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User's Manual

Measles virus IgG Antibody ELISA Kit

REF **DEIA-NS2303-6**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This product is used to qualitatively detect in vitro whether the measles virus IgG antibody titer in human serum, plasma or whole blood samples reaches 200 mIU/ml.

General Description

Measles virus is a spherical, non-segmented, single-stranded negative-sense RNA virus with a diameter of 120 to 125 nm. In virology, it belongs to the genus Morbillivirus in the family Paramyxoviridae. Measles virus is mainly spread from person to person through respiratory droplets, and can also be spread through the air in the form of aerosols. The virus is most contagious in the prodromal stage, and the second-generation incidence rate among susceptible families (and institutions) is high, reaching 90% or more. Since cases can detoxify before and after visiting a doctor, on average, rashes can appear in secondary cases 14 to 15 days after the first case in the family is seen. Almost all primary cases (except for cases where maternal antibodies are still present in the body or immunoglobulins have been injected) show overt infection. There is currently no evidence that immune people can spread the virus without developing symptoms after exposure.

The clinical manifestations are that after respiratory exposure, early clinical symptoms begin to appear after an incubation period of 10 to 12 days. If exposure occurs after skin or mucous membrane damage, the incubation period can be shortened to 2 to 4 days; in patients with suppressed immune function, the incubation period may be extended. The prodromal stage mainly manifests as fever, general fatigue, conjunctivitis, rhinitis and bronchitis (such as cough), which can last for 2 to 4 days. These symptoms are similar to those of general upper respiratory tract infections. Within the next 4 days, the body temperature increased significantly, reaching 40.6°C. Koplik's spots are considered to be a mucosal rash unique to measles. They appear on the buccal mucosa 1 to 2 days before the rash and can last 1 to 2 days after the rash. Fourteen days after exposure to the virus, the patient developed erythematous papules, which spread from the head (face, forehead, hairline, behind the ears and upper neck) through the trunk to the limbs, which took about 3 to 4 days. The rash is usually concentrated on the face and upper body and fades under pressure. In the next 3 to 4 days, the rash began to subside according to the order of presentation, leaving light brown pigmentation.

After natural infection with measles virus, the body can develop long-term (possibly lifelong) immune memory, which includes the continuous production of measles virus-specific antibodies and measles virus-specific CD4+ and CD8+ T lymphocytes in the circulation. Measles vaccine induces humoral and cellular immunity comparable to natural infection, although antibody titers are usually lower. Additionally, infants born to mothers vaccinated against measles had lower average concentrations of maternally transmitted antibodies compared with mothers who had been naturally infected with measles. After vaccination with measles vaccine, transient, measles-specific immunoglobulin IgM antibodies can appear in the blood, while IgA antibodies appear in mucosal secretions; IgG antibodies can persist in the blood for many years. At the same time, the World Health Organization (WHO)) stated in "The Immunological Basis of Immunization Series (Part 7: Measles)" that using the NIBSC second generation international standard (NIBSC code: 66/202) for traceability, the lowest protective level of anti-measles virus IgG antibodies in the human body is 200mIU/ml.

At present, the main methods for detecting measles virus-specific IgG antibodies include: hemagglutination inhibition test (HI), complement fixation test (CF), plaque reduction neutralization test (PRNT), microcytopathy

inhibition test, and enzyme-linked immunosorbent assay. (ELISA) etc.

Principles of Testing

The kit is an indirect enzyme-linked immunosorbent assay.

This kit uses inactivated and purified measles virus antigen to coat the microplate. After the sample is added to the microwell, the anti-measles virus antibody and the solid-phase measles virus antigen in the microwell form an antigen-antibody immune complex. Unbound substances are removed by washing, and horseradish peroxidase-labeled mouse anti-human IgG monoclonal antibody is added. , react with the complex to form an antigen-antibody-enzyme-labeled secondary antibody complex, wash to remove the enzyme conjugate that does not participate in the reaction, and add TMB substrate to develop color. The depth of the color is proportional to the content of the measles virus IgG antibody.

