Performance Evaluation

Analytical Performance

1. The evaluation purpose

This study was aim to evaluate the various performance characteristics (including Positive/Negative Reference Coincidence Rate, Limit of Detection, Precision and analytical specificity) of the kit, and summarize the studies' protocol, results and conclusion to check that if the design standard can be met.

2. The batch numbers and size of kits used for performance evaluation

Batch Numbers of the 3 batches products: 20200306-E, 20200306-1-E, 20200306-2-E respectively.

Size: 20 tests

3. Study protocol

3.1 Anti-SARS-CoV-2 Rapid Test Enterprise References Panel

Anti-SARS-CoV-2 Rapid Test Enterprise References Panel (also called internal controls panel) which used for control of evaluation performance in the R&D process, raw materials inspection, semi-finished product inspection and finished product inspection. In this study, Anti-SARS-CoV-2 Rapid Test Enterprise References Panel will be used to verify the analytical performances in order to meet the criteria.

The content of Test Enterprise References Panel:

Enterprise References Panel			
Components			Acceptance Criteria
Positive References	SARS-CoV-2 IgM	P1-P6	6/6, P
	SARS-CoV-2 IgG	P1-P6	6/6, P
Negative References	SARS-CoV-2 IgM	N1-N10	10/10,N
	SARS-CoV-2 IgG	N1-N10	10/10,N
LoD References	SARS-CoV-2 IgM	L1	N
		L2	P
		L3	P
	SARS-CoV-2 IgG	L1	N
		L2	P
		L3	P
Precision References	SARS-CoV-2 IgM	J1, J2	10/10, P & uniform in visible color
	SARS-CoV-2 IgG	J1, J2	10/10, P & uniform in visible color

3.1.1 Positive reference coincidence rate

Collect separately 6 SARS-CoV-2 IgM and 6 SARS-CoV-2 IgG positive samples by approved Anti-SARS-CoV-2 products;

These 6 positive references for SARS-CoV-2 IgM and 6 Positive references for SARS-CoV-2 IgG will be tested by Anti-SARS-CoV-2 Rapid Test, the results shall be all positive.

3.1.2 Negative reference coincidence rate

Collect separately 10 SARS-CoV-2 IgM and 10 SARS-CoV-2 IgG negative samples with different potential cross-reactivity substances

Negative references for SARS-CoV-2 IgM		Negative references for SARS-CoV-2 IgG	
No.	Potential cross-reactivity substances	No.	Potential cross-reactivity substances
N1	Rheumatoid factor	N1	Rheumatoid factor
N2	*M. pneumoniae* IgM	N2	*M. pneumoniae* IgG
N3	N/A	N3	N/A
N4	*C. pneumoniae* IgM	N4	Rheumatoid factor
N5	RSV IgM	N5	N/A
N6	N/A	N6	RSV IgG
N7	Adenovirus IgM	N7	N/A
N8	N/A	N8	N/A
N9	N/A	N9	Adenovirus IgG
N10	Coxsackie virus group B IgM	N10	N/A

These 10 negative references for SARS-CoV-2 IgM and 10 negative references for SARS-CoV-2 IgG will be tested by Anti-SARS-CoV-2 Rapid Test, the results shall be all negative.

3.1.3 Limit of Detection

Collect 3 strong positive samples for SARS-CoV-2 IgM, mix together and diluted by negative samples into different dilution ratio, which are L2, L3 and L1.

LoD references for SARS-CoV-2 IgM		LoD references for SARS-CoV-2 IgG	
L1	Negative Serum/plasma	L1	Negative Serum/plasma
L2	1:32 dilution ratio of strong positive samples (close to cutoff value)	L2	1:64 dilution ratio of strong positive samples (close to cutoff value)
L3	1:16 dilution ratio of strong positive samples	L3	1:16 dilution ratio of strong positive samples

These 3 LoD references (L1, L2, L3) for SARS-CoV-2 IgM and SARS-CoV-2 IgG will be tested by Anti-SARS-CoV-2 Rapid Test, L1 shall be negative and L2, L3 were all positive.

