Bovine Leptospira hardjo antibody ELISA Kit

Enzyme Immunoassay for the determination of Bovine's antibody to an important polysaccharide epitope Leptospira hardjo in serum and milk

Catalog number: ARG81019

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INTRODUCTION

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.

The 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 ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Leptospira hardjo sugar antigen is used in this kit. The antigen and the Leptospira hardjo sugar antigen specific monoclonal antibody mixture has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any antibody to Leptospira hardjo sugar antigen present is bound by the immobilized antibody.

After washing away any unbound substances, an HRP-conjugated anti-bovine immunoglobulin antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-antigen complexes coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Positive Control	1 vial (lyophilized)	4°C.
Negative control	1 vial (lyophilized)	4°C.
HRP-Antibody conjugate	12 ml (Ready-to-use)	4°C
ELISA buffer	18 ml (Ready-to-use)	4°C
200X Wash buffer	20 ml	4°C
Substrate A	8 ml	4°C
Substrate B	8 ml	4°C
STOP solution	8 ml (Ready-to-use)	4°C
Plastic cover seal	1	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm and a 620 nm as the reference wavelength.
- Round bottomed microtiter plate
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Handle all biological material as though capable of transmitting infectious diseases.
- Do not pipette by mouth.
- Do not eat, drink, smoke or prepare foods, or apply cosmetics within the designated working area.
- TMB substrate (buffer B) is toxic by inhalation, through contact with skin or when swallowed; observe care when handling substrate.
- Store the kit at 4°C at all times. An open packet should be used within 10 days.
- Do not use components past the expiry date and do not mix components from different serial lots.
- Positive and negative controls should be stored at -20 °C in aliquots after reconstitution. Avoid repeated freezing and thawing as this increases non-specific reactivity.

- Buffer, controls, samples and antibody need to be shaken gently before use, in order to dissolve/mix any components that may have precipitated. Gently tap the vials onto the table, so any fluid still retained in the cap falls back into the solution.
- If fluids need to be mixed into the test well, gently shake by tapping the wells with the fingers or resuspend with the last pipette tip used for that particular well. Avoid contamination through spattering and prevent any fluid to enter inside the pipette itself.
- Place the reagents back 2°C- 8°C immediately after use.
- If crystals are observed in the 200X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Wash step: 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. Washing may be done manually or with automatic equipment. Automatic washing equipment usually gives better results.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Milk- Centrifuge samples 1500 x g for 15 min at 4°C. Collect the aqueous fraction and repeat three times. Collect the supernatants and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 200X Wash buffer into distilled water to yield 1X Wash buffer.
- Negative Control (stock solution): Briefly centrifuge the tube. Reconstitute negative control with 1 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use. Avoid repeated freeze-thaw cycles as this increases non-specific reactivity.
- Positive Control (stock solution): Briefly centrifuge the tube. Reconstitute positive control with 0.5 ml distilled water, divide into aliquots, and store after complete dissolving immediately at -20°C until use. Avoid repeated freeze-thaw cycles as this increases non-specific reactivity.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, around 21°C) 15 min before use (without exposing them to direct sunlight or any heat sources and place the reagents back at 4°C immediately after use).

QUALITATIVE - SERUM

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Dilute Negative Control: Dilute stock solution 1:50 in ELISA buffer to make working solution. Make sure to make minimal 125 µl of every dilution to be able to transfer 100 µL to the coated plate.
4. Dilute Positive Control: (for duplicates) Dilute stock solution 1:50 in ELISA buffer to make working solution. Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Dilute Serum: (for duplicates) Dilute serum samples 1:100 in ELISA buffer. Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.

   Dilution Note:

a) For 1:10 dilution: add 5 µL of samples into 45 µL of ELISA buffer, mix well.

b) For 1:100 dilution: add 15 µL of diluted samples from **a)** into 135 µL of ELISA buffer, mix well.

c) For 1:50 dilution: add 3 µL of controls into 147 µL of ELISA buffer, mix well.

