COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold)

Performance Evaluation Report

COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold)

Performance Evaluation Report

Novel coronavirus pneumonia (NCP) is a kind of disease that caused by novel coronavirus.Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. Coronavirus can be excreted through respiratory secretions, transmitted by oral fluid, sneeze, contact, and transmitted through droplets. COVID-19(Sars-CoV-2) Antigen Test is a simple, visual qualitative test that detects novel coronavirus in human nasal swabs. The test is based on immunochromatography and can give a result within 10-15 minutes.

We systematically evaluated the performance of the products, mainly related to limit of detection, specificity, repeatability, difference between batches, etc. The reagents used for the evaluation were three batches of test cassette (Lot: 200401, Lot: 200402, Lot: 200403).

Positive：Two distinct lines appear. One line should always appear in the control line region (C), and another one or two apparent colored line should appear in the test line region(T).

Negative: One colored line appears in the control region (C). No Apparent colored line appears in the test line region(T).

1. Physical property evaluation

1.1 Materials and Method

Take 3 batches of COVID-19 (Sars-CoV-2) Antigen Test Kit (colloidal gold) produced by our company and take 3 test cassettes for each experiment.

Use a vernier caliper to measure the width of each test strip. The width of the strip should be controlled above 2.5mm. Immerse the test paper in the sample solution and start timing with a stopwatch. The liquid transfer speed of each test paper should be no less than 10mm/min. We use the actual measured data as the physical property evaluation index.

1.2 Results and discussion

Each batch number is repeated 3 times. The results are as follows:

Table 1-1 shows the experimental results of the test strips

Test items / Lot		width（mm）	Liquid mobility rate（mm/min）
200401	1	3.01	45.9
	2	3.02	43.4
	3	3.02	48.9
200402	1	3.01	45.8
	2	3.00	44.7
	3	3.02	42.6
200403	1	2.99	43.8
	2	3.02	47.3
	3	3.01	45.5

Summary: According to the above results, it can be seen that the width of the test strips and the liquid migration speed of different models can meet the requirements, and the physical properties completely meet the requirements.

2. The limit of detection

2.1 Materials and method

For the evaluation of LOD with the Ct values of the qRT-PCR, the Ct values of 28 and 31 were tested with the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue with 20 replicates. The Ct values of 28 and 31 of qRT-PCR all indicated the positive results of COVID-19. And the number of the antigen test positive results were analyzed and compared with those of the qRT-PCR.

SARS-CoV-2 Samples (qRT-PCR)	Antigen detection kit (Colloidal gold) DeepBlue	Antigen detection results/ qRT-PCR	Positive%
Ct 28	20	20/20	100 %
Ct 31	20	20/20	100 %

For the evaluation of LOD with the $TCID_{50}$, LOD studies determined the lowest detectable concentration of SARS-CoV-2 at which 100% of all (true positive) replicates test positive. The LOD for the DeepBlue SARS-CoV-2 Ag Test was established using limiting dilutions of gamma-irradiated SARS-CoV-2 (BEIResources DB-1001). The DB-52287 is a preparation of SARS-Related Coronavirus 2 (SARS-CoV2), that has been inactivated by gamma-irradiation at 5 x 10^6 RADs. The material was supplied frozen at a concentration of 2.8 x 10^5 $TCID_{50}$/mL.

The detection limit of the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) was evaluated by the two kinds of indexes including the $TCID_{50}$ of the viral infectivity and the cycle threshold (Ct value) of the real time fluorescent PCR, respectively.

The collected nasal swabs were stored in the virus storage tube and inactivated by the thermal treatment at 56 ℃ for 30 min . Then the inactivated virus samples were extracted of RNA by the commercial extraction kit. Then the extracted RNA samples of the virus were measured with the commercial BGI kit (20203400060). The corresponding virus samples with the tested Ct values were further determined with the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue.

Isolation and identification of the virus. Nasal swabs were collected from patients with confirmed COVID-19 and filtered with 0.22 μM membrane to remove impurity. The supernatants were inoculated onto Vero Cells in a T25 culture flask. After 1h incubation at 37 ℃ with 5% carbon dioxide for binding, the inoculum was removed and replace with fresh DMEM with 2% Fetal Bovine Serum . The cells were incubated at 37 ℃ with 5% carbon dioxide for 72-96 h and monitoring daily to evaluate cytopathic effects (CPE). Finally, the supernatant was harvested and inactivated at 56 ℃ for 30 min . All of the above operations must be performed in the BSL-3 laboratory. The inactivated products were tested for ORF1ab and N gene of SARS-CoV-2 by RT-PCR (BGI, 20203400060), according to the instruction of the kit. Once the result showed positive, the viral particles were collected from culture.

