# *Fasciola* IgM ELISA Kit

**Fasc-M-96**

## Intended Use

The *Fasciola* ELISA test is a semi-quantitative enzyme immunoassay for the detection of IgM antibodies to *Fasciola*, in samples of human serum or plasma. This test is intended to be performed by trained medical technologists only.

## Summary and Explanation

*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]

## Assay Principle

The microwells are coated with *Fasciola* antigen. 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

| Item | Description | Symbol |
|---|---|---|
| Test Strips | Microwells containing *Fasciola* recombinant antigens - 96 test wells in a test strip holder. | MT PLATE |
| Enzyme Conjugate | One (1) bottle containing 11 ml of anti-human IgM (μ chain specific) conjugated to peroxidase. | CONJ |
| Positive Control | One (1) vial containing 2 ml of diluted surrogate positive control. | CONTROL + |
| Negative Control | One (1) vial containing 2 ml of diluted human sera. | 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 with RF Absorbent. | SPECM DIL |
| Stop Solution | One (1) bottle containing 11 ml of 1 M phosphoric acid. | SOLN |

## Statement of Warnings

- **Do not deviate from the specified procedures when performing this assay**. All specimen dilutions, incubation times/temperatures and washings have been optimized for the best performance characteristics. Deviations from the specified procedures may affect the sensitivity and specificity of the assay.
- For *In Vitro* Diagnostic Use Only.
- Do not interchange reagents between kits with different lot numbers.
- Do not use reagents that are beyond their expiration dates. Expiration dates are on each reagent label. Use of reagents beyond their expiration dates may affect results.
- Unused microwells should be stored in the desiccated pouch to protect them from moisture.
- Do not use solutions if they precipitate or become cloudy.
  **Exception**: Wash concentrate may precipitate during refrigerated storage, but will dissolve upon warming.
- Do not add azides to the samples or any of the reagents.
- Controls and some reagents contain Thimerosal as a preservative, which may be irritating to skin, eyes and mucous membranes. In case of contact, flush eyes or rinse skin with copious amounts of water.
- 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 reagents and samples as potentially infectious materials. Negative control has been tested and found negative for Hepatitis B surface antigen and for the antibody to HIV be required test methods. Use care to prevent aerosols and decontaminate any spills of samples.
- Stop solution is a 5% solution of phosphoric acid in water. If spilled on the skin, wash with copious amounts of water. If acid gets into the eyes, wash with copious amounts of water and seek medical attention.

## Storage

- Reagents, strips and bottled components should be stored at 2-8 °C
- Squeeze bottle containing diluted wash buffer may be stored at room temperature (15-25 °C)

## Preparation

- Before use, bring all reagents and samples to room temperature (15-25 °C) and mix.
- (20X) Wash Concentrate may precipitate during refrigerated storage, but will go back into solution when brought to room temperature and mixed. ***Ensure that (20X) Wash Concentrate is completely in solution before diluting to working concentration.*** To dilute (20X) wash concentrate to working dilution, remove cap and add contents of one bottle of Wash Concentrate to a squeeze bottle containing 475 ml of DI water. Swirl to mix. Squeeze bottle should have a narrow tip to optimize washings.

## Specimen Collection And Handling

Serum or plasma may be stored at 2-8 ºC for up to five days. Sample may be frozen below -20 ºC for extended periods. Freezing whole blood samples is not advised. Do not heat inactivate samples and avoid repeated freezing and thawing of samples.

## Sample Preparation

Dilute patient sera 1:100 using the Dilution Buffer (e.g. 5 µl sera and 500 µl dilution buffer).

## Procedure

### Materials Provided

*Fasciola* IgM ELISA Kit

### Materials Required But Not Provided

- Micropipette
- Reagent grade (DI) water
- Graduated Cylinder
- Timer
- Tubes for serum dilution

### Suggested Materials

ELISA plate reader with a 450 nm and a 620 - 650 nm filter

### Proper Temperature

All incubations are at room temperature (15-25 °C)

## Test Procedure

### Notes:

- Ensure all samples and reagents are at room temperature (15-25°C)
- Negative and positive controls are supplied pre-diluted. DO NOT dilute further.

1. Break off number of wells needed (three for controls plus number of samples) and place in strip holder.
2. Dilute patient sera as described above in Sample Preparation Section.
3. Add **100 µl** of the negative control to well #1 and well #2, **100 µl** of the positive control to well #3 and **100 µl** of the diluted test samples to the remaining wells.
4. Incubate at room temperature for **30 minutes**, then wash.*
5. Add **100 µl** of Enzyme Conjugate to each well.
6. Incubate at room temperature for **10 minutes**, then wash.*
7. Add **100 µl** of the Chromogen to each well.
8. Incubate at room temperature for **10 minutes**.
9. Add **100 µl** of the Stop Solution to each well. Mix contents by gently tapping the side of the strip holder.
10. Read within one hour of adding Stop Solution.

*** Washings consist of 5 washings of 300 µl per well for each step with a 30 second dwell time for each wash set. If possible, slap out excessive wash buffer from the wells against absorbent toweling before addition of the next reagent.**

**Proper and thorough washing is key to obtaining accurate and reproducible results.**

## Reading Results

**ELISA Reader:** Zero reader on air. Set for bichromatic readings at 450/620-650 nm.

## 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 0.2 OD units
**Positive** - 0.5 OD units and above

## Interpretation of the Test – ELISA Reader

1 – Calculate the average extinction value by taking the average OD value of the Negative Control.
2 – Add 0.300 to this average extinction value. This value is the cut-off value used in the Sample Index Calculation.

Example:

Negative Control 1 OD = 0.084
Negative Control 2 OD = 0.100
Average is 0.084 + 0.100 = 0.184 / 2 = 0.092 = Average Extinction Value

Cut-off value is the Average Extinction Value + 0.300 (in this example 0.092 + 0.300 = 0.392

3 – Determine the Sample Index by diving the patients OD value by the Cut-off value.

Example:

Patient OD value of 1.225
Cut-off value of 0.392

1.225 / 0.392 = 3.12

4 – Evaluate the Sample Index.

Negative = less than 1.0 Sample Index
Equivocal = 1.0 to 1.5
Positive = greater than 1.5

## Limitations of The Procedure

Diagnosis of *Fasciola* infection should not be made solely based on results of the ELISA *Fasciola* test alone, but in conjunction with other clinical signs and symptoms and other laboratory findings.
Epidemiologic factors, clinical findings, exposure to endemic regions, and other laboratory results should be considered when making a diagnosis.

## 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.