VetLine
Anaplasma ELISA
(ANAVT0850)

Performance Characteristics

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1 Introduction

Anaplasma species are rickettsial bacterial pathogens that reside within host erythrocytes.

Anaplasma causes Anaplasmosis and has worldwide distribution due to their wide range of vectors (ticks).

Anaplasmosis or Tick Borne Fever (TBF) is a rickettsial disease affecting the white blood cells of sheep and cattle, causing anaemia and seasonal “pasture fever”. It is caused by *Anaplasma phagocytophilum* (previously known as *Ehrlichia phagocytophila*). Seasonal Anaplasmosis occurs in cattle that are returned to tick infected pasture in the spring. Anaplasmosis naturally affects primarily sheep and cattle, and less commonly, deer, horses and dogs. Anaplasmosis causes multisystemic disease, causing cardiovascular, gastrointestinal, respiratory, reproductive and neurological signs, and also lymphadenopathy and wasting disease.

Antibodies can be detected using Indirect Immunofluorescence (IFAT), Complement Fixation, Enzyme-linked immunosorbent assay (ELISA) and Immunoelectrophoresis (CIEP).

2 Intended Use

The NovaTec VetLine Anaplasma ELISA is intended for the qualitative determination of antibodies against Anaplasma 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 Anaplasma 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	0.717	2.92
2	24	0.705	2.73
3	24	0.259	2.63

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	11.6	7.55
2	12	15.9	9.91
3	12	6.8	10.40

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 Anaplasma ELISA, internal studies were conducted by NovaTec in comparison to pre-defined samples. Samples are of canine origin.

Materials

VetLine Anaplasma ELISA Lot: ANAVT-002

25 positive canine samples

17 negative canine samples

Results

Total number of canine samples: 42

Table **5**: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Anaplasma ELISA	positive	23	1	**24**
	negative	2	16	**18**
	Σ	**25**	**17**	**42**

Diagnostic Sensitivity canine: 92.00 % (95% confidence interval: 73.97 % - 99.02 %)

Diagnostic Specificity canine: 94.12 % (95% confidence interval: 71.31 % - 99.85 %)

Agreement: 92.85 % (39/42)

Conclusion

The diagnostic sensitivity canine was 92.00 % and the diagnostic specificity canine was 94.12 % (agreement: 92.85 %).