Code: HP001 cysticercosis Ab
96 Test

distribuito da:

Cysticercosis Ab
(Taenia solium)

Cysticercosis

Cysticercosis is an enzyme immunoassay for the detection of specific IgG antibodies against the larval forms of Taenia solium in human serum.

General Information

Cystericercosis is a disease characterized by the harboring of the larval form (cysticerci) of Taenia in any tissue or organ.

Although many sites of infection have been documented, the larvae are often found associated with the central nervous system. Presence of the cysticerci in the brain may cause increased cranial pressure, seizures and altered mental states.

The disease is acquired by ingestion of T. solium eggs. This may happen from a number of different routes such as food contaminated with feces, contaminated water or gastric reflux in tapeworm carriers.

Cysticercosis is rare in most developed nations but is endemic in Latin America, Asia and Africa. Most of the cases of cysticercosis in the developed nations are associated with immigrants from those geographic areas.

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 (such as echinococcosis) is still a problem. All positive serology samples should be confirmed by a more specific test method such as the immunoblot..

Principle of the test

The Cypress Diagnostics' Cysticercosis test is based on the principle of an enzyme Immunoassay.

On the surface of the microtiter wells, inactivated soluble antigens of Taenia solum are bound. Diluted serum samples and controls are pipetted into the wells and incubated at room temperature. Present antibodies bind to the immobilized antigens. Unbound material is removed in a washing step.

In a second step, a POD-conjugated Protein A is added, which has a high affinity to the Fc unit of human IgG. After incubation, unbound conjugate is removed by washing. Substrate (urea peroxide) and chromogen (TMB) are added to the wells and incubated at room temperature. The enzyme bound in the wells converts the colorless substrate/chromogen to a blue color. Addition of stop solution converts the color from blue to yellow. The absorption is measured at 450 nm wavelength.

Precautions

Full compliance of the following good laboratory practices (GLP) will determine the reliability of the results :

1. Prior to beginning the assay procedure, bring all reagents to room temperature (20-25°C).

2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.

3. Replace caps in all the reagents immediately after use. Do not interchange vial stoppers.

4. Discard the internal transparent stoppers, that come in the vials of some of the reagents (Sample Diluent, Wash Solution, etc. once these vials are opened for the first time. The purpose of this stopper is to avoid leakage during transport.

5. Once the assay has been started, all subsequent steps should be completed without interruption and within the recommend time limits.

6. Use a separate disposable tip for each specimen to prevent cross-contamination.

7. Do not use or mix components from different lots.

8. Do not use reagents after expiration date.

9. It is recommended to dispense in a clean test tube the amount of reagent needed for each run in order to prevent possible contamination of the vials content(specially the Substrate A and B solutions). Clean the micropipettes after each reagent dispensing step.

10. All specimens and controls should be run at the same time so that all testing conditions remain the same.

11. The quality control specimens(controls) should be run every time samples are assayed to check the test performance.

12. Check both precision and accuracy of the laboratory equipment used during the procedure (micropippetes, ELISA reader, etc.).

The Health and Safety instructions listed below must be followed :

1.Do not smoke, eat, drink or pipet by mouth in the laboratory.

2.Wear disposable gloves whenever handling patient specimens.

3.Use a 5% sodium hypochlorite solution to wipe up spills.

4. Treat all materials used in the test as if they were infectious. Autoclave all materials for 1 hour at 121,5°C. Add sodium hypochlorite to liquid waste and disposable material in order to reach a final concentration of 5%. Leave the material at least 30 minutes in this solution. Incinerate all disposable material.

5.All serum samples must be considered as potential infectious and must be handled with usual precautions.

6.The positive and negative controls have been tested and found nonreactive to HBsAg, HIV and other infectious agents. As no available test methods can offer, presently, complete assurance that these agents are absent from the sample tested, all the controls must be regarded as potentially infectious and must be handled with usual precautions.

Hydrogen peroxide can cause cauterization. Handle with care.

The stop solution contains 1 M sulfuric acid. Avoid contact with skin and clothing.

