Clinical trial report

Product Name： COVID-19 IgM/IgG Test Kit (Colloidal Gold)

Clinical trial facility (seal): Wuhan Third Hospital

Main researcher (Print and sign)： Jianbin Sun

Head of statistics (Print and sign)： Liang Song

Clinical trial time： In March 2020

Product registration applicant (seal): Xiamen AmonMed Biotechnology Co., Ltd.

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Where the original materials are kept： Wuhan Third Hospital

Report date： March 16, 2020

Explanation

1. Medical institutions in charge of clinical trials should conduct clinical trials in a fair and objective manner in accordance with clinical trial protocols in a serious and responsible manner, and write clinical trial reports.

2. This trial must be conducted by a clinical trial institution with an experienced attending physician or more, or a person with intermediate job title or above who has the relevant work experience.

3. This clinical trial category is clinical validation, and this report should be written with reference to the "Technical Guidelines for Clinical Trials of In Vitro Diagnostic Reagents".

Content

Research Summary

According to the requirements of the "technical guiding principles for clinical tests of in vitro diagnostic reagents" (Announcement No. 16 of 2014), commissioned by Xiamen AmonMed Biotechnology Co., Ltd. ("AmonMed Biotechnology"), Wuhan third hospital conducted a clinical trial on its new coronavirus IgM/IgG antibody assay kit (Colloidal Gold) (abbreviation: assessment reagent) in March 2020. The clinical trial was conducted in a synchronous blind comparative study with the diagnostic criteria (referred to as the control group) in the diagnosis and treatment program for novel coronavirus pneumonia (Trial Version 7) issued by the general office of the national health commission, to investigate the equivalence between the clinical application performance of the test reagent and the diagnostic criteria.

The samples of this clinical trial were plasma samples of 227 subjects. The test results are summarized as follows:

Results of assessment reagents and control group: There were 102 positive samples: (IgM + IgG) positive 95 cases, 80 IgM positive samples, 86 IgG positive samples; 29 early positive samples: (IgM + IgG) positive for 25 cases, 25 IgM positive samples, 18 IgG positive samples; There were 73 cases of intermediate and advanced positive samples: (IgM + IgG) positive in 70 cases, IgM positive in 55 cases, and 68 IgG positive samples. There were 125 negative samples: 123 negative IgM samples and 124 IgG negative samples.

The examination results of 227 clinical stool samples showed that the total (IgM + IgG) sensitivity of the test reagent and the control group was 93.13%, the total IgM sensitivity was 78.43%, and the total IgG sensitivity was 84.31%. (IgM + IgG) Kappa consistency analysis k = 0.951, IgM Kappa consistency analysis k = 0.873, IgG Kappa consistency analysis k = 0.909, and the results of the test reagents and control group were highly consistent.

Conclusion: The diagnostic results of the COVID-19 IgM/IgG Test Kit (Colloidal Gold) from Xiamen AmonMed Biotechnology Co., Ltd. are highly consistent with the diagnostic criteria in the diagnosis and treatment program for novel coronavirus pneumonia (Trial Version 7) issued by the general office of the national health and health commission, that is, it is equivalent to the clinical diagnostic criteria.

Principal researchers of this test

Clinical tester	Position / Title	Responsible for testing content	Current unit
Jianbin Sun	Chief technician	Principal researcher, responsible for the organization of clinical tests, review of clinical test protocols and reports.	Wuhan Third Hospital
Li Xu	Chief technician	Researcher	Wuhan Third Hospital
Wei Wang	Chief technician	Researcher	Wuhan Third Hospital
Youtao Hu	Chief technician	Researcher	Wuhan Third Hospital
Lingling Ke	Chief technician	Researcher	Wuhan Third Hospital
Zhixiang Zhang	Technician	Instrument reagent manager	Wuhan Third Hospital
Xiaoqing Gao	Technician	Quality controller	Wuhan Third Hospital
Hanhong Tang	Deputy chief technician	Data manager	Wuhan Third Hospital
Liang Song	Assistant researcher	Person in charge of statistics	Department of Translation Medicine, Haixi Institute of Chinese Academy of Sciences

