Sheep anti-Salmonella abortus ovis IgG Immunoassay

Product Number # AKE0016

Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Salmonella abortus ovis IgG in sheep serum and plasma.

FOR IN VITRO DIAGNOSTIC USE

NAME AND INTENDED USE

Enzyme-linked immunosorbent assay (ELISA) for the detection of anti-Salmonella abortus ovis IgG in sheep serum and plasma for the diagnosis of abortive salmonellosis infection and evaluation of antibody response to vaccination.

INTRODUCTION

Salmonella enteritidis subsp. enteritidis ser. abortus ovis, a sheep-adapted serotype, causes an infectious disease with abortion as the main symptom, sometimes accompanied with mortality of lambs at term (1). Salmonella abortus ovis infections can be found worldwide, but are particularly common in Europe and Western Asia (2). Salmonella abortion is usually stress related and most ewes are sick and febrile before aborting. There are no specific placental lesions, and the fetus is autolyzed (3). 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 and annual vaccination with dead or living vaccines is advisable in endemic areas (1).

Serological research of antibodies against S. abortus ovis could have a fundamental importance when the infected sheep showing no excretion of the pathogen (4). Moreover a simple, quick and reliable ELISA test could make easier the infection diagnosis and the herds screening. Obviously the assay can be also used to check the immunity response to S. abortus ovis vaccination.

The “Sheep anti-Salmonella abortus ovis IgG Immunoassay”, having a sensitivity (Se) and a specificity (Sp) higher than 95%, satisfies all these requirements.

PRINCIPLE OF THE ASSAY

Microtiter strips coated with S. abortus ovis lipopolysaccharide (LPS) are incubated with collected samples. During this incubation step, anti- S. abortus ovis antibodies are bound to antigens forming specific complexes. Antibody excess is removed by washing and in each well the antigens-antibodies complexes is detected by adding anti-IgG HRP-conjugated globulin. 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 by ELISA microwell plate reader.

LIMITATIONS OF THE PROCEDURE

FOR IN VITRO DIAGNOSTIC USE

- No drugs have been investigated for assay interference.
- The kit should not be used beyond the expiration date on the kit label.
- Any variation in specimen diluent, operator, pipetting technique, washing technique, incubation time or temperature, or kit age can cause variation in binding.

REAGENTS

Microtiter Strips (12 x 8 well strips) coated with S. abortus ovis LPS, blocked with BSA 2%.
Assay Diluent (Buffer A): 50 ml ready to use, with preservative.
Wash Buffer concentrate (Buffer B): 100 ml 10x to dilute to 1000 ml final volume with distilled water, with preservative.

Conjugated Antibody: 50 µl rabbit anti-sheep IgG-HRP conjugated.

Chromogen Solution: 30 ml Buffer C; 30 µl buffer C₁; 2 ABTS tablets.

**STORAGE**

Maintain the kit at 2-4°C.

**OTHER SUPPLIES REQUIRED**

- Microplate reader capable of measuring absorbance at 405 nm.
- Precision pipettes and pipette tips.
- Glass or plastic pipettes.
- Deionized or distilled water.
- Multi-channel pipette, semi-automated or automated microplate washer.
- 1000 ml graduated cylinder for preparation of Wash Buffer.
- Vortex mixer.
- Glass tubes.

**PRECAUTIONS**

- The buffer C₁ provided with this kit is H₂O₂ solution 30% m/m (110 volumes). Causes burns, after contact with skin, wash immediately with plenty of water. Wear suitable protective clothing and eye/face protection.
- Do not interchange components between different kits.
- When using the kit check that the reagent solutions are clear.
- Avoid cross-contamination between serum specimens.
- Do not use the kit after the expiration date.
- Treat all specimens and kit serum-based reagents as potentially infectious.

**SAMPLE COLLECTION AND STORAGE**

- **Serum** – Collect sample in Serum-Use pyrogen/endotoxin free collecting tubes. After blood clotting centrifuge it at approximately 1000 x g for 10 min and remove serum from the red cells.
- **Plasma** - Collect blood in Serum-Use pyrogen/endotoxin free collecting tubes with heparin or EDTA and centrifuge it at 1000 x g for 10 min. Remove plasma rapidly and carefully.
- **Storage** – Samples can be stored at 2–4°C for up 24 hours after collection. For longer periods samples should be stored frozen. Avoid freeze-thaw cycles.
- **Recommendation** - Before assaying thaw completely samples at room temperature. Do not thaw by heating at 37°C or 56°C.

**REAGENT PREPARATION**

Bring all reagents to room temperature before use.

**Assay diluent.** Ready to use, utilized this buffer for sera or plasma dilutions in a clean glass vials.

**Wash Buffer Concentrate.** If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 100 ml of Wash Buffer (Buffer B) concentrate to 1000 ml with distilled water.

**Conjugated antibody.** Prepare immediately before use. Dilute 1:1000 with buffer A, in a clean glass vial, according to the number of wells to be used (100 µl/well). Do not keep this dilution for further experiments.

**Colour solution.** Reconstitute immediately before use. Dissolve one ABTS tablet in 12.5 ml of buffer C and mix with 12.5 µl of buffer C₁. The Colour solution is light sensitive, avoid prolonged exposure to light.
RUNNING PARTIAL PLATES

This ELISA provides the flexibility to run two partial plates on separated occasions. Decide the number of strips you wish to run, leaving the strips to be used in the frame. Remove the unnecessary strips from the frame and store them at 2-4°C.

Reconstitute the Colour solution as previously described using only one ABTS tablet. Care must be taken to ensure that the remaining buffer C and C₁ are not contaminated.

ASSAY METHOD

1. Dilute sera 1:100 in Buffer A.
2. Dispense diluted sera 100 µl/well, in duplicate. Add 100 µl of Buffer A into two wells as reagent blank.
3. Cover the microwells and incubate at 37°C for 60 minutes.
4. Wash the microtiter strips five times with reconstituted Buffer B.
5. Dispense the rabbit anti-sheep IgG HRP-conjugated diluted 1:1000 in Buffer A (100 µl/well).
6. Cover the microwells and incubate at 37°C for 60 minutes.
7. Wash the microtiter strips five times with reconstituted Buffer B.
8. Add 100 µl of reconstitute Colour buffer to each well, including reagent blank wells.
9. Cover the microtiter strips and incubate at room temperature for 30 minutes.
10. Read immediately the absorbance at 405 nm using a microplate reader.

Test negative and positive controls are available and could be ordered separately from Diatheva.

RESULTS

The mean absorbance value for each sample must be interpreted as follow:

<table>
<thead>
<tr>
<th>ABSORBANCE</th>
<th>RESULT</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td>Negative</td>
<td>No detectable antibodies to listeriolysin O by the ELISA test. Such individuals are presumed to be uninfected with Listeria monocytogenes.</td>
</tr>
<tr>
<td>*</td>
<td>Equivocal</td>
<td>Sample should be retested.</td>
</tr>
<tr>
<td>*</td>
<td>Positive</td>
<td>Indicates presence of detectable antibodies to listeriolysin O. The individual may be infected with Listeria monocytogenes.</td>
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*Reference absorbance values are specified in the datasheet supplied with the kit.

REFERENCES