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Leptospira Hardjo Antibody ELISA Kit

Cat. No.:DEIA2332
Pkg.Size:96T

Intended use

This diagnostic test for leptospirosis is intended to identify antibodies against sugar antigens of leptospira in serum and milk samples.

General Description

Leptospira interrogans serovar hardjo and pomona are important pathogens of cattle. Cattle are the primary reservoir hosts for hardjo, which is transmitted by direct contact with contaminated urine and less often through water. Pomona is less host specific and more resistant to environmental conditions. Thus hardjo may be expected to enter a herd through the introduction of infected cattle, whereas pomona may enter either through infected cattle or through contaminated water. (The major recognized site of leptospiral persistence in carrier cattle 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. This ELISA system is intended to use as a rapid screening test for the specific detection of Leptospira hardjo antibodies in serum and milk samples of infected cattle.

Principle Of The Test

An antigen solution antibody mixture is coated to the wells of the microtiter plate. After stabilization and drying plates are vacuum sealed. Diluted milk or serum samples are added to the wells. After incubation and appropriate washing a monoclonal anti-bovine 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.

Reagents And Materials Provided

1. 1 x 96 well microtiter plate coated with monoclonal antibodies
2. 1 vial 100X concentrated HRPO conjugated (anti-bovine) monoclonal antibody
3. 1 x 13 mL Conjugate Diluent
4. 1 x 1 mL inactivated positive control (freeze-dried)
5. 1 x 1 mL inactivated negative control (freeze-dried)
6. 1 x 20 mL wash-solution 200 x concentrated, must be diluted in deionized water before use!
7. 1 x 20 mL ELISA buffer
8. 1 x 7 mL substrate buffer A
9. 1 x 7 mL substrate buffer B
10. 1 x 8 mL stop solution
11. 1 x plastic cover seal

Storage

The kit should be stored at 4°C. After reconstitution, the lyophilized reagents should be used immediately. The test kit is stable until the date given on the label. Positive and negative control sera can be stored in aliquots at -20°C and used until the expire date. Repeated freezing and thawing must be avoided.

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Specimen Collection And Handling

Fresh samples can be used without restrictions. Addition of 0,1% sodium azide to the samples has no influence on the test results. For prolonged storage, samples should be frozen as soon as possible and stored at -25°C until use. Avoid repeated freezing and thawing as this increases non-specific reactivity and decreases end-point titers.

Milk samples - undiluted

For optimal sensitivity pooled milk samples can be tested undiluted.

To avoid false positive reactions defatted samples must be used. Centrifuge the milk samples for 15 minutes at 2500 x g and take a sample from below the fat layer.

Milk samples - diluted

For optimal specificity individual milk samples should be tested at a 1:4 dilution.

Pooled milk samples, collected from up to 25 individual animals, can also be tested at a 1:2 dilution.

The use of diluted milk samples guarantees minimum false positivity.

Serum samples

Individual serum samples should be diluted 1:100 in ELISA buffer.

Assay Steps

WASHING Protocol

In ELISA's un-complexed components must be removed efficiently between each immunological 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 advised to follow the washing procedures outlined below carefully. Both manual washing and washing with automatic equipment can be performed. (Automatic washing equipment usually gives better results).

Manual washing

1. Empty each well by turning the microtitre plate upside down, followed by a firm vertical movement.
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 by a firm short vertical movement.
5. Place the inverted plate on absorbent paper towels and tap the plate firmLy to remove residual washing solution in the wells.
6. Take care that none of the wells dries out before the next reagent is dispensed.

Washing with automatic equipment

When using automatic plate washing equipment, check that all wells can be 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.

TEST Protocol

1. Wash the microtiter plate with washing solution according to the washing Protocol.

The washing solution provided has to be diluted 200x.

Reconstitute positive and negative control with 1 mL bidistilled water, divide in aliquots and store the not immediately used controls at -20°C.

2. Predilute the test sera to be tested 1:10 (10 µL sample ± 90 µL Buffer) in a round bottomed microtiter plate (not supplied in the kit), also predilute the positive and negative control 1:10 (10 µL control ± 90 µL Buffer).

3. Dispense 100 µL ELISA Buffer to wells A1 and B1 (blanks) of the Leptospira hardjo coated plate.

Serum samples: Dispense 90 µL ELISA Buffer to all remaining wells.

Individual milk samples: Dispense only 75 µL ELISA Buffer to all remaining wells.

4. Serum: Transfer 10 µL of the prediluted samples to the wells of the coated microtiter plate already filled with 90 µL ELISA Buffer.

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Milk: Dispense 25 µL from individual milk samples to the wells of the coated microtiter plate already filled with 75 µL ELISA Buffer.
If pooled milk samples are used, dispense 100 µL skimmed milk sample to the coated well don´t add any buffer.
5. Seal and incubate 60 min. at 37°C.
6. Wash as in 1.
7. Dilute the 100X concentrated HRPO conjugated monoclonal antibody 1:100 in conjugate diluent.
Dispense 100 µL diluted conjugated antibody to all wells.
8. Seal and incubate 1 hour at 37°C.
9. Wash as in 1.
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 µL 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.

Reference Values

In order to confirm appropriate test conditions
the mean absorption value of the negative control should be ≤ 0.200 OD units (450 nm).
The mean absorption value of the positive control provided should be ≥ 0.800 OD units (450 nm).

Interpretation of 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 Leptospira hardjo 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: ODsamples ≤ Mean ODnegative control ± 0.150.
Positive: ODsamples ≥ Mean ODnegative control ± 0.150.

- Low prevalence:
A sample is scored Leptospira hardjo 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: ODsamples ≤ Mean ODnegative control ± 0.100.
Positive: ODsamples ≥ Mean ODnegative control ± 0.200.
Doubtful: between mean ODnegative control and 0.200.

Milk- High prevalence:
A sample is scored leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0.050 OD units
Negative: ODsamples ≤ Mean ODnegative control ± 0.075.
Positive: ODsamples ≥ Mean ODnegative control ± 0.075.

- Low prevalence:
A sample is scored Leptospira hardjo negative if the OD value is below or equal to the average OD value of the mean negative control plus 0,050 OD units.
Negative: ODsamples ≤ Mean ODnegative control ± 0.050.
Positive: ODsamples ≥ Mean ODnegative control ± 0.100.
Doubtful: between mean ODnegative control and 0.100.

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Precautions

1. Handle all biological materials as though capable of transmitting Leptospira Hardjo (human pathogen).
2. Do not pipette by mouth.
3. Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated work area.
4. TMB is toxic by inhalation, through contact with skin or when swallowed; observe care when handling the substrate.
5. Do not use components past expire date and do not intermix components from different serial lots.
6. 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.
7. Each well is ultimately used as an optical cuvette. Therefore do not touch the under-surface of the microtiter plate and prevent it form damage and dirt.

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