# Fasciola IgG ELISA

## Fasc-G-96

### Intended Use

For the qualitative screening of serum IgG antibodies to *Fasciola* using an Enzyme Linked Immunoabsorbant Assay (ELISA) technique.

### Summary

*Fasciola* is a hermaphroditic trematode which causes the zoonotic disease Fascioliasis.[1,2,4] 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. Larvae penetrate Glisson's capsule, enter the liver and stay in the liver parenchyma for up to 9 weeks. Eventually, the larvae enter the bile ducts and mature into adult worms and produce eggs.[1]

While in the body, pathological damage begins once the larvae enter the liver parenchyma. Hyperplasia of the bile ducts occurs, likely due to the toxins produced by the larvae. Once matured, the worms cause damage from metabolic by-products, mechanical irritation, and obstruction. Adult worms may be found elsewhere in the body besides the liver, for example in the gallbladder. The worms may also reenter the liver parenchyma and produce abscesses.[1] Some patients may be symptomatic within the first few weeks of infection however, no eggs will be present in feces until the worms have matured, which takes 8 weeks.[1,4] Symptoms include fever, anorexia, weight loss, anemia, diarrhea, and vomiting. Some patients will not experience any symptoms.[1-4] If a patient is found to be seropositve for Fascioliasis, the patient will remain seropositve for several years and will be cross reactive with Schistosomiasis.[1]

The life-cycle of *Fasciola* holds the key to preventing the spread infection. Adult worms within a host, in this case humans, produce eggs inside the body. These eggs are passed through the feces and enter the surrounding environment. The eggs are picked up by certain species of snails, which act as an intermediate host. From there, the cerearіae encyst onto water vegetation.[1] By stressing the dangers of eating uncooked water vegetation in endemic areas, the spread of infection could be reduced.[1,4] Recent estimates report that as many as 2.4 million people are infected worldwide.[3]

### Principle of Procedure

The micro test wells are coated with *Fasciola* 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) and a substrate (hydrogen peroxide) are 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

| Item | Description | Symbol |
|---|---|---|
| Test Strips | Microwells containing *Fasciola* antigens – 96 test wells in a test strip holder. | MT PLATE |
| Enzyme Conjugate | One (1) bottle containing 11ml of Protein A conjugated to peroxidase. | CONJ |
| Positive Control | One (1) vial containing 2 ml of a surrogate positive control. | CONTROL + |
| Negative Control | One (1) vial containing 2 ml of diluted negative human serum | CONTROL − |
| Chromogen | One (1) bottle containing 11 ml of the chromogen tetramethylbenzidine (TMB). | SUBS TMB |
| Wash Concentrate (20X) | Two (2) bottles containing 25 ml of concentrated buffer and surfactant. | WASH BUF |
| Dilution Buffer | Two (2) bottles containing 30 ml of buffered protein solution. | SPECM DIL |
| Stop Solution | One (1) bottle containing 11 ml of 1 M phosphoric acid. | SOLN |

## Precautions

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.

## Storage Conditions

Reagents, strips and bottled components:
Store between 2-8 ºC.
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 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).

## Procedure

**Materials Provided**
*Fasciola* 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 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 10 minutes.
4. Shake out contents and wash 3 times with the diluted wash buffer.
5. Add 100 µl 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. Slap wells against paper towels to remove all of the wash buffer.
8. Add 100 µl of the Chromogen to every well.
9. Incubate at room temperature for 5 minutes.
10. Add 100 µl of the Stop Solution and mix by tapping strip holder.

*** Washings consist of vigorously filling each well to overflowing and decanting contents three (3) separate times. If using automated washers; add 1 minute dwell time between washings and increase number of washes from three to five.**
**For Automated Use: Decrease run times to 7-4-3 minute steps.**

## 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 0.09 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 to 620 nm.
**Positive** - Absorbance reading greater than 0.2 OD units.
**Negative** - Absorbance reading less than 0.2 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 obvious and significant.

## 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

| | | Reference Method* | |
|---|---|---|---|
| | | + | - |
| New Life | + | 12 | 0 |
| | - | 0 | 50 |

Positive Agreement: 100% (12/12)
Negative Agreement: 100% (50/50)

*Reference Method refers to a commercially available ELISA.

Found no cross reaction to known positives of *T. cruzi.*

## References


1. Bruckner, D., Garcia, L. Diagnostic Medical Parasitology. 2nd Edition. American Society for Microbiology, 1993. pp. 309-317.
2. Sampaio Silvia, M. L. et. al. "Antigentic Components of Excretory-Secretory Products of Adult *Fasciola hepatica* Recognized in Human Infections". Am J Trop Med Hyg. Vol. 54 (Sup 3), 1996, pp. 146-148.
3. O'Neill, S. et. al. "Short Report: Immunodiagnosis of Human Fascioliasis using Recombinant *Fasciola hepatica* Cathepsin L1 Cysteine Proteinase". Am J Trop Med Hyg. Vol. 60 (Sup 5), 1999, pp. 749-751.
4. Hillyer, G. Fascioliasis and Fasciolopsiasis. Chapter 90, pp. 856-861.