MONOSCREEN Ab ELISA

Instructions for use
BIO K 211-Fasciola hepatica_NO_(EN)_V06
20/02/2023

Monoscreen AbELISA *Fasciola hepatica*

Reference: BIO K 211

ELISA Kit for the serodiagnosis of fasciolosis

Biwell, indirect test

In vitro and strictly veterinary use

Sample	Individual analysis	Pool analysis*, possible up to
Bovine serum	✓	10
Ovine serum	✓	10
Bovine milk (skimmed** and non-skimmed)	✓	Tank

* This is done in accordance with the legislation in force in your country, the certifying body or the recommendations made by the NRL when they exist. Mixtures must be made volume to volume, i.e., by taking the same volume of each of the sera making up the mixture. // ** centrifugate 20 minutes at 4000 g

Presentation

Product reference	BIO K 211/2	BIO K 211/5
Format	2 plates, strips of 16 wells	5 plates, strips of 16 wells
Reactions	96 tests	240 tests

Composition of the kit

Provided material	BIO K 211/2	BIO K 211/5
Microplates	2	5
Washing solution (20X)	1 X 100 mL	1 X 250 mL
Colored dilution solution (1X)	2 X 100 mL	4 X 100 mL
Conjugate (50X)	1 X 0,6 mL	1 X 1,5 mL
Positive control	1 X 0,5 mL	1 X 0,5 mL
Negative control	1 X 0,5 mL	1 X 0,5 mL
Tracer (1X)	1 X 0,5 mL	1 X 0,5 mL
Single component TMB (1X)	1 X 25 mL	1 X 55 mL
Stop solution (1X)	1 X 15 mL	1 X 30 mL

Revision history

Date	Version	Modifications
16/06/2022	V03	Layout and simplification of the entire manual
05/08/2022	V03	Correction "$\Delta OD_{positive\ serum}$" instead of "$\Delta OD_{sample}$" (I. Interpretation of results)
06/09/2022	V04	Correction of interpretation thresholds for pool samples
24/11/2022	V05	Modification of the conjugate's volumes
20/02/2023	V06	Correction of the validation of results

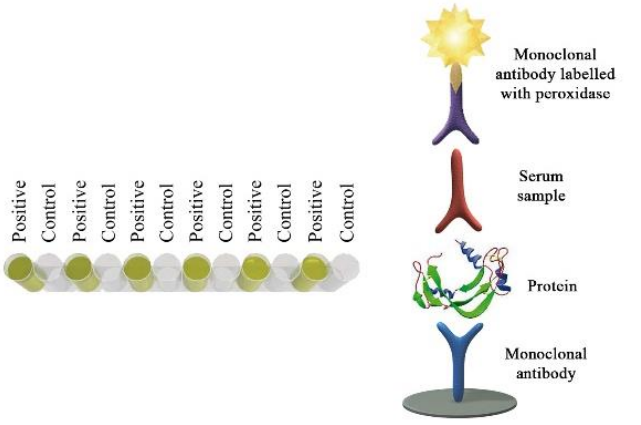
Note : minor changes to typography, grammar and formatting are not included in the revision history.

A. Introduction

Bovine 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 *(Galba 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 infests 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. Diagnosis of *Fasciola hepatica* in cattle can only be made after 8 to 10 weeks by coprological examination of faecal material. However, sometimes even repeated fecal examination cannot indentify any *Fasciola hepatica* infection due to the lack of sensitivity of this method. Acute distomatosis of the sheep is characterized by anemia and sometimes sudden mortality and chronic distomatosis by anemia, reduction of the dairy production, reduction of the average daily profit and oedemas.

