Clinical Validation report of COVID-19 IgG/IgM Rapid Test (Colloidal Gold)

Product name:COVID-19 IgG/IgM Rapid Test Device (Colloidal Gold)

Package Specification:20 tests/kit

Manufacturer: Hangzhou Realy Tech Co., Ltd

I Clinical validation time

This clinical evaluation was conducted from February 2020 to March 4th,2020.

II Background information for clinical evaluation

Since December 2019, Wuhan City, Hubei Province has successively discovered multiple cases of patients with new-type coronavirus pneumonia. With the spread of the epidemic, other cases in China and abroad have also been found.As an acute respiratory infectious disease,the disease has been included in the Class B infectious diseases stipulated in the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases,and is managed as a Class A infectious disease.Based on the current epidemiological investigation, the incubation period is 1-14 days,mostly 3-7 days.

The main manifestations are fever, dry cough, and fatigue. A few patients have symptoms such as nasal congestion, runny nose, sore throat, myalgia and diarrhea. Severe patients usually have dyspnea and / or hypoxemia one week after the onset of symptoms, and severe patients can quickly progress to acute respiratory distress syndrome, septic shock,difficult to correct metabolic acidosis, coagulation dysfunction and multiple organ Functional failure,etc.It is worth noting that in the course of severe and critically ill patients, there may be moderate to low fever, even without obvious fever.

Mild patients showed only low fever, mild fatigue, and no pneumonia. Judging from the current cases, most patients have a good prognosis, and a few patients are critically ill. The elderly and those with chronic underlying disease have a better prognosis. Symptoms in children are relatively mild.

The COVID-19 IgG/IgM Rapid Test Device (Colloidal Gold) developed by our company can help diagnose whether patients are infected with the new coronavirus. It has further enriched the detection methods of new coronavirus, expanded the supply of detection reagents, and fully served the needs of epidemic prevention and control.

III. Test purposes

The COVID-19 IgG/IgM Rapid Test Device(Colloidal Gold) produced by Hangzhou Realy Technology Co., Ltd. was used to verify the feasibility of clinical evaluation and the reliability of test results for Chinese subjects.

The purpose of research of the clinical test is :calculate the consistency percentage of negative/positive and the total consistency percentage and Kappa coefficient by making statisics of and analyzing test results through comparative experimental research.

IV. Test design

1. Test plan selection and reasons

In vitro diagnostic reagents for testing and reference reagents were used to conduct comparative research tests on clinically suspected new-type coronavirus venous whole

blood, serum, and plasma samples, and it was proved that the in vitro diagnostic reagents used in the test can achieve the expected assistance in infection of the new coronavirus.

2. Sample volume required

The total number of clinical trials of this product is not less than 200 cases. The samples is classified into the positive group and the negative grpup as per the test results of the reference product.Meanwhile,the samples shall be tested via the qualitative test strip tested and the reference one and then the test results of the product tested and the reference product shall be compared,with statistical analysis being made.

4.Sample collection, processing and storage

Sample collection: Suitable for human serum, plasma or whole blood samples,including plasma or whole blood samples prepared from commonly used anticoagulants (EDTA,heparin,sodium citrate).

Sample processing: Before testing,slowly return the refrigerated or frozen samples to room temperature and mix them carefully.When clearly visible particulate matter is present in the sample, it should be centrifuged to remove sediment before testing. If the sample contains a large amount of lipid, hemolysis or turbidity, please do not use it, so as not to affect the result judgment.

Sample storage: The serum and plasma samples to be tested are stored at 2-8°C for 5 days.For long-term storage,store at -20°C.Avoid repeated freeze-thaw samples.
Anticoagulated whole blood samples should not be stored for more than 72 hours at room temperature;not more than 7 days at 2 to 8 °C,

5.In vitro diagnostic reagents and reference products for testing

5.1 Test in vitro diagnostic reagents

Name:COVID-19 IgG/IgM Rapid Test Device(Colloidal Gold)

Specification:20 tests/kit

LOT:NO1G06T,NO1G07T,O1G08T

Expiry: August,2020

Storage Conditions: Store in a dry place at 2-30°C, protected from light. After opening the inner package,the test card will become invalid due to moisture absorption.Please use it within 1 hour.