7.Avoid contact of substrate and stop reaction solutions with skin and mucosa (possible irritation, burn or toxicity hazardous). In case of contact, rinse the affected zone with plenty of water.

8. Handling and disposal of chemical products must be done according to good laboratory practices(GLP).

Material provided

Sample buffer, colorless lid yellow dyed.

Volume : 2 vials 50 ml each (pH 7.4). Ready to use

Wahing buffer, brown lid , blue dyed

Volume 1 vial 50 ml (pH 7.4). 20 x conc.

Positive control , red lid, ready to use.

Volume 1 vial 1.2 ml , inactivated human serum

Negative control, colorless lid. Ready to use.

Volume 1 vial 1.2 ml, inactivated human serum

Proteine A Conjugate , red lid, ready to use

Volume 1 vial 6 ml, HRP conjugated Protein A.

Substrate, green lid , ready to use.

Volume 1 vial 6 ml. Urea peroxide.

Chromogen, blue lid, ready to use

Volume1 vial 6 ml, tetramethylbenzidine.

Stop Solution, yellow lid,

Volume 1 vial 6 ml. 1M sulfuric acid

12 Microstrips of 8 wells each coated with inactivated Taenia solium antigen in a resealable foil bag.

1 Instruction manual.

Material needed but NOT provided

Micropipettes for volumes of 10 - 100 µl,

and 100 - 1000µl.

Single or double distilled water for the dilution of the washing solution.

Absorbent paper

Mesuring cylinder

Manual or automatic microwell washer.

Elisa-reader with a 450 nm filter.

Stability

Reagents must be stored at temperatures between 2°C and 8°C and can be used up to the expiry date printed on the labels. Microbial contamination has to be avoided. A quality warranty cannot be given beyond the kit expiration date.

The diluted washing buffer has a shelf life of 4 weeks if stored at 2°-8°C.

Allow reagents and microwell strips to get to room temperature before use. To avoid moisture within the strips, do not take the strips out of the foil bag before having reached room temperature. The foil bag should be opened with a pair of scissors without detaching the fastener. Return any unused strips to the foil bag. Reseal and store them directly at 2°-8°C.

The colorless chromogen must be protected from exposure to direct light to avoid deterioration or coloration by auto-oxidation. If the chromogen has turned blue, the reagent should be discarded.

The following criteria may indicate a reagent deterioration :

A turbidity or a blue coloration of the colorless chromogen prior to its use.

An absorbance value (O.D.) of the negative control at 450 nm >0.3

An absorbance value (O.D.) of the positive control at 450 nm < 0.8

Serum Specimens Information

Fresh Serum or Plasma are required.

Repeated freezing and thawing of the samples as well as microbial contamination must be avoided.

The application of heat treated, lipemic, hemolytic, icteric or turbid samples can lead to wrong results.

The sample material can be stored for up to 1 week at 2°-8°C if the test cannot be carried out immediately. A prolonged storage of samples is possible at –20°C.

If they have been stored at 2°-8°C, diluted samples can be used for up to 7 hours.

Test Procedure

Preliminary comments

Bring all reagents and the microwell strips for at least 30 minutes to room temperature before use.

Mix the reagents well before use. Reproducibility in any EIA procedure depends on exact pipetting, the observance of incubation times and temperature and the consistency of wash sequences.

During the washing steps, take care that all wells are filled with buffer and that the liquid is completely removed from the wells. Do not allow microwells to dry between steps.

Avoid direct sunlight during all incubations. Covering the microtiter plate is recommended.

Except the washing buffer all the reagents are ready to use.

Preparation of the washing buffer

1 part of the concentrated washing buffer is diluted with 19 parts of distilled water. Crystals in the buffer concentrate can be dissolved in a water bath at 37°C.

Add 50 ml of the concentrated washing buffer to a 1000 ml graduated cylinder. Bring the final volume to 1000 ml with distilled or deionized water.

The diluted washing buffer has a shelf life of 4 weeks if stored ate 2°-8°C

Preparation of the samples

Before starting the test, serum samples have to be diluted 1 : 100 with the sample buffer.

e.g. 10µl serum + 990µl sample buffer.

