VetLine

Borrelia ELISA

(BORVT0040)

Performance Characteristics

Table of Contents

1 Introduction

Lyme disease (Borreliosis) is a tick-borne, bacterial disease of domestic animals and humans. It is caused by spirochetes of the Borrelia burgdorferi sensu lato group. In Europe, the most frequently isolated pathogenic genospecies of humans and dogs are Borrelia afzelii, Borrelia garinii and Borrelia burgdorferi sensu stricto.

Borrelia are helically wound, flexible, highly motile bacteria. By rotation of their axial filaments (periplasmatic flagella) they are able to move efficiently in corkscrew fashion through viscous media (serum). Thereby they can disseminate throughout the body within days to weeks of infection.

The pathogens are transmitted by various tick species of the genus Ixodes. In Europe, Ixodes ricinus is the most important vector. However, infestation rates with Borrelia vary depending on the region. In endemic areas of Germany, approximately 3-7 % of the larvae and 10-34 % of nymphs and adult ticks are infected by Borrelia burgdorferi sensu lato.

Natural reservoirs are wild animals, including rodents as well as many other small mammals and birds. The ticks take their meals (blood) from these hosts.

Since dogs frequent the areas ticks live, they are more affected than humans. Typical tick habitats are the edge of the woods, bushes, undergrowth and tall grass; but infected ticks can also be found in public parks.

Symptoms of Lyme disease in dog comprise fever, apathy, loss of appetite and anorexia as well as recurrent and shifting lameness and polyarthritis. The characteristic rash or the circular area of redness around the bite (erythema chronicum migrans) which is seen in man may be absent or is overlooked due to hair coat or dark pigmentation.

2 Intended Use

The NovaTec VetLine Borrelia ELISA is intended for the qualitative determination of antibodies against Borrelia burgdorferi 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 Borrelia 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	0,517	4,00
2	24	1,790	4,23
3	24	2,339	9,04

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	37,2	4,.17
2	12	45,3	9,28
3	12	1,90	9,94

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 against spirochaetes, cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

To evaluate the diagnostic performance of the Novatec VetLine Borrelia ELISA, internal studies were conducted by NovaTec in comparison to predetermined samples.

Material

VetLine Borrelia ELISA Lot: BORVT-091-1 - BORVT-106-1

21 positive canine samples

15 negative canine samples

Results

Total number of samples: 36

Table 3: Diagnostic Sensitivity and Specificity
(Equivocal results were not included in the calculations)

		Demand		
		positive	negative	**Σ**
NovaTec VetLine Borrelia ELISA	positive	19	1	**20**
	negative	1	13	**14**
	Σ	**20**	**14**	**34**

Diagnostic Sensitivity canine: 95,00 % (95 % confidence interval: 75,13 % - 99,87 %)

Diagnostic Specificity canine: 92,86 % (95 % confidence interval: 66,13 % - 99,82 %)

Agreement: 94,12 % (32/34)

Conclusion

The diagnostic sensitivity canine was 95,00 % and the diagnostic specificity canine was 92,86 % (agreement: 94,12 %).