Abbreviations

Xiamen AmonMed Biotechnology Co., Ltd.	AmonMed Biotechnology
COVID-19 IgM/IgG Test Kit (Colloidal Gold)	Assessment reagent
Judgment according to the diagnostic criteria in the "Pneumonitis Diagnosis and Treatment Program for the Printing and Distribution of New Coronavirus Infection (Trial Version 7)" issued by the General Office of the National Health Commission	Control group

Fundamental contents

1. Introduction

1.1 The source, biology and physicochemical properties of the measured object

The COVID-19 IgM/IgG antibody is the first antibody that appears in the human immune system during the infection of the virus. It appears earlier after infection and peaks during the acute or recovery phase.

1.2 Purpose of clinical use and current diagnostic methods for this indication

The clinical population is generally susceptible. The elderly and those with underlying diseases are more ill after infection, and children and infants also affected. Laboratory diagnostic methods of novel coronavirus include virus isolation and culture, serological diagnosis and direct antigen detection. At present, the patients diagnosed as suspected cases are mainly confirmed by nucleic acid test of novel coronavirus or gene sequencing of novel coronavirus.

1.3 The methods, principles, and technical requirements used

Detection principle: The COVID-19 IgG/IgM test kit (Whole Blood/Serum/Plasma) is a lateral flow immunochromatographic assay. The burgundy colored conjugate pad contains colloidal gold conjugated to recombinant COVID-19 antigens conjugated with colloid gold (COVID-19 conjugates) and rabbit IgG-gold conjugates. When a specimen followed by sample diluent is added to the sample well, IgM and/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 and/or anti-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 test contains an internal control (C band) which should exhibit a burgundy colored band of the immunocomplex goat anti-rabbit IgG/rabbit IgG-gold conjugate regardless of the color development on any of the test bands. Otherwise, the test result is invalid and the specimen must be retested with another device.

Detection method: Please read the instruction manual carefully before testing, the samples to be tested, testing reagents and other testing materials are equilibrated to room temperature, and testing should be performed at room temperature. Refer to the kit instructions for the operating steps and test results.

Technical requirements:

(1) The test should be performed as soon as possible after the specimen is collected. If the test cannot be performed in time, the specimen can be stored at 2-8 ° C for no more than 72 hours, and it should be restored to room temperature before testing.

(2) Test the same patient sample in parallel and as far as possible to avoid false positives and false negatives caused by other reasons.

(3) If the reagent is stored at low temperature, it is necessary to return to room temperature before opening the package to avoid moisture absorption.

(4) Avoid exposing the reagent to sunlight and humidity. Store in a dry and cool place at 2-30 ° C.

1.4 Domestic approved products

At present, there are novel coronavirus (2019-ncov) IgM antibody test kit (Colloidal Gold) (Guangdong Hexin Health Technology Co., Ltd. National Machinery Note 20203400199), novel coronavirus (2019-ncov) antibody test kit (Colloidal Gold) (Innotek (Tangshan) Biotechnology Co., Ltd. National Machinery Standard 20203400177), novel coronavirus (2019-ncov) antibody test kit (Colloidal Gold) (Guangzhou Wanfu Biotechnology Co., Ltd. National Machinery Standard 20203400176) and novel coronavirus (2019-ncov) IgM/IgG antibody test kit (Colloidal Gold) (Zhuhai Lizhu Reagent Co., Ltd. National Machinery Note 20203400240), etc. for detection of novel coronavirus (2019-ncov) in China which have been approved to market by the state food and drug administration (CFDA).

2. Research purposes

The evaluation reagent "novel coronavirus IgM/IgG antibody test kit (Colloidal Gold)" was compared with the current clinical diagnostic standard (control group), the coincidence rate of the two test results was calculated, and the effectiveness between the evaluation reagent and the control group was verified. Discover or predict problems that may be encountered in practical applications through clinical trials, and remind users in the instructions.