Source: Hangzhou Realytech Co.,Ltd

5.2 Reference products

Name:COVID-19 IgM Antibody Test Kit(CMIA)

COVID-19 IgG Antibody Test Kit(CMIA)

Manufacturer: Bioscience(Chongqing) Biological Technology Co., Ltd

Storage Conditions: Store in a dry place at 2-8°C,protected from light.

V. Experiment method

1. Collect 200 whole blood samples from patients with positive and negative persons..
2. The test group uses the in vitro diagnostic reagent products for testing, and the control group uses the "reference reagent" for testing.
3. Each whole blood sample needs to be tested in random order using in vitro diagnostic reagents for the test, and the "reference reagent" confirms the results.
4. The operation steps of the in vitro diagnostic reagents for the test are as follows. For details, please refer to the product instruction manual:

Step 1: If the sample is stored refrigerated or frozen, remove the test sample and required reagents from the storage conditions and equilibrate to room temperature (15-30°C). After thawing, mix the samples thoroughly before testing.

Step 2: When preparing for testing, open the aluminum foil bag from the tear. Remove the test card and lay it flat on a horizontal table.

Step 3: Label the sample number on the test card.

Step 4: Whole blood sample: Use a sample gun or a dropper to draw a whole blood sample from the sample tube and add 1 drop (about 20μl) to the sample hole on the test card, and immediately add 2 Drops (about 70～100μL) of sample dilution, and ensure that no air bubbles are generated during the operation.

Step 5: Time counting and interpret the results within 10 minutes.

Note: The detection steps need to be completed under protection against infection.

VI. Statistical methods Methods of statistical analysis of clinical research data

A Methods evaluating clinical performance

Whether various indexes can reach the standards of clinical evaluation shall be judged by calculating the consistency percentage of negative/positive and the total consistency percentage in the test results of the product tested and the reference product, to validate the accuracy and applicability of the product in clinical applications. The product tested shall be subject to tests through the sample of diferent types, with statistics on the results. Meanwhile,different types of sample of the subjects shall be subject to determ nation by the product tested synchronously,and then the determination results of both shall be compared.The test results recorded shall be subject to statistical analysis upon completion of determination of all clinical samples,to calculate the consistency percentage of negative/positive and the total consistency percentage. Afterwards, equivalence of both shall be evaluated as per these statistical indexes

B Statistical method

The products launched on the market shall be subject to comparative study and

evaluation.Kappa inspection: each sample shall be tested with the product tested and the reference product respectively, and then the consistency in statistical results of these two inspection methods shall be compared through Kappa inspection.

The data shall be subiect to Kappa inspection and analysis and the Kappa coefficient shall be calculated.Favorable consistency can be proven if Kappa is＞0.8. The consistency in test results of the product tested and the reference product is evaluated as per the evaluation standards.

VII Standards of clinical evaluation

The coincidence rate shall be calculated by comparing with the reference product whose marketing is approved. The product performance shall meet the following requirements.

1)Coincidence rate of negative: the sample whose test results are negative for both the product tested and the reference product and the proportion in the sample whose test results are negative for the reference product shall be more than 90%.

2)Coincidence rate of positive: the sample whose test results are positive for both the product tested and the reference product and the proportion in the sample whose test results are positive for the reference product shall be more than 90%.

3)Total coincidence rate: the sample whose test results are the same for the product tested and the reference product and its proportion in the total number of sample shall be more than 90%.

Method		COVID-19 IgG Antibody Test Kit(CMIA)		Total Results
COVID-19 IgG Rapid Test Device	Result	positive	negative	
	positive	A	B	A+B
	negative	C	D	C+D
Total Results		A+C	B+D	A+B+C+D

Clinical sensitivity =A/(A+C)*100%
Clinical specificity = D/(B+D)*100%
Accuracy: (A+D)/(A+B+C+D)*100%

Method		COVID-19 IgM Antibody Test Kit(CMIA)		Total Results
COVID-19 IgM Rapid Test Device	Result	positive	negative	
	positive	A	B	A+B
	negative	C	D	C+D
Total Results		A+C	B+D	A+B+C+D

Clinical sensitivity =A/(A+C)*100%

Clinical specificity = D/(B+D)*100%
Accuracy: (A+D)/(A+B+C+D)*100%

If the coincidence rate of positive/negative can meet clinical requirements, two methods or Products are considered as equivalent;If the coincidence rate of positive/negative is greatly different,the clinical scheme should be re-designed.
4)Kappa consisitency analysis shall be adopated for statistical analysis of reference reagents.
The results of the product tested are statistical materials and can be per the table below:

Method		COVID-19 IgG Antibody Test Kit(CMIA)		Total Results
COVID-19 IgG Rapid Test Device	Result	positive	negative	
	positive	A	B	A+B
	negative	C	D	C+D
Total Results		A+C	B+D	A+B+C+D

$P_{0=}$ (A+D)/(A+B+C+D)*100%
P_e=((A+B)(A+C) +(A+B)(B+D)) /$(A+B+C+D)^2$
Kappa:(P_0 - P_e)/(1-p_e)

Method		COVID-19 IgM Antibody Test Kit(CMIA)		Total Results
COVID-19 IgM Rapid Test Device	Result	positive	negative	
	positive	A	B	A+B
	negative	C	D	C+D
Total Results		A+C	B+D	A+B+C+D

$P_{0=}$ (A+D)/(A+B+C+D)*100%
P_e=((A+B)(A+C) +(A+B)(B+D)) /$(A+B+C+D)^2$
Kappa:(P_0 - P_e)/(1-p_e)

If conducting Kappa consistency analysis for the base data above, high consistency can be judged if the Kappa coefficient is >0.8,and both systems are considered as equivalent. Consistency is considered if 0.4<Kappa coefficient <0.8, and the coincidence rate of positive/negative shall be compared, with statistical analysis being made. Two such systems are considered as inconsistent and inequivalent if the Kappa coefficient is <0.4.

VIII Provisions for amendments to clinical validation

In general,the clinical validation should not be changed.Any modification to the project during the test should be explained,and the time,reason,process of change,and whether there is a record of the change are explained in detail and its impact on the evaluation of

the entire research result is explained.

IX. Results and Analysis of Clinical Tests

In total, 200 test samples (125 for male and 75 for female) are included for the unit and all test samples included are tested. Statistics on test results and those of the product tested are as follows:

For IgG:

Method		COVID-19 IgG Antibody Test Kit(CMIA)		Total Results
COVID-19 IgG Rapid Test Device	negative	positive	negative	
	positive	99	0	99
	negative	1	100	101
Total Results		100	100	200

Clinical sensitivity = 99/100*100%=99%

Clinical specificity = 100/100*100%= 100%

Accuracy: (98+100)/(96+1+4+99)*100%=99%

P_e=((100*99)+(99*100))/(200*200)=0.495

Kappa:(P_0 - P_e)/(1-p_e)=0.98

According to the above tabel,100 are proven negative of 100 negative specimens, 99 are proven positive of 100 positive specimens.The sensitivity and accuracy are more than 95%,indicating favorable consistency with the reference product.The Kappa=0.98＞0.8,indicating favorable and high consistency of two methods and equivalence of two such systems.

For IgM:

Method		COVID-19 IgM Antibody Test Kit(CMIA)		Total Results
COVID-19 IgM Rapid Test Device	negative	positive	negative	
	positive	98	1	99
	negative	2	99	101
Total Results		100	100	200

Clinical sensitivity = 98/100*100%=98%

Clinical specificity = 99/100*100%= 99%

Accuracy: (98+99)/(98+2+1+99)*100%=98.5%

P_e=(100*99+99*100)/(200*200)=0.495

Kappa:(P_0 - P_e)/(1-p_e)=0.97

According to the above tabel,99 are proven negative of 100 negative specimens, 98 are proven positive of 100 positive specimens.The sensitivity and accuracy are more than 95%,indicating favorable consistency with the reference product.The Kappa=0.97＞0.8,indicating favorable and high consistency of two methods and equivalence of two such

systems.

X Analysis on Inconsistency in Test Results

NO.	Gender	Age	COVID-19 IgG/IgM Rapid Test Device		COVID-19 IgG Antibody Test Kit(CMIA)	COVID-19 IgG Antibody Test Kit(CMIA)	Clinical Diagnosis
			IgG	IgM	IgG	IgM	
23	F	45	NEG	NEG	POS	POS	Subsequent visit of pneumonia triggered by COVID-19
24	F	66	POS	NEG	POS	POS	Subsequent visit of pneumonia triggered by COVID-19
27	M	56	NEG	POS	NEG	NEG	Non-pneumonia triggered by COVID-19

For those subjected to subsequent visit, IgM in the blood may be degraded and IgG definite diagnosis is more effective.