6. Take 2 wells in antibody-antigen coated plate as substrate controls, add only 100 µl of ELISA buffer to these wells.
7. Add 100 µL of diluted samples, positive and negative controls into respective wells in antibody-antigen coated plate.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µL HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. **Prepare immediately before use.**
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately and use the substrate controls as blank. It is recommended to read the absorbance within 10 min after adding STOP solution.

QUANTITATIVE-SERUM

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Dilute Negative Control: Make a 3-step dilution of the negative control in ELISA buffer starting with 1:50, 1:150, 1:450 and 1:1350 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
4. Dilute Positive Control: Make a 3-step dilution of the positive control in ELISA buffer starting with 1:50, 1:150, 1:450 and 1:1350 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Dilute Serum: Make 3-step dilutions of each sample in ELISA buffer starting with 1:50, 1:150, 1:450 and 1:1350 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.

   Dilution Note:

   a) For 1:10 dilution: add 10 µL of samples/controls into 90 µL of ELISA

buffer, mix well.

b) For 1:50 dilution: add 30 µL of diluted samples/controls from **a)** into 120 µL of ELISA buffer, mix well.

c) For 1:150 dilution: add 50 µL of diluted samples/controls from **b)** into 100 µL of ELISA buffer, mix well.

d) For 1:450 dilution: add 50 µL of diluted samples/controls from **c)** into 100 µL of ELISA buffer, mix well.

e) For 1:350 dilution: add 50 µL of diluted samples/controls from **d)** into 100 µL of ELISA buffer, mix well.

6. Take 2 wells in antibody-antigen coated plate as substrate controls, add only 100 µl ELISA buffer to these well.
7. Add 100 µl of diluted samples, positive and negative controls into respective wells in antibody-antigen coated plate.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. **Prepare immediately before use.**
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately and use the

substrate controls as blank. It is recommended to read the absorbance within 10 min after adding STOP solution.

QUALITATIVE - MILK

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Dilute Negative Control: Dilute stock solution 1:50 in ELISA buffer to make working solution. Make sure to make minimal 125 µl of every dilution to be able to transfer 100 µL to the coated plate.
4. Dilute Positive Control: (for duplicates) Dilute stock solution 1:50 in ELISA buffer to make working solution. Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
5. Dilute Milk: (for duplicates) Dilute Milk samples 1:3 in ELISA buffer. Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.

   Dilution Note:

   a) For 1:3 dilution: add 50 µL of samples into 100 µL of ELISA buffer, mix well.

b) For 1:50 dilution: add 3 µL of controls into 147 µL of ELISA buffer, mix well.

6. Take 2 wells in antibody-antigen coated plate as substrate controls, add only 100 µl of ELISA buffer to these wells.
7. Add 100 µL of diluted samples, positive and negative controls into respective wells in antibody-antigen coated plate.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µL HRP-antibody conjugate solution into each well. Incubate for 60 minutes at 37°C.
11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. **Prepare immediately before use.**
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.
15. Read the OD with a microplate reader at 450 nm immediately and use the substrate controls as blank. It is recommended to read the absorbance within 10 min after adding STOP solution.

QUANTITATIVE-MILK

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it, and use them within 10 days.
2. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (250 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. Take care that none of the wells dry out before the next reagent is dispensed.
3. Dilute Negative Control: Make a 3-step dilution of the negative control in ELISA buffer starting with 1:30, 1:90, 1:270 and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.
4. Dilute Positive Control: Make a 3-step dilution of the positive control in ELISA buffer starting with 1:30, 1:90, 1:270 and 1:810 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.

   Dilution Note:

   a) For 1:5 dilution: add 20 µL of controls into 80 µL of ELISA buffer, mix well.

   b) For 1:30 dilution: add 25 µL of diluted controls from a) into 125 µL of ELISA buffer, mix well.

   c) For 1:90 dilution: add 50 µL of diluted controls from b) into 100 µL of

ELISA buffer, mix well.

d) For 1:270 dilution: add 50 µL of diluted controls from **c)** into 100 µL of ELISA buffer, mix well.

e) For 1:810 dilution: add 50 µL of diluted controls from **d)** into 100 µL of ELISA buffer, mix well.