3.1.4 Precision

Use the strong positive samples in 3.1.3, and diluted by negative samples to prepare precision references.

LoD references for SARS-CoV-2 IgM		LoD references for SARS-CoV-2 IgG	
J1	1:32 dilution ratio of strong positive samples (close to	J1	1:64 dilution ratio of strong positive samples (close to

	cutoff value)		cutoff value)
J2	1:16 dilution ratio of strong positive samples	J2	1:16 dilution ratio of strong positive samples

These 2 precision references (J1, J2) for SARS-CoV-2 IgM and SARS-CoV-2 IgG will be tested with 10 replicates by Anti-SARS-CoV-2 Rapid Test, the result shall be all positive and visible color is uniform.

3.2 Whole blood samples evaluation

3.2.1 Whole blood samples sensitivity

Collect 6 positive whole blood samples (3 for SARS-CoV-2 IgM antibody and 3 for SARS-CoV-2 IgG antibody), centrifuge some amount of this 6 positive whole blood samples to get 6 positive plasma samples (3 for SARS-CoV-2 IgM antibody and 3 for SARS-CoV-2 IgG antibody); These samples are diluted with negative whole blood samples and negative plasma samples respectively in different dilution ratio, the maximum dilution factor of positive samples shall be found out and the results shall met the requirement.

3.2.2 Negative reference coincidence rate for Whole blood samples

Test 20 negative whole samples and its plasma samples, the results shall be no difference.

3.2.3 Positive reference coincidence rate for Whole blood samples

Test 10 positive whole samples and its plasma samples, the results shall be no difference.

3.2.4 Precision for Whole blood samples

Test 1 positive whole samples and its plasma samples with 10 replicates, the results shall be all positive and no significant difference in visual color rendering changes.

3.3 Cross-Reactivity

Determine the cross-reactivity of SARS-CoV-2 IgM by evaluating 159 samples with potentially cross reacting substances:

Cross substance	**No. of samples**	**Cross reactivity**
Endemic human coronavirus (HKU1, OC43, NL63 and 229E)	4	Results shall be no cross-reactivity
Influenza A virus	10	
Influenza B virus	8	
Parainfluenza virus IgM	10	
Respiratory syncytial virus IgM	10	
Mycoplasma pneumoniae IgM	10	
Chlamydia pneumoniae IgM	8	
Coxsackie virus type A16 IgM	4	
Coxsackie virus group B IgM	7	
Adenovirus IgM	10	

CMV IgM	10	
Enterovirus 71 IgM	10	
Toxo IgM	10	
Rubella IgM	10	
HSV-1 IgM	10	
HSV-2 IgM	10	
Measles virus IgM	5	
Mumps Virus IgM	8	
Varicella zoster virus IgM	5	

Determine the cross-reactivity of SARS-CoV-2 IgG by evaluating 175 samples with potentially cross reacting substances:

Cross substance	No. of samples	Cross reactivity
Endemic human coronavirus (HKU1, OC43, NL63 and 229E)	4	Results shall be no cross-reactivity
Influenza A virus	10	
Influenza B virus	8	
Parainfluenza virus IgG	5	
Respiratory syncytial virus IgG	10	
Mycoplasma pneumoniae IgG	10	
Chlamydia pneumoniae IgG	8	
Coxsackie virus group B IgG	5	
Legionella IgG	5	
Adenovirus IgG	10	
EBV-NA IgG	10	
EBV capsid antigen IgG	10	
EBV-EA IgG	10	
CMV IgG	10	
Enterovirus 71 IgG	10	
Toxo IgG	10	
Rubella IgG	10	
HSV-1 IgG	10	
HSV-2 IgG	5	
Measles virus IgG	5	
Mumps Virus IgG	5	
Varicella zoster virus IgG	5	

3.4 Destructive effect of IgM antibody detection

Test 6 pathogen IgM antibody samples, the results shall be all negative.

3.5 Endogenous Interference

The main endogenous interferences are bilirubin, hemoglobin and triglycerides. Endogenous Interferences are evaluated to check the effect of coating membrane's background and test results.

