SARS-CoV-2 Antigen Test Kit
(Colloidal Gold)
Analytical specificity

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1 Overview

This study for the test kit is to determine the analytical specificity in the presence of potential interferents including endogenous and exogenous substances. The batch number information of the kit used in the analysis is shown in the following table:

Table 1 Sample information of the kit

Kit name	Batch No.
SARS-CoV-2 Antigen Test Kit (Colloidal Gold)	20200301, 20200304, 20200307

2 Analytical specificity

Through experiments, the analysis specificity of the SARS-CoV-2 Antigen Test Kit (Colloidal Gold) was clarified, that is, the performance of the sample without novel coronavirus was detected without false positive, and the interference effect of the suspected interference on the sample from the novel Coronavirus sample was evaluated.

2.1 Cross reactivity

2.1.1 Experimental scheme

The cross reactions in the test program to collect a set of potential cross reactants, including common viral and bacterial microorganisms, such as local human coronavirus (HKU1, OC43, NL63 and 229E), parainfluenza 1, 2, 3, 4, influenza A, influenza B, respiratory syncytial virus, a rhinovirus, adenovirus type, enterovirus, haemophilus influenzae, streptococcus pneumoniae, streptococcus pyogenes, candida albicans, Bordetella pertussis, pneumonia mycoplasma, pneumonia chlamydia, eosinophilic lung legionella,

Staphylococcus epidermidis, staphylococcus aureus, Mycobacterium tuberculosis, Pneumocystis jirovecii (PJP)and pooled human nasal wash, etc.The basic information such as the categories and concentrations of the above-mentioned microorganisms is shown in Table 3.

When experimenting, other microbial samples known to have novel Coronavirus negative were first diluted to a certain level of infection concentration (typically, concentration of 10^6 cfu/mL or higher for bacterial, and 10^5 pfu/mL or higher for virus), the test was carried out with reference to the operating instructions of the product, and each sample was repeated for 3 times, and then the specificity analysis was performed.

Table 3 Potential cross-reactant and test concentration

Potential Cross-Reactant	Test Concentration
Human coronavirus 229E (heat inactivated)	1.0×10^5 $TCID_{50}$/mL
Human coronavirus OC43	1.0×10^5 $TCID_{50}$/mL
Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL
Adenovirus	1.0×10^5 $TCID_{50}$/mL
Human Metapneumovirus	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 1	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 2	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 3	1.0×10^5 $TCID_{50}$/mL
Parainfluenza virus 4	1.0×10^5 $TCID_{50}$/mL
Influenza A	1.0×10^5 $TCID_{50}$/mL
Influenza B	1.0×10^5 $TCID_{50}$/mL
Enterovirus	1.0×10^5 $TCID_{50}$/mL
Respiratory syncytial virus	1.0×10^5 $TCID_{50}$/mL
Rhinovirus	1.0×10^5 $TCID_{50}$/mL

HCoV-HKU1	10μg/mL
Human SARS-coronavirus Nucleoprotein	25ng/mL
MERS-CoV Nucleoprotein	0.25ng/mL
Haemophilus influenza	1.5 x 10^6 CFU/mL
Streptococcus pneumoniae	1.5 x 10^6 CFU/mL
Streptococcus pyogenes	1.5 x 10^6 CFU/mL
Candida albicans	1.5 x 10^6 CFU/mL
Bordetella pertussis	1.5 x 10^6 CFU/mL
Mycoplasma pneumoniae	1.5 x 10^6 CFU/mL
Chlamydia pneumoniae	1.5 x 10^6 CFU/mL
Staphylococcus epidermidis	1.5 x 10^6 CFU/mL
Staphylococcus aureus	1.5 x 10^6 CFU/mL
Legionella pneumophila	1.5 x 10^6 CFU/mL
Mycobacterium tuberculosis	1.5 x 10^6 CFU/mL
Pneumocystis jirovecii (PJP)	1.5 x 10^6 CFU/mL
Pooled human nasal wash	100%

2.1.2 Test method

The samples with concentrations in the above table were prepared from the mixture of the novel coronavirus negative mixtures of other microorganisms and normal nasal lotion. Each sample was tested separately using the three batches of the SARS-CoV-2 Antigen Test Kit (Colloidal Gold), and each sample was repeated for three times.

2.1.3 Test result

See Appendix B4 the test results of microbial cross-reaction verification.

2.1.4 Test conclusion

After testing, SARS-CoV-2 Antigen Test Kit (Colloidal Gold) for batches 20200301, 20200304 and 20200307 has cross-reactivity with Human

SARS-coronavirus nucleoprotein at a concentration of 25 ng/mL or more because SARS-CoV has high homology (79.6%) to the SARS-CoV-2, but was not affected under the interference of the above other cross-reactant.

