



User's Manual

Anti-Salmonella abortusovis ELISA Kit



DEIA-WZ1001S



5x96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Salmonella Abortusovis test is an Enzyme-linked immunosorbent assay (ELISA) intended for the detection of IgG anti-Salmonella Abortusovis in sheep serum samples. The test is designed to be used for the detection of abortive salmonellosis infection and evaluation of antibody response to vaccination.

General Description

Salmonella enterica spp. enterica serovar Abortusovis (Salmonella Abortusovis) is a Salmonella serovar host-adapted to sheep, which causes infections that are mainly characterized by abortion as a main symptom. The disease develops in the last weeks of pregnancy, and the pathogenic mechanisms involved have not yet been understood. In the areas endemic for the microorganism, abortion may occur in up to 50% of the ewes in a flock, usually during the first pregnancy. This high incidence of salmonellosis represents a major threat to the flocks and may result in important economic losses in regions that depend on shepherding.

Salmonella Abortusovis is reportable to the World Organization for Animal Health (OIE), but outbreaks are uncommonly described outside a few regions, such as southern Europe and Western Asia.

Diagnosis is made by culture of placenta, fetus, or uterine discharge. Isolation of aborting ewes and destruction of contaminated bedding and of all products of abortion reduce contamination. Prevention is mainly based on vaccination with dead or living vaccines in endemic areas.

The ELISA test may be used for serological identification of infected animals, flocks and herds. However, the test does not distinguish between vaccinated and infected sheep. IgG levels typically appear 1-3 weeks after infection and last several months (up to 10 months after abortion). The test is suitable for automation and hence to large scale testing programmes.

Principles of Testing

Microtiter strips coated with S. Abortusovis lipopolysaccharide (LPS) are incubated with collected samples. During this incubation step, anti - S. Abortusovis antibodies bind to the antigen, forming specific complexes. Antigen-antibody complexes are detected by anti-sheep IgG HRP-conjugated secondary antibody. Revelation step is performed incubating the strips with 2,2'-azino-bis(3- ethylbenzthiazoline-6-sulfonic acid) (ABTS) as chromogen and reading the absorbance values at 405 nm by ELISA microplate reader.

Reagents And Materials Provided

1. Microtiter strips (12 x 8 well strips): 5 microplate coated with S. Abortusovis antigen, preserved with sodium azide 0,02%
2. Buffer A: 50 ml x5 ready to use, with preservative
3. Buffer B (Wash Buffer 10x concentrate): 100 ml x5, to dilute to 1x with distilled water; with preservative
4. HRP-conjugated secondary antibody: 13 ml x5 ready to use, with preservative
5. ABTS Solution: 13 ml x5, ready to use, with preservative

6. Negative control: lyophilized (prepared in Buffer A) ×5
7. Positive control: lyophilized (prepared in Buffer A) ×5

Materials Required But Not Supplied

1. Microplate reader equipped with 405 nm filter
2. Incubator at 37°C (avoid CO₂ that oxidizes the immune complexes) or thermo block
3. Precision pipettes and pipette tips
4. Glass or plastic pipettes
5. Deionised or distilled water
6. Semi-automated or automated microplate washer
7. 1000 mL graduated cylinder
8. Vortex
9. Tubes for diluting samples

Storage

Store the kit at +2/8°C

Specimen Collection And Preparation

Serum - Collect samples in serum-use pyrogen/endotoxin-free collecting tubes. After blood clotting, centrifuge the tubes at approximately 1,000 x g for 10 min and remove sera from the red cells.

Storage - Samples can be stored at 2–4°C for 24 hours after collection. For longer periods, samples should be stored frozen in small aliquots. Avoid freeze-thaw cycles.

Before use, thaw completely samples at room temperature. Do not thaw by heating.

Reagent Preparation

1. PREPARATION OF NEGATIVE AND POSITIVE CONTROLS:

Add 500 µl of distilled water into the vials containing lyophilized positive and negative controls. Vortex for at least 30-60 seconds. Make sure that the controls are completely dissolved and check that no visible aggregates are present.

After to rehydrate, equilibrate negative and positive controls at room temperature for at least 30 minutes before performing the test.

Recommendation: The rehydrated positive and negative controls can be kept frozen at -20 ° C for up to 15 days.