3.6 Autoantibody Interference

Test HAMA antibody, rheumatoid factor, antinuclear antibody (ANA), anti-mitochondrial antibody (AMA), total IgG / IgM, high concentration of specific IgG and IgM samples. The results shall be no interference.

3.7 Drug Interference

Drugs such as α-interferon, zanamivir, Ribavirin, oseltamivir, paramivir, lopinavir, ritonavir abidol, levofloxacin, azithromycin, ceftriaxone, meropenem, tobramycin are spiked into samples. These drugs will be prepared to certain concentration which is near cut-off value (visible color is consistent with L2 which is one of LoD reference with cut-off value in the panel). These drugs will be tested with control group. The results shall be no interference.

3.8 Hook Effect

Collect some clinical positive samples (RNA positive clinical samples) and select one most strong positive sample as the original sample, dilute this sample into different concentration. Test these samples and the results shall be no false negative (hook effect) in the certain concentration range of clinical positive samples.

4. Results

4.1 Enterprise Internal Controls Panel

SARS-CoV-2 IgM test results (20200306-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/
J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

SARS-CoV-2 IgG test results (20200306-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/

J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

SARS-CoV-2 IgM test results (20200306-1-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/
J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

SARS-CoV-2 IgG test results (20200306-1-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/
J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

SARS-CoV-2 IgM test results (20200306-2-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/
J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

SARS-CoV-2 IgG test results (20200306-2-E):

1	2	3	4	5	6	7	8	9	10
N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
-	-	-	-	-	-	-	-	-	-
P1	P2	P3	P4	P5	P6	L1	L2	L3	/
+	+	+	+	+	+	-	+	+	/
J1	J1	J1	J1	J1	J1	J1	J1	J1	J1
+	+	+	+	+	+	+	+	+	+
J2	J2	J2	J2	J2	J2	J2	J2	J2	J2
+	+	+	+	+	+	+	+	+	+

Conclusion for SARS-CoV-2 IgM test results:

Lot No.	Criteria	20200306-E	20200306-1-E	20200306-2-E
Type	/	stipe	stipe	stipe
Size	/	20 tests	20 tests	20 tests
Negative reference coincidence rate	10/10, N	10/10, N	10/10, N	10/10, N
Positive reference coincidence rate	6/6, P	6/6, P	6/6, P	6/6, P
LOD	2/3	2/3	2/3	2/3
Within-run precision	the result were all positive and uniform in visible color			
Between-batch precision	the result were all positive and uniform in visible color			

Conclusion for SARS-CoV-2 IgG test results:

Lot No.	Criteria	20200306-E	20200306-1-E	20200306-2-E
Type	/	strip	stipe	strip
Size	/	20 tests	20 tests	20 tests
Negative reference coincidence rate	10/10, N	10/10, N	10/10, N	10/10, N
Positive reference coincidence rate	6/6, P	6/6, P	6/6, P	6/6, P
LOD	2/3	2/3	2/3	2/3
Within-run precision	the result were all positive and uniform in visible color			
Between-batch precision	the result were all positive and uniform in visible color			

4.2 Whole blood samples evaluation

4.2.1 Whole blood samples sensitivity

6 positive whole blood samples (3 for SARS-CoV-2 IgM antibody and 3 for SARS-CoV-2 IgG antibody) were collected, some amount of 6 positive whole blood samples were centrifuged to get 6 positive plasma samples (3 for SARS-CoV-2 IgM antibody and 3 for SARS-CoV-2 IgG antibody); These samples were diluted with negative whole blood samples and negative plasma samples in different dilution ratio, maximum dilution factor of positive samples were found out.

The results for 3 SARS-CoV-2 IgM positive samples:

Dilution ratio	Sample 1		Sample 2		Sample 3	
	Plasma	Whole blood	Plasma	Whole blood	Plasma	Whole blood
1:1	+	+	+	+	+	+
1:2	+	+	+	+	+	+
1:4	+	+	+	+	+	+
1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+
1:32	+	+	+	+	+	+
1:64	—	—	+	+	+	+
1:128	—	—	—	—	+	+
1:256	—	—	—	—	—	—

Conclusion: the maximum dilution factor of positive whole blood samples is 1:128, the sensitivity met the requirement.