Reagents And Materials Provided

1. Pre-coated plate: 12T×8, inactivated purified measles virus antigen

2. Mouse anti-human IgG monoclonal antibody enzyme label: 10ml × 1 bottle, HRP enzyme-labeled mouse anti-human IgG monoclonal antibody, containing stabilizer and biological preservatives

3. Concentrated washing solution (20×): 30ml×1 bottle, 20 times concentrated phosphate buffer, containing NaCl, Tween-20

4. Sample diluent: 50ml×1 bottle, sodium citrate buffer, stabilizer, etc.

5. Negative control substance: 0.5ml×1 bottle, inactivated human anti-measles virus IgG negative serum, stabilizer

6. Positive control substance: 0.5ml×1 bottle, inactivated human anti-measles virus IgG positive serum, stabilizer

7. Substrate solution A: 5.0ml×1 bottle, the main component is carbamide peroxide

8. Substrate solution B: 5.0ml×1 bottle, the main component is TMB

9. Stop solution: 5.0ml×1 bottle, 0.5M H_2SO_4 solution

10. Ziplock bag: 1 serving

11. Parafilm: 3 sheets

Materials Required But Not Supplied

A microplate reader with a wavelength of 450nm or a fully automatic enzyme immunoassay analyzer.

Storage

1. Store refrigerated at 2-8°C and away from light. Freezing is prohibited. The validity period is 12 months.
2. Unused slats should be immediately placed in a sealed bag for storage. Opened kits should be used within one week.
3. See the label for the production date and expiry date.

Specimen Collection And Preparation

1. Human serum/plasma or whole blood can be used as measurement samples. Anticoagulants (heparin, EDTA, sodium citrate) have no interference with sample test results.
2. Serum/plasma samples can be stable for 4 days when refrigerated at 2-8°C, but it is recommended to be used within 72 hours; whole blood samples can be used immediately; if long-term storage is required, serum/plasma samples can be directly frozen at -20°C. It is stable for one year. The whole blood sample can be diluted with sample diluent and the supernatant is aspirated out. It can be stable for one year when frozen and stored at -20°C. It is recommended that frozen samples be frozen and thawed no more than 3 times.
3. Avoid using hemolyzed and lipemic samples, and do not use contaminated samples.

Assay Procedure

1. Sample preparation:

a. First add 500µl of sample diluent into the 1.5ml centrifuge tube. serial number.

b. Collect 10 µl of whole blood or 5 µl of serum/plasma, add it to the corresponding centrifuge tube, and shake gently immediately to mix.

c. If it is a whole blood sample, place the centrifuge tube at room temperature (or 4°C refrigerator) and wait until all the red blood cells have settled to the bottom of the well. Take the supernatant for testing; if it is a serum or plasma sample, mix it well before use.

2. Take out the kit and samples from the refrigerated environment, place them for 30 minutes, and balance to room temperature; adjust the incubator or water bath to 37±1°C.
3. Prepare washing solution: add 1 times the volume of concentrated washing liquid to 19 times the volume of distilled water, purified water or deionized water, and mix well.
4. Add samples: For each test, set up 1 well for positive control and 1 well for negative control, and add 100 µl of the corresponding control solution to each well; set up 1 well for blank control, and add 100 µl of sample diluent; add diluted samples (full) to each of the remaining wells. Take 100 µl of supernatant from blood samples. Attach a sealing film and incubate at 37°C for 60 minutes. Carefully remove the sealing film and pour out the liquid in the well. Add 300 µl of diluted washing solution to each well, shake slightly for 10 seconds, pour it out, and pat dry. Repeat 5 times. If possible, set the parameters according to the above washing conditions and wash with a plate washer.
5. Add anti-human IgG antibody enzyme label: 100 µl per well (do not add to blank wells). Attach the sealing film and incubate at 37°C for 30 minutes. Carefully remove the sealing film and pour out the liquid in the well. Add 300 µl of diluted washing solution to each well, shake slightly for 10 seconds, pour it out, and pat dry. Repeat 5 times.
6. Color development: Add 50 µl each of substrate solution A and substrate solution B to each well, and mix well. Attach a sealing film and place at 37°C to develop color in the dark for 15 minutes.
7. Determination: Carefully remove the sealing film, add 50 µl of stop solution to each well, and mix well. Within 30 minutes, use a microplate reader with dual wavelength 450nm/630nm to measure the OD value of each well (when using a single wavelength of 450nm, you must use a blank well to zero).

