Fasciola hepatica IgG ELISA

Enzyme immunoassay for the diagnosis of human fascioliasis

96 assays on individual wells for in vitro diagnostic use and for professional laboratory use

BORDIER AFFINITY PRODUCTS

Instructions for use for article N° **9650**
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CE

Intended use:

The Bordier *Fasciola hepatica* IgG ELISA kit is intended for the quantitative detection of IgG antibodies against *Fasciola hepatica* in human serum. Serology is an aid for diagnosis and cannot be used as the sole method of diagnosis.

Background:

Fascioliasis is mainly caused by the trematode *Fasciola hepatica* (liver fluke). Humans can be infected by eating raw watercress or other water plants contaminated with infective parasite larval stages. The immature larval flukes first migrates through the liver parenchyma, causing traumatic liver dysfunction, and later to the bile ducts, where they develop into mature adult flukes, which produce eggs. In the early phase, symptoms can include gastrointestinal problems such as nausea, vomiting, and abdominal pain/tenderness. During the chronic phase, the clinical manifestations may be similar or more discrete, reflecting inflammation and blockage of bile ducts, which can be intermittent. Diagnosis is based on egg detection in stool and a positive result by serological testing.

Principle and presentation:

The kit provides all the material needed to perform 96 enzyme-linked immunosorbent assays (ELISA) on breakable microtitration wells sensitized with ***Fasciola hepatica*** recombinant antigen. Specific antibodies in the sample will bind to these antigens and washing will remove unspecific antibodies. The presence of parasite specific antibodies is detected with a Protein A - alkaline phosphatase conjugate. A second washing step will remove unbound conjugate. Revealing bound antibodies is made by the addition of pNPP substrate which turns yellow in the presence of alkaline phosphatase. Color intensity is proportional to the amount of *Fasciola hepatica* specific antibodies in the sample. Potassium phosphate is added to stop the reaction. Absorbance at 405 nm is read using an ELISA microplate reader.
The test can be performed with automatic systems, but this must be validated by the user.

Material contained in the kit (96 assays):

WELL	9650-01	Breakable ELISA strips sensitized with ***Fasciola hepatica*** recombinant antigen	96	wells
DILB	9650-02	Dilution buffer (10 x) concentrate, coloured purple	50	ml
WASH	9650-03	Washing solution (10 x) concentrate	50	ml
ENZB	9650-04	Enzyme buffer	50	ml
STOP	9650-05	Stopping solution (0.5M K_3PO_4)	25	ml
CONTROL -	9650-06	Negative control serum (20 x), green cap	200	µl
CONTROL -/+	9650-07	Weak positive control serum (cut off, 20 x), yellow cap	200	µl
CONTROL +	9650-08	Positive control serum (20 x), red cap	200	µl
CONJ	9650-09	Protein A - alkaline phosphatase conjugate (50 x), purple cap	300	µl
SUBS	9650-10	Phosphatase substrate (para-nitrophenylphosphate)	20	tablets
		Multipipette reservoir, 25 ml	1	piece
		Frame for ELISA 8-well holder	1	piece

Shelf life and storage:

Store the kit at 2° to 8°C (transport at ambient temperature), avoid long term exposure of the components to direct light. The expiry date and the lot number of the kit are printed on the side of the box. After initial opening, all reagents are stable until the expiry date when stored at 2-8°C.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Dilution tubes. Adhesive tape to cover wells during incubations. Distilled water. Incubator set at 37°C. ELISA reader set at 405 nm. Manual or automatic equipment for rinsing wells. Vortex mixer. Timer.

Preparation of reagents before use:

Bring all reagents to room temperature and mix before use.

ELISA wells: open side of aluminum bag 9650-01 and remove number of wells needed (one for blank, three for controls plus the number of samples). Place sensitized wells in 8-well holder(s). If needed, complete the empty positions in the holder with used wells. Insert holder(s) in the frame in the correct orientation. Reseal open package with desiccant pad.

Dilution buffer: dilute dilution buffer (10 x) concentrate 9650-02, 1/10 in distilled water. This is used for the dilution of controls, samples, and conjugate. The diluted buffer is stable for 2 months at 2-8°C.

