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Clinical research report (I)

1. Name and Lot No. of the kit

Name: Dengue NS1 &IgM /IgG Test (colloidal gold method)

Lot No.: Cassette ： 20140317，20140318，20140319

2. Manufacturer

Name: Henso Medical (Hangzhou) Co., Ltd.

Address: 1-510 TONGPU ROAD, HANGZHOU 310012, CHINA

Research

The Dengue NS1 &IgM/IgG Test (colloidal gold method) developed by Henso Medical (Hangzhou) Co., Ltd., is based on the pilot production and laboratory evaluation of the kit, according to the requirements of In Vitro Diagnostic Reagent Registration Management Measures of the State Food and Drug Administration. The clinical application value of the kit was evaluated by three medical institutions qualified for clinical trials by the State Food and Drug Administration. The three clinical trial medical institutions were Guangdong Center for Disease Control and Prevention, the First Affiliated Hospital of Sun Yat-sen University, and Guangdong Provincial People's Hospital.

The target reagent is used for qualitative detection of dengue NS1 & IgM/IgG antibodies in human serum, plasma or whole blood using capture method and colloidal gold immunochromatography technology, for clinical diagnosis. The test kit was produced by Henso Medical (Hangzhou) Co., Ltd., batch No. 20140317, 20140318, 20140319, valid for 24 months. Reference reagent was Panbio Rapid detection kit for Dengue fever, Batch No. 14231, valid for 18 months. A total of 1084 samples were tested in this clinical study, including 326 NS1 positive samples and 758 NS1 negative samples.

Test results: there were 324 positive samples, 2 of which were inconsistent after comparison. 754 negative specimens, 4 of which were inconsistent. Statistical analysis showed that the sensitivity of the kit was 99.38%, the specificity of the kit was 99.47%, the false positive rate was 0.62% and false negative rate of the kit was 0.53%. And the total coincidence rate was 99.45%. A total of 1142 IgM /IgG samples were detected, including 324 IgM positive samples and 818 IgM negative samples. Results: The results of 322 positive samples were compared with those of the kits, and 2 of them were inconsistent. The results of 814 negative specimens were compared with those of the test kits, and 4 of them were inconsistent. By statistical analysis, the sensitivity of the IgM /IgG was 99.38%, the specificity of IgM /IgG was 99.51%, the false positive rate was 0.62%, the false negative was 0.49% and the total coincidence rate was 99.47%. There are 342 IgG-positive samples and 800 IgG -negative samples. Results: The results of 339 positive samples were compared with those of the kits, and 3 of them were inconsistent. The results of 798 negative specimens were compared with those of the test kits, and 2 of them were inconsistent. By statistical analysis, the sensitivity of the IgM /IgG was 99.12%, the specificity of

IgM /IgG was 99.75%, the false positive rate was 0.82%, the false negative was 0.25% and the total coincidence rate was 99.56%. Statistical analysis showed that the target reagent to be evaluated had a good consistency with the reference reagent.

The results of clinical study showed that the kit was reliable, accurate, simple and had high clinical application value.

Introduction

1.The origin, biological and physicochemical properties of the object to be measured

Dengue fever is an acute infectious disease caused by dengue virus type 1-4 and transmitted by Aedes mosquitoes. Dengue virus IgM antibodies appear 3-5 days after infection, peak at 2 weeks, and persist for 2-3 months. Especially in the first infected IgM antibody increased significantly. It is a class B infectious disease according to the law of China on the Prevention and Treatment of Infectious Diseases. IgG antibodies can appear 10 days after initial infection, 4 days after reinfection, and peak 3W after infection, and can produce immune memory. It is a class B infectious disease according to the law of China on the Prevention and Treatment of Infectious Diseases. This disease occurs in Asia, Oceania, America and Africa, and is a very serious public health problem in tropical and subtropical areas.

There are four serotypes of dengue virus, and there are often alternating epidemics of different serotypes in a given area, which increases the likelihood of dengue haemorrhagic fever and dengue shock syndrome. Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DHS) have a high fatality rate, which not only seriously affects people's health, but also the development of local economy, trade and tourism. Therefore, early diagnosis is extremely important.

