

研究用



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FASCIOLA HEPATICA Ag ELISA KIT

BIO K 201

Fasciolosis caused by the digenic trematode *Fasciola hepatica* is a worldwide parasitic disease common in ruminants. This two-host life cycle parasite is classically found in farms where all conditions for the survival and the multiplication of the snail intermediate host (*Lymnaea truncatula*) are fulfilled. This snail is mainly found in damp meadows (watering-places, brooks, springs,...). *Fasciola* egg shedding occurs with faeces. Hatching follows in water and gives rise to the miracidium which infects the snail. After multiplication in this host, cercariae are eliminated and give rise to infectious metacercariae fixed on a plant holder. Once ingested by a ruminant, young flukes migrate through the liver to reach 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. Zootechnical characteristics are hampered by the disease: decrease in milk yield (-10%), weight loss, intermittent diarrhoea, anemia and fertility problems. One of the main problems in the control of this disease is the lack of sensitive and convenient tests for the diagnosis of *Fasciola* spp. infection in large herds under field conditions, and for monitoring the efficacy of flukicide treatments. Diagnosis of animal fascioliasis is largely based on microscopic demonstration of parasite ova in feces, but *Fasciola* spp. egg shedding is intermittent and irregular. Serological techniques allow the detection of specific antiparasite circulating antibodies but tests of this type are of limited diagnostic value in endemic areas because antibody titers remain at high levels even when animals have been successfully treated. BIO K 201 is a reliable and ultrasensitive method for detecting subnanogram amounts of *F. hepatica* antigens in feces from sheep and cattle, facilitating early diagnosis.

Reliable Results

The use of monoclonal antibody as conjugate ensures excellent specificity and very reliable results.

The use of polyclonal antibody as capture reagent ensures very high sensitivity.

Early diagnosis.

Allows monitoring of flukicide treatments (negative results 1-3 weeks after treatment)

Ease-of-Use

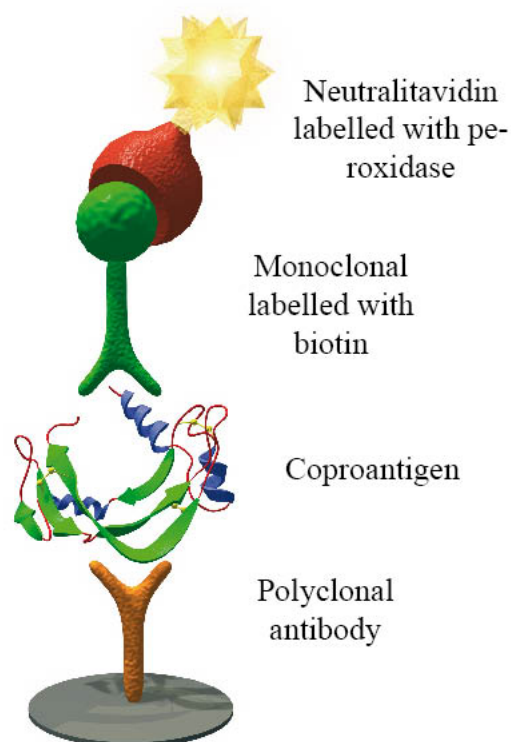
Minimal hands-on-time
Room temperature incubation
Results available in 300 minutes for single or batch testing

Flexibility

Results can be read visually or spectrophotometrically.

EIA Procedure

- 1- Microplate coated with polyclonal antibody
- 2- Add samples and positive control.
Incubate 1 hour at 21°C +/- 3°C
Wash
- 3- Add first conjugate (monoclonal biotin conjugated).
Incubate 1 hour at 21°C +/- 3°C .
Wash
- 3- Add second conjugate (avidin-peroxidase).
Incubate 1 hour at 21°C +/- 3°C .
Wash
- 4- Add chromogen + Substrate
Wait 10 minutes.



Bio-X Diagnostics

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Example of results

A group of 21 four-months old lambs was experimentally infected with variable number of metacercariae (5 to 40) obtained from experimentally infected *Lymnaea truncatula* snails. Six lambs infected with 40 metacercariae were treated with a single oral dose of 10 mg/kg of triclabendazole (Fasinex 5 %) at 14 weeks post infection. Fecal samples were taken from each animal just before infection and then weekly for 18 weeks post infection. At the end of the experiment, all animals were necropsied and all liver parasites collected (Mezo et al. 2004).

