VetLine

Feline Leukemia Virus Antigen ELISA

(FELVT4800)

Performance Characteristics

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1 Introduction

Feline Leukemia Virus (FeLV) belongs to the family of retroviruses, the subfamily of the oncoviruses, and can cause leukemia, anemia and tumours in various organs. The virus is particularly sensitive to environmental influences and can be inactivated by heat, sunlight and the use of disinfectants. For the routine diagnosis of a FeLV infection, the p27 antigen detection is mainly used. The p27 antigen is an antigen that can be detected in serum, plasma, saliva, bone marrow, and in all infected tissues of a FeLV-infected cat. The p27 antigen matters 25-50 % of the particle mass of the virus.

The transmission of the virus occurs through excretions (saliva, excrement, nasal secretion, milk) of FeLV-infected cats. It affects mainly cats that absorb the infectious material through the mucous membranes and wounds. Direct contact between two cats, for example, during hunting or during cleaning, presents the main infectious source. In infected mother animals, pregnancy generally ends with the death of cat puppies, death birth or the birth of infected, life-threatening cat puppies. Cat puppies are particularly susceptible to FeLV infection. With increasing age they can develop a resistance against the virus. Cat leukaemia is a common infection disease worldwide that can be fatal. FeLV infections can take very different disease forms that are associated with nonspecific symptoms, which make a reliable diagnosis more difficult. Healthy cats can also include and transmit pathogens.

The possible disease forms after infection are:

Form 1: Cats undergo a temporary infection. Healthy cats stalk the virus, the infection goes unnoticed.

Form 2: Cats undergo a transient infection. The immune system can not develop a sufficient immune response. Cats develop the typical disease pattern of cat leukaemia. The animals strive within 3-5 years.

Form 3: Cats make a latent infection. The virus is not excreted in this phase.

Form 4: In the case of immunosuppression by stress, the disease can at any time pass into the disease form 2 if viruses are present.

species	disease	symptoms	infection
Feline Leukemia Virus	Feline Leukemia	Unspecific symptoms such as fatigue, loss of appetite, dyspnea, digestive disorders and persistent fever Anemia, flu and pneumonia, thoracic effusions, diarrhea, chronic inflammation of the gums (gingivitis) and mouth (stomatitis), emaciation, various tumor forms (leukemia, lymph sarcoma, fibro sarcomas), abortions of kittens and stillbirths Secondary diseases such as haemobartonellosis, toxoplasmosis, septicemia, fungi, glomerulonephritis due to immune complex formation	By ingestion of infectious material over the mucous membranes and wounds.

The presence of pathogen or infection may be identified by

- Pathogen detection: Virus isolation
  PCR
- Serology: Antigen detection by ELISA, IFT
  Antibody detection by ELISA, Lineblot

2 Intended Use

The NovaTec VetLine Feline Leukemia Virus Antigen ELISA is intended for the qualitative determination of antigens against Feline Leukemia Virus in feline serum.

3 Principle of the Assay

The qualitative immunoenzymatic determination of specific antigens is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiterplates are coated with specific antibodies to bind corresponding antigens of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled antibody conjugate is added. This conjugate binds to the captured antigens. 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 antigens 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 Feline Leukemia Virus Antigen ELISA kit was determined by comparing 12-24 replicates of 2 different samples in one assay (within-run) and by comparing 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	0.202	7.63
2	24	0.330	7.65
3	24	0.803	6.77

Table 2: Between-Run Precision

Sample	n	Mean (NTU)	CV [%]
1	12	6.089	10.61
2	12	7.363	10.91
3	12	13.858	6.62

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 and Dirofilaria.

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

4.3 Diagnostic Sensitivity and Specificity

Introduction

To evaluate the diagnostic performance of the NovaTec VetLine Feline Leukemia Virus Antigen ELISA, the studies were conducted intern by Novatec and external by Futurlab (Italy) in comparison to pre-defined samples.

Samples are of feline origin.

Materials

VetLine Feline Leukemia Virus Antigen ELISA Lot: FELVT-006

Production date: 2018-03 Expiry date: 2019-03-31

57 positive feline serum samples

133 negative feline serum samples

Results

Total number of samples: 190

Table 3: Diagnostic Sensitivity and Specificity

		Demand		
		positive	negative	**Σ**
VetLine Feline Leukemia Virus Antigen ELISA	positive	57	2	59
	negative	0	131	131
	Σ	57	133	190

Diagnostic Sensitivity: 100,00 % (95% confidence interval: 93,73 % - 100,0 %)

Diagnostic Specificity: 98,50 % (95% confidence interval: 94,67 % - 99,82 %)

Agreement: 98,95 % (188/190)

Conclusion

The diagnostic sensitivity was 100,00 % and the diagnostic specificity was 98,50 % (agreement: 98,95 %).