Appendix III

Clinical Evaluation Report of the In Vitro Diagnostic Reagents

Product Name	COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Packaging Specification	25 Tests/Kit
Evaluation Time	Feb. 2020-
Clinical Evaluation Organization	Multi-Center (Specified below)
Sponsor	Zhejiang Orient Gene Biotech Co., Ltd
Contact Information	
Reporting Date	March 12, 2020

Clinical Evaluation Organization List

- Chinese Academy of Sciences University affiliated Ningbo Huamei hospital
- Zhejiang University Affiliated No.1 Hospital
- Ninbo Yinzhou People Hospital
- Wenzhou Central Hospital
- Wuhan No.1 People Hospital
- Wuhan University People Hospital
- Jiande No.1 People Hospital
- Taizhou Centra Hospital
- Zhejiang Province No.2 Prison Hospital
- Children's Hospital Zhejiang University School of Medicine

Appendix III

CONTENT

Appendix III

[Introduction]

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases. Seven coronavirus species are known to cause human disease. Four viruses - 229E, OC43, NL63, and HKU1 - are prevalent and typically cause common cold symptoms in immunocompetent individuals.4 The three other strains - severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and 2019 Novel Coronavirus (COVID-19) - are zoonotic in origin and have been linked to sometimes fatal illness. IgG and IgM antibodies to 2019 Novel Coronavirus can be detected with 2-3 weeks after exposure. IgG remains positive, but the antibody level drops overtime.

The current method for detecting the 2019-nCoV is to detect viral RNA by fluorescent PCR method for qualitative detection of 2019-nCoV nucleic acid.Routine viral nucleic acid detection includes a series of steps needed to do such as nucleic acid extraction and purification, reagent preparation, specimen loading and instrument testing. So as same to the new coronavirus nucleic acid detection reagents that have been used to detect and monitor the disease.Each step requires careful operation by the inspector.It often takes 2-3 hours to get the test results. At the same time, there is a risk of contamination and infection to the experimental operation at each step, which places high requirements on the inspection process and the inspectors.

The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a lateral flow immunochromatographic assay. The test uses anti-human IgM antibody (test line IgM) , anti-human IgG (test line IgG) and rabbit IgG (control line C) immobilised on a nitrocellulose strip. The burgundy colored conjugate pad contains colloidal gold conjugated to recombinant COVID-19 antigens conjugated with colloid gold (COVID-19 conjugates). When a specimen followed by assay buffer is added to the sample well, IgM &/or IgG antibodies if present, will bind to COVID-19 conjugates making antigen antibodies complex. This complex migrates through nitrocellulose membrane by capillary action. When the complex meets the line of the corresponding immobilized antibody (anti-human IgM &/or anit-human IgG) the complex is trapped forming a burgundy colored band which confirm a reactive test result. Absence of a colored band in the test region indicates a non reactive test result.

The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgG and IgM antibodies to 2019 Novel Coronavirusi in human whole blood, serum or plasma.The results tested by The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be read in 10 minutes.This test provides only a preliminary test result can be an alternative testing method to assist the diagnosis to COVID-19 infection

[Purpose]

To evaluate the clinical performance of the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

[Study Design]

The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is supposed to test the specimen(whole blood, serum, plasma) from suspected and confirmed COVID-19 infection patients.

And the results are supposed be compared to COVID-19 Diagnostic Criteria and the medical determination of disease process towards COVID-19 (PCR test results are recommended in clinical to fully evaluate COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)). Adopting 2X2 tabulation and Kappa value so as to evaluate consistency of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) with reference reagent.

If test COVID-19 result is different from COVID-19 Diagnostic Criteria and the medical determination, the PCR results is supposed to be the confirmation

[Evaluation Method]

Specimen selection

To enroll subjects according to *COVID-19 Treatment Plan* (trial implementation 6th edition) by National Health Commission

Specimen Selection Criteria

Complete specimen information, including subjects' age, gender, specimen collection date and clinical diagnosis etc.

