DRG

DRG® Cysticercosis ELISA (EIA-2905)

As of 12 Oct. 2010 rm (Vers. 1.1)

IVD

1 NAME AND INTENDED USE

The DRG Cysticercosis ELISA is an Enzyme Immunoassay for Qualitative determination of anti-Taenia Solium Antibodies in Human Serum.

(For Professional Use Only)

2 SUMMARY AND EXPLANATION OF TEST

Cysticercosis, the harboring of the larval form (cysticerci) of Taenia, may involve any tissue of organ but are often associated with the central nervous system. Presence of the cysticerci in the brain may cause increased cranial pressure, seizure and altered mental states. Cysticercosis is acquired by ingestion of Taenia solium eggs and is rare in the most developed countries but endemic in Latin America, Asia and Africa. Most cases of cysticercosis in the developed countries are associated with immigrants from developing countries [1].

Diagnosis of cysticercosis usually requires multiple methods such as radiography and serology. Although use of cyst vesicular antigen has helped to increase its sensitivity and specificity, significant cross reactions with other diseases is still a problem. Specimens which test positive by serology should be confirmed by a more specific test method such as the immunoblot or other non-serological method. [2-4].

PRINCIPAL OF PROCEDURE

This Cysticercosis test kit is a solid phase enzyme linked immunosorbent system employing plastic wells coated with Taenia solium antigens. Incubation of serum samples in the coated wells results in the binding of anti-Taenia solium antibodies to the immobilized antigens. Subsequent addition of the enzyme conjugate, comprised of horseradish peroxidase, results in the immobilization of peroxidase in direct proportion to amount of Taenia solium antibody present in the serum sample. Unbound enzyme conjugate is washed from the wells and a substrate and chromogen solution is added. The intensity of the color formed as a result of enzyme activity is a direct measure of the anti-Taenia solium antibody present in the serum samples and may be quantified by use of a photometric wells reader at 450 nm wavelength.

3 MATERIALS PROVIDED

The Cysticercosis (Taenia solium) test kit provides the following:

1. Microwell Strips (96 wells): Isolated Taenia solium antigen coated wells. 8 x 12 Strips.
2. Specimen Diluent: buffered and stabilized protein solution, 1 bottle (50 mL)
3. Washing Buffer Concentrate (100x): (10 mL) Prepare working solution by adding 10 mL washing buffer concemtrate into 990 mL distilled water.
4. Enzyme Conjugate: Conjugate to horseradish peroxidase (11 mL).
5. TMB Solution(11 mL): Buffer solution containing peroxide and TMB
6. Stop Solution: 2 N HCl.
7. Negative Control: Diluted human serum containing buffer and preservative (1 mL)
8. Low Position Control: Diluted serum containing anti-Taenia solium antibodies, buffer and preservative (1 mL)
9. High Positive Control: Diluted Serum Containing anti-Taenia solium antibodies, buffer and preservative (1 mL)
10. Well holder for securing individual wells

3.1.1 WARNING AND PRECAUTION

1. The Cystercercosis ELISA kit is designed for in vitro use only.
2. The components of this kit are carefully matched and are intended to be used as an integral unit. Components of different lots should not be used interchangeably.
3. Although all human materials used in the manufacture of this kit have been found negative for Hepatitis B antigen and for antibody to HIV by required test methods, no test can offer complete assurance that infectious agents are not present, and therefore all calibrators, controls and samples should be handled as potentially infectious agents.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Microwell reader capable of measuring optical absorbance at 450 nm.
2. Pipetor with tips for measuring 5, 50, and 100 uL.
3. Clean plastic washing buffer during testing procedure.
4. Clean test tubes of 2 mL capacity for making dilutions of patient samples.

