INTERPRETATION

Calculation of the Ratio

- For control, calculate the mean of the ODs obtained on the plate coated with *L. pomona* and the mean of the ODs obtained on the plate coated with *L. hardjo*.
- Calculate the RATIO for each pathogen by dividing each sample's OD for that pathogen by the positive control's mean OD for that pathogen.

Example:

$$\frac{\text{OD sample } (L.\ pomona)}{\text{Mean OD positive } (L.\ pomona)} = \text{RATIO } (L.\ pomona)$$

$$\frac{\text{OD sample } (L.\ hardjo)}{\text{Mean OD positive } (L.\ hardjo)} = \text{RATIO } (L.\ hardjo)$$

Validity Criteria

The following criteria have to be met in order to validate the test:

- Negative control ratio for each pathogen must be less than 0.25.
- Mean of positive control ODs for each pathogen must be greater than 0.75.

Interpretation:

For each pathogen:

- Sample ratio less than 0.20 is considered negative.
- Sample ratio less than 0.30 but greater or equal to 0.20 is considered suspicious. A second serum sample could be collected 2 weeks later and retested.
- Sample ratio greater or equal to 0.30 is considered positive.

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Leptospira pomona and *hardjo* Antibody Test Kit, ELISA
Bovichek® Lepto HP
Insert

2017-07-24

This is an immunoenzymatic assay for the detection of antibodies against *Leptospira pomona* and *hardjo* in bovine serum.

Leptospirosis is a worldwide zoonotic disease of domestic animals and wildlife. It is caused by a spirochete bacteria classified under the genus *Leptospira*. The taxonomy of the genus is confusing, with a number of species and serovars. In cattle leptospirosis is primarily due to *Leptospira borgpetersenii* serovar hardjo (*L. hardjo*) and *Leptospira interrogans* serovar pomona (*L. pomona*). Both serovars may affect cattle of all ages but clinical signs greatly vary depending on the serovar and the age of the animals.

L. pomona may cause acute infections especially in calves. Calves may demonstrate high fever, anorexia, dyspnea, hemolytic anemia, hemoglobinuria, icterus, and high mortalities. Acute infections of naive cattle with *L. hardjo* are characterized by a sudden drop in milk production. The milk is thick, yellow, and blood-tinged. The udder is typically soft and flabby. *L. hardjo* causes the milker's fever syndrome. Moreover both serotypes may cause abortion which generally occurs in late pregnancy. Stillbirths and birth of premature or weak infected calves also occur.

The diagnosis of leptospirosis especially in adult cattle may be difficult. Culture in special media, PCR, immunohistochemistry techniques and serology may be used. Antibody titers may peak before abortion because the acute infection occurred several weeks previously. Abortion due to *L. hardjo* infections may occur with low or negative serologic titers.

PRINCIPLE OF THE TEST

Controls and prepared bovine serum samples are incubated in wells coated with either *L. pomona* or *L. hardjo* antigens (Ag). The antibodies (Ab) specific to *L. pomona* or *L. hardjo* present in positive serum samples will bind the Ag in the wells. After several washes to eliminate unbound substances, a conjugate (protein G coupled to peroxydase) is added. After incubation, the excess of this conjugate is eliminated by a second series of washes and its attachment to bovine antibodies is revealed with a chromogenous substrate. The conjugate, if present, reacts with the substrate and a blue color develops. The reaction is then stopped and the optical densities are read. The intensity of the color is proportional to the quantity of Ab in the serum samples.

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MATERIAL

Components	Quantity
• 12 strips of 8 wells coated with *L. pomona*	1
• 12 strips of 8 wells coated with *L. hardjo*	1
• Ready-to-use positive control	2.5 mL
• Ready-to-use negative control	2.5 mL
• Concentrated wash solution (10X)*	2 x 100 mL
• Concentrated conjugate	500 - 1000 uL**
• Ready-to-use substrate	25 mL
• Ready-to-use stop solution *	25 mL

* Crystals may form when these solutions are kept at 2-7°C. This will not affect the efficiency of the products. However, crystals have to be dissolved by bringing the solutions to room temperature and agitating.

** Precise volume depends on the lot of conjugate and the recommended working dilution (see section C).

Material Required but not Provided:

- Purified water
- Adjustable single- and multi-channel micropipettes
- Single use micropipettes tips
- ELISA microplate washer (facultative)
- Test tubes for sample dilution
- ELISA 96-well microplate reader equipped with 450 nm filter
- Containers for preparation of solutions

PRECAUTIONS

- For *in vitro* veterinary use only.
- The materials used in this kit must be considered as infectious. Therefore, all waste must be decontaminated before being discarded.
- Do not use the kit after the expiry date indicated on the package.
- Do not mix the reagents or instructions from different serial numbers.
- The sensitivity and specificity of the test are guaranteed only if the procedures are strictly observed.
- Do not expose the substrate to either light or oxidizing agent. Always keep the substrate in a clean plastic container. This solution may cause skin or eye irritation.
- The stop solution contains a strong acid and must be manipulated with caution to avoid contact with skin or mucous membranes.
- Dispose of the wash solution, the substrate and the stop solution according to local regulations for chemicals.
- Keep all reagents at 2-7°C and bring to room temperature before use.

EXECUTION

A. Preparation of Wash Solution

After homogenizing the concentrated wash solution (no evidence of crystals), dilute the 10X concentrated wash solution in purified water at 1/10 (e.g. 50 mL of 10X concentrated wash solution in 450 mL of purified water). Once prepared, the solution (1X) is stable for 1 week at à 2-7°C.

B. Preparation of Samples

Dilute serum samples at 1/200 in 1X wash solution (see section A) (e.g.: 4 µL of serum in 796 µL of 1X wash solution). Make sure to use a new tip for each sample. Also make sure each dilution is properly mixed before being distributed into the wells.

C. Conjugate Preparation

Dilute the conjugate with 1X wash solution (see section A) according to the dilution indicated on the Quality Control Certificate. Dilute the conjugate a few minutes prior to its use and always prepare a fresh solution.

D. Test Procedure

Bring all reagents to room temperature and mix well manually before use.

1. Make a schematic representation of the plates and the distribution of the controls and samples.
2. Dispense 100 µL of ready-to-use positive control, ready-to-use negative control or diluted samples (see section B) into appropriate wells (it is recommended to run control sera in duplicates).
3. Incubate at 23 ± 2°C for 30 minutes.
4. Wash each well 3 times with 300 µL of 1X wash solution (see section A). Throw away all liquid contained in the plate after each wash. After the last wash, dry the plate by tapping it on absorbent paper.
5. Dispense 100 µL of the diluted conjugate (see section C) into each well.
6. Incubate at 23 ± 2°C for 30 minutes.
7. Repeat step 4.
8. Dispense 100 µL of ready-to-use substrate into each well.
9. Incubate in the dark at 23 ± 2°C for 15 minutes.
10. Dispense 100 µL of ready-to-use stop solution into each well.
11. Measure optical density (OD) of each well at 450 nm. The reading should be done no later than 15 minutes after the addition of the stop solution.
12. Calculate the results.

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