Washing solution: dilute washing solution (10 x) concentrate 9650-03, 1/10 in distilled water. You may also use your own washing solution. Avoid buffers containing phosphate which could inhibit the enzymatic activity of the alkaline phosphatase. The diluted washing solution is stable for 2 months at 2-8°C.

Control sera: dilute 10 µl control sera 9650-06 to -08 in 190 µl dilution buffer solution (final dilution 1/20). The diluted control sera are stable for 2 months at 2-8°C.

Conjugate: dilute conjugate 9650-09 in dilution buffer solution (final dilution 1/50). Dilute conjugate on the day of the assay. Do not store diluted conjugate.

Substrate solution: dissolve tablet(s) of phosphatase substrate 9650-10 in undiluted enzyme buffer 9650-04 (1 tablet in 2.5 ml buffer). Vortex until complete dissolution of the tablet(s). Dilute substrate on the day of the assay and protect the tube from direct light. Tablets and substrate solutions should be colourless or should have only a slight yellow tinge. If a tablet or a substrate solution turns yellow, it may have been partially hydrolysed and should be discarded. Do not store the substrate solution.

Stopping solution: use reagent 9650-05 undiluted.

Specimen collection and preparation:

Use human serum. Serum should be stored at 2-8°C if analysed within a few days, otherwise store at -20°C or lower. Avoid repeated freezing and thawing.
Vortex samples and dilute 1/201 in dilution buffer solution (for instance 5 µl sample in 1.0 ml).

Warnings and precautions:

Toxic compounds are found in following concentration:

Component	Reference	Sodium azide (N_aN_3)	Merthiolate
Dilution buffer (10 x)	9650-02	0.1 %	0.02 %
Washing solution (10 x)	9650-03	0.05 %	/
Enzyme buffer	9650-04	0.01 %	/
Control sera (20 x)	9650-06 to -08	0.1 %	0.02 %
Conjugate (50 x)	9650-09	0.1 %	/

At the used concentrations, sodium azide and merthiolate do not have any toxicological risk on contact with skin and mucous membranes.

- The stopping solution 9650-05 (0.5 M K_3PO_4) is irritant.
- The negative, weak positive, and positive control sera (9650-06 to -08) are from rabbits.
- Treat all reagents and samples as potentially infectious material.
- Do not interchange reagents of different lots or Bordier ELISA kits.
- Do not use reagents from other manufacturers with reagents of this kit.
- Do not use reagents after their expiry date.
- Close reagent vials tightly immediately after use and do not interchange screw caps to avoid contamination.
- Use separate and clean pipettes tips for each sample.
- Do not re-use microwells.
- Avoid deterioration of the microwells by mechanical action (tips/cones, nozzles).
- The descriptions of symbols used on the labels can be found on the website www.bordier.ch.

Disposal consideration:

All materials used for this test are generally considered as hazardous waste. Refer to national and regional laws and regulations for the disposal of hazardous waste.

Procedure:

When running the assay, avoid the formation of bubbles in the wells.

Step 1: Blocking:
Fill completely wells with dilution buffer solution.
Incubate for 5 to 15 minutes at ambient temperature (blocking).
Remove dilution buffer by aspiration or by shaking the wells over the sink.

Step 2: Incubation with samples:
Fill the first well of the first strip with 100 µl dilution buffer only (no-serum blank).
Fill the subsequent three wells with respectively 100 µl diluted negative, weak positive (cut off) and positive control serum. For assays of more than 25 samples, we recommend to fill the three last wells with control sera as a duplicate.
Fill remaining wells with the diluted samples (100 µl each).
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Remove sera and wash 4 x with ~ 250 µl washing solution.

Step 3: Incubation with conjugate:
Distribute 100 µl diluted conjugate in each well (including no-serum blank).
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Remove conjugate and wash 4 x with ~ 250 µl washing solution.

Step 4: Incubation with substrate:
Distribute 100 µl substrate solution per well.
Cover wells with adhesive tape and incubate for 30 minutes at 37°C.
Stop the reaction by the addition of 100 µl stopping solution to each well.

Step 5: Measurement of absorbances:
If needed, wipe the bottom of the wells and eliminate bubbles. Measure absorbances at 405 nm within 1 hour after the addition of stopping solution.