The results for 3 SARS-CoV-2 IgG positive samples:

Dilution ratio	Sample 1		Sample 2		Sample 3	
	Plasma	Whole blood	Plasma	Whole blood	Plasma	Whole blood
1:1	+	+	+	+	+	+
1:2	+	+	+	+	+	+
1:4	+	+	+	+	+	+
1:8	+	+	+	+	+	+
1:16	+	+	+	+	+	+

1:32	+	+	+	+	+	+
1:64	+	+	+	+	+	+
1:128	—	—	—	—	+	+
1:256	—	—	—	—	—	—

Conclusion: the maximum dilution factor of positive whole blood samples is 1:128, the sensitivity met the requirement.

4.2.2 Negative reference coincidence rate for Whole blood samples

20 negative whole samples and its plasma samples were tested, the results showed below:

Sample No.	SARS-CoV-2 IgG		SARS-CoV-2 IgM	
	Plasma	Whole blood	Plasma	Whole blood
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	—	—
7	—	—	—	—
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
13	—	—	—	—
14	—	—	—	—
15	—	—	—	—
16	—	—	—	—
17	—	—	—	—
18	—	—	—	—
19	—	—	—	—
20	—	—	—	—

Conclusion: All tested results for whole blood samples were negative, which was same as plasma results. It met requirement.

4.2.3 Positive reference coincidence rate for Whole blood samples

10 positive whole samples and its plasma samples were tested, the results showed below:

Sample No.	SARS-CoV-2 IgG		SARS-CoV-2 IgM	
	Plasma	Whole blood	Plasma	Whole blood

1	+	+	+	+
2	+	+	+	+
3	±	±	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	±	±
9	+	+	+	+
10	+	+	+	+

Conclusion: All tested results for whole blood samples were positive, which was same as plasma results. It met requirement.

4.2.4 Precision for Whole blood samples

1 positive whole samples and its plasma samples were tested with 10 replicates, the results showed below:

Replicates	SARS-CoV-2 IgG		SARS-CoV-2 IgM	
	Plasma	Whole blood	Plasma	Whole blood
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	+	+
10	+	+	+	+

Conclusion: the results were all positive and no significant difference in visual color rendering changes. The precision of whole blood sample met requirement.

4.3 Cross Reactivity

159 samples were detected the cross-reactivity of SARS-CoV-2 IgM, the results are as follow:

Cross substance	**No. of samples**	**Autobio**	**Cross reactivity**
Endemic human coronavirus (HKU1, OC43, NL63 and 229E)	4	Negative	No cross-reactivity
Influenza A virus	10	Negative	No cross-reactivity
Influenza B virus	8	Negative	No cross-reactivity
Parainfluenza virus IgM	10	Negative	No cross-reactivity
Respiratory syncytial virus IgM	10	Negative	No cross-reactivity

Mycoplasma pneumoniae IgM	10	Negative	No cross-reactivity
Chlamydia pneumoniae IgM	8	Negative	No cross-reactivity
Coxsackie virus type A16 IgM	4	Negative	No cross-reactivity
Coxsackie virus group B IgM	7	Negative	No cross-reactivity
Adenovirus IgM	10	Negative	No cross-reactivity
CMV IgM	10	Negative	No cross-reactivity
Enterovirus 71 IgM	10	Negative	No cross-reactivity
Toxo IgM	10	Negative	No cross-reactivity
Rubella IgM	10	Negative	No cross-reactivity
HSV-1 IgM	10	Negative	No cross-reactivity
HSV-2 IgM	10	Negative	No cross-reactivity
Measles virus IgM	5	Negative	No cross-reactivity
Mumps Virus IgM	8	Negative	No cross-reactivity
Varicella zoster virus IgM	5	Negative	No cross-reactivity

Conclusion: these substances have no cross reaction with this assay.

