Clinical Study:
The Diagnostic Kit
for IgG and IgM Antibodies against SARS-CoV-2

1 Clinical trial background

The 2019 novel coronavirus (or "2019-nCov") was detected due to a case of viral pneumonia in Wuhan in 2019. On January 12, 2020 it was named by the World Health Organization. On February 7, 2020, the National Health Commission of the People's Republic of China tentatively named the new coronavirus pneumonia: “Novel Coronavirus Pneumonia" (NCP). On February 18, 2020 the WHO proposed an official name for the new coronavirus disease: “COVID-19”. On February 21, 2020, the National Health Commission revised the English name of "new coronavirus pneumonia" to "COVID-19", which is consistent with the name of the WHO,

The virus causing the disease was named SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV), which is independent of the World Health Organization, and is an international organization that biologically classifies and names viruses and sets standards, having previously named the SARS coronavirus of 2003 and the MERS coronavirus of 2012.

Since December 2019, influenza and related diseases have been continuously monitored in Wuhan, Hubei Province, and a number of cases of viral pneumonia have been detected, all of which have been diagnosed as viral pneumonia/lung infection. The new pneumonia is transmitted from person to person. The National Health Commission has decided to put pneumonia infected with the new coronavirus under the administration of class B of infectious diseases prescribed by law, and to take preventive and control measures for class A infectious diseases.

The SARS-CoV-2 is the seventh type of coronavirus isolated by humans. The virus belongs to the genus β. It has the envelope, and the particles are round or elliptic, often pleomorphic, with a diameter of 60-140nm. Its genetic characteristics are significantly different from those of SARSr-CoV and MERSr-CoV. At present, the homology of Bat sars-like coronavirus (Bat-SL-CoV ZC45) is more than 85%. At present, experts have obtained the whole genome sequence, electron microscope photos of the virus, in vitro isolation culture has been successful. The understanding of the physical and chemical characteristics of coronavirus mainly comes from the study of SARSr-CoV and MERSr-CoV. The virus is sensitive to ultraviolet light; heat-treatment at 56℃ for at least 30 min、ethyl-ether、75% ethanol、chlorine-containing disinfectant、peracetic acid、chloroform and other lipid solvents and can therefore be effectively inactivated by these methods. Chlorhexidine does not inactivate the virus effectively.

The SARS-CoV-2 gene encodes multiple structural proteins, such as the N-protein, E-protein and S-protein, which include multiple antigen epitopes. By using the specific binding between antigen and antibody, the presence of SARS-CoV-2-antigens can be detected by the specific antibody binding with antibodies against SARS-CoV-2-antigens, thus directlydemonstrating the presence of SARS-CoV-2 in the sample.

The SARS-CoV-2 virus particle, as an immunogen, stimulates plasma cells to produce specific antibodies against the SARS-CoV-2-antigens from the virus particle after the virus has infected the human body. Demonstrating the presence of antibodies against SARS-CoV-2-antigens therefore, indirectly prove that the human body has been infected with the SARS- CoV-2. The appropriate sample types for antibody detection reagents are generally blood, including serum, plasma, and whole blood. The detected antibodies are mainly divided into IgM and IgG. At present, there is a lack of systematic research on the production and duration of the synthesis of these two types of antibodies against SARS-CoV-2.

Under normal circumstances, IgM antibody is produced early after infection, quickly produced, the synthesis is only maintained shortly and the disappearance is quick. A positive detection of human IgM against SARS-CoV-2-antigens in the blood can be used as an indicator of an early SARS-CoV-2 infection. IgG antibody production is stimulated later, the maintenance time is long, the disappearance is slow. A positive detection of human IgG in the blood can therefore be used as an indicator of older on-going infection or a previous infection episode.

At the initial stage (the first days) of the infection with SARS-CoV-2, the synthesis of IgM can be rather low, whereas the synthesis of IgM and IgG in the later days of the infection period and the disease will be much higher. It is therefore recommendable also to perform a PCR-test to show nucleic acids from SARS-CoV-2 in this initial phase, despite this type of testing is obviously dependent that you catch a virus particle when you take the swab of the throat – or else you will get a false-negative. But with the combination of the two types of test, there is a very high probability that you will find the infected patients – also in the initial phase. Later, it is only necessary to test for IgM and IgG antibodies against SARS-CoV-2 antigens.

In addition, during the recovery period of infection, IgG continues to rise, and the IgM gradually disappears. Therefore, IgM is less detectable in the recovery period. So, the detection of the combined IgG and IgM antibody response can improve the detectability of different disease stages, and it can also give valuable information about the stage of the infection.

In view of the characteristics and status quo of antigen/antibody detection reagents, their sensitivity and specificity are limited, and cannot be used as the only basis for the diagnosis and exclusion of new pneumonia.

