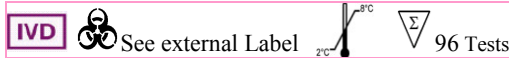


AccuDiag™
Echinococcus IgG
ELISA Kit

REF 8202-35



Test	Echinococcus IgG ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Qualitative : Positive, Negative Control
Sample	5 µL serum
Total Time	~ 20 min.
Shelf Life	12 Months from the manufacturing date
Specificity	91.7%
Sensitivity	97.9%

INTENDED USE

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

SUMMARY AND EXPLANATION

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.

TEST PRINCIPLE

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.

SPECIMEN COLLECTION AND PREPARATION

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).

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing Echinococcus antigens - 96 test wells in a test strip holder.
2. **Enzyme Conjugate:** One (1) bottle containing 11 ml of Protein A conjugated to peroxidase.
3. **Positive Control:** One (1) vial containing 1 ml of diluted positive rabbit serum.
4. **Negative Control:** One (1) vial containing 1 ml of diluted negative human serum.
5. **TMB Substrate Solution:** One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB).
6. **Wash Concentrate 20X:** One (1) bottle containing 25 ml of concentrated buffer and surfactant.
7. **Dilution Buffer:** Two (2) bottles containing 30 ml of buffered protein solution.
8. **Stop Solution:** One (1) bottle containing 11 ml of 1M phosphoric acid.

Materials required but not provided

1. Pippettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water and graduated cylinder
4. Tubes for sample dilution
5. Absorbent paper
6. ELISA plate reader with a 450 nm and a 620-650 nm filter (optional if results are read visually).

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.

ASSAY PROCEDURE

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 TMB 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.

Positive Agreement: 97.9% (46/47)
 Negative Agreement: 91.7% (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)

***CAUTION! WHEN USING AN AUTOMATED OR SEMI-AUTOMATED WASHING SYSTEM THE FOLLOWING MUST BE FOLLOWED. FAILURE TO DO SO WILL RESULT IN INADEQUATE WASHING OF THE WELLS AND MAY LEAD TO FALSE POSITIVE RESULTS!**

Washing Procedure for Auto and Semi-Automated Washers

- Perform five (5) washes per step instead of three
- Set machine to "soak" for one minute between each step
- After each set of washings, slap wells against an absorbent towel.

LIMITATIONS OF PROCEDURE

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

EXPECTED VALUES

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.

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 450/620-650 nm.

PRECAUTIONS

1. 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.
2. 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.
3. 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.
4. Do not add azides to the samples or any of the reagents.

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.

STORAGE

1. Reagents, strips and bottled components should be stored at 2-8 °C
2. Squeeze bottle containing diluted wash buffer may be stored at room temperature.

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.

REFERENCES

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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.

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

PERFORMANCE CHARACTERISTICS

Study #1 – CDC&

		Reference Method *	
		+	-
Diagnostic Automation, Inc.	+	46	1
	-	1	11



ISO 13485
 ISO 9001



**Diagnostic Automation/Cortez
 Diagnostics, Inc.**
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EC REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
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