VetLine
Canine Parvovirus ELISA
(PARVT0370)

Performance Characteristics

NovaTec Immundiagnostica GmbH

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1 Introduction

The canine parvovirus (CPV) infection is a highly contagious viral illness that affects dogs. Parvoviruses are single-stranded DNA viruses with a genome of about 5000 nucleotides. They are one of the smallest known viruses. Without envelope they have a diameter of about 20-25 nm. They show a high physical stability. The capsid is highly resistant and the virions can remain infectious for months to years.

The virus manifests itself in two different forms. The more common form is the intestinal form, which is characterized by vomiting, diarrhea, weight loss, and lack of appetite (anorexia). The less common form is the cardiac form, which attacks the heart muscles of very young puppies, often leading to death. Parvovirus is excreted in the feces. The most common mode of transmission is indirectly. The viruses are taken up oronasal from the contaminated environment or contaminated litter. They are transported via the lymphatic cells of the pharynx in almost all organs. Replication occurs preferably in lymphocytic cells.

During replication, the host cell is destroyed. Via the epithelial cells the virus is transported in the intestinal lumen and is then excreted within the feces.

The majority of cases are seen in puppies that are between six weeks and six months old.

Species	Disease	Symptoms (e.g.)	Transmission route
Canine Parvovirus	Parvovirus infection of dogs	Severe, bloody diarrhea Lethargy Anorexia Fever Vomiting Severe weight loss	Oronasal

Infections may be diagnosed by:

- Cell culture
- PCR
- Serology: Determination of specific antibodies by ELISA

2 Intended Use

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

3 Principle of the Assay

The qualitative immunoenzymatic determination of antibodies against Canine Parvovirus is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Canine Parvovirus 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 Parvovirus 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 Parvovirus 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 Parvovirus 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	0,350	8,96
2	24	1,211	2,60
3	24	0,777	8,61

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	4,532	8,34
2	12	23,000	4,63
3	12	15,241	7,78

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 with closely related pathogens cannot be excluded.

4.3 Diagnostic Sensitivity and Specificity

Introduction

The evaluation of the diagnostic performance of the VetLine Canine Parvovirus ELISA was performed in comparison to well defined Canine Parvovirus samples.

Materials

VetLine Canine Parvovirus ELISA Lot: PARVT-002

56 positive samples

6 negative samples

Results

Total number of samples: 62

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Canine Parvovirus ELISA	positive	55	0	**63**
	negative	1	6	**7**
	Σ	**56**	**5**	**62**

Diagnostic Sensitivity: 98,21 % (95 % confidence interval: 90,45 % - 99,95 %)

Diagnostic Specificity: 100,0 % (95 % confidence interval: 54,07 % - 100 %)

Agreement: 98,39 % (61/62)

Conclusion

The diagnostic sensitivity was 98,21 % and the diagnostic specificity was > 98 % (agreement: 98,39 %).