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Listeria sheep Test

AKE0006

96 Test

Intended use

Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of anti-listeriolysin O (LLO) IgG in sheep serum to diagnose *listeria* infection.

Introduction

Listeria monocytogenes is the causative agent of listeriosis, a severe nervous disease associated with a high case fatality rate. Among the domestic animals, the disease most commonly occurs in ruminants [Cooper J & Walker RD, 1998].
Listeriolysin O (LLO), is a major virulence factor and it was confirmed that it can induces a strong humoral response during infection, even when animals were infected with subclinical infecting doses of L. monocytogenes [Lhopital et al., 1993].

Principle of the assay

Microtiter strips coated with LLO antigen are incubated with collected samples. During this incubation step, anti-LLO 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 410 ±5nm by ELISA microplate reader.

Kit contents

- **Microtiter strips (12 x 8 well strips on single well holding frame):** 1 microplate coated with LLO antigen, preserved with sodium azide 0,02%
- **Buffer A:** 50ml ready to use, with preservative
- **Buffer B** (Wash Buffer 10X concentrate)**:**100ml, to dilute to 1X with distilled water; with preservative
- **HRP-conjugated secondary antibody:** 13ml ready to use, with preservative
- **ABTS Solution:** 13ml, ready to use, with preservative
- **Negative control:** 500µl ready to use with preservative
- **Positive control:** 500µl ready to use with preservative

Material and equipment required (not provided):

- Microplate reader equipped with 405nm filter
- Incubator at 37°C (avoid CO_2 that oxidizes the immune complexes) or thermo block
- Precision pipettes and pipette tips
- Glass or plastic pipettes
- Deionised or distilled water
- Semi-automated or automated microplate washer
- 1000 mL graduated cylinder
- Vortex
- Tubes for diluting samples

Storage

Store the kit at +2/8°C
Shipping at room temperature has no detrimental effect on the performance of this kit

Warnings and Precautions

- Do not interchange components between different kits
- Strict adherence to the test protocol will lead to achieving best results
- When using the kit, check the reagent solutions are clear
- Do not use the kit after expiration date
- Avoid cross-contamination between serum specimens
- Treat all specimens and kit serum-based reagents as potentially infectious

Procedure

LIMITATION

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

SAMPLE COLLECTION AND STORAGE

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.

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

PREPARATION OF REAGENTS

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

Buffer B (Wash Buffer 10X Concentrate): If crystals have formed in the concentrate, warm at room temperature and mix gently until the crystals are completely dissolved. Dilute 100ml of buffer B to 1000ml with distilled water.

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

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.

TEST PROCEDURE

- Dispense 100µl of negative control into duplicate wells
- Dispense 100µl of positive control into duplicate wells
- Dispense 100µl/well of 1:100v/v diluted sera into the appropriate wells
  Note: avoid bubbles in the well
- Check that the volume is the same in the two replicate wells.
- Cover the strips or plate with aluminium foil and incubate at 37±1°C for **60±5 minutes**
- Wash the microtiter strips five times with 380µl of reconstituted Buffer B
  (see paragraph "Reagents Preparation")
- Dispense 100µl/well of HRP-conjugated secondary antibody
- Cover the strips or plate with aluminium foil and incubate at 37°±1C for **60**± **5minutes**.
- Wash the microtiter strips five times with reconstituted Buffer B.
- Add 100 µl/well of ABTS solution
- Cover the microtiter strips and incubate at room temperature (22-27°C) for **20± 5 minutes**
  Note: Avoid light exposure
- Read the absorbance at **405**± **5 nm** using a microplate reader

The final results must be interpreted as follow:

CALCULATIONS:

Controls calculation

Make the absorbance mean of the replicates:

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

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

Validity criteria

Negative control (mean ABS at 405nm)	Positive control (mean ABS at 405nm)
≤ 0.300	1.2 ≤ ABS ≤ 2.2

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

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:

$$S/P = \frac{\textit{sample A (abs mean)} - \textit{NC (abs mean)}}{\textit{PC (abs mean)} - \textit{NC (abs mean)}}$$

The presence or absence of antibody against *L. monocytogenes* is determined by calculating the sample to Positive (S/P) ratio for each sample

INTERPRETATION (cutoff ratio Sample/Positive):

Purpose: Prevalence of infection and demonstration of freedom from infection in individual animals

NEGATIVE	POSITIVE
S/P ≤ 0.420	S/P > 0.420

Purpose: Confirmatory diagnosis of suspect of clinical cases

NEGATIVE	POSITIVE
S/P ≤ 0.450	S/P > 0.450

Purpose : Determination of immune status in individuals or populations post vaccination

NEGATIVE	POSITIVE
S/P ≤ 0.380	S/P > 0.380

Technical assistance

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