XI Discussion and Conclusions

1.discussion

A Results of comparative analysis of the product tested and the reference product:

Test results of the serum sample of the product tested and the reference product: both the coincidence rate of negative/positive and the total coincidence rate are larger than 90%, indicating favorable consistency with the reference product. In the analysis results of Kappa inspection, Kappa was proven >0.8, indicating favorable and high consistency of both methods. Both systems were proven equivalent.

B Statistical analysis results of the product tested for different types of clinical sample

While testing the SARS-CoV-2 antibody through the product tested for different types of clinical sample, the consistency percentages of negative/positive are 100.0% and the total consistency percentage is 100.0%. The Kappa coefficient = 1.00 (>0.8) in the results of Kappa inspection and analysis, indicating favorable and complete consistency of two methods and equivalence of two such systems.

2.Test conclusions

By analyzing the test results of the product tested and the reference product, the consistency percentage of negative/positive and the total consistency percentage are proven high. Moreover, according to the results of statistical analysis, there is no remarkable difference in test results of both, indicating favorable consistency in diagnosis and equivalence of two such systems. Meanwhile, the test results of the product tested for the serum and plasma sample of the same patient are completely identical. Therefore, such product is applicable to qualitative clinical analysis on the SARS-CoV-2 antibody in the serum and plasma sample of humans, and can be used for auxiliary diagnosis of those suffering from pneumonia triggered by COVID-19.

X. Quality control methods

On-site quality control

1）During the course of this study, clinical implementors appointed clinical inspectors to conduct regular on-site supervision visits to the research hospital. Through monitoring visits, it was found that all the contents of the research plan were strictly observed, and the correctness of the research data was also guaranteed. Participating researchers have undergone unified training, unified recording methods and judgment standards. The entire clinical trial process is conducted under strict operation, and the test content is complete and authentic. All observations and findings in the clinical trials have been verified and the data are reliable. The conclusions in the clinical trials are derived from the original data.
2）Quality control of clinical experiment process
During the evaluation, quality control was performed daily to ensure that the product was under control. Strict quality control is performed for each trial to ensure the quality of clinical trials.

XI. Prediction of adverse events

Because the COVID-19 IgG/IgM Rapid Test Device(Colloidal Gold) is an in vitro diagnostic reagent product, no direct contact with patients is required in clinical trials, no test report is provided to patients, and the test results are only used for comparative studies. It involves personal privacy, does not serve as a basis for auxiliary diagnosis, does not bring any risk to the subject, and does not cause adverse events.

References：
1.The "Technical Review Points for the Registration of New Coronavirus Antigen / Antibody Detection Reagents in 2019 (Trial)" issued by the State Drug Administration Medical Device Technical Evaluation Center on February 25, 2020；
2. "Pneumonitis Diagnosis and Treatment Program for New Coronavirus Infection (Trial Version 6)" issued by the National Health Committee on February 19, 2020.

Analytical Sensitivity

Testing Report

1. Purpose

The purpose of this proposal is to provide an validation of the analytical sensitivity. The production of at least three consecutive 2019-nCOV IgG/IgM Rapid Test Device products shall be controlled.

2. General information

Manufacturer:Hangzhou Realy Tech Co.,Ltd.

Product name:2019-nCOV IgG/IgM Rapid Test Device

Catalogue number:K460216D

3. Material

Positive Control:2019-nCOV-IgM：diluted by 6# clinical specimen (600ul clinical specimen and 1240ul covid-19 buffer) which is sixth day of infection;

Positive Control:2019-nCOV-IgG: diluted by 1# clinical specimen (100ul clinical specimen and 19900ul covid-19 buffer) which is 20th day of infection;

Validation lot 1:NO1G01T;

Validation lot 2:NO1G02T;

Validation lot 3:NO1G03T.

4. Method

Tests the low positive control of 2019-nCOV-IgM,2019-nCOV-IgG and negative sample. Each specimen tests in 11 tests. Read the positive result at 10 mi. Do not interpret the result after 15 minutes.

5. Interpretation of results

Positive result: ≥ G3

Negative result:<G3

6. QC Acceptance Criteria

Serum/Plasma specimens: C-line≤ 3min.

Whole blood specimens: C-line≤ 5min.

C-line≥G8 in 10min.

