Clinical research report

1. **Name and Lot No. of the kit**

Name: Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold)

Lot No.: Strip ： 20200217

Cassette ： 20200217

2. **Manufacturer**

Name: Nantong EGENS Biotechnology Co.,Ltd

Address: Building 15，Building 12(west) , No.1692 Xinghu Avenue Nantong Economy & Technology Development Zone Jiangsu Province China

Summary of Research

Entrusted by Nantong Egens Biotechnology Co., Ltd. (hereinafter referred to as “Egens”), Nantong designated admission hospital implemented clinical test on Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) researched and produced by Nantong Egens Biotechnology Co., Ltd. according to *Guiding Principle for Clinical Research Technology of In Vitro Diagnostics Reagent* (CFDA MD(2014) 16).

The kit to be evaluated uses immunochromatography and the principle of Capture ELISA to qualitatively detect 2019-nCoV IgM/IgG Antibodies in human serum (or plasma or whole blood) for clinical auxiliary diagnosis. The test kit is produced by Nantong Egens Biotechnology Co., Ltd.

Lot No. 20200217, Specification: 50t/box, Type: Strip

Lot No. 20200217, Specification: 20t/box, Type: Cassette

Shelf life: 12 months

Strip Type

In this clinical study, a total of 100 samples were tested, including 32 confirmed samples of novel coronavirus and 68 negative samples. Result: among the 32 positive samples, 1 case was inconsistent according to the comparison of test kit results, while the results of 68 negative cases were all in conformance according to the comparison of test kit results. Through statistical analysis, the sensitivity of the assessed kits is 96.88%, the specificity is 100%, the false positive rate is 0%, the false negative rate is 3.12%, and the total conformity rate is 99%.

Cassette Type

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The results of clinical study show that this kit is reliable, accurate, convenient, and has high clinical application value.

Introduction

1. Source, biological and physicochemical properties of analyte

The 2019 novel coronavirus, known as the "2019-nCoV", was found due to viral pneumonia cases in Wuhan in 2019 and was named by the world health organization on January 12, 2020. Coronaviruses are a large family of viruses known to cause colds and more serious illnesses such as Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS). The novel coronavirus is a new strain of coronavirus that has never been found in humans before. China has reported tens of thousands of laboratory-confirmed cases, and the number is rising daily. Most reports have come from Hubei and surrounding provinces, while many cases have also been reported from other provinces and municipalities. Sporadic cases are also being reported in other countries, including Asian and European countries, Australia, the United States (Washington, Illinois, California, Arizona and Massachusetts) and Canada. Human-to-human transmission of 2019-ncov has been confirmed in China and has been found in other countries, including the United States. So far the dissemination risk of this novel coronavirus is not clear.

The main manifestations of the disease are fever, cough, dyspnea, and chest imaging findings of double lung infiltration, and the incubation period is within 14 days after exposure. Although many of the cases reported so far are not severe, about 20% of those diagnosed are in critical condition, with respiratory failure, septic shock or other organ failure requiring intensive care. Most of the deaths were due to underlying complications.

Whole-genome sequencing and phylogenetic analysis showed that the 2019-nCoV is a novel β coronavirus, which belongs to a different evolutionary branch from the severe acute respiratory syndrome (SARS) and MiddleEast respiratory syndrome (MERS) related novel β coronavirus. The 2019-nCoV is very similar to the bat coronavirus, and bats are likely to be the main source, but it is unclear whether it is transmitted directly from bats to humans or through other mechanisms, such as with some intermediate hosts.

2. Expected clinical using purpose and the diagnosis methods applied to such adaptation disease at present

At present, there are mainly nucleic acid detection (RT-PCR) and colloidal gold immunochromatography (GICA). Compared with nucleic acid detection, the rapid diagnostic kit for 2019-nCoV is suitable for samples of serum, plasma and whole blood. It is convenient, rapid and highly sensitive, and suitable for large-scale screening. Results can be obtained within 15 minutes. At the same time, cross contamination between samples can be avoided by using single reagent strips. In addition, it can reduce the exposure risk of health care workers and facilitate early diagnosis and exclusion of suspicious cases.

3. Testing principle and detection method of the product

Test principle: The reagent strip was precoated on colloidal gold pad with gold-labeled novel coronavirus recombinant antigen and mouse IgG. Anti-human IgM μ-chain monoclonal antibody, anti-human IgG antibody and anti-mouse IgG antibody were coated in the test area and control area of nitrocellulose membrane. The IgM/IgG antibody of the novel coronavirus in human serum (or plasma or whole blood) was detected qualitatively by immunochromatography and the principle of Capture ELISA.

