CYPRESS DIAGNOSTICS

Code: HP002 echinococcosis Ab
96 test

distribuito da:
effegiemme diagnostici

Echinococcosis Ab.

Echinococcus IgG test is an enzyme immunoassay for the detection of specific IgG antibodies against Echinococcus in human serum.

2.General Information

Echinococcosis (hydatidosis) is the infection caused by the cestodes of the genus Echinococcus. At the moment four species are known from which three cause different forms of echinococcosis in humans: E.granulosus, E.multilocularis and E.vogeli (only parts of Middle and South America). The life cycle of these worms include two mammalian hosts. The definitive host, where the adult stage occurs, is a carnivore. After ingestion of eggs by herbivores or omnivores, the larvae develop to hydatides and metacestodes in these intermediate hosts. The life cycle is completed, when carnivores devour infected organs of the intermediate hosts.

Humans become infected by egg ingestion and act as accidental host. The infection caused by E. granulosus is known as cystic hydatid disease (CHD) and manifests itself as cysts in various organs, especially the liver and lungs. These cysts may become quite large and contain hundreds or thousands of metacestodes (protoscoleces, part of hydatid sand. The degree of antibody response to the cyst is mediated by its location and degree of calcification. Normally, liver cysts produce a higher antibody response than lung cysts.

E. multilocularis infection is known as alveolar hydatid disease (AHD). The hydatides are much smaller and form an alveolar structure which spread cancerously throughout the infected tissue.

Since no Echinococcus eggs are shed by infected humans, serological determinations are of great importance in the diagnosis of hydatid disease.

Cross reactivity exists to antibodies against cysticercus (Taenia solium) an may complicate diagnosis like in any serological test; Due to this, it is recommended that any sample showing a positive result by ELISA should be confirmed by additional testing, including other serological methods and clinical findings.

3.Principle of the test

On the surface of the microtiter wells, inactivated soluble antigens of Echinococcus 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 peroxidase-conjugated anti-human antibody (anti-IgG) is added. 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 (optional: reference wavelength $\geq$ 620nm).

4.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).

5.Material provided

The reagents in one package are sufficient for 96 determinations. Each test kit contains:

- 1 X 12 Microwell Strips (breakable) with 8 wells each in a frame; coated with Echinococcus cyst soluble antigen; in a resealable foil bag

- 1 X Sample Buffer (50 ml, pH 7,4), ready to use, dyed yellow, colorless lid

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

- 1 X Positive Control (1.2 ml), red lid; inactivated human serum, ready to use.

- 1 X Negative Control (1.2 ml), colorless lid; inactivated human serum, ready to use.

- 1 X Anti-human-IgG-Conjugate (6 ml), red-marked lid; HRP-conjugated mAb (mouse), ready to use

- 1 X Substrate (6ml), green-marked lid; urea peroxide, ready to use

- 1 X Chromogen (6 ml), blue- marked lid; tetramethylbenzidine (TMB), ready to use

- 1 X Stop Solution (6 ml), white lid; 1 N sulfuric acid

- 1 X instructions for use

6.Reagents required but not provided

6.1. Reagents

Distilled or deionized water

6.2. Accessories

- Test tubes

- Vortex mixer

- Micropipets for volumes of 10-100 µl and 100- 1000µl

- Measuring cylinder (1000ml)

- Microplate washer or multichannel pipet

- Microplate reader (450 nm, optional: reference wavelength $\geq$ 620 nm)

- Absorbent paper

7. Warnings and precautions

The control sera (positive and negative control) have been tested for HIV-and HCV-Ab as well as for HbsAg and were found to be negative. However, they as well as the patient samples should be considered potentially contagious and be treated with the necessary safety precautions.

Urea peroxide can cause cauterization. Handle with care.

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

All reagents and materials coming in contact with potential infectious specimens must be treated with disinfectants or autoclaved at 121°C for at least one hour.

An exchange of reagents between kits of different lot numbers is not possible.

8. Storage instructions

All reagents have to be stored at 2° - 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 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 autooxidation. If the chromogen has turned blue, the reagent should be discarded.

9.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

10. Specimen collection and storage

The Cypress Diagnostics Echinococcus IgG EIA has been evaluated for the investigation of human serum samples. 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.

11.Test Procedure

11.1.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 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 reagents are ready to use.

11.2 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 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 at 2-8°C.

11.3 Preparation of the samples

Before starting the test, serum samples have to be diluted 1:25 with the sample buffer.

e.g. 10 µl serum + 240 µl sample buffer

Attention!

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

11.4 First incubation

After insertion of a sufficient number of cavities into the microwell holder, 50 µl of the postive 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.

11.5 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.

If a microplate washer is used, take care that the washer is adjusted to the used microplate type.

11.6 Second incubaton

Add 50 µl for 1 drop of anti-human-IgG-conjugate to all wells. Incubate the plate for 15 min at room temperature.

11.7 Washing

Wash 5 times according to step 10.5

11.8 Third incubation

Add 50 µl or 1 drop of substrate and 50 µl or 1 drop 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 50 µl or 1 drop 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 (optional: reference wavelength $\geq$ 620 nm)

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

Summary of the test procedure

1. Bring all reagents to room temperature.

2. Dilute the washing buffer

3. Dilute the serum samples

4. Pipet 50 µl of the positive control, the negative control (in duplicate) or the diluted samples into the microwells; 15 minutes incubation at room temperature

5. Discard the incubate and wash 5 times with 300 µl of washing buffer

6. Add 50 µl or 1 drop of anti-human-IgG-conjugate; 15 minutes incubation at room temperature

7. Discard the incubate and wash 5 times with 300 µl of washing buffer

8. Add 50 µl or 1 drop each of substrate and chromogen; 15 min incubation at room temperature in the dark

9. After addition of 50 µl or 1 drop stop solution spectophotometric determination at 450 nm (optional: reference wavelength $\geq$ 620 nm)

12. Analysis

12.1 Quality control

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 from the mean absorbance value more than 25%, the test must be repeated.

12.2 Calculation of the sample ratio

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

2. Add 0.150 to the average O.D. The result is the cut-off value of the assay.

3. Calculate the sample ratio by dividing the sample O.D. through the cut-off value.

E.g. negative control well 1 O.D.=0.115
negative control well 2 O.D.=0.125
sample O.D.=0.508

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

$$\text{sample ratio} = \frac{0.508}{0.270} = 1.88$$

12.3 Test result

Tab.1: Valuation of sample ratio

	negative	equivocal	positive
Sample ratio	< 0.9	0.9-1.1	>1.1

13.Remarks about the test procedure and interpretation

The Cypress Diagnostics Echinococcus IgG EIA detects antibodies against the species of Echinococcus. It should be used in suspected cases of echinococcosis. 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 antibody findings cannot exclude an echinococcosis. Due to a low antibody titer at early time of an infection, the test can show negative or equivocal results. If a clinical suspicion subsists, after two to four weeks another patient's sample should be tested.

Significant cross reactivity has been reported with Taenia solium infections. A positive result does not exclude the presence of other pathogens.

Echinococcosis Ab ELISA Ref. HP002

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.

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