LilliTest

Glanders cElisa Test Kit

A competitive enzyme linked immunosorbent assay (ELISA) to determine the presence of *B. mallei* antibody in equine sera.

1. INTRODUCTION

Glanders is a highly contagious disease of equids caused by the Gram-negative bacterium *Burkholderia mallei.* The organism is obligatory aerobic, non-spore forming and non-motile. The disease is currently confined to some areas of Asia, the Middle East, Africa and South America. The disease is characterized by nodules and ulcers in the skin, and by choanal and lung granulomas. Both acute (especially in donkeys) and chronic (especially in horses) forms of the disease occur. No vaccines are currently available against this disease. Glanders is a potential zoonosis (1).

2. PRINCIPLE

The Glanders cELISA kit is a competitive enzyme linked immunosorbent assay (ELISA) to determine the presence of *B. mallei* antibody in equine sera. The test is based on the competition between the specific monoclonal antibody and the antibodies in the serum sample binding to the *B. mallei* antigen.

The presence of antibodies to *B. mallei* in the serum sample will block reactivity of the monoclonal antibody resulting in a reduction in expected colour, following the addition of an enzyme labelled conjugate and substrate /chromogen.

The test plate is read at an optical density (OD) of 492nm.

3. MATERIAL SUPPLIED

Material Supplied	Qty
Pre coated test plate	2 plates
Monoclonal antibody (Concentrated)	25µl
HRP-labelled Conjugate (Concentrated)	25µl
Strong Positive (C++) reference serum (Freeze dried)	1ml
Negative (C-) reference serum (Freeze dried)	1ml
OPD (4mg) tablets	2 no's
Phosphate buffered saline (PBS) powder	2 sachets
Hydrogen peroxide	250µl
Blocking agent (Freeze dried)	1.5ml
Tween -20	250µl
Stop solution	2x6ml

4. MATERIALS REQUIRED BUT NOT INCLUDED IN THE TEST KIT

- Single/multi-channel pipettes
- Disposable plastic tips
- ELISA micro plate reader
- Mille Q water
- Reagent reservoirs
- Measuring cylinders
- Micro plate orbital shaker with temperature control
- Timer

5. PREPARATION OF REAGENTS/ WORKING DILUTIONS AND STORAGE

5.1 WASHING BUFFER

0.01M Phosphate Buffered Saline (PBS), pH 7.4
Empty the contents of one sachet into one litre of distilled/deionized water.

Label and store at +4° C for no longer than one month.

5.2 BLOCKING BUFFER

0.01M Phosphate Buffered Saline, pH 7.4 supplemented with 0.5 % (v/v) blocking agent plus 0.05 % (v/v) Tween 20.
Prepare the blocking buffer by dissolving 500µl blocking agent and 50 µl Tween 20 into 100ml of coating / washing buffer.

Note:
Reconstitute the Blocking agent (freeze dried) with precisely 1.5ml of distilled/deionized water and shake gently until completely dissolved.
The reconstituted blocking agent stock must be stored in its original vial at -20° C

Fresh blocking buffer must be prepared for each test day.

5.3 REFERENCE SERUM

Reconstitute the freeze dried contents of a vial of each reference serum (C++ and C-) with precisely 1ml of distilled/deionized water and shake gently until completely dissolved.
The reconstituted **reference serum stocks** must be stored in its original vial at **-20° C.**

Note:
Care must be taken to ensure the high quality of the distilled /deionized water used for the preparation of your reagents and buffer solutions

OIE Reference Laboratory for Glanders

Lillidale Diagnostics
Holt
Wimborne
Dorset
BH21 7DG
United Kingdom

Tel: +44 (0)1202 848456
Fax: +44 (0)1202 848570
Email: support@lillidale.co.uk
Website: www.lillidale.co.uk

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LilliTest
Glanders cElisa
Test Kit

For in vitro veterinary use only

5.4 MONOCLONAL ANTIBODY

Dilute concentrated monoclonal antibody at 1: 500 in blocking buffer.
The **stock solution of monoclonal antibody** must be stored in its original vial at +2 to +4° C without a significant loss of activity.

Note:
Please consult the Quality control certificate (QCC) included in the kit to determine the appropriate working dilution of the reagents.

5.5 HRP- LABELLED CONJUGATE

Dilute concentrated HRP labelled conjugate at 1: 12,000 in blocking buffer.
The **concentrated conjugate** must be stored in its original vial at +2 to +4° C.

Note:
Please consult the QC certificate (included in this manual) which has accompanied the kit to determine the appropriate working dilution of the reagents.

5.6 SUBSTRATE/CHROMOGEN

Dissolve one tablet of OPD (4mg) in 10ml of distilled/deionized water. Mix very gently (without trapping air bubbles) until completely dissolved.
Add 5µl of hydrogen peroxide. Mix very gently. Store in the dark.

Fresh substrate / chromogen must be prepared for each test day.

Care should be taken to ensure the accurate measurement of reagents by using well calibrated pipettes.

The SUBSTRATE/CHROMOGEN solution should be colourless when prepared. If coloured, it must be discarded and a fresh solution must be prepared.

5.7 STOP SOLUTION

Ready to use.

5.8 TEST SERA

The whole clotted blood sample should be centrifuged at 4000G for 5 minutes, after which the serum can be tested.

Note:
Before testing, sera should be examined for haemolysis (haemolytic sera would be red in colour), lipemic (whitish in colour), and for contamination (cloudiness). In the above mentioned cases, the sera have to be discarded.

If not tested immediately, test sera should be aspirated from the clot and stored at +2 to +4 ^{o}C for 72 hrs. Longer duration of storage requires samples to be frozen at -20°C in appropriately labelled cryopreservation vials.