Viral infectivity assay. Vero cells were grown in a 6-well plate and infected with the virus in duplicates at MOI (multiplicity of infection) of 0.001. Distribute viruses over cells and incubated at 37 °C in 5% CO_2 for

1 hour, one virus made three replications, then washed with PBS. Fresh culture media were replenished up to a total volume of 3 mL and incubated 6-well plate at 37°C in 5% CO_2 for 96 hours. The plate were checked daily from 16 h post-infection onwards until 96 h. Collected three duplicated supernatants from the every infected well at 16 h, 24 h, 40 h, 48 h, 72 h and 96 h, then centrifuge at 500×g for 5 min to remove any cellular debris at room temperature. The clarified supernatant was aliquoted into 1.5 mL tubes and stored at -80 ℃ until needed. The tissue culture infective dose that causes 50% cytotoxicity ($TCID_{50}$) assay was performed with the clarified supernatants harvested from 24 hours to the last time point to quantify the viral infectivity. Resuspend 1×10^6 cells in 10 mL Vero cells grown medium per 96-well plate to be used. Seed 1×10^4 Vero cells per well into 96-well plates in normal Vero cell grown medium and culture overnight at 37 ℃ in 5% CO_2. Dilute clarified supernatants in Vero cell grown medium, and then continued diluting down a 5-fold dilution series in triplicate. Then, 100 µl diluted samples were transferred to each well of cells in the plates in eight duplications. After incubated at 37 °C in 5% CO_2 for 1 hour, the cultures were washed with PBS and fresh media were replenished. CPE was monitored after another 72 h incubation, examined plate and score wells. $TCID_{50}$ was calculated using Reed-Muench method.

2.2 Results and discussion

An initial LOD screening study was performed using a 5-fold serial dilutions (six dilutions in total) of the gamma-irradiated virus made in pooled negative human nasal matrix starting at a test concentration of 2 x 10^4 $TCID_{50}$/mL (as shown in table below) and processed for each study as described. These dilutions were tested in triplicate. The lowest concentration at which all (3 out of 3 replicates) were positive was chosen for LOD Range finding. This $TCID_{50}$/mL was treated as 320.

SARS-CoV-2 tested ($TCID_{50}$/mL)	Test Result
200000	3/3 positive
40000	3/3 positive
8000	3/3 positive
1600	3/3 positive
320	3/3 positive
62	0/3 positive

Using the 32 $TCID_{50}$/mL concentration, the LOD was further refined using a 2-fold dilution series (four dilutions in total) of the gamma-irradiated SARS- CoV-2 virus made in pooled negative human nasal matrix. These dilutions were tested in triplicate. The lowest concentration at which all (3 out of 3 replicates) were positive was treated as the tentative LOD for the DeepBlue SARS-CoV-2 Ag Test. This $TCID_{50}$/mL was still 320.

SARS-CoV-2 tested ($TCID_{50}$/mL)	Test Result
320	3/3 positive
160	0/3 positive
80	1/3 positive
40	0/3 positive

3. Cross reaction

Cross-reactivity of the Deepblue SARS-CoV-2 Ag Test was evaluated by testing a panel of related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen and could potentially cross-react with the Deepblue SARS CoV-2 Ag Test including various microorganisms, viruses and negative matrix. Each organism and virus were tested in the absence or presence of heat inactivated SARS-CoV-2 at 3 x LoD. The final concentration of the organisms and viruses are documented in the Table below (the concentrations of 10^6 CFU/mL or higher for bacteria and 10^5 PFU/mL or higher for viruses is recommended). For a number of microorganisms, the stock concentration was lower than or equal to the recommended testing concentration. In these cases, it was only possible to test these microorganisms at the stock concentration.

