SVANOVIR® *L. intracellularis*/Ileitis-Ab

Lawsonia intracellularis
Antibody Test

Contents	**Art. No. SV-122275**
Microtitre plate Microtitre plates (96 wells) coated with non-infectious *L. intracellularis* antigen (sealed and stored dry)	2 (Strips) 12 x 8
Sealer	2
Conjugate 100 x concentrated, (horseradish peroxidase conjugated anti-*L. intracellularis* monoclonal antibodies)	1 x 350 µL
PBS-Tween Solution 20 x concentrate	1 x 125 mL
Sample Dilution Buffer – ready to use	1 x 125 mL
Conjugate Dilution Buffer – ready to use	2 x 25 mL
Substrate Solution (Tetramethylbenzidine in substrate buffer containing H_2O_2) - STORE IN THE DARK!	1 x 20 mL
Stop Solution - Contains sulphuric acid (2M) – DANGER! 	1 x 10 mL
A. Positive Control Serum - Contains preservatives	1 x 300 µL
B. Negative Control Serum - Contains preservatives	1 x 300 µL

This manual covers the following SVANOVIR® *L. intracellularis*/Ileitis-Ab kit: Article number SV-122275

Lawsonia intracellularis
Antibody Test

Name and Application
SVANOVIR® *L. intracellularis*/Ileitis-Ab is a blocking Enzyme Linked Immunosorbent Assay (ELISA) for the detection of *Lawsonia intracellularis* (*L. intracellularis*) specific antibodies in porcine serum and plasma samples.

General information
Porcine proliferative enteropathy (PPE) is caused by the obligate intracellular organism *L. intracellularis*, a gram-negative small rod shaped bacterium. Infection is characterized by hyperplasia and inflammation of the ileum (ileitis) and colon. Disease is categorized pathologically as a severe acute form called proliferative haemorrhagic enteropathy (PHE) that is more common in young adults (> 4 months old), and a chronic or necrotic form which occurs more frequently, usually during the grower phase, typically in pigs 6- to 20-weeks old. The chronic form is often subclinical and results in reduced performance affecting weight gains, reduces feed intake and causes loss of homogeneity in body weight (Jacobson *et al*. 2003; Guedes 2004).
Not all animals that are infected with *L. intracellularis* shed the bacteria in faeces at detectable amounts and shedding is often intermittent (Knittel *et al*. 1998). Cultivation of the bacterium on traditional media is not possible, thus necropsy and microscopy of epithelial cells was the only diagnostic method for a long time, restricting the diagnostics to post mortem analysis. However, serological antibody detecting tests have proven suitable for the diagnosis of an ongoing infection in the live animal. The application of the ELISA technology for this purpose enables high sample throughputs in comparison to *e.g.* the indirect fluorescent antibody test (IFAT) with comparable results.
To manage and control the disease seroprofiling in combination with vaccination is an effective strategy.

Principle
The kit procedure is based on a solid phase blocking ELISA. In this procedure samples are exposed to non-infectious *L. intracellularis* antigen coated wells on microtitre plates or strips. If *L. intracellularis* antibodies are present in the test sample they will bind to the antigen in the well and block these antigen sites. If *L. intracellularis* antibodies are absent in the test sample these sites will remain free.
The anti-*L. intracellularis* horseradish peroxidase (HRP) conjugate added subsequently bind to free *L. intracellularis* antigen sites in the well. Unbound material is removed by rinsing before the addition of a substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate. A negative result is indicated by the development of a blue colour. The reaction is stopped by addition of stop solution; the colour changes to yellow. The result can be read by a microplate photometer, where the optical density (OD) is measured at 450 nm.

Materials needed but not provided

1. Precision pipettes (range from 10 to 200 µL)
2. Disposable pipette tips
3. Distilled, deionised or any similar high quality water
4. Wash bottle, multichannel pipettor or plate washer. The used washing pressure should not exceed 0.2 bar.
5. Container: 1 to 2 litres for PBS-Tween
6. Microplate photometer, 450 nm filter
7. Plate sealer
8. Incubator +37°C

Specimen information

Individual serum:

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

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.

Conjugate:

Dilute the concentrated conjugate 1:100 in the conjugate dilution buffer. Prepare 1 mL per strip by adding 10 µL conjugate to 990 µL Conjugate 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 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. The Stop Solution contains sulphuric acid, which is corrosive*.
15. All unused biological materials should be disposed according to the local, regional and national regulations.

Recommendations!

There is always a surplus volume for the liquid reagent. The volume mentioned on the label is the minimum obtainable.
Samples can be stored at 2-8°C for a maximum of 7 days. For long term storage samples have to be kept at -20°C.

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 90 µl of Sample dilution buffer to each well that will be used for serum samples and controls.

B. Add 10 µl of Positive Control (Reagent A) and 10 µl of Negative Control (Reagent B) respectively to selected wells. For confirmation purposes it is recommended to run the control sera in duplicates.

C. Add 10 µl of serum sample to selected wells. 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/strip and incubate at 37°C (98.6°F) for 60 minutes.
4. Rinse the plates/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 pre-diluted HRP Conjugate to each well and incubate at 37°C (98.6°F) for 60 minutes.
6. Repeat step #4.
7. Add 100µl of Substrate solution to each well. Incubate for 10 minutes at room temperature, 18-25°C (64-77°F). Begin timing when the first well is filled.
8. Stop the reaction by adding 50µl 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 450nm in a microplate photometer. Measure the OD within 15 minutes after the addition of Stop Solution to prevent fluctuation in OD values.

Calculations

1. Calculate the mean OD values for each of the controls and samples.
2. Calculate the percent inhibition (PI) values for positive control as well as samples, using the following formula:

$$PI = \frac{OD_{\text{Negative Control}} - OD_{\text{Sample or Positive Control}}}{OD_{\text{Negative Control}}} \times 100$$

Interpretation of the results

Criteria for test validity

To ensure validity, control values should fall within the following limits:

OD	**Negative control**	**≥ 0.5**
PI	**Positive control**	**≥ 40%**

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

PI	**< 30**	**Negative**
PI	**≥ 30**	**Positive**

Within the negative range of results any sample presenting a PI-value between 20 and 30 should be considered questionable. A second test on a different sample from the same animal is recommended.

***DANGER: Stop solution (sulphuric acid)**
May be corrosive to metals. Causes skin irritation. Causes serious eye irritation.

Keep only in original container. Wear eye protection/ face protection. Wear protective gloves.
IN CASE OF CONTACT WITH EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician. If eye irritation persists: Get medical advice/ attention.
IN CASE OF CONTACT WITH SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse. If skin irritation occurs: Get medical advice/attention. Absorb spillage to prevent material damage.

Symbols

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

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