SVANOVIR® *F. hepatica*-Ab

Fasciola hepatica
Antibody Test

Contents	**Art. No. SV-104896**
Microtitre plate Microtitre plate (96 wells) coated with non-infectious *Fasciola hepatica* antigen (sealed and stored dry)	2 (Strips) 12 x 8
Conjugate Ready-to-use (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)	1 x 24 mL
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Sample Dilution Buffer Ready-to-use	1 x 120 mL
Substrate Solution ABTS - STORE IN THE DARK!	1 x 50 mL
Stop Solution Contains 1% SDS	1 x 25 mL
A. Positive Control Serum - Contains preservatives	1 x 2.5 mL
B. Negative Control Serum - Contains preservatives	1 x 2.5 mL

This manual covers the following SVANOVIR® *F. hepatica*-Ab kit: Article number SV-104896

Fasciola hepatica Antibody Test

Name and Application

SVANOVIR® *F. hepatica*-Ab is an Enzyme Linked Immunosorbent Assay (ELISA) for the detection of *F. hepatica* specific antibodies in bovine serum/plasma, meat juice and milk (individual and bulk tank milk) samples. The screening for *F. hepatica* antibodies in the above mentioned samples have been demonstrated to be a promising parameter to determine the infection level, thus becoming an instrument to determine the need for anthelmintic control.

General information

Fasciolosis is a parasitic disease of herbivores. One of the major causes of the disease is the common liver fluke *Fasciola hepatica*. *F. hepatica* has a two-host life cycle and adult worms stay in the liver of herbivores. Depending on the degree of infection and the reaction of the host mild to severe damage of the liver tissue occurs. Cattle fasciolosis occurs mostly in form of subclinical infection with no specific clinical signs but gradual/progressive production loss. The economic burden to the ruminant livestock industry comes from reduced milk yield, fertility and meat harvest including discharge of affected livers[1,2]. Additional costs come from anthelmintic treatments and milk-withdrawal periods after treatment. Furthermore fasciolosis can predispose cattle to bacterial infections due to anaerobiers (*e.g. Fusobacterium necrophorum*, *Arcanobacterium pyogenes* or *Clostridia spp.*)[3] or the failure to detect bovine tuberculosis[4]. Last but not least severe infection of *Fasciola spp.* is an animal welfare problem.
The prevalence of *F. hepatica* seems on the increase over the past decade. Regional differences exist and herd-level prevalence varies between 30-80% in affected regions in Western Europe. Also within an affected herd, there is a high variation in the proportion of animals infected and the worm burdens of infected animals.

It is not possible to eradicate *F. hepatica* but it is possible to control the impact of an infection on the production output. Monitoring and controlling herd infection to an acceptable level with minimal loss of production is a new way to manage fasciolosis.

Principle

The kit procedure is based on a solid phase indirect ELISA. In this procedure, samples are exposed to non-infectious *F. hepatica* antigen coated wells of microtitre plates. *F. hepatica* antibodies (if present in the sample) bind to the antigen in the wells. The HRP conjugate added subsequently forms a complex with these *F. hepatica* antibodies. Unbound material is removed by rinsing before the addition of substrate solution. Subsequently a blue-green colour develops which is due to the conversion of the substrate by the conjugate. A positive result is indicated by development of a blue-green colour. The reaction is stopped by adding stop solution. The result can be read by a microplate photometer, where the optical density (OD) is measured at 405 nm.

Materials needed but not provided

1. Precision pipettes
2. Disposable pipette tips
3. Distilled, deionised or any similar hight quality water
4. Wash bottle, multichannel pipettor or plate washer
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 405 nm filter

Specimen information

Serum:

5 µL of serum or plasma is required for each sample well. Fresh, refrigerated, or previously frozen serum or plasma may be tested.

Meat juice:

10 µL of meat juice is required for each sample well. Fresh, refrigerated, or previously frozen meat juice may be tested. The meat juice is collected by placing 5-10 g of skirt muscle in a sample bag or other suitable container. Store it at -20°C overnight. On thawing meat juice will form, gently squeeze the meat sample to release more juice if needed.

Indiviudal, pooled or bulk tank milk samples:

100 µL of skim milk is required for each sample well. Fresh, refrigerated or previously frozen milk may be tested. It is recommended to centrifuge milk samples for 15 minutes at 2000 x g to remove the lipid layer, or leave the milk samples until the fat layer is formed on top of the sample. Pipette under the fat layer.

Preparation of reagents

PBS-Tween Buffer:

Dilute the PBS-Tween Solution 20 x concentrate 1/20 in distilled water. Prepare 500 mL per plate by adding 25 mL PBS-Tween solution to 475 mL distilled water and mix thoroughly.

N.B. Please check that there is no crystal precipitation in the bottle. If crystals are seen, please warm and shake well.

Pre-dilution of samples:

Serum samples

For testing, the serum samples should be pre-diluted 1/100 in Sample Dilution Buffer (*e.g.* 5 µL serum sample into 495 µL of sample dilution buffer).