2 Clinical Trial Design

In accordance with “The technical guidelines for clinical trials of *in vitro* diagnostic reagents” and “The key points for technical review of registration of 2019 novel coronavirus antigen/antibody detection reagents (trial)”, the clinical performance of the product was confirmed by a comparative study between the test-kit and available clinical reference-standards. Due to the sudden outbreak of pneumonia caused by SARS-CoV-2 infection, there are few studies on antibody related to pneumonia caused by SARS-CoV-2 infection. Currently, there are only kits and methods available that have been approved in an emergency, and their performance compared with the clinical diagnosis needs to be further observed and studied.

The comparison between the test kit and the clinical diagnostic results was performed to evaluate the clinical performance of the test kit. The enrolled population was the patients suspected with SARS-CoV-2 pneumonia. Clinical samples were obtained in the principle of complete randomization. and the continuous samples were collected from pneumonia patients with SARS-CoV-2 infection at different time. In addition, nucleic acid detection results (by the PCR-method) was compared at the same time to evaluate if there were any differences between the two methods for their the detection ability and if the time-window for two methods for detecting SARS-CoV-2 infections was comparable. These test was performed to prove that the clinical performance of the test-kit meets the requirements of intended use.

3 Clinical Study Results and Analysis

3.1 Sample inclusion

In this study, performed at Jinyintan Hospital in Wuhan, 319 cases were included, of which 160 cases were confirmed by clinical diagnosis and 159 cases were negative. Continuous samples were collected from 20 patients at different time points.

3.2 Statistics of the test kit and clinical diagnostic results

3.2.1 Comparative analysis of the clinical diagnostic results with test kit results.

The detection results of the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens and clinical diagnostic results are shown in the following table:

		clinical diagnosis results		Total
		Confirmed Diagnosis	Negative Diagnosis	
Test kit results	Positive (+)	159	2	161
	Negative (-)	1	157	158
Total Number		160	159	319

Sensitivity: 99.38%;（95%CI：96.55%~99.89%）

Specificity: 98.74%;（95%CI：95.53%~99.65%）

Total clinical coincidence rate: 99.06% (95%CI：97.27%~99.68%）

3.2.2 Comparative analysis of test reagent results with nucleic acid test results.

The detection results of the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens and the nucleic acid test results (PCR-method) are shown in the following table:

		Nucleic Acid Test Results		Total
		Positive (+)	Negative (-)	
Test reagent results	Positive (+)	158	2	160
	Negative (-)	2	157	159
Total Number		160	159	319

Positive coincidence rate: 98.75%;（95%CI：95.56%~99.66%）

Negative coincidence rate: 98.74%;（95%CI：95.53%~99.65%）

Total coincidence rate: 98.75%;（95%CI：96.82%~99.51%）

3.2.3 Statistical comparisons of test results of homologous samples.

3.2.3.1 A total of 20 samples of serum and plasma samples from the same patient were tested with the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens, and the results of these two kinds of sample were consistent. The data is shown in the following table:

		Serum		Total
		Positive (+)	Negative (-)	
Plasma	Positive (+)	11	0	11
	Negative (-)	0	9	9
Total Number		11	9	20

Positive coincidence rate: 100%; Negative coincidence rate: 100%; Total coincidence rate: 100%

3.2.3.2 A total of 20 samples of serum and whole blood samples from the same patient were tested with the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens, and the results of these two kinds of sample were consistent. The data is shown in the following table:

		Serum		Total
		Positive (+)	Negative (-)	
Whole blood	Positive (+)	11	0	11
	Negative (-)	0	9	9
Total Number		11	9	20

Positive coincidence rate: 100%; Negative coincidence rate: 100%; Total coincidence rate: 100%;

3.2.3.3 A total of 20 samples of plasma and whole blood samples from the same patient were tested with the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens, and the results of these two kinds of sample were consistent. The data is shown in the following table:

		Plasma		Total
		Positive (+)	Negative (-)	
Whole blood	Positive (+)	11	0	11
	Negative (-)	0	9	9
Total Number		11	9	20

Positive coincidence rate: 100%; Negative coincidence rate: 100%; Total coincidence rate: 100%

4 Conclusion

The test reagent for this clinical study was investigated by the manufacturer. This clinical study was designed as a blinded clinical trial.

A total of 339 samples were tested and 339 cases were included for statistical analysis. There were 171 patients with confirmed positive Covid-19 diagnose and 168 patients with confirmed negative Covid-19 diagnose. The results from both the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens and nucleic acid detection were statistically analyzed. The above results show that there is no statistically significant difference between the test kit detecting both IgG and IgM antibodies against SARS-CoV-2-antigens and the clinical diagnostic results.