4 REAGENT PREPARATION

1. Prepare the Working Wash Buffer by adding the entire contents of the Wash Buffer Concentrate to 1000 ml distilled water in a clean plastic wash bottle. Mix gently to dissolve. Store at room temperature.
2. The controls provided in this kit are diluted at time of manufacture. *Do Not Dilute These Controls At Time Of Testing.*

5 STORAGE AND STABILITY

1. Store the kits at 2-8°C and keep microwells in a dry bag with desiccants.
2. Unopened reagents are stable until expiration of the kit. The |TMB Solution should be colorless. If the solution turns blue, it must be replaced. Do not expose these reagents to strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture. Allow to clot and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be assayed immediately, they can be stored at -20°C for at least six months. Avoid repeated freezing and thawing of samples. Azide should not be used as a preservative. Specimens obviously contaminated with bacteria should not be used. Specimens turbid with high lipid concentrations should be clarified prior to assay.

6 PREPARATION FOR ASSAY

1. Bring all reagents and samples to room temperature (24±3°C) and mix gently before beginning the test.
2. Have all reagents and samples ready before the start of the assay. Once the test has begun, it must be performed without any interruption to get the most reliable and consistent results.
3. Use new disposable tips for each specimen.

7 ASSAY PROCEDURE

1. Prepare 1:100 dilutions of test samples by adding 5 uL of sample to 0.5 mL sample dilution in the separate glass tubes.
2. Secure the desired number of coated wells in the holder with sample identification.
3. Dispense 100 uL of the Sample Diluent into well #1 as a blank, the Negative Control into well #2, the High Positive Control into well #3, the Low Positive Control into well #4, and the diluted patient samples into the remaining wells.
4. Incubate for 15 minutes at room temperature.
5. Wash five times with Washing Buffer (300 µL/well/each rinse).
6. Dispense 100 uL Enzyme Conjugate into each well.
7. Incubate for 15 minutes at room temperature.
8. Wash five times with the Washing Buffer (300 µL/well/each rinse).
9. Dispense 100 uL of TMB Solution including the bank well.
10. Incubate for 15 minutes at room temperature.
11. Stop reaction by adding 50 uL of Stop Solution to each well.
12. Zero a microwell reader on the blank and measure the absorbance of each well at 450 nm.

PROCEDURAL NOTES

It is important to wash the microwells thoroughly and remove the last droplets of liquid to achieve optimal results.

1. Pipet all reagent and samples into bottom of the well. Vortex-mixing or shaking of wells after sample and reagent pipetting is not required.
2. The appropriate number of wells should be secured in a holder and all reagent and sample caps should be removed prior to the start of testing. This will permit pipetting at equally timed intervals without interruption. A maximum of 20 patient samples should be assayed at one time in order to minimize error due to timing differences between specimens.

8 QUALITY CONTROL

Each laboratory should utilize internal controls at several levels to monitor assay performance. The controls should be treated as unknown. Results obtained should be in agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservatives.

8.1.1 INTERPRETATION OF RESULTS

Specimens yielding absorbance readings greater than Low Positive at 450 nm should be reported as positive for antibodies against Taenia solium. Absorbances of less than Low Positive are found with specimens having no prior immunological experience with Taenia solium.

8.1.2 LIMITATIONS OF THE PROCEDURE

The results obtained by use of this kit should be used as an aid to diagnose and should not be interpreted as a diagnostic by itself. Should negative results be obtained and other clinical findings suggest infection by Taenia solium, a second serum should be obtained three weeks after the first and testing repeated. Initial testing may have occurred prior to significant antibody production in response to infection.

REFERENCES

1. Flisser, A., and Larralde, C., Cysticercosis. Immunodiagnosis of Parasitic Diseases, Vol. 1, Helminthic Diseases, Ed. Walls and Schantz. Academic Press, pp. 109-161 (1986)
2. Gottstein, et al. Species-specific Immunodiagnosis of Taenia Solium Cysticercosis by ELISA and Immunoblotting, Trop Med Parasi 38, 299-313 (1987)
3. Larralde, C., et al. Reliable serology of Taenia Solium Cysticercosis with Antigens from Cyst Vesticular Fluid: ELISA and Hemagglutination Tests, Am J Trop Med Hyg 35, 965-973 (1986)
4. Tsang, V. et al. An Enzyme Linked Immunoelectrotransfer Blot Assay & Glycoprotein Antigens for Diagnosis of Human Cysticercosis (Taenia Solium), J Infect Dis. 159, 50-59 (1989).