Canine Leptospira Antibody ELISA

A monoclonal-mediated ELISA to detect antibodies against an important polysaccharide epitopes of Canine Leptospira in serum samples

REF EVL-EIAD1013-AB01

User’s Manual
1 INTRODUCTION

Leptospiracanica , Icteroheamoragia and grypotyphosa are important pathogens of Dogs. Dogs are the primary reservoir hosts for, which is transmitted by direct contact with contaminated water and less often through urine. Grypotyphosa is less host specific and more resistant to environmental conditions. Thus may be expected to enter a herd through the introduction of infected Dogs, whereas pomona may enter either through infected Dogs or through contaminated water. (The major recognized site of leptospiral persistence in carrier Dogs is the kidney).

The specificity of the monoclonal antibodies used in this test was also determined by modified microscopic agglutination test (MAT). Monoclonal antibodies are standardized reagents which are suitable for use in catching or detecting ELISA systems. Conventional tests for detecting antibodies give many problems of batch-to-batch variation and interpretation.

The EVL ELISA system is intended to use as a rapid screening test for the specific detection of Canine Leptospira antibodies in serum samples of infected Dogs.

2 INTENDED USE OF THE TESTKIT

This diagnostic test for leptospirosis is intended to identify antibodies against sugar antigens of leptospira in serum and milk samples. In contrast to test systems which make use of unpurified non-specific leptospira antigens, this test uses a monoclonal antibody which catches a specific leptospira sugar antigen. This monoclonal based ELISA has very high specificity and sensitivity.

3 PRINCIPLE OF THE TEST KIT

An antigen solution antibody mixture is coated to the wells of the micro titer plate after stabilization and drying. Plates are vacuum sealed. Diluted milk or serum samples are added to the coated wells. After incubation and appropriate washing a monoclonal anti-Dog conjugate is added and the plates are again incubated. After appropriate washing, substrate is added. Within several minutes the color reaction is stopped and the plates are immediately read at 450 nm.

4 CONTENTS

- 1 x 96 well micro titer plates coated with monoclonal antibody antigen complexes
- 1 x 13 ml HRPO conjugated (anti-dog) monoclonal antibody
- 1 x 1 ml inactivated positive control (Freeze dried)
- 1 x 1 ml inactivated negative control (Freeze dried)
- 1 x 20 ml wash-solution 200 x concentrated, must be diluted in de-ionized water before use!
- 1 x 20 ml ELISA buffer
- 1 x 7 ml substrate buffer A
- 1 x 7 ml substrate buffer B
- 1 x 8 ml stop solution
- 1 x plastic cover seal.

5 HANDLING AND STORAGE OF SPECIMENS.

The kit should be stored at +4°C.

An open packet should be used within 10 days.

Samples may be used fresh or may be kept frozen below -20°C before use.

Positive and negative controls may be stored after reconstitution in aliquots at -20°C and used until the expiry date.

Avoid repeated freezing and thawing as this increases non-specific reactivity.
6 WASH PROTOCOL
In ELISAs, un-complexed components must be removed efficiently between each incubation step. This is accomplished by appropriate washing. It should be stressed that each washing step must be carried out with care to guarantee reproducible inter- and intra-assay results. It is essential to follow the washing procedures outlined below. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.

Manual washing
1. Empty each well by turning the microtiter plate upside down, followed by a firm vertical downward movement to remove the buffer.
2. Fill all the wells with 250 µl washing solution.
3. This washing cycle (1 and 2) should be carried out at least 4 times
4. Turn the plate upside down and empty the wells with a firm vertical movement
5. Place the inverted plate on absorbent paper towels and tap the plate firmly to remove any residual washing solution in the wells.
6. Take care that none of the wells dry out before the next reagent is dispensed

Washing with automatic equipment
When automatic plate washing equipment is used, check that all wells are aspirated completely and that the washing solution is correctly dispensed, reaching the rim of each well during each rinsing cycle. The washer should be programmed to execute at least 4 washing cycles.

7 PREPARATION OF SPECIMENS
- Serum samples:
  Centrifuged (3000 g 10 min) Individual serum samples should be diluted 1:50 in ELISA buffer

8 TEST PROTOCOL
1. Wash the micro titer plate with washing solution according to the washing protocol. The washing solution provided has to be diluted 200x
   Reconstitute positive and negative control with 0.5ml demi water and store -20 C in aliquots
2. Pre dilute the sera to be tested 1:10 (10 µl serum sample + 90 µl buffer) in a round bottomed micro titer plate (not supplied in the test kit), also pre dilute the positive and negative control 1:10 (10 µlpositive/negative + 90 µl buffer)
3. Dispense 100 µl ELISA buffer to wells A1 and B1 (blanks) of the Leptospira coated test plate
   Serum samples:
   Dispense 80 µl ELISA buffer to all remaining wells
4. Serum:
   Transfer 20 µl of pre-diluted samples to the wells of the coated microtiter plate already filled with 80 µl of ELISA-buffer
5. Seal and incubate 60 min. at 37°C.
6. Wash as pointed out in wash protocol.
7. Dispense 100 µl ready to use HRPO-conjugated monoclonal antibody to all wells.
8. Seal and incubate 1 hour at 37°C.
9. Wash as pointed out in wash protocol.
10. With gentle shaking mix equal parts of buffer A and B together. Prepare immediately before use!
    Dispense 100 µl substrate solution to each well. Incubate 10-15 min. at room temperature (21°C.).
11. Add 50 ml stop solution to each well.
12. Read the absorbency values immediately (within 10 min.) at 450 nm.
   Use as a reference wave length 620 nm.
9 PRECAUTIONS
- Handle all biological material as though capable of transmitting Leptospira hardjo (human pathogen).
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer A/B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Optimal results will be obtained by strict adherence to this protocol. Careful pipetting and washing throughout this procedure are necessary to maintain precision and accuracy.
- Each well is ultimately used as an optical cuvette. Therefore, do not touch the under-surface of the microtiter plate and protect it from damage and dirt.

10 VALIDATION OF THE TEST
In order to confirm appropriate test conditions the mean absorption value of the negative control should be < 0.350 OD units (450 nm). The mean absorption value of the positive control provided should be > 0.700 OD units (450 nm).

11 INTERPRETATION OF TEST RESULTS
In general high prevalence is more than 15% positive animals. This prevalence can be used for a certain area (f.i. farm, state or country) depending on elimination campaign or other (government) restrictions.

**Serum**
- **High prevalence:**
  A sample is scored Leptospirahardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.150 OD units.

  Negative: OD samples < OD mean negative control plus 0.250.
  Positive: OD samples > OD mean negative control plus 0.250.

- **Low prevalence:**
  A sample is scored Leptospirahardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.100 OD units.

  Negative: OD samples < OD mean negative control plus 0.150.
  Positive: OD samples > OD mean negative control plus 0.250.
  Doubtful: between mean negative control and 0.250.
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