Calculation

1. Negative control OD450 <0.3, positive control OD450 ≥0.6, the test is established.
2. Cut-Off value = negative control OD450 value × 2.1, S/CO value = $OD450nm_{sample}$/Cut-Off

   0.9≤S/CO <1.1, suspicious, it is recommended to repeat the test;

   S/CO value ≥1.1, positive;

   S/CO value <0.9, negative

Interpretation Of Results

1. Positive result: S/CO value ≥1.1, indicating that the measles virus IgG antibody titer in the sample is not less than 200mIU/ml.
2. Negative result: S/CO value <0.9, indicating that the measles virus IgG antibody titer in the sample is less than 200mIU/ml.
3. Suspicious results: 0.9 ≤ S/CO value < 1.1, indicating that the measles virus IgG antibody in the sample is at a critical value. It is recommended to repeat the test or test again after two weeks to dynamically observe changes in antibody levels.

Precision

CV% < 15%

Specificity

This product is not compatible with rubella virus IgG antibody-positive samples, mumps virus IgG antibody-positive samples, hepatitis A virus IgG-positive samples, hepatitis B virus surface antibody-positive samples, varicella-zoster virus IgG antibody-positive samples, and hepatitis C virus IgG antibody positive samples, Treponema pallidum IgG antibody positive samples, Human immunodeficiency virus IgG antibody positive samples, Epstein-Barr virus IgG antibody positive samples, Mycoplasma pneumoniae IgG antibody positive samples, Cytomegalovirus IgG antibody positive samples, Herpes simplex virus IgG antibody positive samples , Toxoplasma IgG antibody-positive samples cross-reacted. This product has not been studied on non-specific high IgG, non-specific high IgM, and HAMA samples, so clinical use should be avoided.

Interferences

Hemolysis with a hemoglobin concentration of 2.0 mg/ml, lipemia with a triglyceride concentration of 6 mmol/L, and jaundice with a bilirubin concentration of 100 μmol/L have no effect on the test results. Samples containing autoantibodies (rheumatoid factor, antinuclear antibodies) and serum samples from pregnant women will not interfere with this product.

Precautions

1. This product is only for in vitro diagnosis, one-time use, and cannot be reused.
2. Please strictly follow the instructions. Unauthorized changes may cause unreliable results.

3. The same components in our company's kits with different batch numbers and different varieties must not be mixed. Do not mix with reagents from other manufacturers. Do not use reagents that have expired.
4. Required utensils and consumables not provided in the kit: distilled water, purified water or deionized water; 10μl, 200μl, 1000μl pipettes; pipettes; incubator or water bath; containers and measuring tools, etc.
5. Samples should be taken accurately when diluting. When adding samples, a micro-injector should be used to add samples accurately.
6. Do not use tap water to dilute the washing solution or wash the plate.
7. The sealing film can only be used once to avoid cross-contamination.
8. The interval between adding substrate solution A and substrate solution B should not exceed 2 minutes. The light blue substrate solution B visible to the naked eye should be discarded.
9. The stop solution contains H_2SO_4, please pay attention to safety when using it.
10. All samples and waste after testing should be considered infectious and disposed of in accordance with relevant biosafety regulations.

Limitations

It is only used for the auxiliary diagnosis of measles virus infection and the qualitative detection of measles virus IgG antibodies.