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User's Manual

Tetanus toxoid IgG Antibody ELISA Kit

REF **DEIA-NS2303-7**

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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 for the qualitative in vitro detection of tetanus toxoid IgG antibodies in human serum/plasma or whole blood samples.

It is suitable for detecting whether the anti-tetanus toxoid IgG antibody level in normal or susceptible people has reached the full protective titer (0.1 IU/ml) and whether the anti-tetanus toxoid IgG antibody level has reached the successful level of immunity after vaccination with a vaccine containing tetanus toxoid components. Standard (0.1 IU/ml).

General Description

Tetanus is a strictly anaerobic bacillus that can form spores. Tetanus is an infectious bacterial disease caused by Clostridium tetani. Under anaerobic conditions (such as a contaminated necrotic wound), this ubiquitous bacilli produces a highly toxic neurotoxin called tetanospasm toxin. Tetanus spasmotoxin blocks inhibitory neurotransmitters in the central nervous system, causing muscle rigidity and spasms that are typical of tetanus.

The incubation period of tetanus usually ranges from 3 to 21 days (median 7 days, range 0 to >60 days). In most cases, neonatal tetanus can develop between 3 and 14 days after birth. In more than 80% of cases, tetanus presents as a systemic ankylosing disease. Typical features include early facial muscle spasm (trismus and snicker), followed by dorsal muscle spasm (opistotonus) and sudden generalized tonic epilepsy (tetanus spasm). Glottic spasm can induce sudden death. In neonatal tetanus, generalized cramps are often preceded by an inability to suck or feed and excessive crying. The case fatality rate of tetanus ranges from 10% to 70%, depending on treatment, age and general health of the patient. If elderly and infant patients do not receive hospitalization and intensive treatment, the mortality rate can reach almost 100%. In hospitals with the best medical conditions, the mortality rate can be reduced to 10% to 20%.

Tetanus can occur at any age and has obvious seasonality, with its peak occurring in mid-summer or the wet season. Even with modern intensive care conditions, the case fatality rate is very high. The vast majority of tetanus cases are related to childbirth, mostly in developing countries, and are mainly seen in newborns and pregnant women with unclean delivery and poor postpartum hygiene. Tetanus following injury in children and adults is also a serious public health problem.

Laboratory methods for detecting tetanus toxoid IgG antibodies mainly include indirect hemagglutination test, toxin neutralizing antibody test, enzyme-linked immunoassay (ELISA), etc.

Principles of Testing

The kit adopts the principle of enzyme-linked immunoassay indirect method.

This kit uses refined tetanus toxoid antigen to coat the microplate. After the sample is added to the microwell, the anti-tetanus toxoid antibody forms an antigen-antibody immune complex with the solid-phase tetanus toxoid antigen in the microwell. Unbound substances are removed by washing, and horseradish peroxidase-labeled mouse anti-human is added. The IgG monoclonal antibody reacts with the complex to form an antigen-antibody-enzyme-labeled secondary antibody complex. The enzyme conjugate that does not participate in the reaction is washed to remove, and TMB substrate is added to develop color. The depth of the color is consistent with that of the tetanus toxoid IgG antibody. Proportional to the content.

Reagents And Materials Provided

1. Pre-coated plate: 12T×8, purified tetanus toxoid 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-tetanus toxoid IgG negative serum, stabilizer

6. Positive control substance: 0.5ml×1 bottle, inactivated human anti-tetanus toxoid 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, main ingredient is TMB

9. Stop solution: 5.0ml×1 bottle, 0.5M H2SO4 solution

10. Ziplock bag: 1 serving

11. Sealing film: 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 pre-coated boards should be immediately put into a ziplock bag with desiccant and sealed, and the storage time should not exceed one week in an environment of 2~8°C.
3. Any unused reagents of other components should be capped immediately and stored in an environment of 2~8°C. The storage time should not exceed one week.
4. Please refer to the product label for the production date and expiration date of the product.

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 a 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 a 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

The cut-off value of this kit is set at a fully protective titer of tetanus toxoid IgG antibody of 0.1IU/ml (original sample).

1. Positive result: It indicates that the person has been vaccinated with a vaccine containing tetanus toxoid and has produced antibodies. The antibody level is not less than 0.1IU/ml and reaches a complete protective titer.
2. Negative result: It indicates that the patient has not been vaccinated with a tetanus toxoid-containing vaccine or the antibody level produced after vaccination with a tetanus toxoid-containing vaccine is less than 0.1IU/ml, which means the complete protection titer has not been reached.
3. Suspicious results: For suspicious results, it is recommended to repeat the test or test again after two weeks, and dynamically observe changes in antibody levels.

Precision

CV% < 15%

Specificity

This product is not compatible with pertussis toxoid IgG antibody positive samples, diphtheria toxoid IgG antibody positive samples, hepatitis A virus IgG positive samples, hepatitis B virus surface antibody positive samples, varicella-zoster virus IgG antibody positive samples, hepatitis C Viral 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 Cross-reaction occurred between samples and Toxoplasma IgG antibody-positive samples. 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; contain autoantibodies (rheumatoid factor, antinuclear antibodies) Samples and maternal serum samples have no interference 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 qualitative detection of tetanus toxoid IgG antibodies in humans