3.1 For Microorganism:

Test kit batch number: 200401. The results are as follows:

Microorganism	Concentration	Cross-Reactivity (Yes/No)
Adenovirus 3	1 x 10^5 PFU/mL	No (3/3 negative)
Parainfluenza virus Type 2	1 x 10^5 PFU/mL	No (3/3 negative)
Human coronavirus NL63	9.87 x 10^3 PFU/mL	No (3/3 negative)
MERS coronavirus(Pseudovirus, part of ORFlab+N gene)	7930 copies/mL	No (3/3negative)
Human coronavirus 229E	1 x 10^5 PFU/mL	No(3/3 negative)
Human coronavirus OC43	1 x 10^5 PFU/mL	No (3/3 negative)
SARS-COV-2Pseudovirus (N full-length gene)	1 x 10^5 copies/mL	No (3/3 negative)
Enterovirus	1 x 10^5 PFU/mL	No(3/3negative)
Respiratory syncytial virus(A)	1 x 10^5 PFU/mL	No(3/3negative)
Parainfluenza virus Type 3	1 x 10^5 PFU/mL	No(3/3negative)
Parainfluenza virus Type 4a	1 x 10^5 PFU/mL	No(3/3negative)
Influenza A H3N2 (Wisconsin/67/05)	8.82 x 10^4PFU/mL	No(3/3negative)
Influenza A H1N1	1 x 10^5 PFU/mL	No(3/3negative)
Influenza B （VICRTORIA）	2.92 x 10^4 PFU/mL	No(3/3negative)
Rhinovirus(HRVA30）	4.17 x 10^5 PFU/mL	No(3/3negative)
Haemophilus influenzae	1 x 10^6 CFU/mL	No(3/3negative)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	No(3/3negative)
Streptococcus pyogenes	1 x 10^6 CFU/mL	No(3/3negative)
Candida albicans	1 x 10^6 CFU/mL	No(3/3negative)
Bordetella pertussis	1 x 10^6 CFU/mL	No(3/3negative)
Mycoplasma pneumoniae	1 x 10^6 CFU/mL	No(3/3negative)
Chlamydia pneumoniae	1 x 10^6 CFU/mL	No(3/3negative)

Legionella pneumophila	1 x 10^6 CFU/mL	No(3/3negative)
Mycobacterium tuberculosis	1 x 10^6 CFU/mL	No(3/3negative)
Pneumocystis jirovecii	1 x 10^6 CFU/mL	No(3/3negative)
Pseudomonas Aeruginosa	1 x 10^6 CFU/mL	No(3/3negative)
Human Metapneumovirus (hMPV)	1 x 10^5 PFU/mL	No (3/3 negative)
Parainfluenza virus Type 1	1 x 10^5 PFU/mL	No (3/3 negative)
Staphylococcus Epidermidis	1 x 10^6 CFU/mL	No(3/3negative)
Streptococcus Salivarius	1 x 10^6 CFU/mL	No(3/3negative)
Staphylococcus aureus	1 x 10^6 CFU/mL	No(3/3negative)

During the test, each microorganism is directly tested, and the labeled concentration is the test concentration. Each sample is tested 3 times, and the results are all negative to be judged as no cross-reaction.

To estimate the likelihood of cross-reactivity with SARS-CoV-2 of organisms that were not available for wet testing, in silico analysis using the Basic Local Alignment Search Tool (BLAST) managed by the National Center for Biotechnology Information (NCBI) was used to assess the degree of protein sequence homology.
For Human Coronavirus HKU1, homology exists between the SARS- CoV-2 nucleocapsid protein and Human Coronavirus HKU1. BLAST results showed 30 sequence IDs, all nucleocapsid protein, showing homology. Sequence ID AGW27840.1 had the highest alignment score and was found to be 39.1% homologous across 76% of the sequences, this is relatively low but cross-reactivity cannot be fully ruled out.
For SARS-Coronavirus, high homology exists between the SARS-CoV-2 nucleocapsid protein and SARS-Coronavirus. BLAST results showed 68 sequence IDs, mostly nucleocapsid protein, showing homology. Sequence ID AAR87518.1, had the highest alignment score isolated from a human patient and was found to be 90.76% homologous across 100% of the sequence. This is high and cross-reactivity is likely.
For MERS-Coronavirus, high homology exists between the SARS- CoV-2 nucleocapsid protein and MERS-Coronavirus. BLAST results showed at least 114 sequence IDs, mostly nucleocapsid protein, showing homology. Sequence IDs AHY61344.1 and AWH65950.1, had the highest alignment scores isolated from a human patient and were found to be 49.4% and 50.3% homologous across 88% of the sequence. Whilst this potentially represents moderate cross-reactivity testing of the MERS virus at 7930 PFU/mL showed no reactivity (see table above).

