VetLine
Feline Corona Virus (FCoV/FIP) ELISA
(FIPVT0870)

Performance Characteristics

Table of Contents

1 Introduction

Feline Infectious Peritonitis (FIP) is a viral disease of cats caused by certain strains of a virus called the Feline Corona Virus. Most strains of Feline Corona Virus are avirulent, which means that they do not cause disease, and are referred to as Feline Enteric Corona Virus. Cats infected with a virulent FCoV generally do not show any symptoms during the initial viral infection. An immune response occurs with the development of antiviral antibodies. In a small percent of infected cats (5 to 10 %), either by a mutation of the virus or by an aberration of the immune response, the infection progresses into clinical FIP. The virus is then referred to as Feline Infectious Peritonitis Virus (FIPV). With the assistance of the antibodies that are supposed to protect the cat, white blood cells are infected with the virus, and these cells then transport the virus throughout the cat's body. An intense inflammatory reaction occurs around vessels in the tissues where these infected cells locate, often in the abdomen, kidney or brain. It is this interaction between the body's own immune system and the virus that is responsible for the disease. Once a cat develops clinical FIP involving one or many systems of the cat's body, the disease is progressive and is almost always fatal.

Disease	Symptoms
Feline Infectious Peritonitis (FIP)	e.g.: Loss of appetite Weight loss Vomit Diarrhea Disorders of the central nervous system Increase of the abdominal circumference Fluid accumulation in the abdominal cavity Inflammation of the kidneys Inflammation of the inner abdominal organs Anemia

2 Intended Use

The NovaTec VetLine Feline Corona Virus ELISA is intended for the qualitative determination of antibodies against FCoV in feline serum.

3 Principle of the Assay

The qualitative immunoenzymatic determination of antibodies against FCoV is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with Feline Corona Virus 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 Feline Corona 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 Feline Corona 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 Feline Corona 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	0,819	3,84
2	24	0,477	5,92
3	24	0,222	6,12

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	16,544	4,15
2	12	9,818	9,83
3	12	9,136	6,47
4	12	9,147	7,68

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

To evaluate the diagnostic performance of the VetLine Feline immunodeficiency Virus ELISA, external studies were conducted by different laboratories in comparison to pre-defined samples. Samples are of feline origin.

Material

VetLine Feline Corona Virus ELISA Lot: FIPVT-002

Production date: 2017-04 Expiry date: 2018-04-30

66 positive samples

26 negative samples

Results

Total number of samples: 92

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Feline Corona Virus ELISA	positive	66	2	**68**
	negative	0	24	**24**
	Σ	**66**	**26**	**92**

Diagnostic Sensitivity: 100,0 % (95 % confidence interval: 94,56 % - 100,0 %)

Diagnostic Specificity: 92,31 % (95 % confidence interval: 74,87 % - 99,05 %)

Agreement: 97,83 % (90/92)

Conclusion

The diagnostic sensitivity was > 98 % and the diagnostic specificity was 92,31 % (agreement: 97,83 %).