Clinical Evaluation Report of COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)

Product Name	COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Packaging Specification	25 Tests/Kit
Evaluation Time	Feb. 2020-March 2020
Clinical Study Sites	Multi-Center (Specified in study design)
Sponsor	Zhejiang Orient Gene Biotech Co., Ltd
Reporting Date	April , 2020

CONTENT

[Introduction]

Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds 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. The three other strains (SARS-CoV, MERS-CoV, SARS-CoV-2) are zoonotic in origin and have sometimes been linked to fatalities. IgG and IgM antibodies to SARS-CoV-2 can be detected within 1-3 weeks of exposure. IgG remains positive, but the antibody level drops overtime.

The current method for detecting SARS-CoV-2 is to use fluorescent PCR to qualitatively detect SARS-CoV-2 RNA. Routine viral nucleic acid detection includes a series of steps including nucleic acid extraction and purification, reagent preparation, specimen loading and instrument testing. Each step requires careful operation by trained laboratory personnel and it often takes 2-3 hours to get the test results. In addition, there is a risk for contamination and infection to laboratory personnel at each step of the lengthy, involved process.

The COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) is a lateral flow solid phase immunochromatographic assay for the rapid qualitative and differential detection of IgG and IgM antibodies to SARS-CoV-2 in human whole blood, serum or plasma.. The test uses anti-human IgM antibody (test line IgM), anti-human IgG antibody (test line IgG) and rabbit IgG antibody (control line C) immobilized on a nitrocellulose strip. The burgundy colored conjugate pad contains recombinant COVID-19 antigens conjugated with colloid gold (COVID-19 conjugates). When a specimen followed by assay buffer is added to the sample well, if IgM and/or IgG antibodies are present, it will bind to COVID-19 conjugates and make an antigen antibodies complex. This complex migrates through the nitrocellulose membrane by capillary action. When the complex meets the line of the corresponding immobilized antibody (anti-human IgM and/or anit-human IgG) the complex is trapped and forms a burgundy colored band, confirming a reactive test result. The test results can be read in 10 minutes. This test provides only a preliminary test result that can be used as an alternative testing method to assist in the diagnosis of a SARS-CoV-2 infection. Therefore, any reactive specimen with the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) must be confirmed with alternative testing method(s) and clinical findings. Absence of a colored band in the test region indicates a non-reactive test result.

[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 designed to test specimen from suspected and confirmed COVID-19 patients and to compare to COVID-19 diagnostic criteria and the medical determination of disease process (PCR test results are recommended to fully clinically evaluate COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)). To evaluate the consistency of the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) with the reference reagent, 2X2 tabulation and Kappa value are

calculated. If the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) result differs from the COVID-19 diagnostic criteria and the determination of medical disease progress, the PCR results is supposed to be the indication of the true clinical results.

Site #	Name of the site	Location	Principle Investigator
1	Ningbo Huamei Hospital Chinese Academy of Sciences University	Room 402, Building 3, 41 Xibei Street, Haishu District, Ningbo, Zhejiang, China	Zhang Shun
2	Wenzhou Central Hospital	Lucheng District, Wenzhou City, Zhejiang Province.	Xu Xueqin
3	Wuhan 3rd Hospital	No.241 Pengliuyang Road, Wuchang District, Wuhan City,	Sun jianbin

[Evaluation Method]

Specimen Enrollment Basis

Subjects are enrolled according to clinical diagnosis based on the *COVID-19 Diagnosis and Treatment plan* (trial implementation 6th edition) by the National Health Commission.The specimen size can be appropriately adjusted according to actual status of the clinical trial site.

Specimen Enrollment Criteria

- Complete specimen information, including subjects' age, gender, specimen collection date and clinical diagnosis etc. Positive specimen from subjects confirmed with COVID-19.
- Negative specimen from subjects excluded from COVID-19. Negative specimen from subjects cured of COVID-19 (positive to negative after treatment)
- Specimens from patients infected with influenza virus or other lower respiratory infection.
- Considering the characteristics of the new coronavirus epidemic, a certain percentage of retrospective specimens that meet the requirements mentioned in package insert is acceptable.

Specimen Exclusion Criteria

- Specimen volume is inadequate to support the test.
- Specimen collected via an unacceptable method or specimen has expired or deteriorated.

Specimen Elimination Criteria

- Specimen tested by device has quantity deficiency.
- Specimen mistakenly enrolled by operator and/or with unconvincing results and/or 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 specimen 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.
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.

Specimens generation
Whole blood specimen:
Disinfect the blood collection area.
Collect venous blood specimens in clean, dry test tubes pre-embedded with heparin, EDTA or sodium citrate anticoagulants. Mix evenly after collection to prevent clotting.

Plasma specimen:
Disinfect the blood collection area.
Collect venous blood specimens in clean, dry test tubes pre-embedded with heparin, EDTA or sodium citrate anticoagulants. Mix evenly after collection to prevent clotting. Centrifuge and separate the supernatant, which is the plasma specimen.

Serum specimen:
Disinfect the blood collection area.
Collect venous blood in dry, clean, anticoagulant-free test tubes;
Centrifuge the coagulated venous blood specimen, and the obtained supernatant is the serum specimen.