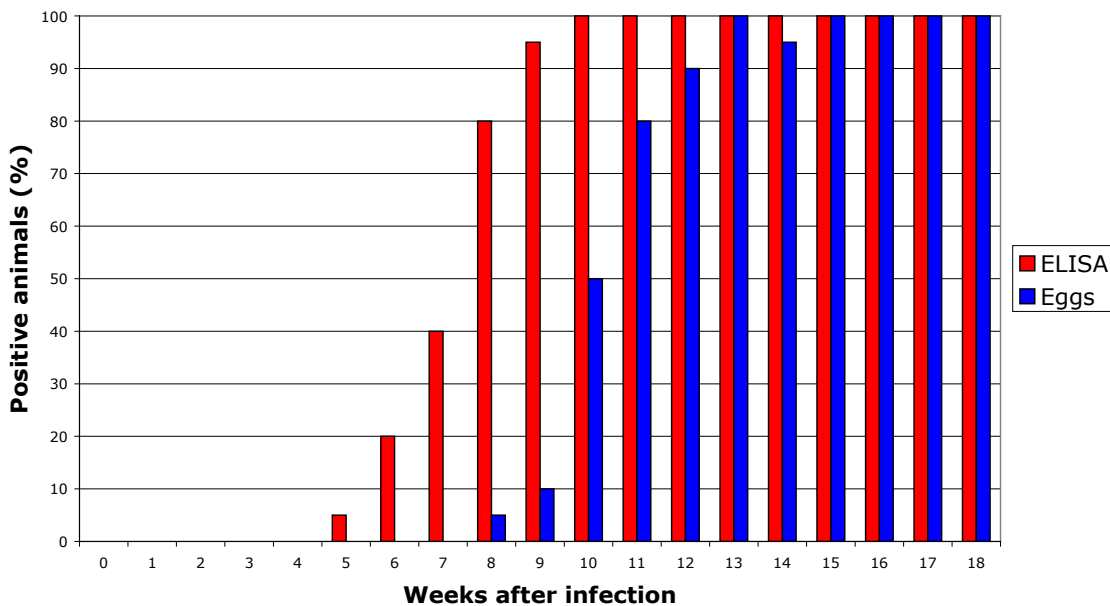
Untreated animals

Weeks post infection	14 weeks	15 weeks	16 weeks	17 weeks	18 weeks
Mean E.P.G. \pm SD	56 \pm 73 (15/15)	64 \pm 93 (15/15)	59 \pm 92 (15/15)	95 \pm 115 (15/15)	100 \pm 102 (15/15)
Mean O.D. \pm SD	2.042 \pm 0.189 (15/15)	1.981 \pm 0.298 (15/15)	2.135 \pm 0.105 (15/15)	2.005 \pm 0.275 (15/15)	2.139 \pm 0.224 (15/15)
Range fluke burden in the liver	ND	ND	ND	ND	1-36 (15)

