Code: HP006 toxocara Ab
96 Test

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Toxocara Ab

Toxocara

Toxocara is an enzyme immunoassay for the detection and semi-quantitative measurement of antibodies Anti - Toxocara Canis in human serum.

General Information

Toxocariasis is an infection caused by migration of roundworm Toxocara larvae to organs and tissues.

Although the genus Toxocara includes many species including T. cati (roundworm of the cat), only T. canis (roundworm of the fox and dog) has been consistently implicated in the disease. Although Toxocara larva migrans has historically been under-reported, it is now believed to be the second most common helminth infection in developed countries.

It is usually acquired by ingestion of soil contaminated with embryonated eggs of Toxocara. Eggs reach the soil through faces of infected cats or dogs (studies of United Kingdom's parks showed 24% of soil samples with eggs). They need from 1 to 3 weeks incubation to get infectious, but they remain viable and infectious on the soil for many months. Desiccation affect them unfavorably.

After their ingestion embryonated eggs free in the gut the larvae, which go across gut's wall and migrate to the liver or lungs through lymphatic and circulatory systems.

Larvae spread from lungs to several organs and damage them by migration and induction of granulamatous lesions.

The disease manifests itself as two distinct forms : visceral larval migrans(VLM) and ocular larval migrans(OLM).

Signs and symptoms of VLM vary from an asymptomatic state with mild eosinophilia to a severe and potentially fatal disorder, including hepatomegaly, hyperglobulinaemia, pulmonary symptoms and fever. In serious cases, the leucocytes count can reach 100.000/mm^3 or more, with 80 - 90% eosinophils.

Symptoms can persist for one year or more. Also pneumonitis or neurological disorder can appear.

Patients with OLM also vary widely in presentation, from asymptomatic states to acute lesions in the eye, including endophthalmitis, caused by penetration of larvae in the eyes, with vision loss on the affected eye ; a retina- damage similar to retinoblastoma can lead to unnecessary eye enucleation.

Incubation period may range from weeks to months, depending on infection intensity, reinfection and patient sensibility.

Distribution is probably worldwide. In the United States and the United Kingdom the disease has been target of greater attention, but there is probably not more prevalence than in other countries. The grave form occurs sporadically in isolated cases within a family, affecting rather children from

14 to 40 months old, though occurs even to older children. Very few times, adults are infected. That lower incidence in adults and older children is rather related with a less exposition to the disease.

Disease reservoir consists of dogs and cats. Almost all cubs are infected by larvae transplacentary and transmammary migration. At 3 to 4 weeks old, the cubs begin to expel eggs in faeces.

The demonstration of the toxocara larvae by means of liver biopsy confirms the clinical diagnosis, but is rarely justified.

Since there is no definitive method to diagnose Toxocara infections, true sensitivity and specificity of serologic tests cannot be accurately determined.

The diagnosis is further complicated by the fact that the antibody response varies widely depending on worm burden and location.

However, numerous studies have shown that immunoassays using a purified excretory antigen from the larval stage have shown dramatically improved sensitivities and specificities when compared to using crude antigens.

Principle of the test

The Cypress Diagnostics' Toxocara semi-quantitative test is based on the principle of an enzyme Immunoassay.

On the surface of the microtiter wells, inactivated excretory antigens of Toxocara canis 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 to the wells 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. Wear disposable gloves .

2. Hydrogen peroxide can cause cauterization. Handle with care. The stop solution contains 1 M sulfuric acid. Avoid contact with skin and clothing.

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.

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

Material provided

12 breakable Microwell strips with 8 wells each in a frame coated with inactivated Toxocara canis antigen , in a resealable foil bag.

1 vial Sample Buffer (50 ml, pH 7.4), ready to use dyed yellow, colorless lid.

1 vial Washing Buffer (50 ml, pH 7.4) 20 x conc., dyed blue, brown lid.

1 vial Positive Control. Volume : 1,2 ml. , red lid, inactivated Human Sera. Ready to use.

1 vial Negative Control. Volume : 1,2 ml. inactivated Human Sera. Colorless lid Ready to use.

1 vial Protein A Conjugate (6 ml) red lid. HRP-conjugated Protein A, ready to use.

1 vial Substrate (6 ml) green lid , urea peroxide, ready to use.

1 vial Chromogen (6 ml) , blue lid. tetramethylbenzidine (TMB) ready to use.

1 vial Stop Solution 6 ml, yellow lid, 1 M sulfuric acid

1 instruction manual.

Material needed but NOT provided

Micropipettes for volumes of 10 µl - 100 µl. and 100µl – 1000µl.

Single or double distilled or deionized water

Test tubes

Measuring cylinder (1000 ml)

Manual or automatic microwell washer.

Vortex mixer

Absorbent paper

Elisa-reader with a 450 nm filter.

