ELISA

for the detection of *Clostridium chauvoei* flagella

EIA0001

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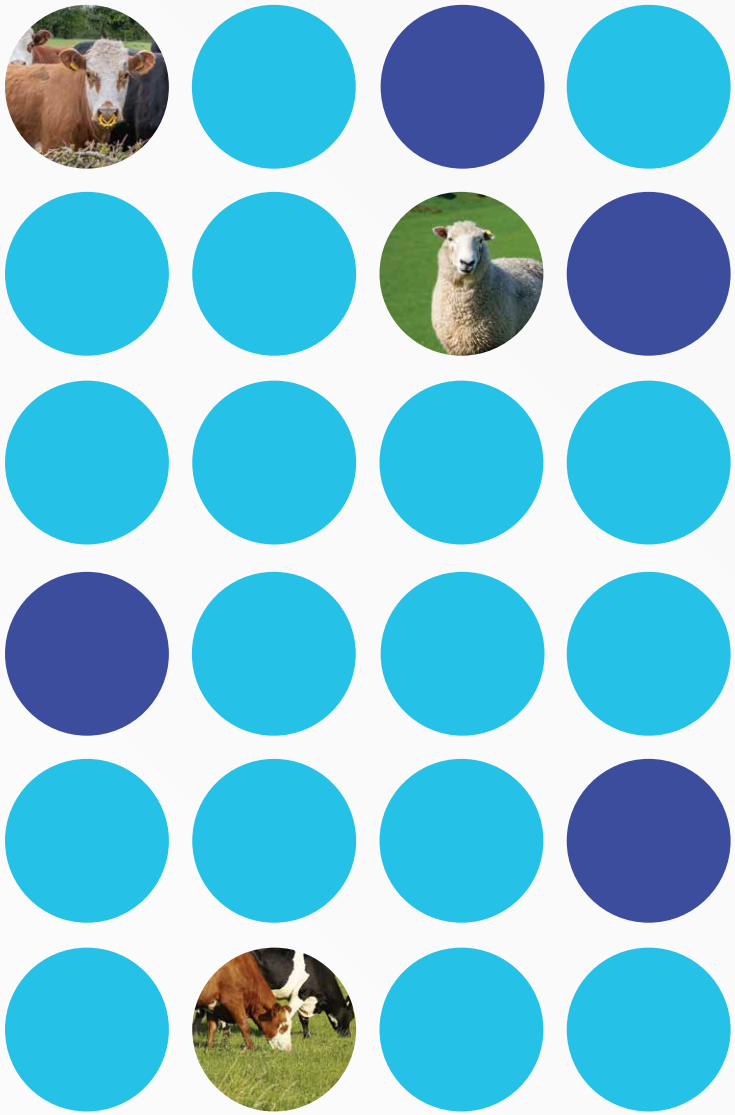
INTRODUCTION

Clostridium chauvoei, a Gram positive, highly pathogenic, strict anaerobic bacterium that is able to sporulate, is the etiologic agent of blackleg, a severe disease of cattle and to a lesser extent of small ruminants. Vaccines1 against blackleg consist of chemically inactivated bacteria of mostly relatively ancient strains, providing outer membrane proteins and flagellar proteins that have been proposed as immunogens and bacterial culture supernatants that are expected to contain the main toxins.



INTENDED USE

This protocol describes the ELISA method used to detect flagellin of *Clostridium chauvoei* to detect flagellin of *Clostridium chauovei* to follow culture through its protective antigen production, perform relative potency test of *C. chauovei* vaccines and monitor the stability of inactivated *C. chauovei* bulk.

This product is intended for research use only.

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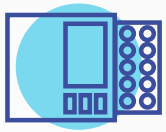
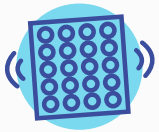
KIT COMPONENTS

- 5 Precoated plates
- 100X Standard flagellin
- Detection antibody
- 100X α-rabbit-HRP antibody
- Substrate solution
- Stop solution
- Diluent solution
- Wash solution salts

EQUIPMENT REQUIRED



Microplate reader, wavelengths (450 nm)



Microplate shaker

Refrigerator, 2°- 7°C

Micropipettes, 10- µL, 200-µL and 1000-µL

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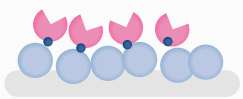
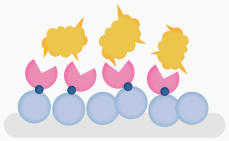
1



Plates are pre-coated with capture monoclonal antibody (V_HH) against flagellin of *C.chauvoei*. Allow room temperature.

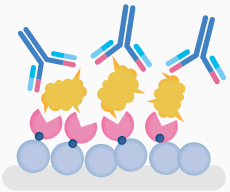
2



60′

Two-fold serial dilutions of standards and samples are added to the wells and binds to the immobilized capture antibody.

