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MONOSCREEN Ag ELISA

# *Fasciola hepatica*

ELISA kit for antigenic diagnosis of *Fasciola hepatica*
Indirect Sandwich test for faeces
Diagnostic test for cattle and sheep
Double wells

## *I - INTRODUCTION*

Bovine fascioliasis caused by the digenic trematode *Fasciola hepatica* (common liver fluke) is a worldwide parasitic disease common in ruminants. This two-host parasite is classically found on farms where all conditions for the survival and reproduction of its intermediate host, the snail *Galba truncatula*, are fulfilled. This snail is found mainly in damp meadows (watering places, brooks, springs, etc.).
Fasciola eggs are shed in the faeces. Hatching follows in water and gives rise to miracidia, which infest the snail. After multiplication in this host, cercariae are eliminated and encyst on aquatic plants as infectious metacercariae. Once ingested by a ruminant, young flukes migrate through the liver to reach the bile ducts. The prepatent period is 8 to 10 weeks. Adults appear in the bile ducts and start to lay eggs.
Liver damage and acute disease (especially in sheep) are caused by migrating immature parasites. Chronic disease occurs in cattle during the biliary phase.
The disease depresses the animals' development and productivity, causing decreases in milk yields (-10%), weight loss, intermittent diarrhoea, anaemia and fertility problems.
Diagnosis of *Fasciola hepatica* in cattle can be made only after 8 to 10 weeks by coprological examination of faecal material. However, sometimes even repeated faecal examinations will fail to identify a *Fasciola hepatica* infection due to the method's lack of sensitivity.
Bio-X Diagnostics' antigenic ELISA kit allows the detection of coproantigens in infested cattle's faecal material. These coproantigens can be found in the faeces even outside the fluke's egg-laying period. Unlike serotests, the samples will test positive with the antigenic assay only if flukes are present in the bile ducts.

## *II - PRINCIPLE OF THE TEST*

Rows A, C, E, and G of the 96-well microplate have been sensitised with a specific polyclonal antibody against *Fasciola hepatica*. This antibody captures the coproantigens in the faecal material. The other rows on the microplate (rows B, D, F, and H) have been sensitised with a polyclonal antibody that is not specific for the parasite. These control rows allow differentiation between a specific immunological reaction and nonspecific binding so as to eliminate false positives.
The faecal material is diluted in dilution buffer and incubated on the microplate for 2 hours at 21°C +/- 3°C.
After this first incubation step, the plate is washed and incubated for 1 hour with the first conjugate (a specific monoclonal antibody against an antigenic determinant of *Fasciola hepatica* coupled to biotin), then the plate is

incubated at 21°C +/- 3°C for 1 hour. The plate is then washed, the second conjugate – a peroxidase-coupled avidine specific to biotin – is applied, and the plate is incubated at 21°C +/- 3°C for another hour. After this second incubation, the plate is washed again and the chromogen (tetramethylbenzidine) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. It is ready to use.
If *Fasciola hepatica* coproantigens are present, the conjugates remain bound in the corresponding microwells and the enzyme catalyses the transformation of the colourless chromogen into a pigmented compound (blue). The intensity of the resulting blue colour is proportionate to the sample's coproantigen titre. The enzymatic reaction can be stopped by acidification (yellow) and the resulting optical density at 450 nm read using a photometer. The signals recorded for the negative control microwells are subtracted from the corresponding positive microwells. A control antigen is provided with the kit so as to validate the test results. This control antigen is composed of lyophilised ground flukes.

## *III - COMPOSITION OF THE KIT*

- **Microplates**: Two 96-well microplates (12 x 8 wells). Rows A, C, E, and G have been coated with specific antibody against *Fasciola hepatica* and rows B, D, F, and H have been coated with the control antibody (polyclonal antibody not specific for the parasite).
- **Washing solution**: One 100-ml bottle of 20x concentrated washing solution. The solution crystallises spontaneously when cold. If only part of the solution is to be used, bring the bottle to 21°C +/- 3°C until disappearance of all crystals. Mix the solution well and remove the necessary volume. Dilute the buffer twentyfold with distilled or demineralised water.
- **Dilution buffer**: One 50-ml bottle of 5x colored, concentrated buffer for diluting samples and conjugate. Dilute this concentrated dilution buffer fivefold with distilled or demineralised water. If a deposit forms at the bottom of the container filter the solution on Whatman filter paper.
- **Conjugate**: One 0.5-ml vial of a 50-fold concentrate of biotin-conjugated anti-*Fasciola hepatica* antibody. The reagent must be diluted fiftyfold in the dilution buffer.
- **Avidine**: One 0.5-ml vial of a 50-fold concentrate of peroxidase-coupled avidine. The reagent must be diluted fiftyfold in the dilution buffer.
- **Positive reference**: Two vials containing the reference antigen. Reconstitute this antigen with 0.5 ml of distilled or demineralised water. The reconstituted reagent may be kept at -20°C. Divide the reconstituted antigen into several portions before freezing in order to avoid repeated freezing and thawing. If these precautions are taken the reagent may be kept for several months.
- **Single component TMB**: One 25-ml bottle of the chromogen tetramethylbenzidine (TMB). Store between +2°C and + 8°C protected from light. This solution is ready to use.
- **Stopping solution**: One 15-ml bottle of the 1 *M* phosphoric acid stop solution.