B. Test principle

The test uses 96-well microtitration plates sensitised by a monoclonal antibody specific to one protein of *Fasciola hepatica*. This antibody is used to trap the protein as well as to purify it from lysate of the parasite. The plate's odd columns (1, 3, 5, 7, 9 and 11) contain the specific protein, whereas the even columns (2, 4, 6, 8, 10 and 12) contain only the monoclonal antibody. This is a genuine negative control to differentiate specific anti-*Fasciola hepatica* antibodies from non-specific ones.
The test blood sera, plasma or milks are diluted in the dilution buffer. The plate is incubated and washed, then the conjugate, a peroxidase-labelled anti-ruminant IgG1 monoclonal antibody, is added to the wells. The plate is then incubated a second time at 21°C+/- 3°C, washed again and the chromogen tetramethylbenzidine (TMB) is added. This chromogen has the advantages of being more sensitive than the other peroxidase chromogens and not being carcinogenic. If specific *Fasciola hepatica* immunoglobulins are present in the test sera or in milk, the conjugate remains bound to the microwell that contains the antigen, and the enzyme catalyses the transformation of the colorless chromogen into a pigmented compound. The intensity of the resulting blue colour is proportionate to the titer of specific antibody in the sample. The signal read off the negative control microwell is subtracted from that of the positive microwell sensitised by the antigen. The interpretation of the results is done by comparing the signals of the samples (serum, plasma, or milk) with those of the positive controls.
Kit allows the analysis of pool of 10 individual sera or plasma. It allows too an analysis of bulk tank milk.



C. Additional material and required equipment (not provided)

- Distilled/demineralized water
- Graduated mono- or multichannel pipettes (2-20µL, 20-200µL et 100-1000µL range) and single-use tips
- Microplate reader (450nm filter)
- Microplate washer
- Incubator at 37±2°C
- Standard laboratory equipment: graduated cylinder, tube rack, lid, …

D. Precautions for use

- The reagents must be kept between +2 et +8°C.
- Unused strips must be stored with the desiccant in the hermetically sealed aluminum envelope.
- Do not use reagents beyond shelf-life date.
- Do not use reagents from other kits.
- Make sure to use distilled/demineralized water.
- The stopping solution contains 1 M phosphoric acid. Handle it carefully.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- Keep the TMB solution away from light.

E. Preparation of solutions

- The solutions are to be prepared extemporaneously.
- The washing solution must be diluted 20-fold in distilled/demineralized water. The cold solution crystallizes spontaneously. Bring the vial to 21±3°C to make sure that all crystals have disappeared; mix the solution well and withdraw the necessary volume.
- The dilution solution is ready to use. The dilution solution is colored in yellow.
- The conjugate must be diluted 50-fold in the dilution solution.
- The stopping solution is ready to use.
- The TMB solution is ready to use. It must be perfectly colorless.

F. Preparation of samples

- **Serum samples** and **kit controls** (positive, negative serum and tracer) must be diluted **100-fold** in the dilution solution and homogenized.
  Avoid using haemolysed samples or containing coagulum.

  Recommended dilution:
  10 µL of sample + 990 µL of dilution solution.

- **Milk** samples must be diluted **4-fold** in the dilution solution and homogenized.

  Recommended dilution:
  25 µL of sample + 75 µL of dilution solution.

G. Procedure

- Bring all the reagents to 21±3°C before use.
- Carefully read through the previous points.

Serum protocol (1/100 dilution)

1. Distribute 100 µL per well of diluted serum samples and kit controls. Cover and incubate the plate at 21 ± 3°C during 60 ± 5 min.

Milk protocol (1/4 dilution)

1. Distribute 100 µL per well of diluted milk samples and diluted kit controls. Cover and incubate the plate at 21 ± 3°C during 60 ± 5 min.

OR

Direct preparation in the kit's microplate

Distribute 75 µL per well of dilution solution. Add 25 µL of pure milk samples per well. Homogenize by pipetting up and down.

Distribute 100 µL of diluted kit controls per well.

Cover and incubate the plate at 21 ± 3°C during 60 ± 5 min.

N.B. : To avoid differences in incubation time between samples of a large series, sample dilutions and reference dilutions can be prepared in a dilution microplate before transfer (100 µL) into the test microplate using a multi-channel pipette.

Joint protocol

2. Remove the content of the microplate. Wash the microplate 3 times with 300 µL of washing solution per well. Avoid the formation of bubbles in the wells between each wash.
3. Distribute 100 µL of diluted conjugate per well. Cover with a lid and incubate the plate at 21 ± 3°C during 60 ± 5 min.
4. Remove the content of the microplate. Wash the microplate 3 times with 300 µL of washing solution per well. Avoid the formation of bubbles in the well between each wash.
5. Distribute 100 µL of TMB solution per well. Incubate at 21 ± 3°C during 10 ± 1 min away from the light, without covering.
6. Distribute the stopping solution at rate of 50 µL per well. The colour changes from blue to yellow.
7. Record the optical densities using a plate spectrophotometer with a 450 nm filter within 5 minutes after adding the stopping solution.