Stability

Reagents must be stored at temperatures between 2°C and 8°C.

Expiration date is the last day of the month of the expiration date found on the box, vials and microstrips labels.

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

Exposure of the reagents to ambient temperatures for short periods does not affect the stability of the reagents. 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 directly at 2°C-8°C.

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

Indication of instability or deterioration of reagents.

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. Serum may be stored at 2°-8°C for op to 1 week. If longer storage is desired, store at -20°C for up to one(1) year. Avoid repeat freezing and thawing of samples, as well as microbial contamination, it may cause false results.

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

Reagents preparation

Vial 5 : Washing Solution. Concentrated 20 times.

Dilute 1 volume of washing solution in 19 volumes of distilled water.

Wash concentrated solution may show crystallization upon storage at 2°-8°C, that will disappear after dilution to working strength or warming in a waterbath 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 between 2°C and 8°C.

Test Procedure

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 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.

1.Preparation of the samples. Before starting the test, serum samples have to be diluted 1 : 50 with the sample buffer.

e.g. 10 µl serum + 490µl sample buffer

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

2.Add 50µl of diluted sample or controls to the appropriate well on the microwell plate. It is recommended to run duplicate of the negative control for greater precision in the results.

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

4.Decant or aspirate all microwells into a waste container with a desinfectant. Ensure complete removal of the liquid form the microwells by tapping the inverted plate onto absorbent paper. Wash the plate 5 times as follows : Add 300µl of diluted washing solution to each well. Discard the content of the wells, turning rapidly the plate over a biohazard container; next, strike the plate, still turned down, against an absorbent or blotting paper. Repeat the above procedure 4 more times.

5.Add 50µl of Protein A conjugate to each well. Gently shake for 15 seconds.

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

7.At the end of the incubation period, discard the content of the wells as outlined in step 4.

Wash the plate 5 times as described in step 4.

8.Add 50 µl of substrate to each well and add 50 µl of chromogen to each well. Gently shake for 10 seconds.

9. Allow the reaction to develop in the dark (e.g. cupboard or drawer ; the chromogen is light sensitive) for 15 minutes at Room Temperature (20°-25°C).

10. Stop the enzyme reaction by adding 50µl of Stop Solution to each well. After careful mixing (soft tapping on the edge of the plate) the absorbance is measured.

15. Read photometrically with the aid of an Elisa reader at a wavelength of 450 nm. Blank it on air.

Remark : Highly positive patient samples can cause dark precipitates of the chromogen.

Quality Control

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

Calculation of Results

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

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

c. Divide the sample O.D. by the value obtained in point b.

d. A ratio :

greater than 1.1 indicates a positive sample

ower than 0.9 indicates a negative sample

between 0.9 and 1.1 indicates an equivocal result.

An equivocal sample 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.

Expected normal values

Each laboratory must establish its own normal ranges based on patient populations and specific geographic areas. Degree of individual antibody response will depend upon such factors as worm burden, time since infection and area of the body infected. Differences in assay techniques can cause variation in the normal values range between laboratories.

e.g. calibrator well 1: O.D. = 0.541
calibrator well 2: O.D. = 0.554
Sample = 1.441

$$\text{Cut off value} = \frac{0.541+0.554}{2} = 0.548$$

$$\text{Sample ratio} = \frac{1.441}{0.548} = 2.63$$

Interpret the results using this sample ratio as follows:

sample ratio < 0.9 = negative result
0.9 < sample ratio < 1.1 = equivocal result
1.1 < sample ratio < 1.5 = low positive result
1.5 < sample ratio < 2.5 = medium positive result
2.5 < sample ratio = high positive control

Limitation of the test

Because serologic methods such as EIA are retrospective indicators of infection by detecting specific IgG antibodies, the results must not be used as the sole criterion for diagnosis.

Results must be interpreted in the context of the total clinical setting, and evaluated as supplementary information.

In cases of samples with a negative result, where a possible infection is suspected, an additional sample should be tested two or three weeks after the initial sample was drawn.

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

This test is a screening procedure only. A positive test result should be investigated further by other methodologies.

Although no specific cross-reactions with other antigens have been recorded to date, reactions with similar organisms cannot be ruled out.

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QUICK REFERENCE TEST PROCEDURE

1. Bring all reagents to room temperature.

Dilute washing solution .

2. Dilute the samples 1 : 50 using the sample diluent.

3. Add 50 µl of diluted samples or controls to each well.

Gently shake for 15 seconds.

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

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

6. Add 50 µl of of Protin A conjugate to each well.

Gently shake for 15 seconds.

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

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

9. Add 50 µl of substrate to each well.

Add 50 µl of Chromogen to each well.

Gently shake for 10 seconds.

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

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

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