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 to be compared to medical diagnosis based on COVID-19 diagnostic criteria and the disease progress (PCR test results are recommended to fully clinically evaluate COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)).

Inconsistent Result Confirmation
On the test COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) result differing from the medical diagnosis based on COVID-19 diagnostic criteria and disease process, detect the specimen with a new candidate test and analyze the possible reasons leading to the results.

Candidate Test
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
- Before the start of the clinical study, clinical study personnel shall be trained by the applicant so that they can be familiar with and master the product operation method, technical performance, quality control operation, etc. to minimize test errors.
- The trial personnel shall operate in strict accordance with the product operation specifications and related requirements to minimize the detection errors.
- The trial shall be organized in strict accordance with Guidelines for Clinical Testing of In Vitro Diagnostic Reagents; the original records shall be kept complete to ensure that the test data is accurate and traceable.

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

Table 1: Clinical Data Analysis

Candidate Test	**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％

The kappa value should be calculated for the clinical data above and the confidence interval (CI) set at 95%. The Kappa value ranges from 0 to 1. The closer the Kappa value is to 1, the more consistent the two tests. On average, if the Kappa value is over 0.75, the candidate test and reference reagent are highly consistent.

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

The above-mentioned statistical analysis method are applied simultaneously for the individual evaluation of each sub-center and the overall sample evaluation.

[Trial Management]

Modification of the plan during the trial

Any modification to the plan during the trial shall be well prescribed in terms of the time, reason, process and whether the corresponding record is available. The impact thereof on the evaluation of the entire trial result shall also be discussed.

Ethical Issues and Instructions Involved in Clinical Trials

- The samples used in clinical trials are the remaining samples after routine testing by the target personnel, and no additional sample is required;
- The test results of this clinical trial are only for clinical research analysis, and are not used as the basis for the follow-up diagnosis and treatment measures or physical evidence identification of the target personnel.
- The subjects will be kept strictly confidential during the implementation of clinical trials, and the names of the subjects will not appear in all public research records and reports. This clinical trial objectively poses no risk to the subjects.

Data Processing and Record Keeping

- Take records of the study in a uniformly printed form. The records should be authentic, standardized and complete.
- Take records of the study with a blue or black writing pen or signature pen. To ensure privacy protections, the name of the subjects shall not appear in the test records, and the name should be replaced by the sample No., hospitalization No. or the initials of the name.
- The test records should not be deleted, modified or added at will. If necessary, contents of revision should be drawn with a slash, and should not be completely blackened to ensure that the records before revision can be identified, and it should be signed by the modifier, indicating the date and reason.
- The test records shall not be copied, photographed or photocopied in any form to other

personnel and units without the unanimous permission of the applicant and the person in charge of the research.

Other Contents that needs explanation

Responsibilities of Clinical Trial Personnel

- Each clinical trial unit should be equipped with the following personnel:
- Principal Investigator: Responsible for trial implementation, technical guidance and management coordination;
- Report Writer: Responsible for writing the clinical summary report.
- Other test personnel are responsible for sample collection, inspection operations, and data recording.

Quality Control

The test unit should have a laboratory quality control and management system. The temperature and humidity of the laboratory should be recorded every day during the test, and the experimental equipment and instruments should be regularly calibrated.

Responsibilities of All Parties

- The applicant for registration should sign a formal clinical trial contract (or agreement) with the research unit before the start of the clinical trial to clarify the responsibilities of both parties.
- The kit provided in this test is only used in this clinical trial, and its test results cannot be used as diagnosis or evidence of the subjects.
- This plan is an attachment to the clinical trial contract (or agreement). After this plan is agreed and signed by all parties, all parties should conduct clinical trials in strict accordance with the requirements of the plan.

[References]

- Quality Management Standards for Medical Device Clinical Trials
- Technical Guidance of In-vitro Diagnostic Clinical Trial,
- COVID-19 Diagnosis and Treatment Plan
- 2019 COVID-19 IgG/IgM Technical Essential of Registration and Review(trial implementation) (trial implementation)

[Report: Results and Analysis]

Test Information

Clinical Study Site 1

Ningbo Huamei Hospital Chinese Academy of Sciences University

Room 402, Building 3, 41 Xibei Street, Haishu District, Ningbo, Zhejiang, China

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

Lot: 20021203

Patient Description

The trial tested samples from a total of 264 target populations, including 142 males, accounting for 53.79%; 122 females, accounting for 46.21%; the male to female ratio was 1.16: 1, maximum age 91 years, minimum age 15 years, average age 57.5 years.

Results Analysis

Candidate Test	**Clinical Diagnosis**		**Total**
	Positive	**Negative**	
Positive	67	3	70
Negative	10	184	194
Total	77	187	264

Positive Agreement＝[a/(a+c)]×100％

＝[67/(67+10)]×100％

＝87.01%

Negative Agreement＝[d/(b+d)]×100％

＝[184/(3+184)]×100％

＝98.40%

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

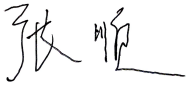
＝[(67+184)/ (67+3+10+184)] ×100％

＝95.08%

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

＝0.88

Reviewed by: Zhang Shun



Clinical Study Site 2
Wenzhou Central Hospital
Address: Lucheng District, Wenzhou City, Zhejiang Province.
Test material: COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Lot: 2002196, 2003206

Patient Description

The trial tested samples from a total of 325 target populations, including 176 males, accounting for 54.15%; 149 females, accounting for 45.85%; the male to female ratio was 1.18: 1, maximum age 91 years, minimum age 2 years, average age 43.1 years.

