



7018 Owensmouth Ave. Suite 103
 Canoga Park, CA, 91303
 Phone: 818-710-1281
 Fax: 818-936-0121
 Email: Info@immunospec.com
www.immunospec.com

Echinococcus

REF

Catalog No.E21-179

IVD

For In Vitro Diagnostics Use

Intended Use

For the qualitative screening of serum IgG antibodies to *Echinococcus* sp. using an Enzyme-Linked Immunosorbent Assay (ELISA) technique.

Summary

Immunospec Echinococcosis (hydatidosis) is the infection caused by cestodes of the genus *Echinococcus*. Humans are potential intermediate hosts and can become infected by ingesting eggs passed in the feces of an infected animal. The resulting disease is called hydatidosis, or hydatid disease.

Four species are known pathogens of the disease: *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli*. The infection caused by *E. granulosus* is referred to as cystic hydatid disease (CHD) and results in cysts in various organs, especially the liver and lungs. These cysts may become quite large and contain hundreds or thousands of scoleces called hydatid sand. The degree of antibody response to these cysts will vary depending on their location and degree of calcification. Liver cysts typically produce a higher antibody response than lung cysts. Infection due to *E. multilocularis* is referred to as alveolar hydatid disease (AHD), and also occurs as cysts that may spread throughout the infected tissue.

Since *Echinococcus* eggs are not shed by infected humans, serological determination has been important in the diagnosis of hydatid disease. A number of tests have been used, including latex agglutination (LA), indirect hemagglutination (IHA), complement fixation (CF), agar gel diffusion (AGD) and enzyme linked immunosorbent assay (ELISA).

Cross reactivity between echinococcosis and cysticercosis (*Taenia solium* infection) will occur to some degree in this assay due to the use of crude antigen. It is recommended that any sample showing a positive result by this test be confirmed by additional testing.

Principle of Procedure

The micro test wells are coated with a crude antigen from an *Echinococcus* cyst. During the first incubation with the diluted patients' sera, any antibodies which are reactive with the antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethylbenzidine or TMB) is added. If the Enzyme Conjugate is present, the peroxidase

will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

Reagents

Test Strips	Microwells containing <i>Echinococcus</i> antigens - 96 test wells in a test strip holder.
Enzyme Conjugate	One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.
Positive Control	One (1) vial containing 2 ml of diluted positive rabbit serum.
Negative Control	One (1) vial containing 2 ml of diluted negative human serum.
Chromogen	One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
Wash Concentrate (20X)	One (1) bottle containing 25 ml of concentrated buffer and surfactant.
Dilution Buffer	Two (2) bottles containing 30 ml of buffered protein solution.
Stop Solution	One (1) bottle containing 11 ml of 1 M phosphoric acid.

Precautions

Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2-8 °C. Crystallization will disappear after dilution to working strength.

Do not use serum that may have supported microbial growth, or is cloudy due to high lipid content. Samples high in lipids should be clarified before use.

Treat all sera as if capable of being infectious. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV by required test methods. This product should be used under appropriate safety conditions that would be used for any potentially infectious agent.

Do not add azides to the samples or any of the reagents.

Storage Conditions

Reagents, strips and bottled components:

Store between 2 – 8 °C.

Squeeze bottle containing diluted wash buffer may be stored at room temperature.

Preparation

Wash Buffer - Remove cap and add contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with a narrow tip opening.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling.

Avoid generating bubbles in the wells during the washing steps.

Collection and Preparation of Serum

Coagulate blood and remove serum. Freeze sample at -20 °C or lower if not used immediately.