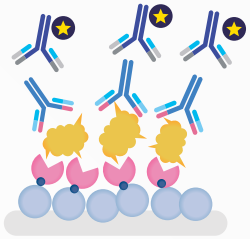
3



60′

Add Rabbit polyclonal detection antibody, an antibody-antigen-antibody “sandwich” is formed.

4



45′

Add Anti-rabbit HRP antibody.

5

Add TMB substrate, allow development of blue color, proportional to the concentration of flagellin.

Add stop solution to finish the reaction.

Absorbance should be read at 450 nm and proceed to **result interpretation**.

ASSAY PROCEDURE

Preliminary notes: *Reagents are not in excess, make sure to take only the required amount. Bring all reagents at room temperature (RT), once is reached, return remaining strips to package, seal tightly and store at 2 to 8 °C. *It is recommended that standards and samples are run in triplicates.

*Note that after received, the standard and conjugate should be store at -20°C, and they are 100x concentrated. *Wash buffer should be prepared prior to test.

1. Dilute bacterin samples and 1X standard flagellin by a factor of 2 in diluent solution. Make serial two-fold dilutions with the diluent solution in the pre-coated plate, starting by 1/2 until 1/128, including a sample blank. For each dilution incubate 100 uL for 60 minutes at RT in agitation.

2. Wash the plate ten times (200 µL/well) with wash solution. Blot plate on absorbent toweling.

3. Place 100 µL of ready to use detection antibody solution in each well. Seal plate and incubate for 60 minutes at RT in agitation.

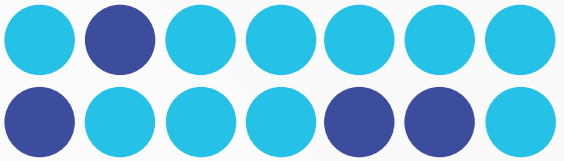
4. Wash the plate five times (200 µL/well) with wash solution. Blot plate on absorbent toweling.

5. Make a 1/100 dilution of HRP conjugated-antibody in the diluent solution. Then, place 100 µL of diluted conjugate in each well. Seal plate and incubate for 45 minutes at RT in agitation.

6. Wash the plate five times (200 µL/well) with wash solution. Blot plate on absorbent toweling. During this step allow substrate solution to reach RT (keep in the dark).

7. Wash the plate five times (200 µL/well) with wash solution. Blot plate on absorbent toweling.

8. Place 100 µL of substrate solution into each well. Seal the plate in dark and incubate at RT for 15 minutes in agitation, or until properly color change has occurred. Place 50 µL of stop solution into each well. Read the plate at 450 nm and proceed to result interpretation.



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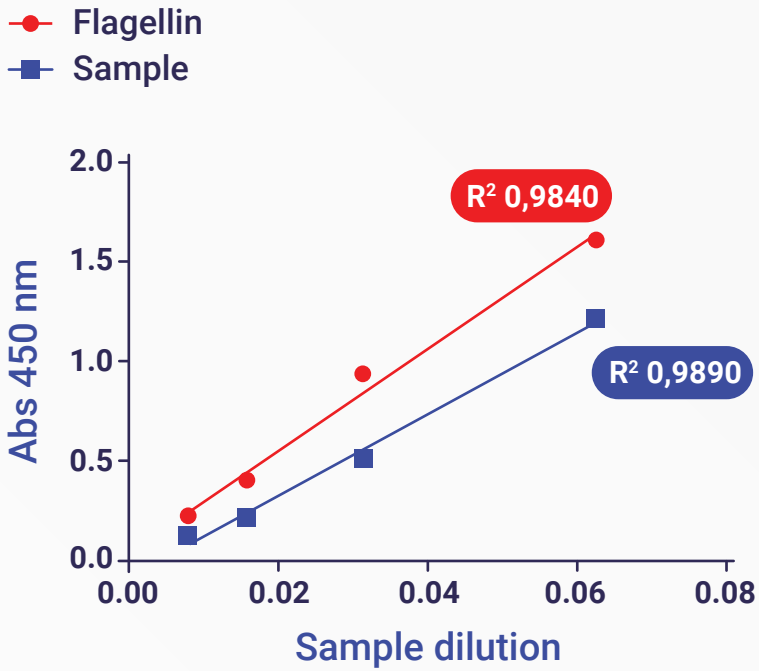
ANALYSIS OF RESULTS

Plot the log of the dilution used for the standard curve and the samples on the x-axis and the corresponding abosrbance on the y-axis. Define the dynamic range of the assay. Lines determined by first-order linear regression must have a correlation coefficient (r^2) of ≥ 0.95. The test's serial dose-response line must show parallelism.



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