VetLine
Canine Distemper Virus ELISA
(MEAVT0330)

Performance Characteristics

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1 Introduction

Canine Distemper Virus is shed in all excretions and secretions, and is spread by direct and indirect contact via ingestion or inhalation of aerosolised droplets. Initial infection of the nasal cavity, pharynx and lungs is followed by a macrophage-associated viraemia to local lymph nodes where the virus replicates. Within one week, all lymphoid tissue is infected, causing lymphopenia. A second viraemic stage then distributes the virus to the surface epithelium of the respiratory, gastrointestinal and urogenital tracts, endocrine tissue and the grey and white matter of the CNS. A biphasic pyrexia is typical of distemper infection: the first fever occurs 3-6 days post-infection and is associated with lymphopenia, and the second peak coincides with widespread viraemia. Further signs depend on both the virus strain and the immune response mounted. In the event of a strong humoral and cellular response, disease may remain subclinical, and if a weak immune response is mounted infection is generally subacute. If the immune response fails, acute disease and potentially death ensues. When clinical disease manifests, this is initially characterized by lethargy, dehydration, anorexia, and weight loss followed by more specific signs depending on the principally affected organ.

Canine distemper is often fatal, but an increased production of virus-neutralizing antibodies can promote the recovery of the animal. However, CDV can persist in the uvea, CNS, lymphoid organs and footpads despite elimination from most organs and the blood. This can result in "old dog encephalitis" in dogs that recovered from acute canine distemper years previously. In this, several neurological episodes occur over weeks to months, and usually culminate in the death of the dog.

Pathogen	Disease	Symptomes
Canine Distemper Virus (CDV)	Canine distemper	Rhinitis Conjunctivitis Leukopenia Pneumonia cough with purulent, bloody sputum Tonsillitis Enteritis Secondary bacterial infections (for example, Bordetella) Neurological symptoms such as tremor, ataxia, nystagmus, optic nerve damage Pustular Dermatitis Hyperkeratosis of the footpads and the nose leather (hard pad disease) Enamel hypoplasia ("distemper teeth") in puppies

Infections may be diagnosed by:

- PCR
- Serology: Detection of antibodies by ELISA

2 Intended Use

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

3 Principle of the Assay

The qualitative immunoenzymatic determination of antibodies against Canine Distemper Virus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Morbillivirus 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 Canine Distemper Virus 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 Canine Distemper Virus 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 Canine Distemper Virus 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	0,314	6,93
2	24	0,931	3,70
3	24	0,718	9,98

Table 2: Between-Run Precision

Sample	n	Mean [NTU]	CV [%]
1	12	33,93	2,76
2	12	28,09	7,46
3	12	3,85	12,11

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 with closely related pathogens cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

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

Materials

VetLine Canine Distemper Virus ELISA Lot: MEAVT-095-1

32 positive samples canine

13 negative samples canine

Results

Total number of samples: 45

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Canine Distemper Virus ELISA	positive	31	1	**32**
	negative	1	12	**13**
	Σ	**32**	**13**	**45**

Diagnostic Sensitivity canine: 96,88 % (95 % confidence interval: 83,78 % - 99,92 %)

Diagnostic Specificity canine: 92,31 % (95 % confidence interval: 63,97 % - 99,81 %)

Agreement: 95,56 % (43/45)

Conclusion

For **canine samples** the diagnostic sensitivity was 96,88 % and the diagnostic specificity was 92,31 % (agreement: 95,56 %).