VetLine

Ehrlichia ELISA

(EHRVT0930)

Performance Characteristics

Table of Contents

1 Introduction 3

2 Intended Use 3

3 Principle of the Assay 4

4 Performance Characteristics 4

4.1 Reproducibility (Precision) 4

4.2 Analytical Specificity 5

4.2.1 Interference from Hemoglobin, Bilirubin and Triglycerides 5

4.2.2 Cross-Reactivity 6

4.3 Diagnostic Sensitivity and Specificity 6

1 Introduction

Canine monocytic Ehrlichiosis (CME) is a canine infectious disease that occurs worldwide in tropical and subtropical regions, as well as in the Mediterranean area of Europe.
It is caused by *Ehrlichia canis*, a gram-negative, obligate intracellular bacterium of the order *Rickettsiales. Ehrlichia canis* is transmitted by the brown dog tick *Rhipicephalus sanguineus.* The incubation period is approximately 1-3 weeks.

The canine monocytic Ehrlichiosis is divided in 3 phases:

Acute phase:
The acute phase lasts about 2-4 weeks. In this phase there is a strong increase of Ehrlichia within the monocytes and lymphocytes of the blood. The symptoms are usually non-specific: fever, loss of appetite, difficulty breathing, anemia, thrombocytopenia and swelling of the lymph nodes can occur.

Subclinical phase:
In this phase, the disease is usually asymptomatic, although high titers of E. canis-specific IgG antibodies and changes in blood values can be detected. The subclinical phase can last from weeks to several years.

Chronic phase:
If not, all pathogens can be eliminated, a chronic illness will result. It often occurs in immunosuppressive dogs. The animals show symptoms such as bone marrow depression, bleeding, neurological diseases, peripheral edema and emaciation.

Canine monocytic Ehrlichiosis is often accompanied by co-infections such as babesiosis and anaplasmosis.

Detection methods:

- Direct detection of pathogens: Giemsa-stained blood smear with evidence of a characteristic accumulation of bacteria in monocytes (morula stage). This detection is only possible in the acute phase of the infection.
- Indirect pathogen detection: detection of antibodies using IFT or ELISA.

2 Intended Use

The NovaTec VetLine Ehrlichia ELISA is intended for the qualitative determination of antibodies against Ehrlichia 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.

Microtiterplates 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 Microtiterplate reader.

4 Performance Characteristics

4.1 Reproducibility (Precision)

Test Description

The reproducibility of the NovaTec VetLine Ehrlichia 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	0,479	2,14
2	24	0,481	4,17
3	24	0,843	2,94

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	16,72	5,20
2	12	16,12	6,62
3	12	18,49	5,51

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

The evaluation of the diagnostic performance of the VetLine Ehrlichia ELISA was conducted at NovaTec with predetermined canine Ehrlichia samples from European accredited laboratories.

Material

VetLine Ehrlichia ELISA	Lot:	EHRVT-002, EHRVT-003
Production date Lot 002: 2018-09	Expiry date:	2019-06-30
Production date Lot 003: 2019-07	Expiry date:	2020-06-30

25 positive canine samples

93 negative canine samples

Results

Total number of canine samples: 118

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Ehrlichia ELISA	positive	23	1	**24**
	negative	2	92	**94**
	Σ	**25**	**93**	**118**

Diagnostic Sensitivity canine: 92,00 % (95% confidence interval: 73,97 % - 99,02 %)
Diagnostic Specificity canine: 98,92 % (95% confidence interval: 94,15 % - 99,97 %)
Agreement: 97,46 % (115/118)

Conclusion

The evaluation of the diagnostic performance of the VetLine Ehrlichia ELISA was conducted at NovaTec.

The diagnostic sensitivity canine was 92,00 % and the diagnostic specificity canine was

98,92 % (agreement: 97,46 %).

Therefore, the acceptance criteria are met.