175 samples were detected the cross-reactivity of SARS-CoV-2 IgG, the results are as follow:

Cross substance	**No. of samples**	**Autobio**	**Cross reactivity**
Endemic human coronavirus (HKU1, OC43, NL63 and 229E)	4	Negative	No cross-reactivity
Influenza A virus	10	Negative	No cross-reactivity
Influenza B virus	8	Negative	No cross-reactivity
Parainfluenza virus IgG	5	Negative	No cross-reactivity
Respiratory syncytial virus IgG	10	Negative	No cross-reactivity
Mycoplasma pneumoniae IgG	10	Negative	No cross-reactivity
Chlamydia pneumoniae IgG	8	Negative	No cross-reactivity
Coxsackie virus group B IgG	5	Negative	No cross-reactivity
Legionella IgG	5	Negative	No cross-reactivity
Adenovirus IgG	10	Negative	No cross-reactivity
EBV-NA IgG	10	Negative	No cross-reactivity
EBV capsid antigen IgG	10	Negative	No cross-reactivity
EBV-EA IgG	10	Negative	No cross-reactivity
CMV IgG	10	Negative	No cross-reactivity
Enterovirus 71 IgG	10	Negative	No cross-reactivity
Toxo IgG	10	Negative	No cross-reactivity
Rubella IgG	10	Negative	No cross-reactivity
HSV-1 IgG	10	Negative	No cross-reactivity
HSV-2 IgG	5	Negative	No cross-reactivity
Measles virus IgG	5	Negative	No cross-reactivity
Mumps Virus IgG	5	Negative	No cross-reactivity
Varicella zoster virus IgG	5	Negative	No cross-reactivity

Conclusion: these substances have no cross reaction with this assay.

4.4 Destructive effect of IgM antibody detection

Test 6 pathogen IgM antibody samples, the results shall be all negative. Perform destructive experiments on 6 samples contain SARS-CoV-2 virus IgM antibodies. After processed with 2-mercaptoethanol by 1:1 dilution, these samples were repeated tested, the SARS-CoV-2 IgM antibodies should be negative.

Results of SARS-CoV-2 IgM

Sample / Additive	1	2	3	4	5	6
Control group	+	+	++	++	+++	+++
Group processed with 2-mercaptoethanol by 1:1 dilution	—	—	—	—	—	—

Conclusion: after processed with 2-mercaptoethanol by 1:1 dilution, the IgM antibodies detections were all negative, which means the requirements can be met.

4.5 Endogenous Interference

Endogenous interference mainly evaluates hemoglobin, triglyceride and bilirubin. Different concentration of hemoglobin, triglyceride and bilirubin were added into negative sample, weakly positive of SARS-CoV-2 IgM and IgG sample and moderately positive of SARS-CoV-2 IgM and IgG sample respectively, then compared these samples with the control group without these endogenous interferent. The results were as follow:

Interferent	Hemoglobin	SARS-CoV-2 IgM	SARS-CoV-2 IgG
Negative sample	32mg/ml	Membrane was moderately red, the results was affected	Membrane was moderately red, the results was affected
	16mg/ml	±	±
	8mg/ml	—	—
	4mg/ml	—	—
	0	—	—
Weakly positive of SARS-CoV-2 IgM and IgG sample	32mg/ml	Membrane was moderately red, the results was affected	Membrane was moderately red, the results was affected
	16mg/ml	±	±
	8mg/ml	±	±
	4mg/ml	±	±
	0	±	±
Moderately positive of SARS-CoV-2 IgM and IgG sample	32mg/ml	Membrane was moderately red, the results was affected	Membrane was moderately red, the results was affected
	16mg/ml	++	++

	8mg/ml	++	++
	4mg/ml	++	++
	0	++	++

Interferent	bilirubin	SARS-CoV-2 IgM	SARS-CoV-2 IgG
Negative sample	0.6mg/ml	Membrane was moderately yellow, the results was affected	Membrane was moderately yellow, the results was affected
	0.3mg/ml	—	—
	0.1mg/ml	—	—
	0	—	—
Weakly positive of SARS-CoV-2 IgM and IgG sample	0.6mg/ml	Membrane was moderately yellow, the results was affected	Membrane was moderately yellow, the results was affected
	0.3mg/ml	±	±
	0.1mg/ml	±	±
	0	±	±
Moderately positive of SARS-CoV-2 IgM and IgG sample	0.6mg/ml	Membrane was moderately yellow, the results was affected	Membrane was moderately yellow, the results was affected
	0.3mg/ml	++	++
	0.1mg/ml	++	++
	0	++	++

