Clinical Trial

Edinburgh Genetics COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined

Trial start date: February 25, 2020
Completion Date: March 20, 2020
Clinical trial location: Hubei Provincial Centre for Disease Control and Prevention

Research Summary

This clinical trial is to investigate and evaluate the performance indicators and clinical application of the COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined. The clinical trial was performed at the Hubei Provincial Centre for Disease Control and Prevention from February 2020 to March 2020 on 127 positive samples from patients who had been clinically diagnosed for at least one week and 145 clinically negative samples.

The 2019-nCoV IgM/IgG antibody detection kit (colloidal gold) produced by Innovita Biological Technology Co. Ltd was used as a control reagent for comparison tests.

The COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined, marketed by Edinburgh Genetics Limited had a positive coincidence rate of 100%, a negative coincidence rate of 98.65%, a positive predictive value of 98.41%, a negative predictive value of 100%, a total coincidence rate of 99.26%, a paired chi-square test P-value ≥0.05 and a Kappa value = 0.9852. The statistical results show that the two reagents are consistent, that is, the test reagent is equivalent to the reagents of the same species on the market. The results of simultaneous detection of homologous serum and plasma, whole blood, and peripheral blood samples using the test reagents show that the test reagent has a positive coincidence rate of 100%, a negative coincidence rate of 100%, a total coincidence rate of 100%, a paired chi-square test P-value ≥0.05 and. Kappa consistency test Kappa value = 1.0, indicating that the results of homologous serum and plasma, whole blood, peripheral blood test are consistent.

Among the 127 clinically confirmed samples, 125 were detected by the test reagents, with a positive detection rate of 98.43% (125/127). 123 cases were detected by the control reagent, with a positive detection rate of 96.85% (123/127). Of the 145 clinically negative samples, 144 were detected by the test reagent with a negative coincidence rate of 99.31% (144/145), while 144 were detected by the control reagent with a positive detection rate of 99.31% (144/145).

Table 1: Clinical trial researchers

Researchers	
Cai Yi, Lead Researcher	Hubei Provincial Centre for Disease Control and Prevention
Cai Yi	Hubei Provincial Centre for Disease Control and Prevention
Zeng Xuehui	Hubei Provincial Centre for Disease Control and Prevention
Mo Qinon	Hubei Provincial Centre for Disease Control and Prevention
Li Lan	Hubei Provincial Centre for Disease Control and Prevention
Huang Zhiyin	

1 Introduction

1.1 Source, biological and physicochemical properties of the new coronavirus

The 2019 new coronavirus (COVID-19) was named on January 12, 2020 by the World Health Organization. Coronaviruses are a large family of viruses that are known to cause colds and more serious diseases such as the Middle East Respiratory Syndrome (MERS) and Severe Acute Respiratory Syndrome (SARS). The new coronavirus is a new coronavirus strain that has never been found in humans before.

Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath, and dyspnea. In more severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. There is currently no specific treatment for diseases caused by the new coronavirus. However, many symptoms can be managed, so they need to be treated according to the clinical situation of the patient. Also, assistive care for infected people can be very effective.

The new Coronavirus (COVID-19) antibody detection kit is used for the rapid and qualitative detection of new coronavirus IgM and IgG antibodies in human serum, plasma, whole blood and peripheral blood. The test results can be observed by naked eyes within 10 minutes, shortening the waiting time for patients.

1.2 Intended use of the product

This kit is used for the qualitative detection of new coronavirus COVID-19 IgM and IgG antibodies in human serum, plasma, whole blood or peripheral blood samples.

1.3 Product Methodology and Principles

The COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined uses a specific antigen-antibody reaction and colloidal gold immunochromatographic technology to detect human serum, plasma, whole blood, peripheral blood samples which include the novel coronavirus IgM and IgG antibodies. When an appropriate amount of test sample is added to the sample well of the reagent card, the sample will move forward along the test card. If the sample contains a novel coronavirus IgM antibody, the antibody binds to the colloidal gold-labelled new coronavirus antigen and the immunity. It also binds to the M-coated anti-human IgM antibody and a change in colour suggests the new coronavirus IgM antibody is positive.

If the sample contains a new coronavirus IgG antibody, the antibody binds to the colloidal gold-labelled new coronavirus antigen, and the immune complex further binds and develops colour on the G-line with the coated anti-human IgG antibody, suggesting a new coronavirus IgG Antibodies are positive.

If neither the M line nor the G line is coloured, a negative result is indicated. The test card also contains a quality control line (C line). Regardless of the results of the M and G lines, the C line must be coloured. If the C line does not appear, the test result is invalid and the sample needs to be retested.

IgM and IgG antibodies are produced at different times after infection. Timing and amount of antibody produced will vary by patient and by case; however a period of at least five days between infection and testing is recommended in order for the antibodies to develop. The test should not be used in isolation for clinical diagnosis.

2 Purpose of this clinical research

The COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined, marketed by Edinburgh Genetics Limited was compared with an equivalent product to show that the test reagents are of equal standards. The results were also compared to the clinically diagnosed statistics in order to estimate the sensitivity and specificity of the testing kit.