H. Validation of results

The test can only be validated if:

- The difference between the optical density (OD) readings of the odd and even wells of the positive serum is greater than 0,800.

  Positive serum: $OD_{odd\ well} - OD_{even\ well} > 0{,}800$

- the difference between the optical density (OD) readings of the odd and even wells of the negative serum is less than 0,300.

  Negative serum: $OD_{odd\ well} - OD_{even\ well} < 0{,}300$

I. Interpretation of results

1. Calculate for each sample its « *delta OD* » by subtracting the optical density of the even well from the odd wells.
   Example: $\Delta OD_{sample} = Sample_{A1\ well} - Sample_{A2\ well}$
2. Calculate for each sample its coefficient (S/P %) using the following formula:

$$S/P\ \% = \frac{\Delta OD_{sample}}{\Delta OD_{positive\ serum}} * 100$$

	Results	Status	
Individual sample	S/P % < 10%	0	No infestation
	10% ≤ S/P % <15%	±	Equivocal
	15% ≤ S/P % <45%	+	Weak infestation
	45% ≤ S/P % <75%	++	Medium infestation
	S/P % > 75%	+++	Strong infestation
Pool sample	S/P % < 5%	0	No infestation
	5% ≤ S/P % <15%	±	Equivocal
	15% ≤ S/P % <45%	+	Weak infestation
	45% ≤ S/P % <75%	++	Medium infestation
	S/P % > 75%	+++	Strong infestation

Get the interpretation of your results quickly and easily using **AnalysiScreen**, our free online platform, available on our website: https://www.biox.com.

ANALYSISCREEN

AnalysiScreen™ is the new module for reading and interpreting all types of Monoscreen™ and Multiscreen™ ELISA plates. **AnalysiScreen™** is :

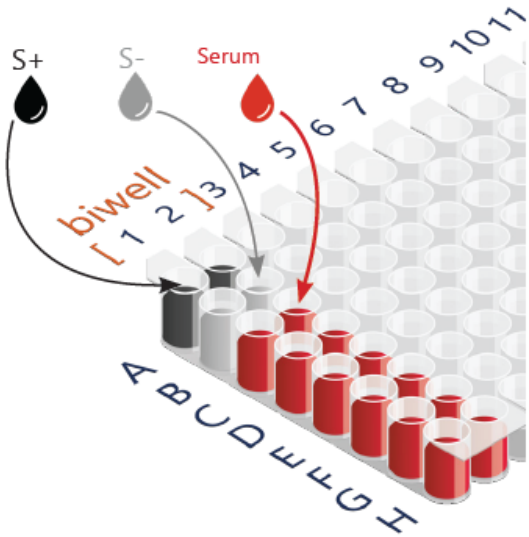
- Free
- Accessible online via our website : https://www.biox.com
- Updated in real time
- Compatible with all Bio-X Diagnostics plate designs
- Very easy to use

SCAN ME

Notes*

Serum protocol

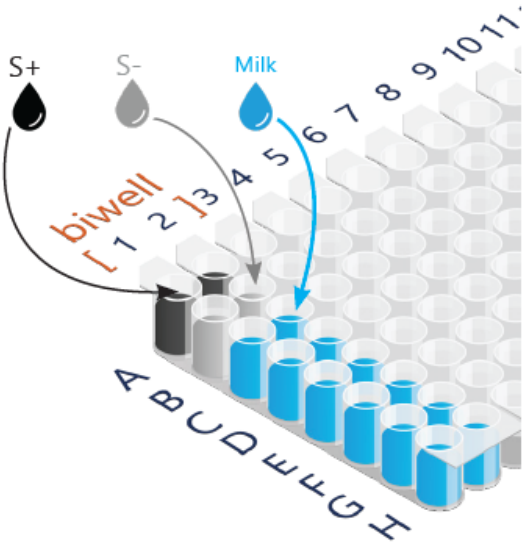
1 Dilution of samples 1/100
Dilution of the kit controls (positive and negative serum) 1/100



Kit microplate

Milk protocol

1 Dilution of samples 1/4
Dilution of the kit controls (positive and negative serum) 1/100



Kit microplate

Joint protocol

2 Add 100 µL of conjugate

3 Add 100 µL of TMB

4 Add 50 µL of stop solution

5 Record the optical densities

* Notes do not replace the instructions for use of which they are a synthesis.

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