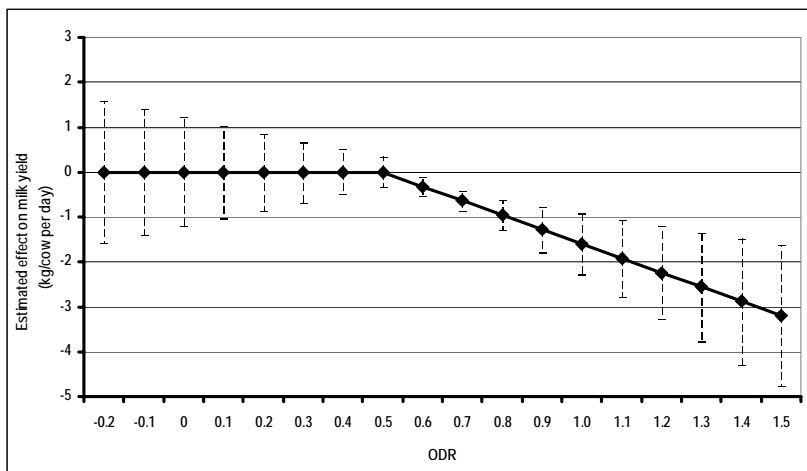


Interpretation of bulk tank milk samples

Figure 1

To assess the importance of the 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

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

This manual covers the following *O. ostertagi*-Ab ELISA kits: Article number 10-2940-02.

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Ostertagia ostertagi antibody ELISA (*O. ostertagi*-Ab) SVANOVIR®

ELISA test for the detection of *Ostertagia ostertagi* antibodies
in bovine milk samples

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. 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.

Principle

The SVANOVIR® *O. ostertagi*-Ab ELISA Kit is designed to detect bovine *O. ostertagi*-specific antibodies in milk. The kit procedure is based on a solid-phase indirect Enzyme Linked Immunosorbent Assay (ELISA). In this procedure, milk samples are exposed to non-infectious *O. ostertagi* antigen coated onto wells of microtiter strips. *O. ostertagi* antibodies (if present in the sample) bind parasite 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 result can be read by a microplate photometer, where the optical density (OD) is measured at 405 nm and 492nm.

Contents

- 2 *O. ostertagi* antigen coated microtiter strips (sealed and stored dry)
- 2 Lyophilized HRP Conjugate (horseradish peroxidase conjugated anti-bovine IgG monoclonal antibodies)
- 1 PBS-Tween Solution 20x concentrate (125ml)
- 1 Substrate Solution ABTS (50ml) – STORE IN THE DARK!

- 1 Stop Solution (25ml) – contains 1% SDS
- 1 Positive Control Milk – 0.1% Merthiolate (2,5ml)
- 1 Negative Control Milk – 0.1% Merthiolate (2,5ml)

Material needed but not provided

1. Precision pipettes (range from 5 to 200 µl)
2. Disposable pipette tips
3. Distilled water
4. Wash bottle
5. 1 Container; 1 to 2 litres for PBS-Tween
6. Microplate photometer, 405nm and 492nm filter

Specimen information

Milk: 100µl of skim milk is required for each sample well. 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 PBST 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.

HRP Conjugate: Reconstitute the lyophilized HRP Conjugate with 11.5 ml PBS-Tween Buffer. Add the buffer carefully to the bottle. Leave the solution one minute and mix thoroughly on a shaker. Prepare immediately before use. The remaining reconstituted conjugate can be stored at -20°C and thawed and refrozen up to 3 times.

Precautions

1. Carefully read and follow all instructions.
2. Store the kit and all reagents at +2 to +8°C (35 to 45°F).
3. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
4. Handle all materials according to the Good Laboratory Practice.
5. Do not mix components or instruction booklets from different test kits.
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 milk controls on each plate or test strip series.
12. Use only distilled water for preparation of reagents.
13. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendation!

The volume of the reagents is sufficient for at least 8 separate test occasions.
 Strips with broken seal can be stored at +2 to +8°C for up to 4 weeks.
 Reconstituted conjugate may not be stored in refrigerator.

Procedure

1. All reagents should equilibrate to room temperature 18 to 25°C (64 to 77°F) before use.
2. **Controls and samples**
 - A. Add 100µl of Positive Control Milk and 100µl of Negative Control Milk samples to selected wells. For confirmation purposes it is recommended to run the control samples in duplicates.
 - B. Add 100µl of Milk Test Samples to selected wells. For confirmation purposes it is recommended to run the samples in duplicates.
 - C. Add 100µl PBS-Tween Buffer to wells selected for conjugate/substrate control. For confirmation purposes it is recommended to run the control samples in duplicates.
3. Seal the plate/strip and shake thoroughly. Incubate for 1 hour at room temperature (18 to 25°C).
4. Rinse the plate/strip 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 100µl of PBS-Tween Buffer to wells selected for substrate control. For all other wells, add 100µl of the reconstituted HRP conjugate.
6. Seal the plate and incubate at room temperature for 1 hour (18 to 25°C).
7. Repeat step #4.
8. Add 100µl substrate solution to each well. Incubate for 30 minutes in the dark at room temperature.
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. Shake the plate thoroughly and measure optical density (OD) at 405 nm and 492 nm in a spectrophotometer.

Calculations

To calculate the final OD value, subtract the OD value at 492 nm from the OD value at 405 nm as follows:

$$OD_{\text{sample or ctrl}} = OD_{405} - OD_{492}$$

Calculation of ODR value

$$ODR = \frac{OD_{\text{sample or ctrl}} - OD_{\text{Neg ctrl}}}{OD_{\text{Pos ctrl}} - OD_{\text{Neg ctrl}}}$$

Interpretation of the results

Criteria for test validity

To ensure the test validity, the following results should be obtained for milk controls:

Positive Control Milk

$$OD_{405-492} > 0.75 \text{ (mean value)}$$

Negative Control Milk

$$OD_{405-492} < 0.30 \text{ (mean value)}$$

For control milk samples (both positives and negatives) in duplicates, the $OD_{405-492}$ value for each single duplicate should not differ more than 25% from the mean value of the two duplicates

For interpretation of data, see figure 1.