Do not heat inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: Make a 1:64 dilution of patients' sera using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

Procedure

Materials Provided

Echinococcus Serology Microwell ELISA Kit

Materials Required But Not Provided

Pipettes
Squeeze bottle for washing strips (narrow tip is recommended)
Reagent grade water and graduated cylinder
Tubes for sample dilution
Absorbent paper

Suggested Materials

ELISA plate reader with a 450 nm and a 650 to 620 nm filter (optional if results are read visually)

Performance of Test

1. Break off number of wells needed (two for controls plus number of samples) and place in strip holder.
2. Add 100 µl (or two drops) of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1:64) test samples to the remaining wells.
Note: Negative and positive controls are supplied prediluted. Do not dilute further.
3. Incubate at room temperature (15 to 25 °C) for 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 2 drops of Enzyme Conjugate to each well.
6. Incubate at room temperature for 5 minutes.
7. Shake out contents and wash 3 times with wash buffer.
8. Slap wells vigorously against paper towels to remove excess moisture.
9. Add 2 drops of the Chromogen to every well.
10. Incubate at room temperature for 5 minutes.
11. Add 2 drops of the Stop Solution and mix by tapping strip holder.

Reading of Results

Visually: Look at each well against a white background (e.g. paper towel) and record as clear or +, ++ or +++ reaction.
ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/650-620 nm.

Test Limitations

Serologic results are an aid in diagnosis but cannot be used as the sole method of diagnosis.

Quality Control

The use of controls allows validation of kit stability. The kit should not be used if any of the controls are out of range. Expected values for the controls are:

Negative - 0.0 to 0.3 OD units
Positive - 0.5 OD units and above

Troubleshooting

Negative control has excessive color after development.

Reason: inadequate washings.

Correction: wash more vigorously. Remove excessive liquid from the wells by tapping against an absorbent towel. Do not allow test wells to dry out.

Interpretation of Results - ELISA Reader

Zero ELISA reader on air. Read all wells at 450/650-620 nm.

Positive - Absorbance reading equal to or greater than 0.3 OD units.

Negative - Absorbance reading less than 0.3 OD units.

A negative OD reading indicates that the patient has no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.

Interpretation of Results -Visual

Compare results to the controls. A sample should be interpreted as positive if the degree of color development is significant and obvious.

Expected Results

The number of individuals showing positive results can vary significantly between populations and geographic regions. If possible, each laboratory should establish an expected range for its patient population.

Performance Data

Study #1 – CDC&P

		CDC&P	
		+	-
IMMUNOSPEC	+	46	1
	-	1	11

Sensitivity of 97.8% (46/47)

Specificity – Normal 91.6% (11/12)

Liver Cancer 66.6% (8/12)

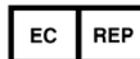
Liver Abscess 66.6% (6/9)

Strongyloides 50% (3/6) (Strong cross reaction)

Ascaris (0/6) (Strong cross reaction)

References

1. Schantz, P. and B. Gottstein. "Echinococcosis (Hydatidosis)." *Immunodiagnosis of Parasitic Diseases, Vol. 1, Helminthic Diseases*. Ed. Walls and Schantz. Academic Press, 1986. pp. 69-107
2. Krogstad D., G. Visvesvara R. Walls, J. Smith. "Tissue Helminths." *Manual of Clinical Microbiology*, 4th Ed.. American Society for Microbiology, Washington, DC. 1985, pp. 657-659
3. Kagan, I. "Serodiagnosis of Parasitic Diseases." *Manual of Clinical Laboratory Immunology*. 3rd Ed.. American Society for Microbiology, Washington, DC. 1986, p. 472
4. Ash, L. and T. Orihel. "Parasites - A Guide to Laboratory Procedures and Identification." 1987, ASCP Press, pp. 176-177
5. Coltorti, E. "Standardization and Evaluation of an Enzyme Immunoassay as a Screening Test for the Seroepidemiology of Human Hydatidosis." *Am J Trop Med Hyg* #35(5), 1986, pp. 1000-1005
6. Coltorti, E. et. al. "Field Evaluation of an Enzyme Immunoassay for Detection of Asymptomatic Patients in a Hydatid Control Program." *Am J Trop Med Hyg*, #38(3), 1988, pp. 603-607



European Authorized

Representative:

CEpartner4U, Esdoornlaan 13, 3951DB Maarn
The Netherlands. Tel.: +31 (0)6.516.536.26



Manufacturer:

IMMUNOSPEC CORPORATION
7018 Owensmouth Ave.#103
CanogaPark, CA. 91301 USA
(818) 710-1281

PLE21-179

Revision date: 12/2010

PN2050