When a positive IgM sample is tested, IgM antibodies to the novel coronavirus in serum (or plasma, or whole blood) samples combine with novel coronavirus recombinant antigens in colloidal gold to form compounds. Due to chromatography, the compounds move forward along the strip, and when passing through the test area, they bind to the pre-coated anti-human IgM μ-chain monoclonal antibodies to form the "Au-2019-nCoV Ag-2019-nCoV IgM Ab-anti-human IgM Ab" sandwich complexes, thus showing a burgundy line in the test area. The free gold-labeled mouse IgG was combined with the anti-mouse IgG antibody in the control area, thus showing a burgundy line in the control area. As for the negative samples, a burgundy line only show in the control area.

When a positive IgG sample is tested, IgG antibodies to the novel coronavirus in serum (or plasma, or whole blood) samples combine with novel coronavirus antigens in colloidal gold to form compounds. Due to chromatography, the compounds move forward along the strip, and when passing through the test area, they bind to the pre-coated anti-human IgG antibodies to form the

"Au-2019-nCoV Ag-2019-nCoV IgG Ab-anti-human IgG Ab" sandwich complexes, thus showing a burgundy line in the test area. The free gold-labeled mouse IgG was combined with the anti-mouse IgG antibody in the control area, thus showing a burgundy line in the control area. As for the negative samples, a burgundy line only show in the control area.

Test method:

Completely read the manual of the products before test.

Strip Type (figure 1)

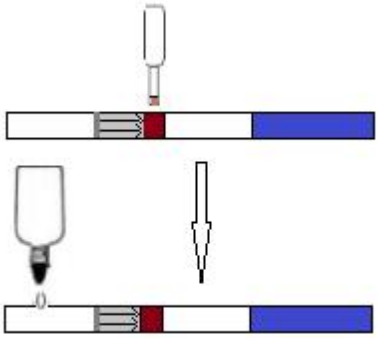

图 一

1. Restore the test strip (without opening the foil bag) to room temperature.

2. Remove the foil pouch from the box and open it, take out the strip, and lay it flat on a table.

3. Draw 2μl of serum/plasma sample or 4μl of whole blood sample with a quantitative dropper and add to the exposed purplish colloidal gold of the strip (arrow pointing). Then add 2 drops of diluent vertically to the sample pad at the lower end of the strip.

4. The experimental results were interpreted and recorded in 10 minutes, but were not valid in 15 minutes. (when a strongly positive sample is tested, a positive result can appear in 1-3 minutes.)

Cassette Type (figure 2)

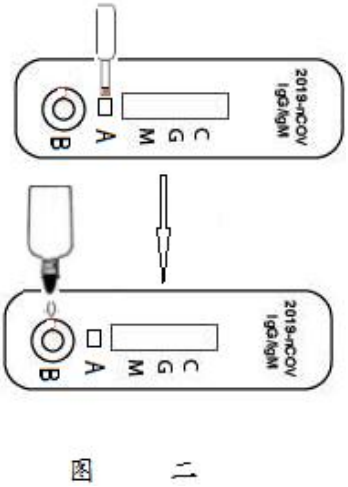

图 二

1. Restore the test strip (without opening the foil bag) to room temperature.

2. Remove the foil pouch from the box and open it, take out the strip, and lay it flat on a table.

3. Draw 5μl of serum/plasma sample or 10μl of whole blood sample with a quantitative dropper and add to hole A. Then add 2 drops of diluent vertically to hole B

4. The experimental results were interpreted and recorded in 10 minutes, but were not valid in 15 minutes. (when a strongly positive sample is tested, a positive result can appear in 1-3 minutes.)

1. Test purpose

The purpose of the Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) clinical trial is to verify the accuracy of this product in clinical test by verifying a certain number of confirmed samples of novel coronavirus, so as to judge whether the safety and effectiveness requirements of the marketed products have been met.

2. Test Management

The Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) is developed and produced by Nantong Egens Biotechnology Co., Ltd., and the clinical evaluation was conducted by Nantong designated admission hospital.