2.2 Interfering substances

Interference test evaluation is an important basis for evaluating the effectiveness of products to be marketed. Considering the sample collection process and the presence of suspicious interferences in the samples of drug users, it may interfere with the results; the suspected interfering substances selected in the interference test plan of this product include mucin, blood, pus, nasal spray, antibiotic tobramycin injection, etc. Interference screening was conducted through the design of interference test and the interference effect evaluation of relevant interfering substances was conducted.

2.2.1 Selection of interfering substances

The test collected a group of potential interferences, including mucin, blood, pus, and several drugs commonly available to relieve nasal congestion and pharynx congestion, dry nose, irritation, asthma and allergy symptoms. See Table 4 Interfering substance information.

Table 4 Interfering substance information

Substance	Interfering substance
Endogenous interfering substances	Mucin
	Blood
	Pus

Exogenous interfering substances	Oxymetazoline
	Dexamethasone
	Sulfur
	Zanamivir
	Mupirocin
	Tobramycin

2.2.2 Sample preparation

1）Preparation of basic samples

The novel Coronavirus culture was selected as the basic sample pool, and the mixture of the novel Coronavirus culture and the interfering substance was mixed in proportion, so that the final concentration of the mixed interfering sample would be at the detection limit level of the minimum critical value.

2）Preparation of samples containing interfering substances

A. Test samples containing mucin: a certain amount of mucin dry powder was weighed and dissolved in each basic sample pool, so that the final concentration was 10g/L.The test concentration was selected according to the following criteria: the normal value of human salivary mucin was 1.36±0.45g/L, and the normal value of serum mucin was 710-870mg/L, which was equivalent to 10 times the amount of normal salivary mucin.

B. Test samples containing blood: mix 10% of human whole blood samples into each basic sample pool. The test concentration is selected on the basis of the maximum amount of blood that can be carried during the sample collection.

C. Preparation of pus samples: 5% of pus samples were mixed into each

basic sample pool. Test concentration selection basis: the maximum amount of pus that may be carried during sample collection.

D. Preparation of hydroxymetazoline samples: a certain volume of hydroxymetazoline hydrochloride spray (specification: 10ml: 5mg) was mixed into each basic sample pool to make the effective concentration of hydroxymetazoline reach 0.375mg/mL. Test concentration selection basis: Conventional dosage of hydroxymetazoline hydrochloride spray was 0.05mg - 0.15mg/time. It was assumed that the drug had not been absorbed by tissue mucosa and completely remained in the samples after treatment. According to the test instructions of this product, the maximum effective concentration of hydroxymetazoline in the treatment solution was about 0.375mg/mL when the samples were extracted with 400 L sample solution.

E. Preparation of samples containing dexamethasone: Dexamethasone sodium phosphate injection (specification: 1ml:5mg) of a certain volume was mixed into each basic sample pool respectively to make the effective concentration of the active ingredient dexamethasone reach 2.5mg /L.Test concentration selection basis: Dexamethasone was assumed to be given intravenously, and the normal dose was no more than 20 mg each time. According to the worst condition test, i.e., the human absorption rate was assumed to be 50%, and the blood volume was calculated as 4L, then the blood concentration was 2.5 mg/L after administration.

F. Preparation of sulfur samples: sulfur ointment (0.1g of sublimated sulfur per gram) was dissolved in an appropriate amount of hot water and diluted to a

certain concentration as the mother liquor. Then, an appropriate amount of solubilized solution was taken and mixed into each basic sample pool to make the sublimated sulfur concentration reach 50mg/mL.Selecting test concentration: sulfur ointment conventional dosage is about 0.1g to 0.2g/day (sublimation sulfur content from 0.01 g to 0.02 g), assumption, the medicine is absorbed by the mucosal drug not fully residues in sample, according to the product inspection instructions, with 400μL sample processing liquid leaching sample, the processing of sublimed sulfur and effective concentration up to about 50 mg/mL.

G. Preparation of zanamivir samples: dissolve a certain amount of relenza samples (zanamivir powder) in each basic sample cell to make the effective concentration of zanamivir, the main component, reach 1.25 mg/L.Test concentration selection basis: The conventional dosage of zanamivir was 10mg inhaled orally each time, assuming that the human absorption rate was 50%, and calculated according to the human blood volume of 4L, the blood concentration after administration was 1.25 mg/L.

H. Preparation of samples containing Mupiroxacin: The mupiroxacin ointment (the active component mupiroxacin content is 2%) was dissolved in an appropriate amount of hot water and diluted to a certain concentration as the mother liquor, and then an appropriate amount of solution was taken and mixed into each basic sample pool to make the effective concentration of Mupiroxacin reach 5mg/mL. Selecting test concentration, dosage of mupirocin ointment routine is about 0.1 g/time (mupirocin content was about 2 mg), assuming that

the drug, the drug is not absorbed by the mucosal completely residues in the sample, according to the product inspection instructions, with 400μL sample processing liquid leaching sample, the processing of mupirocin effective concentration up to about 5 mg/mL.