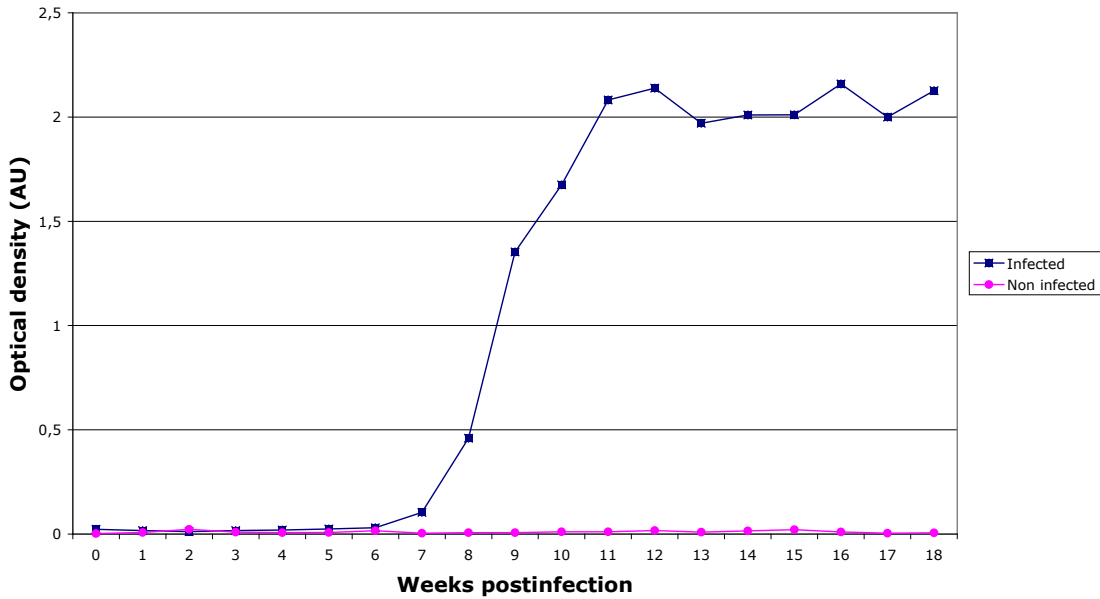
Treated animals

Weeks post infection	14 weeks	15 weeks	16 weeks	17 weeks	18 weeks
Mean E.P.G. \pm SD (*)	67 \pm 65 (5/6)	0 (0/6)	5 \pm 3 (5/6)	12 \pm 13 (2/6)	45 \pm 59 (2/6)
Mean O.D. \pm SD (*)	1.873 \pm 0.294 (6/6)	0.530 \pm 0.06 (1/6)	0.13 \pm 0.025 (1/6)	-	-
Range fluke burden in the liver	ND	ND	ND	ND	0 (0)

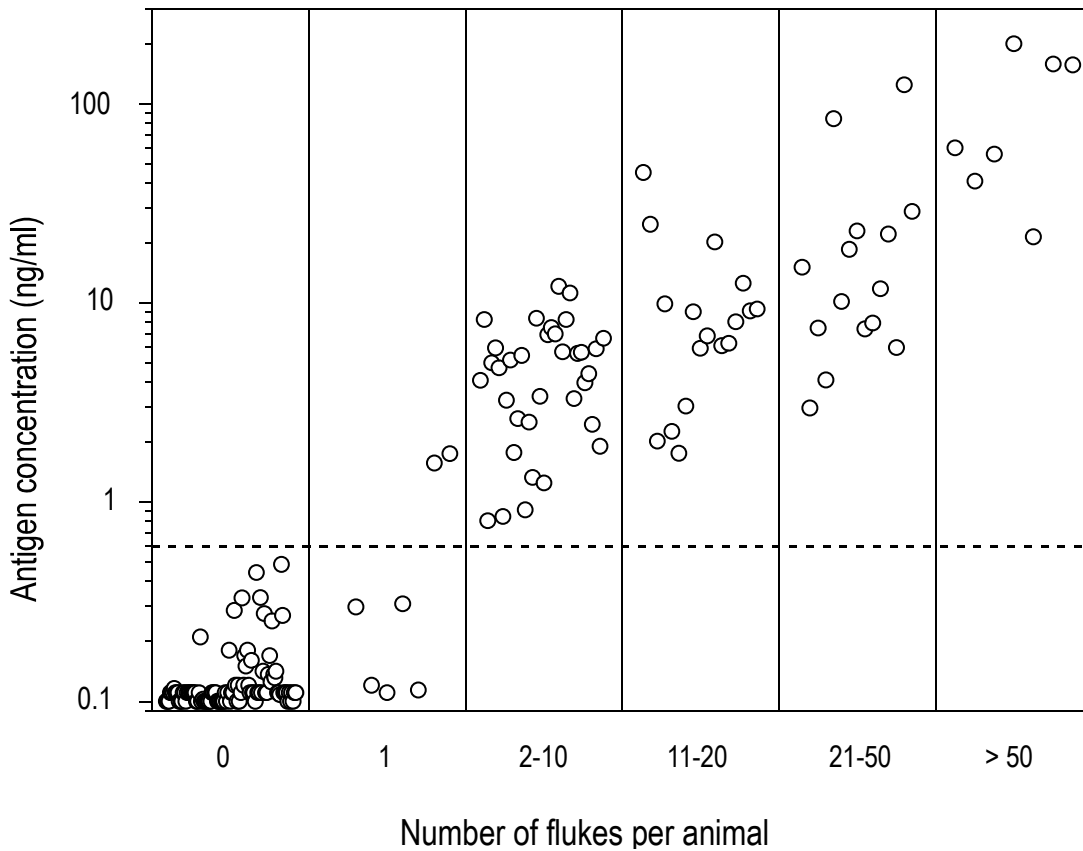
(*): Mean O.D. values and mean E.P.G. counts were calculated using data from positive animals only.



Percentages of experimentally infected lambs testing positive by ELISA and by fecal examination for eggs, at different times after infection (Mezo et al. 2004).



Mean optical density values obtained in ELISA of fecal supernatants from lambs experimentally infected with metacercariae (n = 15) or non infected (n = 6). (Mezo et al. 2004).



Fasciola hepatica coproantigen concentrations measured by ELISA in fecal supernatants from 180 cows killed at the slaughterhouse. The dashed line is the detection limit in the ELISA assay (0.6 ng/ml, corresponding to the lowest concentration giving an OD reading 0.15 higher than the cutoff. (Mezo et al. 2004).

