Biocare™ Diagnostics

Anti-TP ELISA Kit

Enzyme-linked immunosorbent assay for the detection of total antibody to Treponema pallidum in serum

Catalog #: BS1012B

INTRODUCTION

Syphilis is a disease, usually sexually transmitted, caused by infection with the spirochete Treponema pallidum (T. pallidum). Infection is systemic from the outset and the disease is characterized by periods of latency, often in excess of twenty years. These features, together with the fact that T. pallidum cannot be isolated in culture, mean that serological techniques play a major role in the diagnosis of syphilis and treatment follow up.

The procedures most commonly used to screen for antibodies to T. pallidum in clinical diagnostic laboratories are based upon their reaction with non-treponemal lipoidal antigens (the reagin tests). Reagin tests, such as the RPR or VDRL, can be used to test serial dilutions of the serum specimen. The end point values from sequentially obtained serum samples decline following successful treatment until after a period of several months the patient will usually become regain test non-reactive.

Clinical diagnostic serum specimens which are reactive in regain tests are typically confirmed using treponemal tests such as the Microhaemagglutination-T.pallidum (MHA-TP) procedure, where erythrocytes coated with T. pallidum antigens agglutinate in the presence of specific antibodies. In contrast to the nontreponemal tests, treponemal test reactivity will persist following treatment in approximately 85% of the cases for the life of the patient.

Donors of blood and/or plasma for transfusion are screened for T.pallidum antibodies using either reagin or treponemal tests. The detection of T.pallidum antibodies is used to help identify donors who present an increased risk of transmitting blood-borne infections.

Anti-TP ELISA Kit is a solid-phase simultaneous immunoassay to detect antibody against T.pallidum. Microwells are coated with T.pallidum recombinant antigen p15, p17 and p47. A serum specimen is added to the microwells together with Horseradish Peroxidase (HRP) conjugated T.pallidum. After incubation, the complex of antigen-antibody-antigen (HRP-conjugated T.pallidum, anti- T.pallidum antibody and T.pallidum on the wells) will be formed. Thus, the amount of HRP- T.pallidum bound to the well is proportional to the concentration of anti- T.pallidum antibody in the specimen.

The unbound enzyme conjugates will be washed away and then the chromogen substrate solution containing urea peroxide is added to the wells. A blue color is developed in proportion to the amount of anti-T.pallidum antibody in the specimens. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of controls and specimens is determined by using EIA reader with wavelength set at 450 nm.

REAGENTS

Materials provided with the kits:

1. Microtiter Plate: One microplate with 96 wells coated with recombinant T.pallidum antigen p15, p17 and p47.
2. Negative Control: One vial of 0.5ml anti-TP Negative Control.
3. Positive Control: One vial of 0.5ml containing human anti-TP antibody.
4. Recombinant HBcAg: 6 ml
5. Enzyme Conjugate: One bottle of 6 ml containing HRP-conjugated-T.pallidum antigen p15, p17 and p47 for 96 tests.
6. Wash Buffer Concentrate (20X): One bottles of 30 ml for 96 tests. The buffer should be diluted 20 times with distilled water before use.
7. Substrate Solution A: One bottle of 6 ml HRP Substrate for 96 tests.
8. Substrate Solution B: One bottle of 6 ml TMB Chromagen Substrate for 96 tests.
9. Stop Solution: One bottle of 6 ml 2N Sulfuric Acid

Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Must not use kit beyond the expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipet reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before deposing.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURES

1. Allow all components to reach room temperature before use.
2. Dispense one drop (50 ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control., and 50ul of serum or plasma samples into respective wells
3. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well..
4. Place the microtiter plate into a humidified box and incubate at 37°C for 60 min.
5. Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
6. Add one drop (50 ul) of Substrate Solution A (HRP substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
7. Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Positive: P/N value is equal to or greater than 2.1

Negative: P/N value is less than 2.1

$$P/N\ value = \frac{OD\ value\ of\ specimen}{Average\ OD\ value\ of\ Negative\ Control}$$

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE ASSAY

1. Anti-TP EIA is limited to the detection of total antibody against TP in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells completely before adding the washing solution.

3. As with all diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by physician is needed for a final diagnosis.
4. Do not use reagents from different tests that will cause incorrect results.
5. Following the procedure instruction closely, especially the incubation time and temperature.

RELATED READING MATERIALS

1. World Health Organization Technical Report Series. No.674 (1982) Treponemal infections.
2. Schroeter A.L., Lucas J.B., Price E.V., and Falcone V.H. (1972). Treatment for early syphilis and reactivity of serologic tests. Journal of the American Medical Association, 221: 471-476.
3. Immunoassays for Detection of Immunoglobulins G and M to Treponema pallidumin Syphilis. Clinical Microbiology, 28, 1704-1707.
4. Young H., Moyes A., McMillan A. and Robertson D.H. (1989). Screening for treponemal infection by a new enzyme immunoassay. Genitourinary Medicine, 65:72-78.
5. U.S. Department of Health and Human Services. Biosafety in microbiological and biomedical laboratories. HHS Publication (NH) 88-8395, Washington U.S. Government Printing Office, May1988.
6. World Health Organization Laboratory Biosafety Manual, Geneva, World Health Organization, 1983.
7. Advisory Committee on Dangerous Pathogens. Categorization of pathogens according to hazard and
categories of containment. London, HMSO, Second Edition, 1990.

FOR CLINICAL USE

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Manufacturer:

Biocare Diagnostics Ltd.
6F, Building B, 108 Xinghua Road
Xiangzhou, Zhuhai, China 519000
Tel: +86-756-8238560
E-mail: info@ivdbiocare.com
Website: www.ivdbiocare.com

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