Positive specimen from subjects confirmed with COVID-19 infection

Negative specimen from subjects without infection

Negative specimen from subjects cured from COVID-19 infection

Specimens from patients infected with influenza virus or lower respiratory infection

Specimen Exclusion Criteria

Specimen volume is inadequate to support the test

Specimens collected not as required or that expired or deteriorated

Specimen Elimination Criteria

Specimen tested by device with quality deficiency

Specimen mistakenly enrolled by operator or/that with unconvincing results or/that can not be traced

Specimen Collection

1. COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using either whole blood, serum or plasma.
2. Separate serum or plasma from blood as soon as possible to avoid hemolysis. Use only clear, non-hemolyzed specimens.
3. Testing should be performed immediately after specimen collection. Do not leave the specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
4. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
5. If specimens are to be shipped, they should be packed in compliance with local regulations covering the

transportation of etiologic agents.

Reference Reagent Selection

According to *2019 COVID-19 IgG/IgM Technical Essential of Registration and Review(trial implementation)* and *Technical Guidance of In-vitro Diagnostic Clinical Trial,* the test results are supposed be compared to COVID-19 Diagnostic Criteria and the medical determination of disease process towards COVID-19 (PCR test results are recommended in clinical to fully evaluate COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma))

Inconsistent Result Confirmation

If test COVID-19 result is different from COVID-19 Diagnostic Criteria and the medical determination, the PCR results is supposed to be the confirmation

Test Reagent

COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Test procedure

Perform the test according to the test procedure in the package insert

Quality Control

NEGATIVE: The colored line in the control line region (C) changes from blue to red. No line appears in the test line regions M or G. The result is negative.

IgM POSITIVE:

The colored line in the control line region (C) changes from blue to red, and a colored line appears in test line region M. The result is anti-COVID-19 IgM positive.

IgG POSITIVE:

The colored line in the control line region (C) changes from blue to red, and a colored line appears in test line region G. The result is anti-COVID-19 IgG positive.

IgG and IgM POSITIVE:

The colored line in the control line region (C) changes from blue to red, and two colored lines appear in test line regions M and G. The result is anti-COVID-19 IgM and IgG positive.

INVALID:

Control line is still completely or partially blue, and fails to completely change from blue to red. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Data Statistics

Appendix III

The agreement rates and kappa values of IgG and IgM to the reference reagent are supposed be analyzed.

Table 2 x 2

Test Reagent	**Reference Reagent**		**Total**
	Positive	**Negative**	
Positive	a	b	a+b(γ_1)
Negative	c	d	c+d(γ_2)
Total	a+c(C_1)	b+d(C_2)	a+b+c+d (N)

Positive agreement ＝[a/(a+c)]×100％

Negative agreement＝[d/(b+d)]×100％

Total agreement＝[(a+d)/(a+b+c+d)] ×100％

Kappa value should be carried out for the above-mentioned clinical data and the confidence interval(CI) is 95%. The Kappa value is from 0 to 1. the closer to 1 the Kappa value, the more consistent the two tests. Averagely, if the Kappa value is over 0.75 ,the test reagent and reference reagent are highly consistent.

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Kappa= $\frac{N(a+d)-(\gamma_1 C_1+\gamma_2 C_2)}{N^2-(\gamma_1 C_1+\gamma_2 C_2)}$

2. [Results and Analysis]

Appendix III

In the study design,the agreement rates and kappa values of IgG and IgM are supposed to be analyzed respectively, however in the state of COVID-19 emergency, it is hard to work out the results of IgG and IgM respectively in short time, the decision was made to analyze the positive and negative results according to results interpretation as table below currently. if IgG or IgM is positive, positive is recorded; if IgG and IgM are both positive, positive is recorded;

Results Interpretation	
Results by Orient Gene Test	**Results Interpretation and Calculation**
IgM Positive or IgG Positive	Positive
IgM and IgG Positive	Positive
IgM and IgG Negative	Negative

2.1 Test Information

Test site 1

Chinese Academy of Sciences University affiliated Ningbo Huamei hospital
Address: No 655, Huancheng Road, Ningbo City, Zhejiang Province.

