates of each sample is selected as the estimated LOD.

4.2.1.2. Validation of lowest LOD

The novel coronavirus antigen positive culture is diluted with the negative sample to be close to the estimated LOD, and 4 concentrations are diluted in 20 replicates, respectively. The test results are shown in the table below.

Table 41: Determination of Lowest LOD

Sample	Matrix	Concentration (Copies/mL)	Positives detected (+/+)	Positive rate detected
C25	Sample extraction solution	1×10^5	20/20	100%
C26		5×10^4	20/20	100%
C27		2×10^4	18/20	90%
C28		1×10^4	12/20	60%
C29		1×10^5	20/20	100%
C30		5×10^4	20/20	100%
C31		2×10^4	15/20	75%
C32		1×10^4	9/20	45%
C33		1×10^5	20/20	100%
C34		5×10^4	20/20	100%
C35		2×10^4	15/20	75%
C36		1×10^4	10/20	50%
C37	saliva	1×10^5	20/20	100%
C38		5×10^4	20/20	100%
C15		2×10^4	16/20	80%
C16		1×10^4	9/20	45%
C17		1×10^5	20/20	100%
C18		5×10^4	19/20	95%
C19		2×10^4	14/20	70%
C20		1×10^4	10/20	50%

C21		1×10^5	20/20	100%
C22		5×10^4	18/20	90%
C23		2×10^4	13/20	65%
C24		1×10^4	9/20	45%

The test results show that the lowest LOD of the kit is 5×10^4Copies/mL.

4.2.1.3. Verification of lowest LOD

Three cultures are diluted with the above 2 kinds of diluent to 5×10^4Copies/mL. Then, three batches of kits are used for the testing.

Table 41: Verification Results of Three Batches of Trial-produced Kits

Matrix	Number	Measured concentration	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
Sample extraction solution	C25	5×10^4Copies/mL	20/20	20/20	20/20
saliva	C26	5×10^4Copies/mL	20/20	20/20	20/20
Sample extraction solution	C27	5×10^4Copies/mL	20/20	20/20	20/20
saliva	C28	5×10^4Copies/mL	20/20	20/20	20/20
Sample extraction solution	C29	5×10^4Copies/mL	20/20	20/20	20/20
saliva	C30	5×10^4Copies/mL	20/20	20/20	20/20

The test results show that 3 samples with the lowest LOD concentrations are tested in 20 replicates, respectively, and the detection rate is 90%~100%, indicating that the lowest LOD of three batches of trial-produced kits meets the requirements.

4.2.2. Lowest LOD of antigen

4.2.2.1. Determination of estimated LOD of antigen concentration

Table 43: Determination of estimated LOD

Matrix	Number	Concentration	Positives detected (+/+)	Positive rate detected
Sample extraction solution	NP01	1ng/mL	3/3	100%
	NP02	100pg/mL	3/3	100%
	NP03	10pg/mL	0/3	0%

	NP04	1pg/mL	0/3	0%
saliva	NP05	1ng/mL	3/3	100%
	NP06	100pg/mL	3/3	100%
	NP07	10pg/mL	0/3	0%
	NP08	1pg/mL	0/3	0%

The results show that the antigen dilution of 100 pg/mL is selected as the estimated LOD, and gradient dilutions are performed around the estimated LOD. The samples are tested in 20 replicates, respectively. The dilution with the positive detection rate of 90%-100% is selected as the lowest LOD of the kit.

4.2.2.2. Determination of the lowest LOD of antigen concentration

Table 44: Determination of Lowest LOD

Sample	Number	Concentration	Positives detected (+/+)	Positive rate detected
Sample extraction solution	NP09	100pg/mL	20/20	100%
	NP10	50pg/mL	20/20	100%
	NP11	25pg/mL	11/20	55%
	NP12	10pg/mL	0/20	0%
saliva	NP13	100pg/mL	20/20	100%
	NP14	50pg/mL	20/20	100%
	NP15	25pg/mL	10/20	50%
	NP16	10pg/mL	0/20	0%

The test results show that the lowest LOD of the kit is 50pg/mL.