Interferent	Triglyceride	SARS-CoV-2 IgM	SARS-CoV-2 IgG
Negative sample	10mg/ml	±	±
	5mg/ml	—	—
	2.5mg/ml	—	—
	0	—	—
Weakly positive of SARS-CoV-2 IgM and IgG sample	10mg/ml	±	±
	5mg/ml	±	±
	2.5mg/ml	±	±
	0	±	±
Moderately positive of SARS-CoV-2 IgM and IgG sample	10mg/ml	++	++
	5mg/ml	++	++
	2.5mg/ml	++	++
	0	++	++

Conclusion: according to the results above, 8mg/mL of hemoglobin, 0.3mg/mL of bilirubin and 5mg/mL have no effect with this assay.

4.6 Autoantibody Interference

Autoantibodies were collected and tested by SARS-CoV-2 IgM and SARS-CoV-2 IgG, the results were as follow:

Sample Type	Amount/Conc.	SARS-CoV-2 IgM	SARS-CoV-2 IgG
HAMA antibody	10	—	—
Total IgG/IgM	/	—	—
Rheumatoid Factor (RF)	100IU/mL	—	—
Antinuclear antibodies	Titer: 1:160	±	±
Antinuclear antibodies	Titer: 1:320	—	—
Anti-mitochondrial antibody	80U/mL	—	—
nCoV IgM antibody	Titer: 1:128	+++	—
nCoV IgG antibody	Titer: 1:256	—	++++

Conclusion: according to the results above, HAMA antibodies, 100IU/mL of RF, 1:320 of Antinuclear antibodies, 80U/mL of Anti-mitochondrial antibody, total IgG/IgM have no effect with this assay, 1:128 of nCoV IgM antibodies has no effect with SARS-CoV-2 IgG antibody and 1:256 nCoV IgG antibodies has no effect with SARS-CoV-2 IgM antibody.

4.7 Drugs Interference

Some clinical drugs for respiratory tract, inflammation and antiviral were selected to perform drugs interference experiment. These drugs were prepared to certain concentration, and then added into samples near cut-off value (visible color is consistent with L2 which is one of LoD reference with cut-off value in the panel).

Drug	2019-nCoV IgM antibody	2019-nCoV IgG antibody
Control group	±	±
α-interferon	±	±
Zanamivir	±	±
Ribavirin	±	±
Oseltamivir	±	±
Peramivir	±	±
Lopinavir	±	±
Ritonavir	±	±
Arbidol	±	±
Azithromycin	±	±
Certriaxone	±	±
Meropenem	±	±
Tobramycin	±	±
Levofloxacin	±	±

Conclusion: these 13 clinical drugs have no effect with this assay.

4.8 Hook Effect

Some clinical positive samples (RNA positive clinical samples) were collected and one most strong positive sample was selected as the original sample, it was diluted into different concentration, the results will be from strong positive to weak positive. These samples were tested and the results were as follow：

Dilution	SARS-CoV-2 IgM sample	SARS-CoV-2 IgG sample
1:512	—	—
1:256	—	±
1:128	±	+
1:64	+	+++
1:32	+++	+++
1:16	++	++
1:8	+	++
1:4	+	+
1:2	±	±
Original sample	±	±

Conclusion: according to the results above, SARS-CoV-2 IgM sample can be detected as positive between original sample and 1:128 dilutions, in this range of concentration, and the results were no false negative (hook effect). SARS-CoV-2 IgG sample can be detected as positive between original sample and 1:256, dilutions, in this range of concentration, and the results were no false negative (hook effect).

5. Conclusions

Performance characteristics (including Positive/Negative Reference Coincidence Rate, Sensitivity, Precision, analytical specificity and HooK Effect) of the kit can meet the requirements, and the detection requirements of SARS-CoV-2 IgM and IgG can be met.