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		Cow 1	Cow 2	Cow 3	Cow 4
	Week 14	2.320	2.530	2.150	0.940
Triclabendazole	Week 15	0.019	0.04	0.01	0.05

Four cows were infected with 300 metacercariae at week 0. They were treated with Triclabendazole at week 14. Optical densities were followed at week 14 and week 15. None of these treated animals had flukes in the liver at the slaughterhouse.

Conclusions

The Fasciola Antigen ELISA detection kit (BIO K 201) is a reliable, robust and ultrasensitive method for sheep and cattle, capable of detecting subnanogram amounts of specific excretory-secretory antigens in feces.

Diagnosis of animal fascioliasis is largely based on microscopic detecting Fasciola hepatica infections in demonstration of parasite ova in feces, but Fasciola spp. Egg shedding is intermittent and irregular and does not begin until 10-12 weeks postinfection or later (Zimmerman et al., 1982). Besides its low sensitivity, which can result in underdiagnosis of weak infections (Conceição et al., 2002), coproscopy is a laborious technique requiring each sample to be examined individually by training personnel so that it is not suitable for analysing large herds.

Immunological techniques using selected excretory-secretory antigens from Fasciola spp. Allow the detection of specific antiparasite circulating antibodies within 3-5 weeks of the infection (Mezo et al., 2003) and can readily be automated. Nevertheless, tests of this type are of limited diagnostic value in endemic areas because antibody titers remain at high levels even when animals have been successfully treated (Sanchez-Andrade et al., 2001)

Literature cited

Conceição M. A. R., M. Durao, I. H. Costa, and J.M. Correia Da Costa. 2002. Evaluation of a simple sedimentation method (modified McMaster) for diagnosis of bovine fascioliasis. *Veterinary Parasitology* 105: 337-343

Mezo M., M. Gonzalez-Warleta, and F.M. Ubeira. 2003. Optimized serodiagnosis of sheep fascioliasis by FPLC fractionation of Fasciola hepatica excretory-secretory antigens. *Journal of Parasitology* 89: 843-849

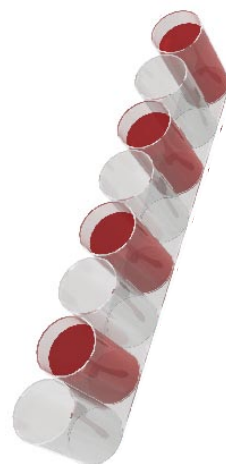
Mezo M., M. Gonzalez-Warleta, C. Carro, and F.M. Ubeira 2004 An ultrasensitive capture ELISA for detection of Fasciola hepatica coproantigens in sheep and cattle using a new monoclonal antibody (MM3) *J. Parasitology* 90(4). 2004 pp 845-852

Zimmerman, G. L., L. W. Jen, J. E. Cerro, K. L. Farnsworth and R. B. Wescott 1982. Diagnosis of Fasciola hepatica infections in sheep by an enzyme-linked immunosorbent assay. *American Journal of Veterinary Research* 43: 2097-2100

BIO K 201 FASCIOLA HEPATICA ANTIGEN ELISA

- 2 plates of 12 strips for a total of 96 tests
- 2 vials of positive control
- 1 vial of conjugate I
- 1 vial of conjugate II
- 1 bottle of washing solution
- 1 bottle of dilution buffer
- 1 drop-dispenser bottle of chromogen
- 1 bottle of substrate solution
- 1 bottle of stopping solution

1 year of stability at 4°C



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Bio-X Diagnostics

BIO-X BOVINE *FASCIOLA HEPATICA* ANTIGEN ELISA KIT

(Sandwich indirect assay for faeces)

KIT FOR ANTIGENIC DIAGNOSIS OF *FASCIOLA HEPATICA* IN FAECES BY ELISA

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 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)
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- Incubate the plate at 21°C +/- 3°C for 2 hours on a plate agitator. Cover with a lid.
- 6- Rinse the plate with the washing solution, prepared as instructed in “Composition of the kit” as follows: Empty the microplate of its contents by flipping it over sharply over a sink. Tap the microplate upside down against a piece of clean absorbent paper to remove all the liquid. Fill all the used wells with the washing solution using a spray bottle or by plunging the plate in an appropriately dimensioned vessel, then empty the wells once more by turning the plate over above a sink. 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- Incubate at 21°C +/- 3°C for one hour. Cover with a lid.
- 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- Incubate at 21°C +/- 3°C for one hour. Cover with a lid.
- 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.
- 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 crystallize 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.

$$\text{Val} = \frac{\text{Delta DO sample} * 100}{\text{Delta DO pos}}$$

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

VIII – ORDERING INFORMATION

BIO-X *FASCIOLA HEPATICA* ANTIGEN ELISA KIT:

2 X 48 tests

BIO K 201/2