3. Test management

One person from Xiamen AmonMed Biotechnology Co., Ltd. and the clinical trial unit will be responsible for the management and communication of the experiment. Xiamen AmonMed Biotechnology Co., Ltd. is responsible for providing clinical trial products and assigning clinical supervisors, while the clinical trial unit is responsible for the implementation and data management of the trial.

Before the start of the trial, Xiamen AmonMed Biotechnology Co., Ltd. appointed clinical monitors and clinical trial leaders to organize clinical trial participants to convene a clinical trial initiation meeting, carefully familiarize themselves with the trial protocol, and uniformly record methods and judgment standards. During the test, the inspectors regularly conduct on-site inspection visits to the clinical verification participating units to ensure that all contents of the test protocol are strictly adhered to and the research materials are filled in correctly. The professional departments participating in clinical trials shall establish standard operating procedures and quality control procedures for experimental observation indicators, and conduct clinical trials in strict accordance with relevant operating procedures. The main investigator supervises and checks the entire research process of the trial. The test operator shall truthfully, detailedly and carefully record all contents according to the requirements for filling in the original records of the clinical trial, including the test name, test purpose, date, test results, signatures of relevant personnel, etc. to ensure that the records are true, complete and traceable. All observations and findings in clinical trials should be verified to ensure reliable data. In order to ensure that the conclusions in the clinical trial are derived from the original data, there are corresponding data management measures in the clinical trial and data processing stages, and each test item must adopt the national legal unit of measurement.

After the clinical study is completed, the above management files are organized and archived.

4. Test design

4.1 Description of overall test design and protocol

After the sequence of the selected samples is encoded, the test results of the test reagents Xiamen AmonMed Biotechnology Co., Ltd.'s COVID-19 IgM/IgG Test Kit (Colloidal Gold)] used by the test operators are compared with the diagnostic results of the diagnostic criteria in the diagnosis and treatment program for novel coronavirus pneumonia (Trial Version 7) issued by the general office of the national health and health commission, while the information of the subjects providing the samples remains blind. According to the comparison between the test results of the company's kit and the diagnostic standard, the positive coincidence rate and negative coincidence rate of the experimental group and the control group were counted. By calculating and comparing the performance of coincidence rate of the company's products with that of the control group, the conclusions of sensitivity and specificity are drawn.

The test results of the test reagent are interpreted according to the requirements of the

instruction manual. If the results are consistent, the test results are recorded. When the results of the two tests are inconsistent, the test operator shall make a full analysis by combining the epidemiological background, clinical symptoms, disease outcome and other information of the patients. After the results were confirmed, the blindness was exposed and the clinical application performance of the AmonMed's kit was evaluated. The equivalence between the reagent and the clinical diagnostic criteria was investigated.

4.1.1 Comparative test of serum, plasma and whole blood

The same subject collects serum, plasma, and whole blood at the same time. Under the condition that the subject information provided by the sample was kept in a blind state, the test operator used the evaluation reagent to conduct tests according to the instructions. The test results of serum, plasma and whole blood from the same subject were compared with those of the control group.

4.2 Test design and test method selection

4.2.1 Sample size and basis for determining sample size

The assessment reagent is the second type of in vitro diagnostic reagent for protein detection. According to the guiding principle of clinical trials in vitro diagnostic reagent technology, clinical studies of the total sample at least 500 and no less than 3 (including 3) tclinical test units to carry out clinical trials. For products with special purposes, clinical trials can be carried out in the centers for disease control and prevention, specialized hospitals, inspection and quarantine centers and drug rehabilitation centers and other institutions that meet the requirements.

With reference to the "technical guidelines for clinical trials of in vitro diagnostic reagents", according to the purpose of clinical research, the number and characteristics of patients in clinical trial units, and statistical requirements, the number of samples to be undertaken by Wuhan Third People's Hospital for this clinical trial shall be not less than 200 cases (IgM-positive and IgG-positive were not less than 80 cases).