Interpretation:

Subtract the value of the no-serum blank from all measured values. When applicable calculate the mean absorbance values of duplicated serum controls. The test is valid if the following criteria are met:

- Absorbance (A) of positive control > 1.200
- A of weak positive control > 8 % of A of positive control
- A of negative control < 8 % of A of positive control
- A of no-serum blank < 0.350

Quality controls of current lots are published on our website: www.bordier.ch.

The antibody concentration of the weak positive (cut off) serum 9650-07 has been set to discriminate optimally between sera of clinically documented cases of fascioliasis and healthy human sera.
The cut off index of a sample is defined, after subtraction of the no-serum blank, as:

$$\text{Index} = \frac{\text{Absorbance sample}}{\text{Absorbance cut off serum}}$$

The result is **negative** when the index of the analyzed sample is lower than 1.0. In this case, the IgG antibody concentration against ***Fasciola hepatica*** antigen is clinically non-significant.
The result is **positive** when the index of the analyzed sample is higher than 1.0. In this case, the IgG antibody concentration against ***Fasciola hepatica*** antigen is considered as clinically significant. It indicates that the patient has had a contact with the parasite.
A grey zone could be defined by each laboratory according to its patients population. In case of borderline or doubtful results, we recommend repeating the test again 2-4 weeks later with a fresh sample.
In case of positive or doubtful result, we recommend performing a confirmation test (most often by western blot) if such a test is available or required by national regulations.

Analytical performances:

Analytical specificity:

A specificity of 97% was found with 30 sera of patients with other parasitic infections. Cross-reactivity mainly occur in patients with alveolar echinococcosis.
No positive or negative interference was observed with supraphysiological concentrations of hemoglobin, lipids or bilirubin in sera supplemented with interferents.

Precision:

Repeatability were assessed by testing 2 human serum samples in 24 wells on 1 assay.
Reproducibility were assessed by testing the 2 human serum samples on 10 differents assays.

	Repeatability		Reproducibility	
	Sample 1	**Sample 2**	**Sample 1**	**Sample 2**
Average (absorbance)	0.459	1.491	0.469	1.547
Standard deviation (absorbance)	0.023	0.089	0.028	0.069
Variation coefficient (%)	5.0	6.0	5.9	4.5

The following performances cannot be evaluated because there is no certified reference material for this analysis:

- Analytical sensitivity (limits of detection and quantitation)
- Accuracy
- Trueness
- Measuring range
- Linearity

Clinical performances:

Diagnostic sensitivity:

A sensitivity of 77% was found with 13 sera from patients suffering from fascioliasis.

Diagnostic specificity:

A specificity of 99 % was found with 99 sera of blood donors (Swiss). A specificity of 98% was found with 100 sera from patients of an infectiology unit (Swiss).

Positive and negative predictive value:

A PPV of 77% and a NPV of 98% were found with the populations mentioned above.

Expected values in normal and affected populations:

In a normal population of 99 Swiss blood donors and 100 sera from a Swiss infectiology unit, the expected Index value is 0.46. In an affected population of 13 sera from patients suffering from fascioliasis, the expected Index value is 5.01.

Incidents :

Any serious incident occurring in connection with the device shall be notified to the manufacturer and to the competent authority of the Member State in which the user and/or the patient is established.

Limitations:

Diagnosis of an infectious disease should not be estabished on the basis of a single test results. A precise diagnosis should take into consideration endemic situation, clinical history, symptomatology, imaging as well as serological data.
In immunocompromised patients and newborns, serological data are of limited value.

References:

Figueroa-Santiago, O., Delgado, B. and Espino, A.M. (2011) Fasciola hepatica saposin-like protein-2-based ELISA for the serodiagnosis of chronic human fascioliais. Diagnostic Microbiology and Infectious Disease 70, 355-361.
Gottstein B, Schneeberger M, Boubaker G, Merkle B, Huber C, et al. (2014) Comparative Assessment of ELISAs Using Recombinant Saposin-Like Protein 2 and recombinant Cathepsin L-1 from Fasciola hepatica for the Serodiagnosis of Human Fasciolosis. PLoS Negl Trop Dis 8(6): e2860. doi:10.1371/journal.pntd.0002860

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