The Dengue IgM/IgG Test (colloidal gold method) developed by Henso Medical (Hangzhou) Co., Ltd. is used for qualitative detection of dengue IgM/IgG antibodies in human serum, plasma or whole blood for clinical diagnosis. On the basis of trial production and laboratory evaluation of the kit, Guangdong Provincial Center for Disease Control and Prevention, the First Affiliated Hospital of Sun Yat-sen University and Guangdong Provincial People's Hospital were

commissioned to conduct clinical trials. The specific assessment was carried out by three clinical trial medical units. The objective was to obtain relevant experimental data through controlled experiments, calculate relevant clinical evaluation indicators and conduct appropriate statistical analysis, so as to provide scientific basis for the clinical application of the kit and ensure that the reagent meets clinical requirements and play a positive role in promoting it.

2. Intended clinical purpose and current diagnostic methods for this indication

Common detection methods for dengue virus are virus separation, serology, molecular biology, colloidal gold immunochromatography and so on. Among them, colloidal gold immunochromatography technology has highly specificity sensitivity. It does not need the assistance of any instrument and equipment, but only needs one step operation, which is simple and fast. Generally, the results can be determined in 15 minutes, thus being widely used. Dengue IgM/IgG Test (colloidal gold method) developed by Henso Medical (Hangzhou) Co., Ltd.. is used colloidal gold immunochromatography technology to detect dengue IgM/IgG antibody.

3. Product testing principle and method

Testing Principle:

The reagent is pre-coated with gold labeled dengue recombinant antigen and mouse monoclonal antibody on colloidal gold pad, and coated with anti-human IgM μ chain monoclonal antibody, anti-human IgG antibody and anti-mouse IgG antibody on the fiber nitrate membrane test line and control line respectively. The principle of immunochromatography and capture method is applied. Qualitative detection of dengue IgM/ IgG antibodies in human serum (or plasma, or whole blood).

When detecting IgM positive samples, the dengue IgM antibody in serum (or plasma, or whole blood) samples combines with the dengue recombinant antigen in colloidal gold to form a complex. Due to the chromatography, the complex moves forward along the strip. Au-dengue Ag-dengue IgM Ab-anti-human IgM Ab complex is formed by combining with the pre-coated anti-dengue IgM μ chain monoclonal antibody after crossing the test line, and the purplish red band is displayed at the test line. The free gold mouse monoclonal antibody is combined with anti-mouse IgG antibody at the control line. In order to show the purplish red strip in the control

line. The negative specimens showed only purplish red bands at the control line.

When detecting IgG-positive samples, dengue IgG antibody in serum (or plasma, or whole blood) samples combines with dengue antigen in colloidal gold to form a complex. Due to the chromatography, the complex moves forward along the strip. Au-dengue Ag-Dengue IgG Ab-anti-human IgG Ab sandwich is formed by combining with the pre-coated anti-human IgG antibody after passing the test line, so that the purplish red band is displayed at the test line. The free gold mouse monoclonal antibody is combined with anti-mouse IgG antibody at the control line. In order to show the purplish red strip in the control line. The negative specimens showed only purplish red bands at the control line.

Testing Method:

Before testing, you must read the instructions of this product completely.

1. Restore the test strip and the sample to room temperature (20-30℃).
2. Take out the reagent card from the original package, add 1 drop sample (about 5μl) to the sample well with a dropper, and then add 1 drop (about 50μl) sample diluent.
3. The experimental results were observed and recorded within 15 minutes, and the results showed no clinical significance after 20 minutes.

1. Study Purpose

Dengue IgM/IgG Test (colloidal gold method) is with the purpose of clinical trials with varieties approved products of a certain number of typical clinical samples were blinded clinical experiment, to evaluation indices such as kits for examination and assessment of sensitivity and specificity, verify the accuracy in the determination of the product in the clinical, to judge whether to achieve the safety and effectiveness of products on the market.

2. Study Management

The Dengue NS1 & IgM/IgG Test (colloidal gold method) in this clinical trial was developed and produced by Henso Medical (Hangzhou) Co., Ltd., and the clinical trial was conducted by three medical institutions, namely Guangdong Center for Disease Control and Prevention, the First Affiliated Hospital of Sun Yat-sen University and Guangdong Provincial People's Hospital. Before the implementation of the trial, Henso Medical (Hangzhou) Co., Ltd. and the relevant

personnel of the three clinical trial units shall discuss and sign the clinical trial agreement according to the Technical Guidelines for Clinical Research on In Vitro Diagnostic Reagents and other relevant provisions, and jointly design the clinical trial scheme, and define the purpose, content and responsibilities of the trial.

The test adopts blind method, and the examiners who participate in the assessment have been trained in technology, so they are inspectors with high level and rich experience.

Test specimens and relevant data shall be managed by special personnel. All data are computer archived and saved in multiple copies.

