Lillidale Diagnostics

LilliTest

T. evansi Indirect Elisa

# Indirect Elisa for the detection of *Trypanosoma evansi* antibody in camel sera

## 1. INTRODUCTION

Camel trypanosomosis (surra), caused by *Trypanosoma evansi*, is the most important single cause of morbidity and mortality in camels in arid zones. The disease, transmitted non-cyclically by haematophagus flies (e.g. Tabanids) is endemic in Africa, Asia and South America. Other than camels also domesticated livestock is affected. Because of the wide geographic range of surra, its control has attracted international attention, with a focus on formulating and implementing effective strategies aimed at increasing productivity and achieving a decrease in mortality and morbidity. Therefore, emphasis is placed on accurate diagnosis of surra, treatment with effective trypanocidal drugs and the use of vector control methods in the control and management of this disease.

## 2. PRINCIPLE

The Camel Surra Elisa kit is based on the indirect enzyme linked immunosorbant assay (iELISA) to determine the presence of *T. evansi* antibody in camel sera. In this procedure, sera are added to Immulon 2 HB ELISA plate wells coated with non-infectious *T.evansi* antigen. If antibodies directed to the *T.evansi* antigen are present in the serum sample, they will bind to the parasitic antigen in the wells. After the addition of horseradish peroxidase (HRP) conjugate it binds the antigen – antibody complex. Unbound material is removed by rinsing before the addition of the substrate solution. Subsequently a blue colour develops which is due to the conversion of the substrate by the conjugate.

A positive result is indicated by the development of blue colour. The reaction is stopped by the addition of the stop solution; the colour changes to yellow. The result can be read visually or by a micro plate photometer, where the optical density (OD) is measured at 450nm. OD values from the samples are compared to those of the positive and negative controls in parallel.

This test is developed and produced by Central Veterinary Research Laboratory CVRL-UAE.

## 3. MATERIAL SUPPLIED

| Material Supplied | Qty |
|---|---|
| Dynatech IMMULON 2 HB ELISA plate (uncoated) | 5 plates |
| *T. evansi* antigen | 25µl |
| Carbonate bi carbonate capsules pH9.6 | 5 |
| HRP-labelled Conjugate (Concentrated) | 25 µl |
| Strong Positive (C++) reference serum (Freeze dried) | 0.5ml |
| Weak Positive (C+) reference serum (Freeze dried) | 0.5ml |
| Negative (C-) reference serum (Freeze dried) | 0.5ml |
| Phosphate buffered saline (PBS) powder | 5 sachets |
| Milk Powder | 100gms |
| Tween -20 | 5ml |
| Phosphate Citrate Buffer with Sodium perborate capsules | 1capsule |
| 3,3',5,5'- Tetramethylbenzidine dihydrochloride (TMB) tablets | 5tabs |
| Stop solution | 60ml |

## 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 washer
- Micro plate orbital shaker with temperature control
- Timer

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LilliTest
Surra (*T. evansi*)
Positive Serum
Camel Origin
For in vitro veterinary use only

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## 5. WORKING DILUTIONS AND STORAGE

### 5.1 COATING BUFFER

0.05M Carbonate-bicarbonate buffer (PBS), pH 9.6 at 25°C
Dissolve contents of one capsule into 100ml of distilled/deionized water.
Label and store at +4°C for no longer than two weeks.

### 5.2 ANTIGEN PREPARATION

Dilute concentrated antigen at 1: 5000 in coating buffer pH 9.6
ie., To test one plate , prepare the working dilution of antigen by mixing 2µl of the Tryps antigen in 10ml of coating buffer.
Dispense 100µl of the working dilution of the antigen to the Immulon 2HB plates and incubate overnight at +2 - +4°C.

The **concentrated antigen** must be stored in its original vial at **-20°C.** Thawing and refreezing of the concentrated antigen does not result in a significant loss of activity.

### 5.3 BLOCKING BUFFER

0.01M Phosphate Buffered Saline, pH 7.4 supplemented with 0.05 %( v/v) Tween 20 and 5% milk powder.
Prepare the blocking buffer by dissolving 5gm milk powder and 50 µl Tween 20 into 100ml of Phosphate Buffered Saline (PBS)
ie., To test 1 plate prepare 250ml of blocking buffer. Dissolve 12.5gms of milk powder 125µl of Tween -20 into 250ml of PBS.
**Note:**
**Fresh blocking buffer must be prepared for each test day.**

### 5.4 PRE DILUTION OF SAMPLES

**For testing, the samples and controls should be pre-diluted to 1/500 in the blocking buffer (for example, 10µl of sample and controls into 4.990 µl of blocking buffer)**

### 5.5 HRP- LABELLED CONJUGATE

Dilute concentrated HRP labelled conjugate at 1: 10,000 in blocking buffer.
The **concentrated conjugate** must be stored in its original vial at -20°C. Thawing and refreezing of the concentrated antigen does not result in a significant loss of activity.
ie., To test one plate , prepare the working dilution of conjugate by mixing 1µl of the HRP labelled conjugate in 10ml of blocking buffer.