3.1.2 For pooled human nasal wash:

We purchased common nasal washes on the market for testing, including Chinese brands and foreign brands. The detailed information of each nasal wash is as follows:

Name of Nasal Wash	Manufacturer	Drug concentration	Cross-Reactivity (Yes/No)
Fluticasone Propionate Nasal Spray	GlaxoSmithKline	0.05%(g/g)	No (3/3 negative)
Ribavirin Spray	Jewim Pharmaceutical(Shandong)Co.,Ltd.	25mg/g	No (3/3 negative)
Budesonide Nasal Spray	AstraZeneca AB	0.64mg/ml	No (3/3 negative)

Naphazoline Hydrochloride Nasal Drops	Sinopharm Sanyi(Wuhu)Co.,Ltd.	1mg/ml	No (3/3negative)

During the test, each nasal wash is mixed in equal volume, and then tested directly from the mixed solution. After testing 3 times, all 3 times are negative before it can be judged as no cross-reaction.

3.2 Microbial Interference Studies

Microbial interference in the Deepblue SARS-CoV-2 Ag Test was evaluated by testing a panel of related pathogens, high prevalence disease agents and normal or pathogenic flora to demonstrate that false negatives do not occur when SARS-CoV-2 is present in a specimen with other microorganisms including various microorganisms, viruses and negative matrix. Each organism and virus were tested in the absence or presence of heat inactivated SARS-CoV-2 at 3 x LoD. The final concentration of the organisms and viruses are documented in the Table below (the concentrations of 10^6 CFU/mL or higher for bacteria and 10^5 PFU/mL or higher for viruses is recommended). For a number of microorganisms, the stock concentration was lower than or equal to the recommended testing concentration. In these cases, it was only possible to test these microorganisms at the stock concentration.

Take 20 normal human samples and mix them with the diluent. Each sample is confirmed to be negative by PCR. Mix 20 negative samples to obtain a negative matrix.

Take inactivated virus samples (source: Anhui Provincial Center for Disease Control and Prevention), with a concentration value of 2.8×10^5 $TCID_{50}$/ml. First, the virus stock solution was diluted 10 times with negative matrix, then 10 times, and then 1.5 times, to obtain a 6 times LoD sample with a concentration of 1867$TCID_{50}$/ml.

Each microorganism and interfering substance is purchased, and the labeled concentration is the initial concentration. Mix each microorganism and interfering substance with a sample of 6 times the LoD concentration to obtain a 3 times the LoD sample, and the dilution factor is 2.

Microorganism	Concentration	Interference (Yes/No)
Parainfluenza virus Type 1	1 x 10^5 PFU/mL	No(3/3 positive)
Parainfluenza virus Type 2	1 x 10^5 PFU/mL	No(3/3 positive)
Parainfluenza virus Type 3	1 x 10^5 PFU/mL	No(3/3 positive)
Parainfluenza virus Type 4a	1 x 10^5 PFU/mL	No(3/3 positive)
Adenovirus (e.g. C1 Ad. 71)	1 x 10^5 PFU/mL	No(3/3 positive)
Human Metapneumovirus (hMPV)	1 x 10^5 PFU/mL	No(3/3 positive)
Influenza A H3N2(Wisconsin/67/05)	8.82 x 10^4 PFU/mL	No(3/3 positive)
Influenza A H1N1	1 x 10^5 PFU/mL	No(3/3 positive)
Haemophilus influenzae	1 x 10^6 CFU/mL	No(3/3 positive)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	No(3/3 positive)
Streptococcus pyogenes	1 x 10^6 CFU/mL	No(3/3 positive)
Influenza B (Malaysia/2506/04)	2.92 x 10^4 PFU/mL	No(3/3 positive)
Enterovirus	1 x 10^5 PFU/mL	No(3/3 positive)
Respiratory syncytial virus	1 x 10^5 PFU/mL	No(3/3 positive)
Rhinovirus	4.17 x 10^5 PFU/mL	No(3/3 positive)

