Bovichek® Lepto HP

Leptospira pomona and *hardjo* Antibody Test Kit, ELISA

The first commercial ELISA kit for the detection of *Leptospira Pomona* and *L. Hardjo* antibodies in cattle

MAJOR ADVANTAGE OF ELISA

• **uses non-hazardous reagents** • **is sensitive and specific** • **quick results** (90 min.) •
• **results can be interpreted objectively is performed at room temperature** •

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 uL
Ready-to-use substrate	25 mL
Ready-to-use stop solution	25 mL

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

IMPORTANT:

- Requires a permit to release veterinary biologics from CFIA in Canada. This product is not licensed by CFIA and any claims made have not been substantiated by CFIA.
- Requires a Research and Evaluation import permit from the USDA in the US. This product is not licensed by the USDA and any claims made have not been substantiated by the USDA.

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BOVICHEK® LEPTO

What is **Leptospirosis**?

Leptospirosis is a bacterial disease caused by a spirochete bacteria classified under the genus *Leptospira*. The taxonomy of this genus is complex, with a large number of species, serovars, and types.

Leptospirosis in cattle

In cattle leptospirosis is primarily due to *Leptospira borgpetersenii* serovar *Hardjo* (*L. Hardjo*) and *Leptospira interrogans* serovar *Pomona* (*L. Pomona*). Both Leptospira types may affect cattle of all ages but clinical signs vary greatly depending on the type and the age of the animals.

L. pomona may cause acute infections especially in calves. These may demonstrate as high fever, anorexia, dyspnea, hemolytic anemia, hemoglobinuria, icterus, and death.

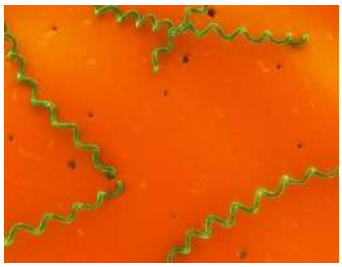
Acute infections of naive (non immune) cattle with *L. hardjo* are characterized by a sudden drop in milk production and abnormal milk.

Moreover both types may cause abortion. Abortions usually occur in late pregnancy several weeks after the initial infection. Stillbirths and birth of weak calves may occur. Abortion rates range from up to 30% in herds not previously infected to 5% in herds where the infection is endemic.

Clinical signs of leptospirosis are more apparent during the initial infection of a herd. A natural immunity then establishes and the herd enters a chronic inactive state of infection in which few signs are seen. However, all new susceptible animals that enter the herd will suffer from an acute infection.

Bovine leptospirosis as a zoonosis

Bovine leptospirosis is a zoonotic disease. Farmers, veterinarians and slaughterhouse workers are the main risk groups. The disease in humans is usually acquired from contact with infected urine, aborted foetus or contaminated water. Clinical signs are flu-like, with headaches and fever, occasionally progressing into meningitis.



Diagnosis of bovine leptospirosis

The diagnosis of bovine leptospirosis is difficult. History and clinical signs may be suggestive but diagnosis needs to be confirmed by laboratory testing. Two groups of diagnostic tests may be used. One group is designed to detect leptospires (culture), leptospiral antigens (IHC techniques) or leptospiral nucleic acids (ISH and PCR techniques). Detection of Leptospires from blood, cerebrospinal fluid, urine and milk can be attempted in acute cases. Urine has to be used in chronic cases.

The other group of diagnostic test is intended to detect anti-leptospiral antibodies. The most commonly used serological assays are the Microscopic Agglutination Test (MAT) and the ELISA. The MAT is usually considered the "gold standard." Unfortunately the test is hazardous to perform because of the need to maintain live cultures of pathogenic serovars. It is also difficult to standardize; considerable variations in results have been observed among laboratories.

Numerous ELISA have been developed for detecting anti-leptospiral antibodies using various culture extracts or recombinant proteins as antigens. Some of them use monoclonal antibodies against specific epitopes in competitive assays. The ELISA offers several advantages over the MAT. It uses non-hazardous reagents, it is sensitive and specific; and it can be automated. Rigorous quality control criteria ensure the reproducibility of the results which are interpreted objectively.

Interpretation of serological results is difficult. There is a lack of consensus over what titer should be used as a cut-off. Results are also complicated by cross-reactions between serovars especially in the early stage of infection. High antibody levels with consistent symptoms are suggestive of an acute infection but a rising titer is necessary for a definitive diagnosis. However antibody titers may peak before abortion because the acute infection occurred several weeks previously. Abortion due to *L. Hardjo* infections may also occur with low or negative titers.

Bovichek® Lepto ELISA

It is an enzyme immunoenzymatic assay (ELISA) designed for the detection of IgG antibodies against *L. Pomona* and *Hardjo* in bovine serum. The kit consists of 12 x 8 wells coated with *L. Pomona* and 12 x 8 wells coated with *L. Hardjo*, *L. Pomona* and *Hardjo* positive and negative controls, washing buffer, anti-IgG enzymatic conjugate (HRPO), substrate and stop solution.

All reactions are performed at **room temperature**. Controls and serum samples are first incubated for **30 minutes** in antigen coated wells. The antibodies specific to *L. Pomona* or *L. Hardjo* present in positive serum samples will bind to the corresponding antigen in the wells. After several washes to eliminate unbound substances, an anti-bovine IgG enzymatic conjugate is added. After **30 minutes** of incubation, the excess of this conjugate is removed by a second series of washes and its attachment to bovine antibodies is revealed with a chromogenic substrate. The conjugate, if present, reacts with the substrate and a blue color develops. The reaction is stopped after **15 minutes** and a yellow color appears.

The optical densities (OD) are read using a plate reader with a 450 nm filter. OD of the samples are compared to those of the *L. Pomona* and *L. Hardjo* positive controls using a S/P ratio (sample OD/ positive control OD). OD and S/P are proportional to the quantity of IgG antibodies present in the samples.

References

1. **Anonymous.** Leptospirosis in Cattle. http://nadis.org.uk/EEDA/Leptospirosis%20in%20Cattle%28EEDA%29.pdf
2. **Anonymous.** Leptospirosis. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009. http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.01.09_LEPTO.pdf
3. **Bolin C.A.** Leptospirosis in cattle: Disease Review and Update. Proc. North American Veterinary Conference, 2005. http://www.ivis.org/proceedings/navc/2005/LA/001.pdf?LA=1

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