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at 2-8°C (36-46°F).
3. All reagents should equilibrate to room temperature 18-25°C (64-77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction manuals from different test kit batches.
6. Care should be taken to prevent contamination of kit components.
7. Do not use test kit beyond date of expiry.
8. Do not eat, drink or smoke where specimens or kit reagents are handled.
9. Use a separate pipette tip for each sample.
10. Do not pipette by mouth.
11. Include positive and negative serum controls on each plate or test strip series.
12. Use only distilled, deionised or any similar high quality water for preparation of reagents.
13. When preparing the buffers, etc., measure the required volume.
14. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

The volume of the reagents is sufficient for at least 10 test occasions.
Strips with broken seal can be stored at 2-8°C (36-46°F) for up to 4 weeks.

Procedure

1. All reagents should be equilibrate to room temperature 18-25˚C (64-77˚F) before use. Label each strip with a number.
2. In duplicates add 100 µL of Positive Control Serum (reagent A) and 100 µL of Negative Control Serum (reagent B) respectively, into selected wells.
3. Add samples

Serum samples

Add 100 µL of the pre-diluted sample to selected wells. The samples can be tested individually or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

Continue at step #4.

Meat juice samples

A. Add 90 µL of the Sample Dilution Buffer to each well that will be used for meat juice samples.

B. Add 10 µL of the meat juice samples into appropriate wells. The samples can be tested individually or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

Continue at step #4.

Milk samples

Add 100 µL of skim milk sample to the selected wells. The samples can be tested individually or in duplicates. However for confirmation purposes it is recommended to run the samples in duplicates.

Continue at step #4.

4. Shake the plate thoroughly. Seal the plate/strip and incubate:
   a. Serum samples: At 37˚C (98.6˚F) for 1 hour or at 2-8˚C (36-46˚F) over night (16-20 hours).
   b. Meat juice and milk samples: At 37˚C (98.6˚F) for 1 hour.
5. Rinse the plate/strips 4 times with PBS-Tween Buffer. At each rinse cycle fill up the wells, empty the plate and tap hard to remove all remains of fluid.
6. Add 100 µL of Conjugate to each well and incubate at 37˚C (98.6˚F) for 1 hour.
7. Repeat step #5.
8. Add 100 µL Substrate Solution to each well. Incubate for 30 minutes in the dark at room temperature 18-25˚C (64-77˚F). Begin timing when the first well is filled.
9. Stop the reaction by adding 50 µL of Stop Solution to each well and mix thoroughly. Add the Stop Solution in the same order as the Substrate Solution in step #8.
10. Measure the optical density (OD) of the controls and samples at 405 nm in a microplate photometer (use air as blank). Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculations

Calculate the mean OD value for the controls and samples.
Calculate the ODR values for the control as well as samples, using the following formula:

$$ODR = \frac{OD_{\text{Sample or Control}} - OD_{\text{Negative Control}}}{OD_{\text{Positive Control}} - OD_{\text{Negative Control}}}$$

N.B: For meat juice samples, calculate the mean ODR of up to 5 individual samples before interpretation of the test results.

Interpretation of the results

Criteria for test validity

To ensure validity, the duplicate of the OD values of the positive control should not differ more than 25% from the mean value of the two duplicates. Additionally, the control values should fall within the following limits:

$OD_{Positive\ Control}$ > 1.1

$OD_{Negative\ Control}$ < 0.3

Should any of these criteria not be fulfilled, the test is invalid. For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of samples

Short incubation (1 hour)

Sample	ODR	Interpretation
Serum	< 0.4 ≥ 0.4	No or low liver fluke burden Infection with liver fluke with likely production losses
Meat juice*	< 0.5 ≥ 0.5	No or low liver fluke burden Infection with liver fluke with likely production losses
Individual milk	< 0.4 ≥ 0.4	No or low liver fluke burden Infection with liver fluke with likely production losses Benefits from treatment
Bulk tank milk	< 0.3 0.3 - 0.6 ≥ 0.6	No or low liver fluke burden Contact with liver fluke, no interference with production Infection with liver fluke with likely production losses

*Based on mean value of ODR of up to 5 individual meat juice samples.

Long incubation (over night incubation)

Sample	ODR	Interpretation
Serum	< 0.6 ≥ 0.6	No or low liver fluke burden Infection with liver fluke with likely production losses

References

1. Charlier, J., Duchateau, L., Claerebout, E., Williams, D., Vercruysse, J. (2007): Associations between anti-*Fasciola hepatica* antibody levels in bulk-tank milk samples and production parameters in dairy herds. Prev. Vet. Med. 78, 57-66
2. Charlier, J., De Cat, A. , Forbes, A., Vercruysse, J. (2009): Measurement of antibodies to gastrointestinal nematodes and liver fluke in meat juice of beef cattle and associations with carcass parameters. Vet. Parasitol. 166, 235-240.
3. Sohair, I. B and Eman, M.N. (2009): Histopathological and bacteriological studies on livers affected with fascioliasis in cattle. J. Comp. Path. & Clinic. Path., 22, 19 – 45
4. Claridge J, Diggle P, McCann CM, Mulcahy G, Flynn R, McNair J, Strain S, Welsh M, Baylis M, Williams DJ. (2012): *Fasciola hepatica* is associated with the failure to detect bovine tuberculosis in dairy cattle. Nature communications, DOI: 10.1038/ncomms1840.

Symbols

REF	Article No.
LOT	Serial (batch) No.
	Temperature limit
	Expiry date
	Corrosive
	Number of tests
	See manual
	Manufacturer

License holder

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