Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Fasciola gigantica ELISA Kit

Cat # 8120-35

IVD See external Label 2°C 8°C 96 Tests

Test	Fasciola gigantica ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Sample	100 µL diluted sample
Total Time	~ 50 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	98.7%

INTENDED USE

The Diagnostic Automation, Inc. Fasciola gigantica ELISA test is an enzyme immunoassay for the qualitative screening of serum Ig antibodies to *Fasciola gigantic.*

SUMMARY AND EXPLANATION

Fasciola is a hermaphroditic trematode which causes the zoonotic disease Fascioliasis. Humans become infected with the disease by ingesting uncooked watercress and other aquatic vegetation on which metacercariae are encysted. Once inside the body, the metacercariae excyst in the small intestine and migrate into the peritoneal cavity through the intestinal wall. Eventually, the larvae enter the bile ducts and mature into adult worms and produce eggs.

Both *Fasciola hepatica* and *Fasciola gigantica* infect humans. *F. hepatica* typically occurs worldwide in temperate regions while *F. gigantica* tend to occur in more tropical areas. Where these regions overlap, *Fasciola* with characteristics of both *hepatica* and *gigantica* have been reported.

Both of DAI Fasciola kits have very strong cross reactions with *hepatica* and *gigantica* but do use different antigens that are more reactive with each individual species. Thus it is recommended that the lab determine the *Fasciola* species for its' testing population and choose that assay. For the region where both species overlap, it may be prudent to use both the *hepatica* and *gigantica* assays.

TEST PRINCIPLE

The micro test wells are coated with *Fasciola gigantica* ES antigen. During the first incubation with the diluted patients' sera, any antibodies that 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 and turn 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 º C or lower if not used immediately.

Do not heat inactivate serum and avoid repeated freezing and thawing of samples.
Test samples: Make a 1:100 dilution of patient's sera using the dilution buffer (e.g. 5 µl sera and 495 µl dilution buffer).

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. **Plate:** Microwells containing *Fasciola gigantica* ES antigens – 96 test wells in a test strip holder.
2. **Enzyme Conjugate**: One (1) bottle containing 11ml of anti-human IgG + IgM 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. **Chromogen:** 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 1 M phosphoric acid.

Materials required but not provided

1. Pipette
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water and graduated Cylinder
4. Sample Dilution Tubes
5. Absorbent paper

Suggested Materials

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

PRECAUTION

Do not use solutions if they precipitate or become cloudy. Wash concentrate may show crystallization upon storage at 2-8 ºC. 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 azide to the samples or any of the reagents.

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.

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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 of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1:100) 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 ºC) for 30 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 100 ul of Enzyme Conjugate to each well.
6. Incubate at room temperature for 10 minutes.
7. Shake out contents and wash 3 times with wash buffer. Slap wells against paper towels to remove all of the wash buffer.
8. Add 100 ul of the Chromogen to every well.
9. Incubate at room temperature for 10 minutes.
10. Add 2 drops of the Stop Solution and mix by tapping strip holder.

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.

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 to 620 nm.
Positive - Absorbance reading greater than 0.20 OD units.
Negative - Absorbance reading less than 0.20 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.

Visual

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

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.

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 0.09 OD units
Positive - 0.5 OD units and above

PERFORMANCE CHARACTERISTICS

		Reference Method *	
		+	-
Diagnostic Automation,Inc.	+	**18**	**1**
	-	**0**	**81**

Positive Agreement: 100% (18/18)
Negative Agreement: 98.7% (81/82)

*Reference Method refers to a reference Laboratories' internal ELISA.

CROSS REACTIVITY

This assay is strongly reactive with patient's positive for *Fasciola hepatica*. The choice of whether to use DAI's *Fasciola hepatica* or *Fasciola gigantica* should depend on the laboratories geography and patient population. (See Summary Section).

LIMITATIONS OF PROCEDURE

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

STORAGE CONDITIONS

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.

REFERENCES

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4. Hillyer, G. Fascioliasis and Fasciolopsiasis. Chapter 90, pp. 856-861.

ISO 13485
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EC REP	**CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu**

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