Note:
Please consult the QC certificate (included in this manual) which has accompanied the kit to determine the appropriate working dilution of the reagents. This is necessary because different batches of reagents may vary in their optimal working dilutions.

6. PLATE LAYOUT

C++ Strong positive reference serum
C- Negative reference serum
Cm Monoclonal antibody control
Cc Conjugate control
Bc Blocking buffer control
1- 40 Test sera in duplicates

	1	2	3	4	5	6	7	8	9	10	11	12
A	C++	Cm	1	5	9	13	17	21	25	29	33	37
B	C++	Cm	1	5	9	13	17	21	25	29	33	37
C	C++	Cm	2	6	10	14	18	22	26	30	34	38
D	C-	Cm	2	6	10	14	18	22	26	30	34	38
E	C-	Cc	3	7	11	15	19	23	27	31	35	39
F	C-	Cc	3	7	11	15	19	23	27	31	35	39
G	Bc	Cc	4	8	12	16	20	24	28	32	36	40
H	Bc	Cc	4	8	12	16	20	24	28	32	36	40

7. TEST/ ASSAY PROCEDURE

Unless otherwise stated volumes used are 50µl /well, and washes are performed using 235µl of wash buffer / well.

7.1 Addition of blocking buffer
Add 40µl of blocking buffer to the reference control wells (C++, C-) and test wells.

Add 50µl of blocking buffer to monoclonal antibody control wells (Cm).

Add 100µl of blocking buffer to the conjugate control (Cc) and to the blocking buffer control wells (Bc).

7.2 Add to the C++, C- and test sera wells 10µl of the respective controls and test sera.

7.3 Add 50µl of the working dilution of monoclonal antibody to all the wells except the conjugate control (Cc) wells and blocking buffer control wells (Bc). Incubate plates for 1 hr at 37° C with shaking at rpm 400.

7.4 Wash plates 3 times with 235µl wash buffer.

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Lillidale Diagnostics
Holt
Wimborne
Dorset
BH21 7DG
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7.5 Add 50µl of working dilution of conjugate solution to the entire wells except the blocking buffer control wells (Bc). Add 50µl blocking buffer to the blocking buffer control wells and incubate plates for 1 hr at 37° C with shaking at rpm 400.

7.6 Wash plates 3 times with 235µl wash buffer.

7.7 Add 50µl of freshly prepared substrate/ chromogen solution to all wells and incubate for 12 (+/-2) minutes **without shaking** at room temperature in dark. Stop test reaction at the end of the prescribed incubation time with 50µl/well of the **ready to use** stop solution provided.

7.8 Read optical density at 492nm using an ELISA plate reader.

Note:
DO NOT USE PLATE WASHERS FOR WASH STEPS, MANUAL WASHING ADVISED
The plate reader should be turned on and allowed to warm up for at least 15 minutes before reading the test plate. This warm up period is necessary to ensure the uniformity of reading for all test plates.
Before reading the test plate, ensure that there are no bubbles in any of the wells, as this will cause optical aberrations. Ensure that there is no condensation or smudges (e.g. finger prints) on the bottom of the test plate. If necessary, rupture any bubbles using a clean pipette tip and wipe the bottom of the plate clean with a soft cloth.

8. ASSAY PERFORMANCE AND INTERPRETATION OF RESULTS.

Measure the optical density (OD) of the wells at 492nm within 10 - 15 minutes after colour development has been stopped.

Calculate the mean of wells monoclonal control (Cm) and blocking buffer control (Bc).

Calculate the corrected OD_{492} of all test samples by subtracting the mean OD_{492} of the blocking buffer control (Bc).

The percentage inhibition (PI) of the reference sera and test sera are calculated according to the formula below;-

$$PI = 100 - \left\{ \frac{\text{Mean } OD_{492} \text{ test serum}}{\text{Mean } OD_{492} \text{ monoclonal antibody (Cm)}} \right\} x100$$

Validation criteria

The test is valid if -

- the percentage inhibition (PI%) of the positive control ≥70%
- the percentage inhibition (PI%) of the negative control <50%
- the mean OD value of the monoclonal antibody control is OD is >0.60 - < 1.60 @ 492 nm

NOT MEETING ANY OF THESE CRITERIA IS REASON TO DISCARD THE RESULTS OF THAT SPECIFIC TEST PLATE.

INTERPRETATION OF PERCENTAGE INHIBITION

PI ≥ 60%	Positive
PI 51% - 59%	Ambiguous
PI ≤ 50%	Negative

IT IS RECOMMENDED TO RETEST ANY POSITIVE /AMBIGUOUS SAMPLES.

Quality Control Certificate (QCC)

It is good laboratory practice to plan your tests in advance. Check that all equipment is available and working, and that all reagents and control sera and test sera are prepared and thawed if necessary.

Care should be taken to ensure the accurate measurement of reagents using well calibrated pipette.

For each pipetting operation, new and clean disposable tips must be used.

Care must be taken to ensure the high quality of the distilled /deionized water used for the preparation of your reagents and buffer solutions.

Only Immuno-plate/ strips provided with the kit should be used for the test and they are not reusable.

Fresh blocking buffer must be prepared for each test day.

The substrate solution (OPD/H_2O_2) should be stored in the dark at all times.

Before reading the test plate, ensure that there are no bubbles in any of the wells, as this will cause optical aberrations. Ensure that there is no condensation or that there are no smudges (e.g. finger prints) on the bottom of the test plate. If necessary, rupture any bubbles using a clean pipette tip and wipe the bottom of the plate clean with a soft cloth.

The plate reader should be turned on and allowed to warm up for at least 15 minutes before reading the test plate. This warm up period is necessary to ensure the uniformity of reading for all test plates.

Ordering information:

LilliTest Glanders cElisa, 2 plates, 80 tests, Cat#VE-3001

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