5. **Dilute Milk**: Make 3-step dilutions of each sample in ELISA buffer starting with 1:3, 1:9, 1:27 and 1:81 in a round-bottomed microtiter plate (not supplied). Make sure to make minimal 125 µL of every dilution to be able to transfer 100 µL to the coated plate.

   Dilution Note:

   a) For 1:3 dilution: add 50 µL of samples into 100 µL of ELISA buffer, mix well.

   b) For 1:9 dilution: add 50 µL of diluted samples from **a)** into 100 µL of ELISA buffer, mix well.

   c) For 1:27 dilution: add 50 µL of diluted samples from **b)** into 100 µL of ELISA buffer, mix well.

   d) For 1:81 dilution: add 50 µL of diluted samples from **c)** into 100 µL of ELISA buffer, mix well.

6. Take 2 wells in antibody-antigen coated plate as substrate controls, add only 100 µl ELISA buffer to these well.
7. Add 100 µl of diluted samples, positive and negative controls into respective wells in antibody-antigen coated plate.
8. Seal and incubate for 60 minutes at 37°C.
9. Wash as according to step 2.
10. Add 100 µl HRP-antibody conjugate solution into each well. Incubate for

60 minutes at 37°C.

11. Wash as according to step 2.
12. Mix equal part of substrate A and substrate B with gentle shaking. **Prepare immediately before use.**
13. Add 100 µl of mixed substrate reagent to each well. Incubate for 10-20 minutes at room temperature in dark. Make sure the negative control does not become too dark.
14. Add 50 µl of Stop Solution to each well.

Read the OD with a microplate reader at 450 nm immediately and use the substrate controls as blank. It is recommended to read the absorbance within 10 min after adding STOP solution.

VALIDATION OF THE TEST

Qualitative:

The results are valid if the following criteria are met:

- The mean value (MV) of the measured OD value for the Positive Control (PC) must be ≥ 0.800
- The MV of the measured OD value for the Negative Control (NC) must be ≤ 0.200

In case of invalid assays the test should be repeated after a thorough review of the instructions for use.

Calculation

Calculate the mean values (MV) of the measured OD for the Negative Control (NC) and the Positive Control (PC).

The ratio (S/P) of sample OD to mean OD of the positive control is calculated according to the following equation:

$$S/P = \frac{OD_{sample} - MV\ OD_{NC}}{MV\ OD_{PC} - MV\ OD_{NC}}$$

Quantitative:

In order to confirm appropriate test conditions the OD of the positive control should be ≥ 0.800 OD units (450 nm) and give an endpoint titer of ≥ 150. The negative control should be ≤ 0.200 OD units (450 nm) and give an endpoint titer of ≤ 50.

INTERPRETATION OF TEST RESULTS

Qualitative: (Positive – Negative)

The results are valid if the following criteria are met:

- A sample with the S/P ratio < 0.34 is negative
  (Specific antibodies to Leptospira could not be detected)
- A sample with the S/P ratio ≥ 0.34 is positive
  (Specific antibodies to Leptospira were detected)

Quantitative: (End point titer)

Serum:

- The ELISA titer can be calculated by constructing a curve and using a cut-off line (dilution 1:50 → 1:150→ 1:450→ 1:1350→ 1:4050, etc., total 8 dilutions of 3 steps) OD on Y-axis and titer on X-axis.
- ELISA titers can be calculated using as cut-off 2.5 times OD value of negative control at 1:50.

Milk:

- The ELISA titer can be calculated by constructing a curve and using a cut-off line (dilution 1:3 → 1:9→ 1:27→ 1:81→ 1:243, etc., total 8 dilutions of 3 steps) OD on Y-axis and titer on X-axis.
- ELISA titers can be calculated using as cut-off 2.5 times OD value of negative control at 1:30.