

SVANOVIR® *O. ostertagi*-Ab

Ostertagia ostertagi
Antibody Test

Contents	Art. No. SV-104897
Microtitre plate Microtitre plate (96 wells) with non-infectious <i>O. ostertagi</i> 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
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® *O. ostertagi*-Ab kit:
Article number SV-104897

Ostertagia ostertagi

Antibody Test

Name and Application

SVANOVIR® *O. ostertagi*-Ab is an Enzyme Linked Immunosorbent Assay (ELISA) for the detection of antibodies against *O. ostertagi* in bovine bulk tank milk samples.

The screening for *Ostertagia ostertagi* antibodies in cattle milk 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

Ostertagia ostertagi is one of the most important gastrointestinal nematodes for cattle worldwide. The parasite has a direct life cycle with two different phases: the first free-living phase on pasture and the second phase as a parasite in the host, infesting the abomasum. As *Ostertagia ostertagi* is present on pasture, all grazing cattle in temperate climate regions are exposed to the parasite.

First-stage larvae develop from parasite eggs in the faecal pats and through moulting develop to second-stage larvae. Ingestion of grass contaminated with third stage larvae, that have developed from the second stage larvae but still enclosed in a sheath, will lead to the actual infestation. Third stage larvae are typically penetrating the gastric glands of the abomasums within 6 hours after ingestion, where development to the fourth adult stage occurs. These adult worms then emerge to the abomasal lumen. Nematode infestations in adult cows are predominantly subclinical, but may lead to a decrease in milk production. It is important to determine the infestation level and the necessity for anthelmintic treatment.

Principle

The kit procedure is based on a solid-phase indirect ELISA. In this procedure, milk samples are exposed to non-infectious *O. ostertagi* antigen coated wells of microtiter strips. *O. ostertagi* antibodies (if present in the sample) bind to the antigen in the wells. The HRP conjugate added subsequently forms a complex with these *O. ostertagi* 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 water, deionised or any similar high 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

Milk:

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.

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 8 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 equilibrate to room temperature 18-25°C (64-77°F) before use. Label each strip with a number.
2. Add samples
- A. Add 100 µL of Positive Control Serum (Reagent A) and 100 µL of Negative Control Serum (Reagent B) to selected wells. For confirmation purposes it is recommended to run the control sera in duplicates.
- B. Add 100 µL of skim milk sample to the selected well. The samples can be tested in singlicates or in duplicates. However, for confirmation purposes it is recommended to run the samples in duplicates.
3. Shake the plate thoroughly. Seal the plate/strips and incubate for 1 hour at room temperature, 18-25°C (64-77°F).
4. Rinse the plate/strips 3 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.
5. Add 100 µL of HRP Conjugate to each well. Seal the plate/strips and incubate for 1 hour at room temperature, 18-25°C (64-77°F).
6. Repeat step #4.
7. 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.
8. 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 #7.
9. 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:

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

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:

$$\text{OD}_{\text{Positive Control}} > 0.8$$

$$\text{OD}_{\text{Negative Control}} < 0.4$$

For interpretation of data, see figure 1.

For invalid tests, technique may be suspect and the assay should be repeated.

Interpretation of bulk tank milk samples

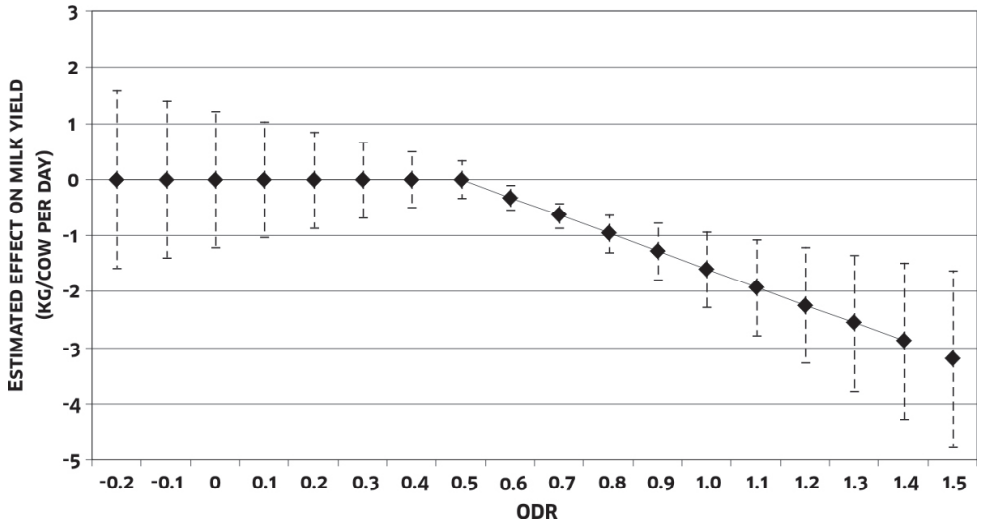










Figure 1

To assess the importance of the gastrointestinal nematode infestation in a specific herd, the herd's bulk tank milk ODR should be plotted on the line. The probable effect of this level of infestation pressure on the herd average milk yield can be read on the Y-axis.

References

1. Sanchez J., Dohoo I., Nødtvedt A., Keefe G., Markham F., Leslie K., DesCôteaux L., Campbell J., 2002. A longitudinal study of gastrointestinal parasites in Canadian dairy farms The value of an indirect *Ostertagia ostertagi* ELISA as a monitoring tool. *Vet. Parasitol.* 107, 209-226.
2. Charlier J., Duchateau L., Claerebout E., Vercruyse J., 2005. Assessment of the repeatability of a milk *Ostertagia ostertagi* ELISA and effects of sample preparation. *Preventive Veterinary Medicine* 68, 277-288.
3. Charlier J., Claerebout E., Duchateau L., Vercruyse J., 2005. A survey to determine relationships between bulk tank milk antibodies against *Ostertagia ostertagi* and milk production parameters. *Veterinary Parasitology* 129, 67-75.
4. Charlier J., Duchateau L., Claerebout E., Vercruyse J., 2007. Predicting milk-production responses after an autumn treatment of pastured dairy herds with eprinomectin. *Veterinary Parasitology* 143, 197-390.

Symbols

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

The test has been developed in co-operation with the Department of Virology, Parasitology and Immunology- Faculty of Veterinary Medicine, Ghent University, Belgium



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