Results Analysis

Candidate Test	**Clinical Diagnosis**		**Total**
	Positive	**Negative**	
Positive	105	2	107
Negative	3	215	218
Total	108	217	325

Positive Agreement＝[a/(a+c)]×100％

＝[105/(105+3)]×100％

＝97.22%

Negative Agreement＝[d/(b+d)]×100％

＝[215/(2+215)]×100％

＝99.08%

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

＝[(105+215)/ (105+2+3+215)] ×100％

＝98.46%

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

＝0.97

Reviewed by: Xue Xueqing

徐雪琴

Clinical Study Site 3
Wuhan 3rd Hospital
Address: No.241 Pengliuyang Road, Wuchang District, Wuhan City,
Test material: COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma)
Lot: 2003206

Patient Description

The trial tested samples from a total of 349 target populations, including 182 males, accounting for 52.1%; 167 females, accounting for 47.9%; the male to female ratio was 1.18: 1, maximum age 98 years, minimum age 7 years, average age 59.8 years.

Results Analysis

Candidate Test	**Clinical Diagnosis**		**Total**
	Positive	**Negative**	
Positive	147	4	151
Negative	22	176	198
Total	169	180	349

Positive Agreement＝[a/(a+c)]×100％

＝[147/(147+22)]×100％

＝86.98%

Negative Agreement＝[d/(b+d)]×100％

＝[176/(4+176)]×100％

＝97.78%

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

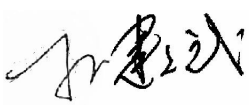
＝[(147+176)/ (147+22+4+176)] ×100％

＝92.55%

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

＝0.85

Reviewed by: Sun Jianbin



Total Results Analysis

The trial tested samples from a total of 938 target populations, including 500 males, accounting for 53.30%; 438 females, accounting for 46.70%; the male to female ratio was 1.14: 1, maximum age 98 years, minimum age 2 years, average age 53.37 years.

Candidate Test	Clinical Diagnosis		Total
	Positive	Negative	
Positive	319	9	328
Negative	35	575	610
Total	354	584	938

Positive agreement
=[a/(a+c)]×100%
=[319/(319+35)]×100%
=90.11% (95% CI:86.52%~93.02%)

Negative agreement
=[d/(b+d)]×100%
=[575/(9+575)]×100%
=98.46% (95% CI:97.09%~99.29%)

Total agreement
=[(a+d)/(a+b+c+d)] ×100%
=[(319+575)/(319+35+9+575)] ×100%
=95.31% (95% CI: 93.75%~96.57%)

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

Kappa=0.90

Compared with the reference reagent, the positive agreement was 90.11% (95% CI:86.52%~93.02%), the negative agreement was 98.46% (95% CI:97.09%~99.29%) and total agreement was 95.31% (95% CI: 93.75%~96.57%). The kappa value of the consistency analysis was 0.90.

Analysis of Inconsistent Results

In this study, there were 44 specimens with candidate test results and reference reagent results that differed, including 35 false negatives and 9 false positives.

The following factors may cause false negatives:

1. The concentration of antibody in the specimen is lower than the limit of detection.

2. Improper operation, i.e. addition of inadequate specimen volume.
3. Improper reading time, i.e. reading the results earlier than the designed time.

The following factors may cause false positives:

1. Some substances in human blood may cause false positives.
2. Improper operation, i.e. adding too much specimen.
3. Improper reading time, i.e. reading the results later than the designed time.

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

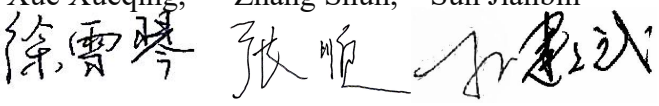
Discussion

The results show that the testing reagent (COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) and reference reagent have equivalent effectiveness in detecting SARS-CoV-2 when tested in the same clinical specimens. Compared with the reference reagent, the positive agreement was 90.11% (95% CI:86.52%~93.02%), the negative agreement was 98.46% (95% CI:97.09%~99.29%) and total agreement was 95.31% (95% CI: 93.75%~96.57%). The kappa value of the consistency analysis was 0.90. 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 SARS-CoV-2.

Conclusion

The results of the COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) have high consistency with the clinical diagnosis. In addition, COVID-19 IgG/IgM Rapid Test Cassette (Whole Blood/Serum/Plasma) has different characteristics that it is stable, easy to store, simple and fast to operate, and it requires no instrument assistance. It is a good method to use this test to do the preliminary screening for COVID-19 in various medical institutions.

Reviewed by: Xue Xueqing, Zhang Shun, Sun Jianbin



Date: April，2020