### 5.6 SUBSTRATE/CHROMOGEN

#### PREPARATION OF NAP

The contents of one capsule of phosphate Citrate buffer with Sodium perborate dissolved in 100ml will yield a 0.05Mphosphate-citrate buffer, pH 5.0 containing 0.03% sodium perborate.
Make aliquots of 10 ml and store at -20°C

#### Preparation of working solution of SUBSTRATE/CHROMOGEN

Dissolve one tablet of TMB in 10ml of phosphate Citrate buffer with Sodium perborate. Mix very gently (with out trapping air bubbles) until completely dissolved. Store in the dark.
**Fresh substrate / chromogen must be prepared for each test day.**
The SUBSTRATE/CHROMOGEN solution should be colourless when prepared. If coloured, it must be discarded and a fresh solution must be prepared.

### 5.7 WASHING BUFFER

0.01M Phosphate Buffered Saline (PBS), pH 7.4 at 25°C with 0.05% Tween 20.
Empty the contents of one sachet into one liter of distilled/deionized water to make 0.01M Phosphate Buffered Saline (PBS), pH 7.4
Add 500µl Tween 20 to the 0.01M Phosphate Buffered Saline (PBS), to prepare the wash buffer.

### 5.8 REFERENCE SERUM

Reconstitute the freeze dried contents of a vial of each reference serum (C++, C+ and C-) with precisely 0.5ml 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.

### 5.9 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 hemolysis (hemolytic 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 - +4°C for 72 hrs. Longer duration of storage requires samples to be frozen at -20°C in appropriately labelled cryopreservation vials.

### 5.10 STOP SOLUTION

Stop solution is supplied as ready to use.

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## 6. PLATE LAYOUT

**C++** Strong positive reference serum
**C+** Weak positive reference serum
**C-** Negative reference serum
**Bc** Blocking buffer control
1- 40 Test sera in duplicates

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

## 7. TEST/ ASSAY PROCEDURE

Unless otherwise stated volumes used are 100µl /well, and washes are performed using 235µl of wash buffer / well.

**7.1** All reagents should equilibrate to room temperature 18 to 25 $^{o}$C (64 to 77 $^{o}$F) before use. Label the plate according to the plate layout.

**7.2** Wash plates 3 times with 235µl wash buffer

**7.3** Pre dilute serum samples 1/500 with the blocking buffer

**7.4** Add 100µl of Controls and pre diluted test samples to selected wells.

**7.5** Incubate plates for 1 hr at 37 $^{o}$C with shaking at rpm 400.

**7.6** Wash plates 3 times with 235µl wash buffer.

**7.7** Add 100µl of freshly prepared conjugate in blocking buffer.

**7.8** Incubate plates for 1 hr at 37 $^{o}$C with shaking at rpm 400

**7.9** Wash plates 3 times with 235µl wash buffer.

**7.10** Add 100µl of substrate/ chromogen solution to all wells and incubate for 12 (+/-2) minutes **with shaking** at 37 $^{o}$C in dark. Stop test reaction at the end of the prescribed incubation time with 100µl/well of the **ready to use** stop solution provided.

**7.11** Measure the optical density (OD) at **450nm** using an ELISA plate reader. Measure the OD within 15 minutes after the addition of stop solution to prevent fluctuation in OD values.

***Note*: 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 450nm within 10 - 15 minutes after colour development has been stopped.
Calculate the corrected $OD_{450}$ of all test samples by subtracting the mean $OD_{450}$ of the blocking buffer control (Bc).

To ensure the assay validity, the Positive control should have corrected OD value greater than 0.5.
The negative control should have a corrected OD value less than 0.3.

FOR INVALID TESTS, TECHNIQUE MAY BE SUSPECT AND ASSAY SHOULD BE REPEATED.

The mean $OD_{450}$ of blocking buffer control Bc: 0.036 - 0.046

Mean OD of Negative Control: OD Value <0.3 @ 450 nm

Mean OD of Positive Control: OD Value > 0.5 - < 1.5 @ 450nm

Mean OD of Strong Positive Control: OD Value > 1.5 @ 450 nm

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## INTERPRETATION OF RESULTS

| | OD @450nm |
|---|---|
| **Strong positive** | >1.5 |
| **Positive** | 0.5-1.5 |
| **Doubtful** | 0.3-0.5 |
| **Negative** | <0.3 |

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

Please ensure all the reagents and test plates are equilibrated to room temperature (18-25 $^{o}$C) before usage.

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.

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

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

<u>Fresh blocking buffer must be prepared for each test day.</u>

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 *T. evansi* Elisa, 5 plates, 200 tests, Cat#VE-3003

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