| | BIO K 201/2 |
|---|---|
| Microplates | 2 |
| Washing solution | 1 X 100 ml (20 X) |
| Colored Dilution buffer | 1 X 50 ml (5 X) |
| Conjugate | 1 X 0.5 ml (50 X) |
| Avidine | 1 X 0.5 ml (50 X) |
| Positive reference | 2 X 0.5 ml (1 X) freeze-dried |
| Single component TMB | 1 X 25 ml (1 X) |
| Stop solution | 1 X 15 ml (1 X) |

## *IV - ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED*

Distilled water, graduated cylinders, beakers, plastic tubes, tube rack, dispenser tips, reagent reservoir for multichannel pipettes, lid, adhesive for microplates, graduated automatic (mono- and multichannel) pipettes, microplate reader, microplate shaker and microplate washer (optional)

## *V - PRECAUTIONS FOR USE*

- This test may be used for "in vitro" diagnosis only. It is strictly for veterinary use.
- The reagents must be kept between +2°C and +8°C. The reagents cannot be guaranteed if the shelf-life dates have expired or if they have not been kept under the conditions described in this insert.
- The concentrated wash solution and dilution buffer may be stored at room temperature. Once diluted, these solutions remain stable for six weeks if kept between +2°C and +8°C.
- Unused strips must be stored immediately in the aluminium envelope, taking care to keep the desiccant dry and the envelope's seal airtight. If these precautions are taken, the strips' activity can be conserved up to the kit's shelf-life date.
- Do not use reagents from other kits.
- The quality of the water used to prepare the various solutions is of the utmost importance. Do not use water that may contain oxidants (e.g., sodium hypochlorite) or heavy metal salts, as these substances can react with the chromogen.
- Discard all solutions contaminated with bacteria or fungi.
- The stop solution contains 1 M phosphoric acid. Handle it carefully.
- All materials and disposable equipment that come in contact with the samples must be considered potentially infectious and be disposed of in compliance with the legislation in force in the country.
- To guarantee the reliability of the results, one must follow the protocol to the letter. Special care must be taken in observing the incubation times and temperatures, as well as measuring the volumes and dilutions accurately.

## *VI – PROCEDURE*

1- Bring all the reagents at 21°C +/- 3°C before use.
2- Remove the microplate from its packaging.
3- Dilute the faecal material in the dilution buffer (2 g + 2 ml for cattle and 0.5 g + 2 ml for ovine). Centrifuge 10 min. at 1,000 g. Collect supernatants.
4- Add 100-µl aliquots of the diluted samples to the wells as follows: sample 1 in wells A1 and B1, sample 2 in wells C1 and D1, etc. Proceed in the same manner for the positive reference (example: G1 and H1).
5- Cover the plate with a lid and incubate at 21°C +/- 3°C for 2 hours on a plate agitator.
6- Rinse the plate with the washing solution prepared as instructed in the section "Composition of the Kit". To do this, dispose of the microplate's contents by flipping it sharply over a container filled with an inactivating agent. Let the microplate drain upside-down on a sheet of clean absorbent paper so as to eliminate all liquid. Add 300 µl of the washing solution, and then empty the plate once again by flipping it over above the containment vessel. Repeat the entire operation at least two more times, taking care to avoid the formation of bubbles in the microwells. After the plate has been washed three times proceed to the next step.
7- Dilute the necessary amount of the biotin-linked anti-*Fasciola hepatica* conjugate **fiftyfold** in the reagent dilution buffer (20 µl of conjugate + 980 µl of the reagent dilution buffer per strip).
8- Add 100 µl of the diluted anti-*Fasciola hepatica* conjugate solution to each well.
9- Cover with a lid and incubate the plate at 21°± 3°C for one hour.
10- Wash the plate as described in Step 6.
11- The avidine-peroxidase conjugate is liquid and must be diluted **fiftyfold** in the reagent dilution buffer (20 µl of conjugate + 980 µl of the reagent dilution buffer per strip).
12- Add 100 µl of the diluted peroxidase-linked conjugate solution to each well.
13- Cover with a lid and incubate the plate at 21°± 3°C for one hour.
14- Wash the plate as described in Step 6.
15- Add 100 µl of the chromogen solution to each well on the plate. The chromogen solution must be absolutely colourless when it is pipetted into the wells. If a blue colour is visible, this means that the solution in the pipette has been contaminated. Incubate at 21°C +/- 3°C and away from light for 10 minutes. Do not cover. This time interval is given as a guideline only, for in some circumstances it may be useful to lengthen or shorten the incubation time.
16- Add 50 µl of stop solution per microwell. The blue colour will change into a yellow colour.
17- Read the optical densities in the microwells using a plate reader and a 450 nm filter. Results must be read fairly soon after the stopping solution has been added since the chromogen may cristallize in wells with strong signals and distort the results accordingly.