4.2.2.3. Verification of the lowest LOD of antigen concentration

N protein is diluted with the sample extraction solution in kit to obtain the sample with the concentration of 50pg/mL, which is tested with three batches of trial-produced kits in 20 replicates.

Table 45: Verification Results of Three Batches of Trial-produced Kits

Number	Measured concentration	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
NP17	50pg/mL	20/20	20/20	19/20
NP18	50pg/mL	20/20	19/20	20/20

The test results show that the samples with the lowest LOD concentrations are tested in 20 replicates, respectively, and the detection rate is 90%~100%, indicating that the lowest LOD of three batches of trial-produced kits meets the requirements.

4.2.3. Lowest LOD of virus titer

4.2.3.1. Determination of estimated LOD

Table 46: Determination of estimated LOD

Matrix	Number	Concentration ($TCID_{50}$/mL)	Positives detected (+/+)	Positive rate detected
Sample extraction solution	S01	5000	3/3	100%
	S02	500	3/3	100%
	S03	50	2/3	66.67%
	S04	5	0/3	0%
	S05	5000	3/3	100%
	S06	500	3/3	100%
	S07	50	1/3	33.33%
	S08	5	0/3	0%
	S09	5000	3/3	100%
	S10	500	3/3	100%
	S11	50	2/3	66.67%
	S12	5	0/3	0%
saliva	S13	5000	3/3	100%
	S14	500	3/3	100%
	S15	50	0/3	0%
	S16	5	0/3	0%
	S17	5000	3/3	100%
	S18	500	3/3	100%
	S19	50	0/3	0%
	S20	5	0/3	0%
	S21	5000	3/3	100%
	S22	500	3/3	100%
	S23	50	0/3	0%
	S24	5	0/3	0%

The results show that the titer of 500 $TCID_{50}$/mL with positive test results for all 3 replicates of each sample is selected as the estimated LOD. The samples are gradiently diluted close to the estimated LOD in 20 replicates, respectively. The dilution with the positive detection rate of 90%-100% is selected as the estimated LOD of the kit.

4.2.3.2. Qualification of Lowest LOD

Table 47: Determination of Lowest LOD

Matrix	Number	Concentration ($TCID_{50}$/mL)	Positives detected (+/+)	Positive rate detected
Sample extraction solution	S25	500	20/20	100%
	S26	400	20/20	100%
	S27	300	20/20	100%
	S28	200	20/20	100%
	S29	100	11/20	55%
	S30	500	20/20	100%
	S31	400	20/20	100%
	S32	300	20/20	100%
	S33	200	20/20	100%
	S34	100	7/20	35%
	S35	500	20/20	100%
	S36	400	20/20	100%
	S37	300	20/20	100%
	S38	200	20/20	100%
	S39	100	9/20	45%
saliva	S40	500	20/20	100%
	S41	400	20/20	100%
	S42	300	20/20	100%
	S43	200	20/20	100%
	S44	100	5/20	25%
	S45	500	20/20	100%
	S46	400	20/20	100%
	S47	300	20/20	100%
	S48	200	20/20	100%
	S49	100	1/20	5%
	S50	500	20/20	100%
	S51	400	20/20	100%
	S52	300	20/20	100%
	S53	200	20/20	100%
	S54	100	4/20	20%

The test results show that the lowest LOD of the virus titer of the kit is 200 $TCID_{50}$/mL.

4.2.3.3. Verification of Lowest LOD

Table 48: Verification Results of Three Batches of Trial-produced Kits

Number	Concentration ($TCID_{50}$/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003

S55	200	20/20	20/20	20/20
S56	200	20/20	20/20	20/20
S57	200	20/20	20/20	20/20
S58	200	20/20	20/20	20/20
S59	200	20/20	20/20	20/20
S60	200	20/20	20/20	20/20

The test results show that 3 samples with the lowest LOD concentrations are tested in 20 replicates, respectively, and the detection rate is 90%~100%, indicating that the lowest LOD of three batches of trial-produced kits meets the requirements.