I. Preparation of samples containing tobramycin: A certain volume of Tobramycin sulfate injection (specification: 2 ml: 80 mg) was mixed into each basic sample pool to make the effective concentration of tobramycin reach 0.8 mg/L.The test concentration was selected according to the following conditions: assume intramuscular injection or intravenous infusion of 1mg/kg for 30 minutes and 30 to 60 minutes, respectively, and the peak blood concentration was 3.7 g/mL. Under the worst conditions, the peak blood concentration was 740μg/mL, assuming the individual weight was 200 kg.

2.2.3 Experimental method

Three batches of SARS-CoV-2 Antigen Test Kit (Colloidal Gold) were used to test above prepared with interfering substance samples, repeat the test three times; The base solution of the interfering substance was also determined as a control; Statistical test results and comparison of interference between the experimental group and the control group whether there is a difference.

2.2.4 Experimental results

See Appendix B5 the test results of interfering substances.

2.2.5 Test conclusion

According to the test results, within the concentration range tested by the SARS-CoV-2 Antigen Test Kit (Colloidal Gold) for batch 20200301, 20200304

and 20200307, mucin, blood, pus, nasal spray and antibiotic toblamycin injection had no significant influence on the test results.

3 Appendix

Appendix B4 The test results of microbial cross-reaction verification

Microbial cross reaction				
Lot	20200301			
Potential Cross-Reactant	Test Concentration	Cross-Reactivity (Yes/No)		
		1st	2st	3st
Human coronavirus 229E (heat inactivated)	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus OC43	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Adenovirus	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human Metapneumovirus	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 1	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 2	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 3	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 4	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Influenza A	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO

Influenza B	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Enterovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Respiratory syncytial virus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
HCoV-HKU1	10 μ g/mL	NO	NO	NO
Human SARS-coronavirus Nucleoprotein	25ng/mL	YES	YES	YES
MERS-CoV Nucleoprotein	0.25ng/mL	NO	NO	NO
Haemophilus influenza	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pyogenes	1.5 x 10^6 CFU/mL	NO	NO	NO
Candida albicans	1.5 x 10^6 CFU/mL	NO	NO	NO
Bordetella pertussis	1.5 x 10^6 CFU/mL	NO	NO	NO
Mycoplasma pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Chlamydia pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Legionella pneumophila	1.5 x 10^6 CFU/mL	NO	NO	NO

Staphylococcus epidermidis	1.5 x 10^6 CFU/mL	NO	NO	NO
Staphylococcus aureus	1.5 x 10^6 CFU/mL	NO	NO	NO
Mycobacterium tuberculosis	1.5 x 10^6 CFU/mL	NO	NO	NO
Pneumocystis jirovecii (PJP)	1.5 x 10^6 CFU/mL	NO	NO	NO
Pooled human nasal wash	100%	NO	NO	NO
Lot	20200304			
Potential Cross-Reactant	Test Concentration	Cross-Reactivity (Yes/No)		
		1st	2st	3st
Human coronavirus 229E (heat inactivated)	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus OC43	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus NL63	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Adenovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Human Metapneumovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 1	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 2	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 3	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO

Parainfluenza virus 4	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Influenza A	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Influenza B	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Enterovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Respiratory syncytial virus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
HCoV-HKU1	10 μ g/mL	NO	NO	NO
Human SARS-coronavirus Nucleoprotein	25ng/mL	YES	YES	YES
MERS-CoV Nucleoprotein	0.25ng/mL	NO	NO	NO
Haemophilus influenza	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pyogenes	1.5 x 10^6 CFU/mL	NO	NO	NO
Candida albicans	1.5 x 10^6 CFU/mL	NO	NO	NO
Bordetella pertussis	1.5 x 10^6 CFU/mL	NO	NO	NO
Mycoplasma pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO

Chlamydia pneumoniae	1.5×10^6 CFU/mL	NO	NO	NO
Legionella pneumophila	1.5×10^6 CFU/mL	NO	NO	NO
Staphylococcus epidermidis	1.5×10^6 CFU/mL	NO	NO	NO
Staphylococcus aureus	1.5×10^6 CFU/mL	NO	NO	NO
Mycobacterium tuberculosis	1.5×10^6 CFU/mL	NO	NO	NO
Pneumocystis jirovecii (PJP)	1.5×10^6 CFU/mL	NO	NO	NO
Pooled human nasal wash	100%	NO	NO	NO
Lot	20200307			
Potential Cross-Reactant	Test Concentration	Cross-Reactivity (Yes/No)		
		1st	2st	3st
Human coronavirus 229E (heat inactivated)	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus OC43	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human coronavirus NL63	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Adenovirus	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Human Metapneumovirus	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 1	1.0×10^5 $TCID_{50}$/mL	NO	NO	NO