## *VII – INTERPRETING THE RESULTS*

Calculate the net optical density of each sample by subtracting from the reading for each sample well (A, C, E, G) the optical density of the corresponding negative control (B, D, F, H).
Proceed in the same way for the positive control antigen.
The test is validated only if the positive control antigen yields a difference in the optical density at 10 minutes that is greater than the validation value given on the QC data sheet.

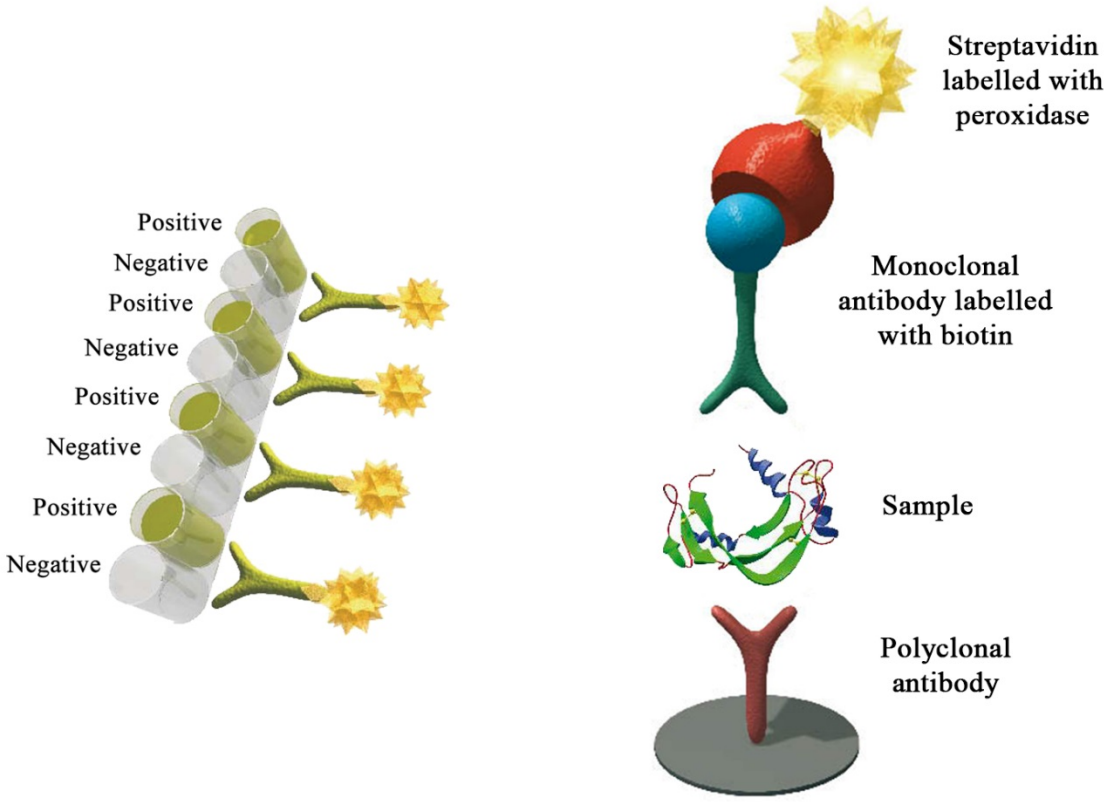
Divide the signal read for each sample well by the corresponding positive control signal and multiply this result by 100 to express it as a percentage.

$$\text{Val(ue)} = \frac{\text{Delta OD Sample} * 100}{\text{Delta OD positive}}$$

Using the first table in the quality control procedure, determine each sample's status (positive, negative).

## *VIII – ORDERING INFORMATION*

Monoscreen AgELISA *Fasciola hepatica* 2 X 48 tests BIO K 201/2

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