4.2.2 Sample selection basis and inclusion criteria

Inclusion criteria:

(1) The sample size is sufficient (greater than 0.5ml), and the age is not limited;

(2) It includes confirmed cases of pneumonia with novel coronavirus infection (including some convalescent cases), suspected cases, excluded cases, etc. The confirmed cases should include patients with different disease processes (such as early onset, middle

stage, late treatment/convalescent patients). In addition, continuous samples collected at different times of some patients with pneumonitis infected by the new coronavirus should be included, including samples of various stages of the new coronavirus nucleic acid and antigen / antibody from negative to positive in this case.

Exclusion criteria:

(1) The time or information for sample collection is unclear;

(2) Insufficient samples due to mistakes in the test operation;

(3) Before the test operation, it was found that the specimens were contaminated during the preservation process, such as fibrin residues, precipitated particles, hyphae, and mycelia.

Rejection criteria:

(1) Anyone who lacks the information required for the original records of any clinical study before the statistics;

(2) The test result is judged as "invalid result" (see the definition of invalid result in the product instruction manual);

(3) Samples collected by the same patient on the same day or on different days can only be enrolled once per patient.

4.2.3 Sample collection, storage, and transportation methods

Whole blood, serum, and plasma samples can be used for testing.

Whole blood samples: According to the "venous blood collection method" of the "National Clinical Test Operating Procedures", use a disposable vacuum blood collection tube containing EDTA anticoagulant to collect venous blood, shake well and set aside, and use it as soon as possible after collection. If it cannot be detected in time after blood collection, it should be stored at 2 ~ 8 ℃, not more than 5 days.

Serum and plasma samples: refer to the "National Clinical Test Operating Procedures" venous blood collection method, use a disposable vacuum blood collection tube without anticoagulant to collect venous blood, and isolate and obtain serum. If the test cannot be performed in a timely manner, the serum samples can be stored in a refrigerator at 2 ~ 8 ° C, and should be stored frozen at -20 ° C for more than 7 days. It can be stored at -20 ° C for 6 months. Please return to room temperature before testing to avoid repeated freeze-thaw.

4.2.4 Details of products for clinical research

(1) Assessment reagents: COVID-19 IgM/IgG Test Kit (Colloidal Gold)

Manufacturer: Xiamen AmonMed Biotechnology Co., Ltd.

Specifications: 25 tests/kit

Lot number: 3120200301

Production Date: 2020.03.01

Storage conditions and validity: Store at 2 ~ 30 ℃, the validity of the product is 18 months.

4.2.5 Quality control methods

In the same laboratory, uniform technical methods are used, and the same batch of test personnel strictly evaluates the reagent operation requirements.

4.2.6 Statistical analysis of clinical trial data

(1) Calculate the negative-positive coincidence rate and total coincidence rate of the assessment reagents. The specific method is as follows: (take Table 1 as an example)

Table 1. Test results of ____ samples

Assessment reagent	Control reagent		Total
	Positive（+）	Negative（-）	
Positive（+）	a	b	a+b
Negative（-）	c	d	c+d
Total	a+c	b+d	a+b+c+d

Positive coincidence rate =a/(a+c)×100%

Negative coincidence rate =d/(b+d) ×100%

Total consistent rate =（a+d）/(a+b+c+d)×100%

(2) Use SPSS software or use the following formula to perform kappa consistency analysis (report the specific value of Kappa value) on the four grid tables in Table 1 to examine the consistency of the test results of the test reagents and control reagents. Kappa value (k) judgment: k> 0.75, good consistency; 0.40≤k≤0.75, good consistency; k <0.40, poor consistency.

