Clinical Evaluation Report of the In Vitro Diagnostic Reagents

Product Name	StrongStep® SARS-CoV-2 IgM/IgG Antibody Rapid Test (Latex immunochromatography)
Packaging Specification	20 Tests/Kit
Evaluation Time	March. 2020- April. 2020
Clinical Evaluation Organization	Wuhan Huoshenshan Hospital (Address: Near to Zhiyin Lake, Caidian District, Wuhan)
Sponsor	N/A
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CONTENT

[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 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 StrongStep® SARS-CoV-2 IgM/IgG Test utilizes the principle of Immuno-chromatography. Each device contain two strips, SARS-CoV-2 specific recombinant antigen are immobilized on the nitrocellulose membrane of each strip in the test window of the test device, Mouse anti-human IgM and human IgG antibodies conjugate with colored latex beads are immobilized on the conjugate pad of the two strips respectively, As the test sample flows through the membrane within the test device, the colored Mouse anti-human IgM and human IgG antibodies latex conjugate complexes with human antibodies (IgM and/or IgG) This complex moves further on the membrane to the test region where it is captured by SARS-CoV-2 specific recombinant antigen, if SARS-CoV-2 virus IgG/IgM antibodies present in the sample, leading to formation of a colored band, which indicates a positive test results. Absence of this colored band in the test window indicates a negative test result. This complex moves further on the membrane to the control region where it is captured by goat anti-mouse form red control line, a built-in control line will always appear in the test window when the test has performed properly, regardless of the presence or absence of anti- SARS-CoV-2 virus antibodies in the specimen.

The StrongStep® SARS-CoV-2 IgM/IgG Test is a solid phase immunochromatographic assay for the rapid, qualitative and differential detection of IgM and IgG antibodies to 2019 Novel Corona virus in human whole blood, serum or plasma. The results tested by The StrongStep® SARS-CoV-2 IgM/IgG Test can be read in 15 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 StrongStep® SARS-CoV-2 IgM/IgG Test.

[Study Design]

The StrongStep® SARS-CoV-2 IgM/IgG Test 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 PCR test(Sansure BioTech Inc). Adopting 2X2 tabulation and Kappa value so as to evaluate consistency of StrongStep® SARS-CoV-2 IgM/IgG Test 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 7th 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. StrongStep® SARS-CoV-2 IgM/IgG Test 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 IgM/IgG 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 StrongStep® SARS-CoV-2 IgM/IgG Test)

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

StrongStep® SARS-CoV-2 IgM/IgG Test

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). 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), 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), 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), 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 absence. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Operator training

6 operators were include in this clinical trial, all the operators were trained to familiar with the clinical trial protocol and how to use this device.

Data Statistics

The agreement rates and kappa values of IgM and IgG 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.

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

2. [Results and Analysis]

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;

2.1 Test Information

Test material: Sars-CoV-2 IgM/IgG Test, Lot: 2003025

Clinical sample: N= 675 positive serum specimens (46 Homologous fingerstick sample were also tested)

78 negative serum specimens (30 Homologous fingerstick sample were also tested)

Results

IgM

	Clinical diagnosis		
StrongStep® SARS-CoV-2 IgM Test		Positive	Negative
	Positive	485	0
	Negative	190	78
	Agreement	71.9%（485/675）	100%(78/78)

IgG

	Clinical diagnosis		
StrongStep®SARS-CoV-2 IgG Test		Positive	Negative
	Positive	630	1
	Negative	45	77
	Agreement	93.3%(630/675)	98.7%(77/78)

Fingerstick sample (Whether IgM or IgG positive regard as positive)

	Clinical diagnosis		
StrongStep®SARS-CoV-2 Test		Positive	Negative
	Positive	43	0
	Negative	3	30
	Agreement	93.5%(43/46)	98.7%(77/78)

Total(Whether IgM or IgG positive regard as positive)

	Clinical diagnosis		
StrongStep® SARS-CoV-2 Test		Positive	Negative
	Positive	631	1
	Negative	44	77
	Agreement	93.5%（631/675）	98.7%(77/78)

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

Kappa=0.7413(0.6704～0.8122)
Positive agreement: 93.5% (91.4%～95.2%)
Negative agreement: 98.7% (93.1%～99.9%)
Total agreement: 94.0% (92.1%～95.6%)

2.2 Analysis of Inconsistent Results

In this study, there were 45 specimens with test reagent results and reference reagent results that differed, including 44 false negative and 1 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.

2.3 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 for IgM, IgG and total is 71.9%, 93.3% and 93.5% respectively; the Negative agreement for IgM, IgG and total is 100%, 98.7% and 98.7% respectively; The kappa value of the consistency analysis was 0.7413(0.6704～0.8122).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. Fingerstick sample can also be used in this test.