VetLine
Leptospira ELISA
(LEPVT0660)

Performance Characteristics

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1 Introduction

Leptospirosis is probably the most widespread zoonosis in the world. It is caused by infection with spirochete bacteria of the genus *Leptospira* and affects humans as well as a broad spectrum of animal hosts (e.g. dogs, pigs, cattle). The incidence is significantly higher in warm climate countries than in temperate regions. The disease is seasonal, with peak incidence occurring in summer or fall in temperate regions, where temperature is the limiting factor in survival of leptospires, and during rainy seasons in warm climate regions, where rapid desiccation would otherwise prevent survival. Natural reservoirs for the pathogenic *Leptospira interrogans* include rodents as well as a large variety of domesticated mammals. Leptospires occupy the lumen of nephritic tubules in their natural host and are shed into the urine. Transmission can occur when humans or animals are directly or indirectly exposed to the urine of infected animals or a urine-polluted environment. Leptospires gain entry into the blood stream via cuts, skin abrasions or mucous membranes through contact with moist soil, vegetation, contaminated water and also by handling infected animal tissues or ingestion of food and water. The incubation period is usually 5-14 days, with a range of 2-30 days. The spectrum of clinical symptoms is extremely wide. The vast majority of leptospiral infections are either subclinical or result in very mild illness and recover without any complications. Clinical manifestations of leptospirosis range from mild influenza-like symptoms to severe life-threatening disease forms, characterized by jaundice, renal failure, bleeding and severe pulmonary hemorrhage. Acute kidney injury (AKI) is the most commonly recognized disease in dogs, accounting for more than 90 % of reported cases of leptospirosis. Hepatic disease occurs concurrently in 10 % - 20 % of dogs with AKI. *Leptospira pomona* and *Leptospira hardjo* cause a febrile syndrome, loss of appetite, and, in dairy cows, a large drop in - even the suppression of - milk secretion for a period ranging from two to ten days. *L. pomona* causes important reproductive problems in female breeding pigs spreading slowly through the herd. *L. tarassovi* causes a similar syndrome as *L. pomona* but tends to be milder and to spread more slowly. The clinical presentation of leptospirosis is biphasic, with the acute or septicemic phase lasting about a week, followed by the immune phase, characterized by antibody production and excretion of leptospires in the urine. Most of the complications of leptospirosis are associated with localization of leptospires within the tissues during the immune phase.

The presence of pathogen resp. infection may be identified by

- Pathogen detection: dark-field microscopy of a culture from blood, urine, cerebrospinal fluid or tissue PCR
- Serology: microscopic agglutination test (MAT), ELISA

2 Intended Use

The NovaTec VetLine Leptospira ELISA is intended for the qualitative determination of antibodies against Leptospira in veterinary serum.

3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.

The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec VetLine Leptospira ELISA kit was determined by comparing 12-24 replicates of at least 2 different samples in one assay (within-run) and by comparing at least 2 different samples assayed in 12 different runs (between-run).

In accordance with the procedures prescribed in the instruction, the mean ($\bar{x}$) and the standard deviation (s) of the absorbance values (E) were determined.

The CV value [%] was then calculated using the following formula:

$$CV = s/\bar{x} \times 100\ \%$$

Acceptance Criterion: CV < 15 %

Results

Table 1: Within-Run Precision

Sample	n	Mean (E)	CV [%]
1	24	0,176	11,78
2	24	1,237	7,50
3	24	0,324	8,47

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	3,63	3,68
2	12	10,89	4,93
3	12	30,98	3,85

Conclusion

The acceptance criterion was met for all samples.

4.2 Analytical Specificity

4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides

Introduction

Increased concentrations of possible interference materials such as bilirubin, triglycerides and hemoglobin may interfere with immunoassays creating false-negative or false-positive results.
In order to investigate this topic a literature research in combination with investigation of nearly 100 parameters were performed.

Material and Test Condition

Different members of the NovaLisa® and VetLine ELISA line were used including assays for the detection of different antibody isotypes (IgA, IgM, IgM, IgM + IgM, total antibody with protein A/G) to bacteria, viruses, fungi, parasites and worms as well as an antigen ELISA for the detection of TSH, Dirofilaria and FeLV.

Defined positive resp. negative or equivocal samples were used.

A certain amount of the potentially interfering substance was added to each sample. The final concentration of each substance was in a pathological range as also described by competitors (10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides). The results obtained with the sample with added “interfering substance” should be 60-140 % of the result of the untreated sample in order to fulfil the specifications.

Conclusion

The internal specifications of 60-140 % were always fulfilled.
Interferences with hemolytic, lipemic or icteric samples were not observed up to a concentration of 10 mg/ml hemoglobin, 0.5 mg/ml bilirubin and 5 mg/ml triglycerides.
These results are also in agreement with literature data.

This conclusion is valid for the NovaLisa® as well as for the VetLine version of the assays.

Dimeski, G. (2008) Interference Testing, Clin Biochem Rev 29, S43 – S48
Tate, J. and Ward, G. (2004) Interferences in Immunoassay, Clin Biochem Rev 25, 105 – 120

4.2.2 Cross-Reactivity

Cross-reactions cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

To evaluate the diagnostic performance of the VetLine Leptospira ELISA, external studies were conducted by Megacor (Austria) in comparison to well defined Leptospira samples. Samples are of canine origin.

Materials

VetLine Leptospira ELISA — Lot: LEPVT-004
Production date: 2015-10 — Expiry date: 2016-10-31

9 positive samples canine

7 negative samples canine

Results

Total number of canine samples: 16

Table **3**: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Leptospira ELISA	positive	8	0	**8**
	negative	1	7	**8**
	Σ	**9**	**7**	**16**

Diagnostic Sensitivity canine: 88,89 % (95% confidence interval: 51,75 % - 99,72 %)

Diagnostic Specificity canine: 100,0 % (95% confidence interval: 59,04 % - 100,0 %)

Agreement: 93,75 % (15/16)

Conclusion

The diagnostic sensitivity canine was 88,89 % and the diagnostic specificity canine was 100,0 % (agreement: 93,75 %).