Chlamydia pneumoniae	1 x 10^6 CFU/mL	No(3/3 positive)
Legionella pneumophila	1 x 10^6 CFU/mL	No(3/3 positive)
Mycobacterium tuberculosis	1 x 10^6 CFU/mL	No(3/3 positive)
Pneumocystis jirovecii	1 x 10^6 CFU/mL	No(3/3 positive)
Pseudomonas Aeruginosa	1 x 10^6 CFU/mL	No(3/3 positive)
Candida albicans	1 x 10^6 CFU/mL	No(3/3 positive)
Bordetella pertussis	1 x 10^6 CFU/mL	No(3/3 positive)
Mycoplasma pneumoniae	1 x 10^6 copies/mL	No(3/3 positive)
Staphylococcus Epidermidis	1 x 10^6 CFU/mL	No(3/3 positive)
Streptococcus Salivarius	1 x 10^6 CFU/mL	No(3/3 positive)
Human coronavirus 229E	1 x 10^5 PFU/mL	No(3/3 positive)
Human coronavirus OC43	1 x 10^5 PFU/mL	No(3/3 positive)
Human coronavirus NL63	9.87 x 10^3 PFU/mL	No(3/3 positive)
MERS coronavirus	7930 copies/mL	No(3/3 positive)
Staphylococcus aureus	1 x 10^6 CFU/mL	No(3/3 positive)
Fluticasone Propionate	0.05%(g/g)	No(3/3 positive)

Each sample is tested 3 times, and the results are all negative to determine that there is no interference with samples with 3 times the LOD concentration.

3.3 Endogenous Interference Studies

A study was performed to demonstrate that potentially interfering substances that may be found in the upper respiratory tract in symptomatic subjects (including over the counter medications) do not cross-react or interfere with the detection of SARS-CoV-2 in the Deepblue SARS-CoV-2 Ag Test.

Take 20 normal human samples and mix them with the diluent. Each sample is confirmed to be negative by PCR. Mix 20 negative samples to obtain a negative matrix.

Take inactivated virus samples (source: Anhui Provincial Center for Disease Control and Prevention), with a concentration value of 2.8×105 TCID50/ml. First, the virus stock solution was diluted 10 times with negative matrix, then 10 times, and then 1.5 times, to obtain a 6 times LoD sample with a concentration of 1867TCID50/ml.

The source, concentration, detection method and results of each endogenous interfering substance are as follows:

Zicam Cold Remedy

We purchased Zicam Cold Remedy nasal spray through the Internet and added it to the negative matrix and 3x LOD samples for research.

We selected Zicam Cold Remedy nasal spray as the research object, and added 50 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 5%.

We added 100 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 10%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Alkalol Nasal Wash

We purchased Alkalol Nasal Wash nasal spray through the Internet and added it to the negative matrix and 3x LOD samples for research.

We selected Alkalol Nasal Wash nasal spray as the research object, and added 100 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 10%.

We added 200 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 20%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Sore Throat Phenol Spray

We purchased Ribavirin Spray and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Jewim Pharmaceutical(Shandong)Co.,Ltd..

We selected Ribavirin Spray as the research object, and added 150 microliters of nasal spray to 1 ml of negative matrix as a negative interference test.The concentration is 15%.

We added 300 microliters of nasal spray to 1 ml of negative matrix as a negative interference test.The concentration is 30%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Blood (human)

We purchased Hemoglobin and added it to the negative matrix and 3x LOD samples for research. The manufacturer is SIGMA-ALDRICH,Co..CAS number is 9008-02-0.

Weigh 50mg of hemoglobin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 5%.

Weigh 100mg of hemoglobin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 10%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Mucin

We purchased Mucinex and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Reckitt Benckiser Healthcare.

Weigh 5mg drug and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 5mg/ml.

Weigh 10mg drug and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 10mg/ml. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Naso GEL

We purchased Neilmed NasoGel through the Internet and added it to the negative matrix and 3x LOD samples for research.

We selected Neilmed NasoGel as the research object, and added 50 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 5%.

We added 100 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 10%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

CVS Nasal Drops (phenylephrine)

We purchased Fluticasone Propionate Nasal Spray and added it to the negative matrix and 3x LOD samples for research. The manufacturer is GlaxoSmithKline.

We selected Fluticasone Propionate Nasal Spray as the research object, and added 150 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 15%.

We added 300 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 30%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Afrin (Oxymetazoline)

We purchased Naphazoline Hydrochloride Nasal Drops and added it to the negative matrix and 3x LOD samples for research. The manufacturer is GlaxoSmithKline.

We selected Naphazoline Hydrochloride Nasal Drops as the research object, and added 150 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 15%.

We added 300 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 30%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

CVS Nasal Spray (Cromolyn)

We purchased Ribavirin Spray and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Jewim Pharmaceutical(Shandong)Co.,Ltd..

We selected Ribavirin Spray as the research object, and added 150 microliters of nasal spray to 1 ml of negative matrix as a negative interference test.The concentration is 15%.