5.Result of Hook effect

Table 49: Statistics of Verification Results of Positive saliva Samples with Different Concentrations of Novel Coronavirus Antigens (+ for Positive)

Sample No.	Sample concentration (Copies/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
Cultures1	2×10^8	+	+	+
	5×10^7	+	+	+
	5×10^5	+	+	+
	5×10^4	+	+	+
Cultures2	2×10^8	+	+	+
	5×10^7	+	+	+
	5×10^5	+	+	+
	5×10^4	+	+	+
Cultures3	2×10^8	+	+	+
	5×10^7	+	+	+
	5×10^5	+	+	+
	5×10^4	+	+	+
N protein	Concentration (Copies/mL)	Test results of Lot 202008001	Test results of Lot 202008002	Test results of Lot 202008003
Protein dilution	1000ng/mL	+	+	+
	100ng/mL	+	+	+

sample	10ng/mL	+	+	+
	1ng/mL	+	+	+

The test results show that when the concentration of the novel coronavirus in the sample tested with the kit is not higher than 2×10^8 Copies/mL and the protein presence was less than 1000ng/mL.

IVConclusions

Three batches of trial-produced kits are used to test enterprise references P1~P6, and the test results all meet the requirements. Three batches of trial-produced kits are used to test 10 samples from different regions, and the repeatability and LOD all meet the requirements. The specificity analysis results show that there are interfering substances in the samples, such as mucin (60mg/dL), 20% (v/v) human blood, phenylephrine (2mg/mL), oxymetazoline (2mg/mL), sodium chloride (with preservative) (20mg/mL), beclomethasone (20mg/mL), dexamethasone (20mg/mL), flunisolide (20μg/mL), triamcinolone acetonide (2mg/mL), budesonide (2mg/mL), mometasone (2mg/mL), fluticasone (2mg/mL), antiviral drug interferon-α (800IU/mL), zanamivir (20mg/mL), ribavirin (10mg/mL), oseltamivir (60ng/mL), peramivir (1mg/mL), lopinavir (500mg/mL), ritonavir (60mg/mL), arbidol (70ng/mL), antibiotic levofloxacin (10μg/mL), azithromycin (1mg/L), ceftriaxone (40μg/mL), meropenem (200mg/mL), antibacterial drug tobramycin (0.6mg/mL), and allergic symptom reliever histamine hydrochloride (5mg/mL), with no significant effect on the detection of the kits within the above concentrations. There are no cross-reactions with pathogen samples of human coronavirus HCoV-OC43, HCoV-229E, HCoV-HKU1, HCoV-NL63; novel influenza A H1N1 virus (2009); seasonal H1N1 influenza virus, H3N2, H5N1, H7N9; influenza B Yamagata and Victoria; respiratory syncytial virus; parainfluenza virus; rhinoviruses A, B, and C; adenovirus types 1, 2, 3, 4, 5, 7, and 55; enteroviruses A, B, C, and D; EB virus; measles virus; human cytomegalovirus; rotavirus; norovirus; mumps virus; varicella-zoster virus; and mycoplasma pneumoniae. Twenty saliva samples from healthy persons are tested, and the test results are all negative. The kit has good test specificity.

Three batches of trial-produced kits are used to test the strongly positive sample, the medially positive sample, the critically positive sample, and the negative sample, and the intra-lot/inter-lot, intra-day/inter-day, inter-operator/inter-location repeatability of the kit is good. The lowest LODs of the test samples, antigens, and virus titer are 5×10^4Copies/mL, 50pg/mL, and 200$TCID_{50}$/mL, respectively.

The HooK test results show that there is no HooK effect when the concentration of the novel coronavirus in the sample tested with the kit is not higher than 2×10^8 Copies/mL and the protein presence was less than 1000ng/mL.