7. Results

Lot	2019-nCOV-IgM	2019-nCOV-IgG	Negative Sample
	IgM	IgM/IgG	IgM/IgG
NO1G01T	+	1/+	1/1

Lot	2019-nCOV-IgM	2019-nCOV-IgG	Negative Sample
	IgM	IgM/IgG	IgM/IgG
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
NO1G02T	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
NO1G03T	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-
	+	+	-/-

Lot	2019-nCOV-IgM	2019-nCOV-IgG	Negative Sample
	IgM	IgM/IgG	IgM/IgG
	+	+	-/-

8. Conclusion

From the results of low positive control test, the test show 100% positive of IgM and IgG with the control.

The 2019-nCOV IgG/IgM Rapid Test Device can show good performance using the positive and negative samples.

Analytical Specificity

Testing Report

1. Purpose

The purpose of this study is to provide a validation of these substances whether have interference to the 2019-nCOV IgG/IgM Rapid Test Device.

2. General information

Manufacturer:Hangzhou Realy Tech Co.,Ltd.

Product name:2019-nCOV IgG/IgM Rapid Test Device

Catalogue number: K460216D

3. Material

Validation lot 1: NO1G01T;

Validation lot 2: NO1G02T;

Negative Control: N1;

Positive Control: 2019-nCOV-IgM L;

Positive Control: 2019-nCOV-IgG L;

Clinical specimen: HIV positive specimen; HBsAg positive specimen; Hcv positive specimen; syphilis positive specimen; FLU positive specimen; RSV positive specimen; ADENO positive specimen; MP(Mycoplasma pneumoniae) positive specimen;

Interfering analytes: triglyceride, ascorbic acid, hemoglobin, bilirubin, total cholesterol sample.

4. Method

Test each clinical specimen with triple tests. Read the positive result at 10 min and negative result at 15 min. Do not interpret the result after 15 minutes.

5 analytes are spiked with QC standard samples (N-1, 2019-nCOV-IgM L, 2019-nCOV-IgG L) to a certain concentration below. Test each specimen with triple tests. Read the positive result at 10 min and negative result at 15 min. Do not interpret the result after 15 minutes.

Hemolytic sample will be shocked in 30 seconds.

The analytes are as follows:

Analytes	Concentration
Triglyceride	100mg/dL
Ascorbic acid	20mg/dL
Hemoglobin	1000mg/dL

Analytes	Concentration
Bilirubin	100mg/dL
Total cholesterol	6mmol/L

5. Interpretation of results

Positive result: ≥ G3

Negative result:<G3

6. QC Acceptance Criteria

No Interfering Substances with the validation lot.

Negative samples T<G3; Positive samples T≥G3

Serum/Plasma specimens: C-line appear≤ 3min.

Whole blood specimens: C-line appear≤ 5min.

C-line≥G8 in 10min.

7. Result

Validation lot1:NO1G01T

Analytes	Concentration	Spiked plasma standard								
		N1			2019-nCOV-IgM L			2019-nCOV-IgG L		
		IgM/IgG			IgM			IgG		
Triglyceride	100mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Ascorbic acid	20mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Hemoglobin	1000mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Bilirubin	100mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Total cholesterol	6mmol/L	-/-	-/-	-/-	+	+	+	+	+	+
HIV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
HCV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
HBV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
SYP positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
FLU positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
RSV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
ADE positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
MP positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A

C-line State:10″;

C-line Appearing Time: ≤ 1′07″.

Validation lot2:NO1G02T

Analytes	Concentration	Spiked plasma standard								
		N1			2019-nCOV-IgM L			2019-nCOV-IgG L		
		IgM/IgG			IgM			IgG		
Triglyceride	100mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Ascorbic acid	20mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Hemoglobin	1000mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Bilirubin	100mg/dL	-/-	-/-	-/-	+	+	+	+	+	+
Total cholesterol	6mmol/L	-/-	-/-	-/-	+	+	+	+	+	+
HIV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
HCV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
HBV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
SYP positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
FLU positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
RSV positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
ADE positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A
MP positive	N/A	-/-	-/-	-/-	N/A	N/A	N/A	N/A	N/A	N/A

C-line State:10″;

C-line Appearing Time:≤1′13″.

8. Conclusion

The results indicated that these interfering substances and Related virus antibody positive specimens have no interference phenomenon to 2019-nCOV IgG/IgM Rapid Test Device.