3 Test management

The party undertaking the clinical trial was the Hubei Provincial Centre for Disease Control and Prevention. The Hubei Provincial Centre for Disease Control and Prevention is a medical institution with clinical verification qualifications and has experience in clinical research of medical devices and in vitro diagnostic reagents.

3.1 Lead researchers

Cai Yi

3.2 Researchers
Mo Qinong, Zeng Xuehui, Li Lan

3.3 Statistics and data management
The test results were recorded and photographed by the researchers. After the data was un-blinded, the statistical results were processed. The test record form, photos of the test results, and clinical trial reports were prepared in duplicate and kept separately by the clinical trial unit and the reporting unit.

3.4 Problems in clinical research and countermeasures
No incident occurred during the clinical trial.

4 Experiment design

4.1 Description of overall test design and scheme
Control reagents and assessment reagents were used to conduct comparative tests on clinical samples simultaneously. This test uses a paired design of the synchronous blind method. After a random compilation of the collected samples, each sample is tested separately in the control reagent and the assessment reagent. Statistical analysis was performed to calculate the positive coincidence rate, negative coincidence rate, positive predictive value, negative predictive value, overall coincidence rate, paired chi-square test, and Kappa consistency test to determine the equivalence of the test reagent and the control reagent.

4.2.1 Sample size and the basis for determining sample size
Clinical tests were performed at the Hubei Provincial Centre for Disease Control and Prevention. There were 127 patients with clinically confirmed new coronavirus pneumonia and 145 negative samples, a total of 272 samples. The 127 patients had been clinically diagnosed for at least one week prior to testing. Serum, plasma, whole blood, and peripheral blood samples were collected each from 50 patients and used to evaluate whether the test results were different when testing different matrix samples.

4.2.2 Sample selection basis, selection criteria, exclusion criteria
(1) Sample source

- Clinical samples were collected at the Hubei Provincial Centre for Disease Control and Prevention.

(2) Selection criteria

- Basic medical records of the patient;
- The control test sample type is serum; the homology test sample type is plasma, whole blood and peripheral blood;
- The sample must be fresh and free of clots and air bubbles;

- The amount of the test substance contained in the sample should be as dispersed as possible, and clinical samples with normal and abnormal values of the test substance should be included;
- Positive samples were taken from confirmed COVID-19 cases at least ten days after infection.

(3) Exclusion criteria

- The sample has a clot and the sample has air bubbles;
- The sample is not fresh;
- Samples are not kept as required;

(4) Elimination criteria

1) Specimens that could not complete the entire test process due to human factors;
2) Severe hemolysis, hyperlipidemia, erosive blood and other abnormal blood samples.

4.2.3 Storing samples

All samples should be tested immediately after collection. If not possible, serum, plasma samples can be stored for 3 days at 2-8 °C, and 1 month at -20 °C after collection. The samples taken from the refrigerator must be returned to room temperature before testing and without repeated freeze-thaw. After the collection of whole blood samples and peripheral blood samples, they can be stored at 2-8 °C for 1 day, and should not be frozen.

4.2.4 Determination of contrast reagents

As a similar product which has been put on the market, the control reagent for this clinical trial was the new coronavirus (2019-nCoV) IgM/IgG antibody detection kit (colloidal gold method) produced by Innovita Biological Technology Co. Ltd.

4.2.5 Test design

This test was designed using the synchronous blind method. After the samples were entered into the group, the samples were randomly set aside. Each sample was divided into two. The samples were “blinded” according to the random coding table. The “blinded” samples were compiled. The test personnel of the assessment group and the control group were respectively issued testing instructions to obtain the test results. The statistical person in charge then compared and analysed the test results to determine the equivalence and consistency of the test reagent and the control reagent.

4.2.6 Quality control methods

Each test card has a quality control line (C line) on the NC membrane. The C line must be coloured during clinical testing. If the C line develops colour, the test is valid. If the C line does not develop colour, the test is invalid.

4.2.7 Sample selection

A total of 272 serum samples were collected, of which 127 were clinically diagnosed with new coronavirus pneumonia (65 male samples and 62 female samples), 145 negative samples (70 male samples and 75 female samples). A total of 50 homologous samples were

collected, including 30 positive samples (16 male samples and 14 female samples) and 20 negative samples (10 male samples and 10 female samples).

Positive samples were from patients known to have shown symptoms of the disease for at least ten days.

4.2.9 Statistical Analysis Methods and Data Judgement

4.2.9.1 Positive coincidence rate, negative coincidence rate, positive predictive value, negative predictive value, and total coincidence rate are calculated using the following statistical tables and methods:

		Control testing kit		Total
		Positive	Negative	
Target testing kit	Positive	a	b	
	Negative	c	d	
Total				

Positive coincidence rate = a / (a + c) * 100%
Negative coincidence rate = d / (b + d) * 100%
Positive predictive value = a / (a + b) * 100%
Negative predictive value = d / (c + d) * 100%
Total coincidence rate = (a + d) / (a + b + c + d) * 100%

The test results of two reagents and two types of samples are required to have a positive coincidence rate, a negative coincidence rate, a positive predictive value, a negative predictive value, and a total coincidence rate of not less than 95%.