Parainfluenza virus 2	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 3	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Parainfluenza virus 4	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Influenza A	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Influenza B	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Enterovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Respiratory syncytial virus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
Rhinovirus	1.0 x 10^5 $TCID_{50}$/mL	NO	NO	NO
HCoV-HKU1	10 µ g/mL	NO	NO	NO
Human SARS-coronavirus Nucleoprotein	25ng/mL	YES	YES	YES
MERS-CoV Nucleoprotein	0.25ng/mL	NO	NO	NO
Haemophilus influenza	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Streptococcus pyogenes	1.5 x 10^6 CFU/mL	NO	NO	NO
Candida albicans	1.5 x 10^6 CFU/mL	NO	NO	NO

Bordetella pertussis	1.5 x 10^6 CFU/mL	NO	NO	NO
Mycoplasma pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Chlamydia pneumoniae	1.5 x 10^6 CFU/mL	NO	NO	NO
Legionella pneumophila	1.5 x 10^6 CFU/mL	NO	NO	NO
Staphylococcus epidermidis	1.5 x 10^6 CFU/mL	NO	NO	NO
Staphylococcus aureus	1.5 x 10^6 CFU/mL	NO	NO	NO
Mycobacterium tuberculosis	1.5 x 10^6 CFU/mL	NO	NO	NO
Pneumocystis jirovecii (PJP)	1.5 x 10^6 CFU/mL	NO	NO	NO
Pooled human nasal wash	100%	NO	NO	NO

Appendix B5 the test results of interfering substances.

Interference substances verification								
Lot	20200301							
Interfering substances	**working concentration**	**Result**						**Whether the color rendering is consistent**
		1		**2**		**3**		
Basic sample		+	L2	+	L2	+	L2	
Purified mucin	10 g/L	+	L2	+	L2	+	L2	Yes

Human blood	10%(v/V)	+	L2	+	L2	+	L2	Yes
Pus	5%(v/V)	+	L2	+	L2	+	L2	Yes
Oxymetazoline	0.375 mg/mL	+	L2	+	L2	+	L2	Yes
Dexamethasone	2.5 mg/L	+	L2	+	L2	+	L2	Yes
Sulfur	50 mg/mL	+	L2	+	L2	+	L2	Yes
Zanamivir	1.25 mg/L	+	L2	+	L2	+	L2	Yes
MuPirocin	5 mg/mL	+	L2	+	L2	+	L2	Yes
Tobramycin	0.8 mg/L	+	L2	+	L2	+	L2	Yes
Lot	20200304							
Interfering substances	**working concentration**	**Result**						**Whether the color rendering is consistent**
		1		**2**		**3**		
Basic sample		+	L2	+	L2	+	L2	
Purified mucin	10 g/L	+	L2	+	L2	+	L2	Yes
Human blood	10%(v/V)	+	L2	+	L2	+	L2	Yes
Pus	5%(v/V)	+	L2	+	L2	+	L2	Yes
Oxymetazoline	0.375 mg/mL	+	L2	+	L2	+	L2	Yes

Dexamethasone	2.5 mg/L	+	L2	+	L2	+	L2	Yes
Sulfur	50 mg/mL	+	L2	+	L2	+	L2	Yes
Zanamivir	1.25 mg/L	+	L2	+	L2	+	L2	Yes
MuPirocin	5 mg/mL	+	L2	+	L2	+	L2	Yes
Tobramycin	0.8 mg/L	+	L2	+	L2	+	L2	Yes
Lot	20200307							
Interfering substances	**working concentration**	**Result**						**Whether the color rendering is consistent**
		1		**2**		**3**		
Basic sample		+	L2	+	L2	+	L2	
Purified mucin	10 g/L	+	L2	+	L2	+	L2	Yes
Human blood	10%(v/V)	+	L2	+	L2	+	L2	Yes
Pus	5%(v/V)	+	L2	+	L2	+	L2	Yes
Oxymetazoline	0.375 mg/mL	+	L2	+	L2	+	L2	Yes
Dexamethasone	2.5 mg/L	+	L2	+	L2	+	L2	Yes
Sulfur	50 mg/mL	+	L2	+	L2	+	L2	Yes
Zanamivir	1.25 mg/L	+	L2	+	L2	+	L2	Yes

MuPirocin	5 mg/mL	+	L2	+	L2	+	L2	Yes
Tobramycin	0.8 mg/L	+	L2	+	L2	+	L2	Yes