We added 300 microliters of nasal spray to 1 ml of negative matrix as a negative interference test.The concentration is 30%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Tamiflu (Oseltamivir phosphate)

We purchased Oseltamivir Phosphate and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 204255-11-8.

Weigh 5mg of Oseltamivir Phosphate and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 500mg/dL.

Weigh 10mg of Oseltamivir Phosphate and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 1000mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Budenoside

We purchased Budenoside and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 131918-64-4.

Weigh 6.3mg of Budenoside and dissolve it in 100ml of negative matrix as a negative interference test. The concentration is 0.00063mg/dL.

Weigh 12.6mg of Budenoside and dissolve it in 100ml of negative matrix as a negative interference test. The concentration is 0.00126mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Biotin

We purchased Biotin and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 58-85-5.

Weigh 35mg of Budenoside and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 0.35mg/dL.

Weigh 70mg of Budenoside and dissolve it in 1ml of negative matrix as a negative interference test. The

concentration is 0.7mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Tobramycin

We purchased Tobramycin and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 32986-56-4.

Weigh 330mg of Tobramycin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 3.3mg/dL.

Weigh 660mg of Tobramycin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 6.6mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Mupirocin

We purchased Mupirocin and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 12650-69-0.

Weigh 15mg of Mupirocin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 0.15mg/dL.

Weigh 30mg of Mupirocin and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 0.3mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Dextromethorphan

We purchased Dextromethorphan and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 125-71-3.

Weigh 15.6mg of Dextromethorphan and dissolve it in 100ml of negative matrix as a negative interference

test. The concentration is 0.00156mg/dL.

Weigh 31.2mg of Dextromethorphan and dissolve it in 100ml of negative matrix as a negative interference test. The concentration is 0.00312mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Dexamethasone

We purchased Dexamethasone and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 50-02-2.

Weigh 120mg of Dexamethasone and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 1.2mg/dL.

Weigh 240mg of Dexamethasone and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 2.4mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Methanol

We purchased Methanol and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd..

We selected Methanol as the research object, and added 50 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 5%.

We added 100 microliters of nasal spray to 1 ml of negative matrix as a negative interference test. The concentration is 10%. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Acetylsalicylic Acid

We purchased Acetylsalicylic Acid and added it to the negative matrix and 3x LOD samples for research.

The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 50-78-2.

Weigh 30mg of Acetylsalicylic Acid and dissolve it in 0.1ml of negative matrix as a negative interference test. The concentration is 3mg/dL.

Weigh 60mg of Acetylsalicylic Acid and dissolve it in 0.1ml of negative matrix as a negative interference test. The concentration is 6mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Diphenhydramine

We purchased Diphenhydramine and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 147-24-0.

Weigh 7.74mg of Diphenhydramine and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 0.0774mg/dL.

Weigh 15.48mg of Diphenhydramine and dissolve it in 1ml of negative matrix as a negative interference test. The concentration is 0.1548mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

Benzocaine

We purchased Benzocaine and added it to the negative matrix and 3x LOD samples for research. The manufacturer is Seebio Biotech (Shanghai) Co.,Ltd.. CAS number is 94-09-7.

Weigh 1500mg of Benzocaine and dissolve it in 0.1ml of negative matrix as a negative interference test. The concentration is 150mg/dL.

Weigh 3000mg of Benzocaine and dissolve it in 0.1ml of negative matrix as a negative interference test. The concentration is 300mg/dL. Mix the nasal spray with the 6x LOD sample in equal volume as the interference study of the 3x LOD sample.

Each sample is tested 3 times, all negative test results should be negative, and all positive test results should be positive.

The final concentration of the substances tested are documented in the Table below.