4.2.9.2 Paired Chi-Square Test
The paired Chi-square test was performed on the test results of the two reagents and two types of samples. If the P-value is ≥0.05, there is no significant difference in the test results of the two reagents and two types of samples.

4.2.9.3 Kappa
The Kappa test is performed on the detection results of two reagents and two types of samples. If the Kappa value is ≥0.8, the detection results of two reagents and two types of samples are considered to have a good consistency.

4.2.9.4 Consistency evaluation of homologous serum, plasma, whole blood and peripheral blood samples
The serum, plasma, whole blood, and peripheral blood samples from the same tester were tested simultaneously with the test reagents. The serum test results were matched with the plasma test results, whole blood test results, and peripheral blood test results to match the

positive coincidence rate and negative coincidence rate. Overall coincidence rate, paired Chi-square test, Kappa test, are used to determine the consistency of the reagents when testing different matrix samples and to determine whether there are significant differences.

4.2.10 Modification of the plan during the study
None.

5 Test process

Before performing the test, the operator collects the blinded samples from the sample manager. If the samples are taken from the refrigerator, the samples are returned to room temperature before testing. Two operators use the test reagents and control reagents to test the clinical samples and record the blind code and the corresponding test results. After the test, treat the used reagent cards as medical waste.

6 Clinical research results and analysis

6.1 Statistical analysis of the test results of the control reagents and assessment reagents

6.1.1 Positive coincidence rate, negative coincidence rate, positive predictive value, negative predictive value, and total coincidence rate, as shown in Table 3:

		Control testing kit		Total
		Positive	Negative	
Target testing kit	Positive	124	2	126
	Negative	0	146	146
Total		124	148	272

Positive coincidence rate = 124 / 124 *100% = 100%
Negative coincidence rate = 146 / 148 * 100% = 98.65%
Positive predictive value = 124 / 126 * 100% = 98.41%
Negative predictive value = 146 / 146 * 100% = 100%
Total coincidence rate = 270/272 * 100% = 99.26%

The negative predictive value and the total coincidence rate were 100%, 98.65%, 98.41%, 100%, and 99.26% - all higher than 95%.

Among the 127 clinically confirmed samples of new coronavirus patients, 125 were detected by the test reagents, with a positive detection rate (sensitivity) of 98.43% (125/127), and 123 cases were detected by the control reagent, with a positive detection rate of 96.85% (123/127). Of the 145 clinically negative samples, 144 were detected by the test reagent,

and the negative coincidence rate (specificity) was 99.31% (144/145), while 144 were detected by the control reagent, and the positive detection rate was 99.31% (144/145).

The test reagents detected two more positive samples than the control reagents. Both samples were clinically confirmed patients with new coronavirus pneumonia and IgM single positive, indicating that in early patients with new coronavirus pneumonia, the test reagents were better.

6.1.2 Paired Chi-square test with test results of test reagents and control reagents
The results show that the test results of the test kit test reagents were paired with a chi-square test with a P-value ≥ 0.05. The overall coincidence rate of the test kit control reagents was the same, and there was no significant difference in test results.

6.1.3 Kappa consistency check
The results show that the Kappa consistency test of the test kit control reagent test result yields Kappa value = 0.9852, indicating that the test kit control reagent test results have a good consistency.

7 Conclusion

The COVID-19 Colloidal Gold Immunoassay Testing Kit, IgG/IgM Combined and the reference have a positive coincidence rate of 100%, the negative coincidence rate of 98.65%, the positive predictive value of 98.41%, negative predictive value of 100%, the total coincidence rate of 99.26%, paired chi-square test P-value ≥0.05, Kappa consistency test Kappa value = 0.9852. The statistical results show that the two reagents are consistent - that is, the test reagent is equivalent to similar reagents on the market.

The results of simultaneous detection of homologous serum and plasma, whole blood, and peripheral blood samples using the test reagents show that the test reagent tests serum, plasma, whole blood, and peripheral blood samples with a positive coincidence rate of 100%, a negative coincidence rate of 100%, and a total coincidence rate 100%, paired chi-square test P-value ≥0.05, Kappa consistency test Kappa value = 1.0, indicating that the results of homologous serum and plasma, whole blood, peripheral blood test are consistent.

Among the 127 clinically confirmed samples of new coronavirus patients, 125 were detected by the test reagents, with a positive detection rate of 98.43% (125/127). 123 cases were detected by the control reagent, with a positive detection rate of 96.85% (123/127). Of the 145 clinically negative samples, 144 were detected by the test reagent, and the negative coincidence rate was 99.31% (144/145), while 144 were detected by the control reagent, and the positive detection rate was 99.31% (144/145).

8 References

[1] "Provisions for Clinical Trials of Medical Devices"
[2] Technical Guiding Principles for Clinical Trials of In Vitro Diagnostic Reagents
[3] "Medical Statistics"