2. PREPARATION OF REAGENTS:

Bring selected strips and all the provided reagents at room temperature at least 30 minutes before use.

Buffer B (Wash Buffer 10× Concentrate): If crystals have formed in the concentrate, warm at room

temperature and mix gently until the crystals are completely dissolved. Dilute 100 ml of buffer B to 1000 ml with distilled water.

Important: The performance of the kit may decrease if reagents are not properly prepared.

3. SAMPLE PREPARATION

Vortex sample 3-5 seconds

Avoid taking any aggregates present on the bottom of the tube

Check that the tip does not become blocked during the sampling

If the sample is slimy, cut the tip before withdrawing

Dilute each test sample 1:100v/v in Buffer A

Recommendation: Avoid any foam or bubbles in the sample.

Assay Procedure

1. Dispense 100 µl of negative control into duplicate wells
2. Dispense 100 µl of positive control into duplicate wells
3. Dispense 100 µl/well of 1:100v/v diluted sera into the appropriate wells

Note: avoid bubbles in the well.

4. Check that the volume is the same in the two replicate wells
5. Cover the strips or plate with aluminium foil and incubate at $37 \pm 1^\circ\text{C}$ for 60 ± 5 minutes
6. Wash the microtiter strips five times with reconstituted Buffer B (see paragraph "Reagents Preparation")
7. Dispense 100 µl/well of HRP-conjugated secondary antibody
8. Cover the strips or plate with aluminium foil and incubate at $37 \pm 1^\circ\text{C}$ for 60 ± 5 minutes
9. Wash the microtiter strips five times with approximately 300 µl reconstituted Buffer B.
10. Add 100 µl/well of ABTS solution
11. Cover the microtiter strips and incubate at room temperature ($22-27^\circ\text{C}$) for 25 ± 5 minutes

Note: Avoid light exposure

12. Read the absorbance at 405nm using a microplate reader

Calculation

Controls calculation

Make the absorbance mean of the replicates:

Negative Control (NC) mean = NC R1 (405 nm) + NC R2 (405 nm) /2

Positive Control (PC) mean = PC R1 (405 nm) + PC R2 (405 nm) /2

Validity criteria

Negative control (mean ABS at 405 nm) ≤ 0.250

Positive control (mean ABS at 405 nm) ≥ 1.11

For invalid assays, technique may be suspect, and the assay should be repeated following a thorough review of the product information.

Samples calculation

Make the absorbance mean of the replicates:

Note: The absorbance variation between the two samples replicate of the same sample is not acceptable if it is higher than 0.250 abs at 405nm. Re-test the sample.

Apply the calculation:

$$S/P = (\text{Sample A (ABS Mean)} - \text{NC (ABS Mean)}) / (\text{PC (ABS Mean)} - \text{NC (ABS Mean)})$$

The presence or absence of antibody to Salmonella Abortusovis is determined by calculating the sample to Positive (S/P) ratio for each sample.

Interpretation Of Results

Cutoff ratio Sample/Positive:

Demonstrate freedom from infection in a defined population: Historical freedom

NEGATIVE: $S/P \leq 0.516$

POSITIVE: $S/P > 0.516$

Confirmatory diagnosis of suspect of clinical cases:

NEGATIVE: $S/P \leq 0.520$

POSITIVE: $S/P > 0.520$

Determinate immune status individual animals or populations post-vaccination

NEGATIVE: $S/P \leq 0.250$

POSITIVE: $S/P > 0.250$

Performance Characteristics

Intra-assay CV: $\leq 20\%$

Inter-assay CV: $< 19\%$

Specificity

The kit was tested on a panel of serum samples positive for other bacterial species that reveal a similar LPS O antigen or species identified as responsible for abortion in sheep. The obtained results indicated that the kit is very specific. No cross-reactions were observed.

Precautions

1. Do not interchange components between different kits
2. Strict adherence to the test protocol will lead to achieving best results

3. When using the kit, check the reagent solutions are clear
4. Do not use the kit after expiration date
5. Avoid cross-contamination between serum specimens
6. Treat all specimens and kit serum-based reagents as potentially infectious

Limitations

1. For laboratory use only
2. No drugs have been investigated for assay interference.
3. Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature, or kit age can cause variation in binding.

