VetLine

Leishmania ELISA

(LEIVT0310)

Performance Characteristics

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1 Introduction

Leishmaniasis is an infectious disease transmitted by sand flies and caused by various species of Leishmania. The parasites can infect both humans and canines, and the resulting condition is known as visceral leishmaniasis. The disease is particularly common in Mediterranean basin (e.g., Italy, Spain and Portugal), the Balkans, central and southwest Asia, north and northwest China, north and sub-Saharan Africa, and parts of Central and South America. The domestic dog seems to be the main reservoir for human visceral leishmaniasis, rendering disease control that much more vital.

In dogs clinical manifestations include chronic wasting, epistaxis, diarrhea, conjunctivitis, ocular signs (anterior uveitis, retinitis), severe muscle atrophy, swollen limbs and joints, lameness, lymphadenopathy, polyarthritis, and protein-losing nephropathy, which may lead to renal failure. Assessment of renal function in all infected dogs is critically important.

Infection may be identified by:

- Microscopy
- Serology: IFA, ELISA

2 Intended Use

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

3 Principle of the Assay

The qualitative immunoenzymatic determination of antibodies against Leishmania is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Leishmania antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase (HRP) labelled Protein A/G conjugate is added. This conjugate binds to the captured Leishmania specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) Substrate Solution which gives a blue reaction product. The intensity of this product is proportional to the amount of Leishmania specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec VetLine Leishmania 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	0,463	4,25
2	24	1,245	5,95
3	24	0,727	3,59

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	27,34	4,.94
2	12	29,33	6,54
3	12	1,23	14,19

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 Novatec 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, especially Trypanosoma spec., cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

To evaluate the diagnostic performance of the Novatec VetLine Leishmania ELISA, internal studies were conducted by NovaTec in comparison to predetermined samples. Specimens used are serum or plasma from European dogs.

Materials

Novatec VetLine Leishmania ELISA Lot: LEIVT-046-1

Production date: 2014-12 Expiry date: 2015-12-31

Novatec VetLine Leishmania ELISA Lot: LEIVT-047-1

Production date: 2015-02 Expiry date: 2016-08-31

Results

Total number of samples: 294

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
NovaTec VetLine Leishmania ELISA	positive	114	8	**122**
	negative	5	167	**172**
	Σ	**119**	**175**	**294**

Diagnostic Sensitivity canine: 95,80 % (95 % confidence interval: 90,47 % - 98,62 %)

Diagnostic Specificity canine: 95,43 % (95 % confidence interval: 91,19 % - 98,01 %)

Agreement: 95,58 % (281/294)

Conclusion

The diagnostic sensitivity canine was 95,80 % and the diagnostic specificity canine was 95,43 % (agreement: 95,58 %).