Test material:

COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot: 2002189

Reference Reagent:

Results Analysis

Test Reagent	**Reference Reagent**		**Total**
	Positive	**Negative**	
Positive	17	0	17
Negative	3	0	3
Total	20	0	20

Positive agreement
＝[a/(a+c)]×100％
＝[17/(17+3)]×100％
＝85%

Test site 2
Zhejiang University Affiliated No.1 Hospital

Address: No. 79 Qinchun Road, Hangzhou City, Zhejiang Province.

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot: 2002189

Reference Reagent:
Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	103	0	103
Negative	9	14	23
Total	112	14	126

Positive agreement
＝[a/(a+c)]×100％
＝[103/(103+9)]×100％
＝91.96%

Negative agreement
＝[d/(b+d)]×100％
＝[14/(0+14)]×100％
＝100%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(103+14)/ (103+0+9+14)] ×100％
＝92.86%

Test site 3
Ninbo Yinzhou People Hospital
Address: No. 251 East Baizhang Road, Ninbo City, Zhejiang Province

Appendix III

Test material: COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:
Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	10	0	10
Negative	1	60	61
Total	11	60	71

Positive agreement
＝[a/(a+c)]×100％
＝[10/(10+1)]×100％
＝90.96%

Negative agreement
＝[d/(b+d)]×100％
＝[60/(0+60)]×100％
＝100%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(10+60)/ (10+1+0+60)] ×100％
＝98.59%

Test site 4
Wenzhou Central Hospital
Address: Lucheng District, Wenzhou City, Zhejiang Province.

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Appendix III

Lot:

Reference Reagent: Shanghai bojie

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	78	0	78
Negative	2	0	2
Total	80	0	80

Positive agreement
＝[a/(a+c)]×100％
＝[78/(78+2)]×100％
＝97.5%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(78+0)/ (78+2+0+0)] ×100％
＝97.5%

Test site 5
Wuhan No.1 People Hospital
Address: No. 215 Zhongshan Avenue Qiaokou District, Wuhan City, Hubei Province

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent: Huada Bio

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	0	2	2
Negative	0	98	98
Total	0	100	100

Negative agreement
＝[d/(b+d)]×100％
＝[98/(2+98)]×100％
＝98%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(0+98)/(0+2+0+98)] ×100％
＝98%

Test site 6

Wuhan University People Hospital

Address: No. 238 Jiefang Road, Wuchang District,Wuhan City, Hubei Province.

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	34	1	35
Negative	1	63	64
Total	35	64	99

Positive agreement
＝[a/(a+c)]×100％
＝[34/(34+1)]×100％
＝97.14%

Negative agreement
＝[d/(b+d)]×100％
＝[63/(1+63)]×100％
＝98.44%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(34+63)/(34+1+1+63)] ×100％
＝97.98%

Test site 7
Jiande No.1 People Hospital
Address: No. 599 Yanzhou Avenue, Xinanjiang Street, Jiande City, Zhejiang Province.

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	0	1	1
Negative	0	79	79
Total	0	80	80

Negative agreement
＝[d/(b+d)]×100％
＝[79/(1+79)]×100％
＝98.75%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(0+79)/(0+0+1+79)] ×100％
＝98.75%

Test site 8

Taizhou Centra Hospital

Address: No. 999 DongHai Avenue, Jiaojiang District, Taizhou City,Zhejiang Province

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	2	0	2
Negative	0	79	79
Total	2	79	81

Positive agreement
＝[a/(a+c)]×100％
＝[2/(2+0)]×100％
＝100%

Negative agreement
＝[d/(b+d)]×100％
＝[79/(0+79)]×100％
＝100%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(2+79)/(2+0+0+79)] ×100％
＝100%

Test site 9

Zhejiang Province No.2 Prison Hospital

Address: No. 3 Qiushan Street, Linpin Town, Yuhang District, Hangzhou City,Zhejiang Province.

