

Echinococcus IgG ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Determination of *Echinococcus granulosus* IgG
level in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-Hydatid-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Echinococcus spp. are 1-6 mm long cestode parasites with 3 to 5 segments. The two species *E. granulosus* and *E. multilocularis* are principally responsible for human disease. These species are etiologic agents of Hydatid disease a zoonotic infection of worldwide distribution in man and other intermediate hosts. The parasite mainly affects canines like wolves, dogs, foxes as its definitive host and man as intermediate host also infected coincidentally by contact with dog feces. The adult worms live in small intestine of definitive host and their mature proglottids containing numerous eggs are released into the environment through the host feces. If eggs are ingested by intermediate host like human, sheep, etc.. they will hatch in small intestine and released oncospheres will penetrate into the lumen wall and entered into the mesenteric blood circulation. They carried throughout the body by blood circulation and mainly affect liver and lungs where they produce Hydatid cysts. The cysts also have been reported from other body organs like brain, bones, heart and other organs. Most of the patients are asymptomatic for years but symptoms of disease arise from compression of adjacent host structures due to gradually enlarging hydatid cyst. Echinococcosis is potentially dangerous disease of human and untreated cases are highly fatal.

Nowadays various serological tests including IHA, IFA and ELISA have been used for serological diagnosis of hydatid disease.

The degree of immune response not only affect by the parasite but other factors like cyst location and size also affect the degree of response. Studies have been shown that bone and liver cysts have higher antibody response than those located in lungs, spleen and brain.

Test Principle

The test principle is based on indirect ELISA test. In this technique, microplate wells are coated with certain amount of Echinococcus antigens. Diluted serum samples are added into the microplate wells and allowed to react with Echinococcus antigens. If specific antibodies were presented in serum they will bind to the solid phase Echinococcus antigens. After washing the microplate wells and removing unbound antibodies, HRP labeled secondary antibody (anti –human IgG- horseradish peroxidase (HRP) conjugate) is added into the wells. The wells are washed again with washing solution to remove unbound labeled antibodies. A solution of TMB is then added and incubated for 15 minutes, resulting in the development of a blue color which is directly proportional to immune complex formation in the wells. The color development is stopped by the addition of stop solution and measured spectrophotometrically at 450 nm.

Materials provided with the kit:

1. Antigen coated wells(1 plate, 96 wells):microtiter wells coated with *Echinococcus granolusus* antigens.
2. Sample diluent (2 vials, each 50 ml) : Contains phosphate buffer solution with protein as stabilizer and Cathon CG as preservative.
3. Enzyme conjugate (1vial, 12 ml) : Contains polyclonal anti human IgG labeled with HRP, ready to use.
4. Negative control (2 ml / vial) : Negative pooled sera in buffer containing protein as stabilizer and Cathon CG as preservative, ready to use.
5. Positive serum control (1 ml / vial): Positive pooled sera contains anti *Echinococcus granolusus* antibodies diluted in buffer containing protein as stabilizer and Cathon CG as preservative. Ready to use.



6. Wash solution (1 vial, 50 ml) : Contains phosphate buffer solution with 0.05 % tween 20 as detergent, pH=6 concentrated (20X).
7. Chromogen substrate reagent (1 vial, 12 ml) : Contains tetramethyl benzidine and hydrogen peroxide, ready to use.
8. Stop solution (1 vial / 12 ml) : Contains 1 molar hydrochloric acid, PH< 1.
9. Cardboard sealer.

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C.

Materials required but not provided:

- ELISA reader with 450 nm filter.
- Precision micropipettes.
- Distilled water.
- Disposable pipette tips.
- Absorbent paper or paper towel.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.
3. Dilute specimens 1:101 with sample diluent (i.e. 10 µl of specimen with 1 ml of sample diluent).

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, anti HCV and anti HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Assay Procedure

1. Secure the desired number of microplate wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Dispense 100 µl of serum controls (the serum controls are ready to use) and diluted specimen samples in appropriate wells according to following order:
 - Use the first well as blank (BL)
 - Use the next two wells for negative control (NC).
 - Use one well for the positive control (PC).
 - Use the remaining wells for specimens.
3. Cover the microplate wells with cardboard sealer tightly. Incubate wells for 30 min. at room temperature (R.T).
4. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of working wash solution).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.