Interfering Substance	Concentration	Interference (Yes/No)
Zicam Cold Remedy	5% v/v	No (3/3 Negative, 3/3 Positive)
Homeopathic (Alkalol)	10 % v/v	No (3/3 Negative, 3/3 Positive)
Sore Throat Phenol Spray	15% v/v	No (3/3 Negative, 3/3 Positive)
Blood (human)	5%	No (3/3 Negative, 3/3 Positive)
Mucin	5 mg/mL	No (3/3 Negative, 3/3 Positive)
Naso GEL (NeilMed)	5% v/v	No (3/3 Negative, 3/3 Positive)
CVS Nasal Drops (phenylephrine)	15% v/v	No (3/3 Negative, 3/3 Positive)
Afrin (Oxymetazoline)	15% v/v	No (3/3 Negative, 3/3 Positive)
CVS Nasal Spray (Cromolyn)	15% v/v	No (3/3 Negative, 3/3 Positive)
Tamiflu (Oseltamivir phosphate)	500 mg/dL	No (3/3 Negative, 3/3 Positive)
Budenoside	0.00063 mg/dL	No (3/3 Negative, 3/3 Positive)
Biotin	0.35 mg/dL	No (3/3 Negative, 3/3 Positive)
Tobramycin	3.3 mg/dL	No (3/3 Negative, 3/3 Positive)
Mupirocin	0.15 mg/dL	No (3/3 Negative, 3/3 Positive)
Dextromethorphan	0.00156 mg/dL	No (19/20 Negative, 3/3 Positive)
Dexamethasone	1.2 mg/dL	No (3/3 Negative, 3/3 Positive)
Methanol	150 mg/dL	No (19/20 Negative, 3/3 Positive)
Acetylsalicylic Acid	3 mg/dL	No (3/3 Negative, 3/3 Positive)
Diphenhydramine	0.0774 mg/dL	No (3/3 Negative, 3/3 Positive)
Benzocaine	150 mg/dL	No (3/3 Negative, 3/3 Positive)

3.4 Summary:

Experiments have confirmed that the above-mentioned clinically commonly used substances have no effect on the detection performance of COVID-19(Sars-CoV-2) Antigen test kit.

4. Repeatability

4.1 Method

Take the same batch test papers. Use negative sample and positive control to test. Both sample was tested 10 times. The results of 10 replicates should be consistent.

4.2 Results and discussion

Table 4-1, Table 4-2, and Table 4-3 are the experimental results of the test strips.

Table4-1 Lot：200401

Sample types / Test times	negative sample	positive control
1	-	+
2	-	+
3	-	+
4	-	+

5	-	+
6	-	+
7	-	+
8	-	+
9	-	+
10	-	+

Table4-2 Lot：200402

Test times \ Sample types	negative sample	positive control
1	-	+
2	-	+
3	-	+
4	-	+
5	-	+
6	-	+
7	-	+
8	-	+
9	-	+
10	-	+

Table4-3 Lot：200403

Test times \ Sample types	negative sample	positive control
1	-	+
2	-	+
3	-	+
4	-	+
5	-	+
6	-	+
7	-	+
8	-	+
9	-	+
10	-	+

4.3 Summary:

According to the above results, we can see that the results are consistent. The results of negative samples are all negative. The results of positive control are all positive.

5. Inter-batch difference

5.1 Method

Take 3 batchs test papers. Use negative sample and positive control to tset. Both sample was tested 10 times.

The results of all batchs should be consistent.

Table 5-1

Lot \ Sample types		negative sample	positive control
200401	1	-	+
	2	-	+
	3	-	+
	4	-	+
	5	-	+
	6	-	+
	7	-	+
	8	-	+
	9	-	+
	10	-	+
200402	1	-	+
	2	-	+
	3	-	+
	4	-	+
	5	-	+
	6	-	+
	7	-	+
	8	-	+
	9	-	+
	10	-	+
200403	1	-	+
	2	-	+
	3	-	+
	4	-	+
	5	-	+
	6	-	+
	7	-	+
	8	-	+
	9	-	+
	10	-	+

5.2 Summary:

It can be seen from the experiment that the three batches of test paper are tested, and each batch number is tested for 10 pieces, totaling 30 pieces. The results are consistent by the 30 items. Uniformity can indicate that the difference between the test is good.

6. Hook effect

6.1 Method

The serial increased concentrations of SARS-CoV-2 samples were tested with the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue. No impact on test performance or hook effect at high concentrations was observed up to1.4 x 10^5 $TCID_{50}$/mL of gamma-irradiated SARS-CoV-2 with the DeepBlue SARS- CoV-2 Ag Test.

Test Dilution	Concentration ($TCID_{50}$/mL)	Mean Signal (ADC Units)
1	0	495
2	62.5	26100.6
3	250	63013.8
4	1000	83451.8
5	1.4 x 10^5	86220

The tested results of the recombinant N protein reference material with the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue were recorded and compared.