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	0	0	0
Negative	0	35	35
Total	0	35	35

Negative agreement
＝[d/(b+d)]×100％
＝[35/(0+35)]×100％
＝100%

Total agreement
＝[(a+d)/(a+b+c+d)] ×100％
＝[(0+35)/(0+0+0+35)] ×100％
＝100%

Test site 10
Children's Hospital
Zhejiang University School of Medicine
Address: No. 3333 Binshen Road, Binjiang District, Hangzhou City, Zhejiang Province.

Appendix III

Test material:COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Lot:

Reference Reagent:

Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	1	0	1
Negative	0	11	11
Total	1	11	12

Positive agreement
=[a/(a+c)]×100%
=[1/(1+0)]×100%
=100%

Negative agreement
=[d/(b+d)]×100%
=[11/(0+11)]×100%
=100%

Total agreement
=[(a+d)/(a+b+c+d)] ×100%
=[(1+11)/(1+0+0+11)] ×100%
=100%

2.2 Total Results Analysis

Test Reagent	Reference Reagent		Total
	Positive	Negative	
Positive	245	4	249
Negative	16	439	455
Total	261	443	704

Positive agreement
=[a/(a+c)]×100%
=[245/(245+16)]×100%
=93.87%

Negative agreement
=[d/(b+d)]×100%
=[439/(4+439)]×100%
=99.10%

Total agreement
=[(a+d)/(a+b+c+d)] ×100%
=[(245+439)/(245+4+16+439)] ×100%
=97.19%

$$\frac{N(a+d)-(\gamma_1 C_1+\gamma_2 C_2)}{N^2-(\gamma_1 C_1+\gamma_2 C_2)}$$

Kappa=

Kappa=0.94

Positive agreement: 93.87% (95%CI:90.24%~96.46%)
Negative agreement: 99.10% (95%CI:97.70%~99.75%)
Total agreement: 97.19% (95%CI:95.65%~98.26%)

Compared with the reference reagent, the positive agreement was 93.87% (95%CI:90.24%~96.46%), the negative agreement was 99.10% (95%CI:97.70%~99.75%) and total agreement was 97.19% (95%CI:95.65%~98.26%). The kappa value of the consistency analysis was 0.94 (95%CI:95.65%~98.26%).

2.3 Analysis of Inconsistent Results

In this study, there were 18 specimens with test reagent results and reference reagent results that differed, including 15 false negative and 3 false positive.

These factors may cause false negative

- The concentration of antibody in human body is lower than the limit of detection
- Improper operation, like add inadequate specimen
- Improper reading time, like reading the results earlier than the designed time.

These factors may cause false positive

- Some substances in human blood may cause false positive

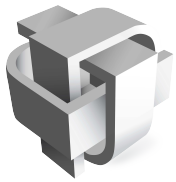
- Improper operation, like adding too much specimen
- Improper reading time, like reading the results later than the designed time.

The repeated test was not carried out due to objective reasons.

2.4 Conclusion

The results show that the testing reagent and reference reagent have equivalent effectiveness in detecting COVID-19 when tested in the same clinical specimens. Compared with the reference reagent, the positive agreement was 93.87% (95%CI:90.24%~96.46%), the negative agreement was 99.10% (95%CI:97.70%~99.75%) and total agreement was 97.19% (95%CI:95.65%~98.26%). The kappa value of the consistency analysis was 0.94 (95%CI:95.65%~98.26%). The results of the clinical evaluation show that the two reagents (methods) have a high degree of consistency and equivalent sensitivity and specificity in detecting COVID-19.

Clinical studies from more sites is being carried on.



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