Before implementation of the test, Nantong Egens Biotechnology Co., Ltd. and representatives of Nantong designated admission hospital should discuss together. According to relevant regulations of *"Clinical Research Technical Guidelines and Rules of IVD Reagents"* etc., both parties should sign clinical test protocol and design clinical test scheme to clarify test purpose, content and responsibilities of both parties.

Before the start of clinical research, participants in the research must be familiar with and master the operation of the product, technical performance, etc., so as to do their utmost to take control of the experimental error as well as unify record method and judgment standard;

Researcher should fill in every item in detail and faithfully according to record chart to make sure content of record chart is complete, true and reliable; all observed results should be verified to ensure every conclusion in clinical trial is derived from the original records; there should be corresponding data management measures in clinical trial and data processing phases.

During the clinical research any other situation outside the scheme shall be settled by the parties through negotiation

3. Test Content

3.1. Selection of test subjects

1) Age and gender are not limited

2) Can provide enough test specimens as required

3) Confirmed samples of novel coronavirus

4) Consider cases of suspected influenza, respiratory syncytial virus, enterovirus, adenovirus and other viral infections

3.2. Information of the test reagent

The test reagent is Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) produced by Nantong Egens Biotechnology Co., Ltd. There are two types, including strip-type and cassette-type, and one batch of each type is used.

Strip type: specification is 50t/box; batch number is 20200217, valid for 12 months, the preservation condition is 2-30 ° C, dry and light-avoiding.

Cassette type: specification is 20t/box; batch number is 20200217, valid for 12 months, the preservation condition is 2-30 ° C, dry and light-avoiding.

3.3. Sample collection and serial number

Serum samples were collected intravenously in the conventional way. The samples to be tested within five days can be stored at 4℃, and the samples can be stored at -20℃ for at least three months. Avoid repeated freezing-thawing of samples as far as possible.

Number the samples (001-100) to avoid sample disorder.

3.4. Test procedures

3.4.1. Read instruction book

Sample provider first should read the instructions for the diagnostic kit carefully, to learn about sample adding, time for result determination and the basis of result interpretation.

3.4.2. Specific operations

The researchers tested the numbered samples with the test reagent and recorded the test results faithfully after the corresponding number in the clinical trial registration form. Samples should be used as soon as possible after collection. Samples to be tested within five days can be stored at 4 ℃. The specimens can be stored at -20 ℃ for at least three months. Avoid repeated freezing-thawing of samples as far as possible.

4. Arrangement and analysis of clinical test results

4.1. Arrangement and analysis of strip-type diagnostic kit results

For the strip-type diagnostic kits assessed, the specification is 50t/box, batch number is 20200217,

and the number of samples tested is 100. The results are as follows:

Assessed reagent – Strip-type diagnostic kit		Clinical diagnosis	
		+	–
	+	31	0
	–	1	68

The results indicate that among the 32 positive samples, 1 case was inconsistent according to the comparison of test kit results, while the results of 68 negative cases were all in conformance according to the comparison of test kit results. Through statistical analysis, the sensitivity of this kit is 96.88%, the specificity is 100%, the false positive rate is 0%, the false negative rate is 3.12%, and the total conformity rate is 99%.

4.2. Arrangement and analysis of strip-type diagnostic kit results

For the cassette-type diagnostic kits assessed, the specification is 20t/box, batch number is 20200217, and the number of samples tested is 100. The results are as follows:

Assessed reagent – Cassette-type diagnostic kit		Clinical diagnosis	
		+	–
	+	31	0
	–	1	68

The results indicate that among the 32 positive samples, 1 case was inconsistent according to the comparison of test kit results, while the results of 68 negative cases were all in conformance according to the comparison of test kit results. Through statistical analysis, the sensitivity of this kit is 96.88%, the specificity is 100%, the false positive rate is 0%, the false negative rate is 3.12%, and the total conformity rate is 99%.

5. Discussion and Conclusion

In the process of test, the researchers carefully read the instructions and independently completed the operation of the assessed reagent kits to avoid the result error caused by improper operation. They filled in the test record faithfully, thus ensuring the reliability of the data.

Through the analysis of the test data, we can see that the two types (strip type, cassette type) of the Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) produced by Nantong Egens

Biotechnology Co., Ltd. are highly consistent with the results of confirmed samples. A high coincidence rate indicates that the Diagnostic kit for 2019-nCoV IgM/IgG Antibodies (Colloidal Gold) produced by Nantong Egens Biotechnology Co., Ltd. is reliable, accurate, safe, convenient, stable and has high clinical application value.