5．Clinical research results and analysis

5.1 Comparison of test results between test reagents and control group

Table 2. (IgM + IgG) test results of 227 positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	

Positive (+)	95	2	97
Negative (-)	7	123	130
Total	102	125	227

Table 3. IgM test results of 227 positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	80	2	82
Negative (-)	22	123	145
Total	102	125	227

Table 4. IgG test results of 227 positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	86	1	87
Negative (-)	16	124	140
Total	102	125	227

Table 5. (IgM + IgG) test results of 29 early positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	25	0	25
Negative (-)	4	0	4
Total	29	0	29

Table 6. (IgM + IgG) test results in 73 cases of intermediate and advanced positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	70	0	70
Negative (-)	3	0	3
Total	73	0	73

Table 7. IgM test results of 29 early positive samples

Assessment	Control group	Total

reagent	Positive (+)	Negative (-)	
Positive (+)	25	0	25
Negative (-)	4	0	4
Total	29	0	29

Table 8. IgM test results of 73 middle and late positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	54	0	54
Negative (-)	19	0	19
Total	73	0	73

Table 9. IgG test results of 29 early positive samples

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	18	0	18
Negative (-)	11	0	11
Total	29	0	29

Table 10. IgG test results in 73 positive patients

Assessment reagent	Control group		Total
	Positive (+)	Negative (-)	
Positive (+)	68	0	68
Negative (-)	5	0	5
Total	73	0	73

Evaluation reagent and control group results:

There were 102 positive samples: (IgM + IgG) positive 95 cases, 80 IgM positive samples, 86 IgG positive samples. There were 29 early positive samples: (IgM + IgG) positive for 25 cases, 25 IgM positive samples, 18 IgG positive samples, and 73 intermediate and advanced positive samples: (IgM + IgG) positive for 70 cases and IgM positive for 55 cases. , 68 cases of IgG positive samples.

There were 125 negative samples: 123 negative IgM samples and 124 IgG negative samples.

The comparative analysis of the test results of the test reagents and the control group is as follows:

(1) Sensitivity:

Total (IgM + IgG) sensitivity: 95/102 × 100% = 93.13%

Early (IgM + IgG) sensitivity: 25/29 × 100% = 86.21%

Mid-late stage (IgM + IgG) sensitivity: 70/73 × 100% = 95.89%

Total IgM sensitivity: 80/102 × 100% = 78.43%

Early IgM sensitivity: 25/29 × 100% = 86.21%

Mid-late IgM sensitivity: 55/73 × 100% = 75.34%

Total IgG sensitivity: 86/102 × 100% = 84.31%

Early IgG sensitivity: 18/29 × 100% = 62.07%

Middle-late IgG sensitivity: 68/73 × 100% = 93.15%

(2) Specificity:

IgM specificity: 123/125 × 100% = 98.40%

IgG specificity: 124/125 × 100% = 99.20%

(3) Kappa consistency analysis

(IgM + IgG) Kappa consistency analysis:

k = 0.951,

It can be considered that the test results of the test reagent and the control group are consistent (k = 0.951> 0.75).

Kappa consistency analysis of IgM:

k = 0.873,

It can be considered that the Hb test results of the test reagent and the control group are consistent (k = 0.873> 0.75).

Kappa Consistency Analysis of IgG:

k = 0.909.

It can be considered that the Tf test results of the test reagent and the control group are consistent (k = 0.909> 0.75).

6. Discussion and conclusion

A total of 227 subject samples were tested in this clinical trial. The test process was strictly performed in accordance with the clinical trial protocol. The test results are summarized as

follows:

1．The examination results of 227 clinical stool samples showed that the total reagent (IgM + IgG) sensitivity was 93.13%, the total IgM sensitivity was 78.43%, the total IgG sensitivity was 84.31%, and the Kappa consistency analysis of (IgM + IgG) was k = 0.951 Kappa consistency analysis of IgM k = 0.873, Kappa consistency analysis of IgG k = 0.909, and the results of the test reagent and control group were highly consistent.

Conclusion: the measurement results of the COVID-19 IgM/IgG Test Kit (Colloidal Gold) of AmonMed Biotechnology Co., Ltd. and the results of the "Pneumonia Diagnosis and Treatment Scheme for Printing and Distribution of New Coronavirus Infection (Trial Version 7)" issued by the General Office of the National Health and Health Commission. The diagnosis results of the confirmed diagnosis standards are highly consistent, that is, they are equivalent to the clinical confirmed diagnosis standards.