Storage Condition

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use reagents beyond the kit expiration date.

Specimen Collection and Preparation



6. Add 100 µl of Enzyme conjugate solution into the microplate wells except well labeled "blank".
7. Cover the plate with cardboard sealer tightly. Leave wells for 30 minutes at room temperature.
8. Repeat steps 4 and 5.
9. Dispense 100 µl of chromogen/substrate to each well.
10. Incubate the microplate wells at room temperature and dark for 15 minutes.
11. Add 100 µl of stop solution to the wells to stop reaction.
12. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available).

3. Those samples with OD values of higher than cut-off value must be considered as positive for specific anti-Echinococcus IgG antibody.
4. Those specimens with OD values of lower than cut-off value should be considered as negative for specific anti-Echinococcus IgG antibody.
5. Those specimens with OD values of too close to cut off value should be re-tested with fresh specimen after a 2-4 weeks to rule out possible Echinococcus infection.

Validity of the Assay

The assay is to be considered valid if:

1. The OD 450 nm of the blanking well is lower than 0.1. Higher values indicate chromogen /substrate contamination. In such a case, repeat the assay carefully checking the reagent.
2. After subtracting the blank, the mean OD value for the Negative control is lower than 0.20. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The value of positive control is higher than the 0.6 OD 450nm. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available) Subtract OD value of blank from all controls and tests OD.
2. to calculate cut off value for the test, use following formulae:

$$\text{Cut-off} = \text{N.C. mean OD (450 nm)} + 0.25$$

Performance Characteristics

Sensitivity & Specificity

A total of 36 patients suspected Echinococcus infection with clinical signs and symptoms related to Hydatid cyst disease were evaluated. Of these, 11 were confirmed positive and 25 were negative by commercial ELISA kit. The ELISA test results were compared to the commercial kits.

		Pishtaz teb ELISA kit		
Commercial ELISA kit		Positive	Negative	Total
	+	10	1	11
	-	1	24	25
Total				36

Relative Sensitivity = $10 / 11 = 91\%$

Relative Specificity = $24 / 25 = 96\%$

Relative Accuracy = $34 / 36 = 94\%$

Correlation Test

175 patient sera were tested by Pishtaz teb ELISA kit and a reference ELISA kit. 5 sera were positive and 167 were negative by both methods (98 % agreement).

		Hydatid IgG ELISA kit Pishtaz teb		
Reference ELISA kit		+	-	Total
	+	5	2	7
	-	1	167	168
Total		6	169	175



3. Reproducibility

It has been calculated on the negative and positive controls tested in replicates in different days. CV's between 3-10% have been obtained dependent on their OD values at 450 nm .

Table no. 1 (Intra-assay)

	No. of Tests	Means OD	SD OD	CV%
Negative control	24	0.06	0.004	6.6
Positive control	24	2.35	0.074	3.1

Table No. 2 (Inter-assay)

	No. of Tests	Means OD	SD OD	CV%
Negative control	10	0.07	0.0069	9.8
Positive control	10	2.41	0.108	4.4

*Each test has been run in duplicate




References:

- 1-Craig, P. S., M. T. Rogan, and M. Campos-Ponce. 2003. Echinococcosis: disease, detection and transmission. Parasitology 127(Suppl.):S5-S20.
- 2-Eckert, J., F. J. Conraths, and K. Tackmann. 2000. Echinococcosis: an emerging or re-emerging zoonosis? Int. J. Parasitol. 30:1283-1294.
- 3-Gonzalez-Sapienza, G., C. Lorenzo, and A. Nieto. 2000. Improved immunodiagnosis of cystic hydatid disease by using a synthetic peptide with higher diagnostic value than that of its parent protein, *Echinococcus granulosus* antigen B. J. Clin. Microbiol. 38:3979-3983.



Echinococcus IgG ELISA Test Procedure

Step 1




Reagent	Blank	Serum Control	Sample
			
Serum Control	None	100 µl	None
Diluted Sample	None	None	100 µl

Cover the microplate wells with cardboard sealer tightly and incubate them for 30 minutes at R.T.


Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.






Enzyme conjugate			
	None	100 µl	100 µl

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at RT.


Step 3




Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.



Chromogen-substrate solution			
	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature and dark.


Step 4

Stop Solution			
	100 µl	100 µl	100 µl

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).