Any other circumstance outside the plan during the experimental study shall be settled by both parties through negotiation.

3. Study Content

3.1 Selection of test subjects

（1） Age and gender are not limited;

（2） Those who can collect enough test specimens as required;

（3） Positive candidates are preferentially selected until the requirements are met;

（4） Some cases of Japanese Encephalitis, Yellow Fever, Plasmodium Falciparum Malaria, Plasmodium Vivax Malaria and Hepatitis E Disease were considered.

3.2 Target reagent and reference reagent information

Three batches of the Dengue NS1 & IgM/IgG Antibody Test manufactured by Henso Medical (Hangzhou) Co., Ltd.

Cassette, the specification is 25T/box, the batch number is 20140317, 20140318, 20140319, valid for 24 months, the storage condition is 2-30℃ dry storage away from light.

The reference reagent was Panbio Rapid Detection kit for Dengue fever from Australia, lot no. 14231, valid for 18 months.

3.3 Methods of sample collection, storage and transportation

a) Venous blood samples are collected under aseptic conditions and the serum or plasma is separated as soon as possible to avoid hemolysis.

b) Fresh specimens should be used as far as possible. If the specimen cannot be tested in time, it can be refrigerated at 2-8℃ for 7 days. Long-term storage should be frozen at -20℃, avoid repeated freezing and thawing.

c) Conventional 0.2mg/ml heparin, 150mmol/L EDTA-2Na and 2.5% sodium citrate anticoagulant did not affect the detection results.

d) Hemolysis specimens are not suitable for this reagent.

e) Adding 0.1% NaN3 into the specimens did not affect the results.

3.4 Study Process

Sample providers should first read the instructions of target reagents and reference reagents carefully to understand how to add samples, the time of result judgment and the basis of result interpretation.

1、Restore the test cassette and sample to room temperature (20-30℃).

2. Take out the reagent cassette from original package.

NS1: Add a drop sample (about 25μl) to the sample well with a dropper, and then add another drop (about 50μl) of sample diluent.

IgM /IgG: Add a drop sample (about 5μl) to the sample well with a dropper, and then add another drop (about 50μl) of sample diluent.

3、The results were observed and recorded within 15 minutes, but were not clinically significant after 20 minutes.

Note: Reagent cards should be used as soon as possible within 1 hour after they are removed from the original package, especially in a room temperature above 30℃ or in a highly humid environment.

4. Collation and analysis of clinical trial results

There are three batch numbers of cassette test kits in the target reagent, lot number: 20140317, 20140318, 20140319.

NS1: A total of 1084 samples were tested. A blind and controlled trial design was adopted in this clinical study. Including 326 NS1 positive samples and 758 NS1 negative samples. Results: There were 324 positive samples, and 2 of them were inconsistent. 754 negative specimens, 4 of

them were inconsistent. Statistical analysis showed that the sensitivity of target reagent was 99.38%, specificity of target reagent was 99.47%, false positive rate of target reagent was 0.62%, false negative rate of target reagent was 0.53%, and the total coincidence rate was 99.45%.

IgM /IgG: A total of 1142 samples were tested. A blind and controlled trial design was adopted in this clinical study. Including 324 IgM positive samples and 818 IgM negative samples.

Results: There were 322 positive samples, and 2 of them were inconsistent. 814 negative specimens, 4 of them were inconsistent. Statistical analysis showed that the sensitivity of target reagent was 99.38%, specificity of target reagent was 99.51%, false positive rate of target reagent was 0.49%, false negative rate of target reagent was 0.62%, and the total coincidence rate was 99.47%. Statistical analysis showed that the target reagent to be evaluated had a good consistency with the reference reagent.

5. Discussion and Conclusion

Before the clinical trial, all sample providers read and signed the informed letter. During the whole process, no unit or individual was forced or threatened to require them to participate in the trial, and they all followed the principle of voluntary participation.

During the test, the test subjects (including sample providers and researchers) carefully read the instructions, independently completed the operation of the target reagent and reference reagent, avoided the result error caused by improper operation, and filled in the test record truthfully, thus ensuring the reliability of data.

Through the analysis of the experimental data, it can be seen that the Dengue IgM/IgG Test (colloidal gold method) produced by Henso Medical (Hangzhou) Co., Ltd. is highly consistent and coincidence with the reference reagent (Australian Panbio rapid detection of Dengue fever reagent), indicating that, The Dengue IgM/IgG Test (colloidal gold method) produced by Henso Medical (Hangzhou) Co., Ltd. has met the safety and effectiveness requirements of registered products in the United States.