Test sample	Recombinant N protein Concentration (ng/mL)	Mean Signal (ADC Units)
1	0	490
2	0.1	540
3	2.65	2490
4	26.5	26780
5	265	78950
6	2650	89650
7	26500	96530
8	265000	97650

6.2 Conclusion

For the results of the conducted studies in this research of detection limit (LOD) and hook effect, it is easy to come to the conclusion that, under the current optimized conditions, the LOD of the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue can be of Ct 31 by q RT-PCR and 32 of $TCID_{50}$/mL, respective.

For the hood effect analysis, when the concentration of the target COVID-19 (Sars-CoV-2) is as high as 1.4 x 10^5 $TCID_{50}$/mL, there is no observed hook effect at this high concentration. Meanwhile, for the evaluation with the recombinant N protein reference materials, it is noticed that as high as 265000 ng/ml

of N protein of COVID-19 does not induce the noticeable hook effect for the direct antigen detection with the COVID-19 (Sars-CoV-2) Antigen Test Kit (Colloidal gold) manufactured by the DeepBlue.

7. Conclusion

Test item	Technical requirements	Conclusion	
Appearance	The test kit shall be clear, no burrs, no damage and no pollution.	Pass	
Width	The width shall not be less than 2.5mm	Pass	
Free Flow Rate	Not less than 10 mm/min when tested with internal quality control.	Pass	
Detection Limit	The LOD concentration is 320 $TCID_{50}$/ml.	Pass	
Cross reaction	Shall not cross-react with Coronavirus (HKU1,OC43,NL63,229E), Influenza A virus (H1N1, H3N2, H5N1, H7N9), Influenza B virus, Chlamydia pneumoniae, Rhinovirus(A,B,C), Adenovirus(1,2,3,4,5,7,55), Enterovirus(A,B,C,D), Epstein-Barr virus, Measles virus, Human cytomegalovirus, Rotavirus, Norovirus, Mumps virus, Varicella-zoster virus, Respiratory syncytial virus, Mycoplasma pneumoniae, Escherichia Coli. Shall not cross-react with pooled human nasal wash.	Pass	
Repeatability	The results should be consistent and the coloration degree should be consistent when detecting the positive control solution by 10 kits of the same batch.	Pass	
Reproducibility	To conduct repeatability test on 3 batches, results shall meet the requirements of Repeatability test.	Pass	
Microbial Interference	Shall not cross-react with Parainfluenza virus Type 1,Parainfluenza virus Type 2, Parainfluenza virus Type 3, Parainfluenza virus Type 4a, Adenovirus (e.g. C1 Ad. 71), Human Metapneumovirus (hMPV), Influenza A H3N2(Wisconsin/67/05), Influenza A H1N1, Haemophilus influenzae, Streptococcus pneumoniae, Streptococcus pyogenes, Influenza B (Malaysia/2506/04), Enterovirus, Respiratory syncytial virus, Rhinovirus, Chlamydia pneumoniae, Legionella pneumophila, Mycobacterium tuberculosis, Pneumocystis jirovecii, Pseudomonas Aeruginosa, Candida albicans, Bordetella pertussis, Mycoplasma pneumoniae, Staphylococcus Epidermidis, Streptococcus Salivarius, Human coronavirus 229E, Human coronavirus OC43, Human coronavirus NL63, MERS coronavirus	Pass	
Endogenous Interference	Zicam Cold Remedy	5% v/v	Pass
	Homeopathic (Alkalol)	10 % v/v	
	Sore Throat Phenol Spray	15% v/v	
	Blood (human)	5%	
	Mucin	5 mg/mL	
	Naso GEL (NeilMed)	5% v/v	
	CVS Nasal Drops (phenylephrine)	15% v/v	
	Afrin (Oxymetazoline)	15% v/v	
	CVS Nasal Spray (Cromolyn)	15% v/v	

	Tamiflu (Oseltamivir phosphate)	500 mg/dL	
	Budenoside	0.00063 mg/dL	
	Biotin	0.35 mg/dL	
	Tobramycin	3.3 mg/dL	
	Mupirocin	0.15 mg/dL	
	Dextromethorphan	0.00156 mg/dL	
	Dexamethasone	1.2 mg/dL	
	Mucinex	5%	
	Methanol	150 mg/dL	
	Acetylsalicylic Acid	3 mg/dL	
	Diphenhydramine	0.0774 mg/dL	
	Benzocaine	150 mg/dL	
Hook effect	When the concentration of the target COVID-19 (Sars-CoV-2) is as high as 1.4×10^5 $TCID_{50}$/mL, there is no observed hook effect at this high concentration.	Pass	