The controls included in the kit are ready to use and must not be diluted.

First incubation

After insertion of a sufficient number of cavities into the microwell holder, 50µl of the positive control, the negative control and the diluted sera are pipetted into the wells and incubated for 15 min at room temperature. It is recommended to include two negative controls in each run.

Washing

Decant or aspirate all microwells into a waste container with a disinfectant. Ensure complete removal of the liquid from the microwells by tapping the inverted plate onto absorbent paper. Then wash all wells 5 times with 300 µl of prepared washing buffer. Be sure to remove residual washing solution by firmly tapping the inverted microwells on absorbent paper after single washing steps.

Second incubation

Add 50 µl of protein A conjugate to all wells.

Incubate the plate for 15 min at room temperature.

Washing

Wash 5 times according to step 10.5

Third incubation.

Add 50 µl of substrate and 50µl of chromogen into each well. Incubate the plate for 15 min at room temperature in the dark. Following the incubation , the reaction is stopped by adding 50µl of stop solution to each well.

After careful mixing (soft tapping on the edge of the plate), the absorbance is measured in a microplate reader at 450 nm.

Highly positive patient samples can cause dark precipitates of the chromogen

Expected Control Values

For the quality control, positive control and negative control (double determination) must be carried along with each test procedure. The test was carried out correctly, if the positive control shows an absorbance value (O.D) at 450 nm greater than 0.8 . The mean absorbance value of the negative control at 450 nm must show a value lower than 0.3. If the single measurements diverge form the mean absorbance value more than 25%, the test must be repeated.

Calculation of Results

a. Calculate the average O.D. of the negative controls.

b. Add 0.150 to the value above. This value is the cut-off value of the assay.

c. Divide the sample O.D. by the value

obtained in point b.

A ratio greater than 1.1 indicates a positive sample ;

A ratio lower than 0.9 indicates a negative sample.

A ratio between 0.9 and 1.1 indicates an equivocal result.

An equivocal result must be retested with a fresh new sample. In case the same equivocal result is obtained, the test must be repeated with a new sample after 2-4 weeks.

e.g.:

Negative control well 1 O.D. = 0.115

Negative control well 2 O.D. = 0.125

Sample O.D. = 0.508

$$\text{Cut-off value} = \frac{0.115 + 0.125}{2} + 0.150 = 0.270$$

$$\text{Sample ratio} = \frac{0.508}{0.270} = 1.88 > 1.1, \text{ positive !}$$

Limitations of the test

The Cysticerciosis EIA kit detects specifically antibodies against the larval forms of Taenia solium. It should be used in suspected cases of trichinosis. Assay results should always be interpreted in connection to the clinical diagnosis and other diagnostic results.

Antibody response is highly variable in regard to parasite location and each individual.

Negative findings cannot exclude a cysticercosis. Due to low antibody titer at a very early time of infection, the test can show negative or equivocal results. If a clinical suspicion subsists, after 2 to 4 weeks another patient's sample should be tested.

Significant cross reactivity has been reported with Echinococcus infections. A positive result does not exclude the presence of tother pathogens.

Rev. Leuven, 10.00

Cysticercosis Ab ELISA Ref. HP001

QUICK REFERENCE TEST PROCEDURE

1. Bring all reagents to room temperature.

2. Dilute the washing buffer

3. Dilute the serum samples.

4. Pipet 50µl of the positive control, of the negative control (in duplicate) and the diluted samples into the microwells.

5. Incubate 15 minutes at Room Temperature (20-25°C)

6. Throw the well content and wash 5 times with 300µl of washing buffer.

7. Add 50µl of protein A conjugate.

8. Incubate 15 minutes at Room Temperature (20-25°C)

9. Throw the well content and wash 5 times with 300µl of washing buffer.

10. Add 50 µl each of substrate and chromogen.

11. Incubate 15 minutes at Room Temperature (20- 25°C) in the dark.

12. Add 50 µl of Stop Reaction Solution to each well.

13. Read Optical Densities with an ELISA reader with a 450 nm filter.