# VetLine

# Feline Immunodeficiency Virus (FIV) ELISA

# (FIVVT0750)

Performance Characteristics

# Table of Contents

# 1 Introduction

The feline immunodeficiency virus (FIV) is a retrovirus that causes immune deficiency in cats. Within the family of retroviruses FIV belongs to the lentivirus.

FIV infections can be found worldwide. In Germany, the prevalence is between 2 % and 3 %. For Europe, different values between 3 % and 30 % are published with higher infection rates when looking on stray cats. In the US, the prevalence rates are between 1-26 %, in Japan at up to 44 %.

5 subtypes of FIV (A-E) can be distinguished from each other. Their nucleotide sequences in the *env* gene, which encodes the proteins of the viral envelope, shows up to 20 % variation from each other. In general, subtype A is more prevalent in Europe and subtype B in Japan.

The main mode of transmission of FIV is by bite wounds. Particularly at risk are stray cats who are involved in rival fights. In particular uncastrated cats, showing aggressive territorial behavior within a high population density, are exposed to a higher risk of infection.

The FIV is excreted primarily via the saliva. By bite injury the virus enters the bloodstream and infects the T-lymphocytes. There the virus replicates and leads to a weakening of the immune system of the cat.

FIV has, like all retroviruses, a high degree of adaptation to its host. Transferability of FIV to humans can be excluded.

FIV infection can be divided into four phases:

| | **Symptomes** | **Duration** |
|---|---|---|
| **Stage 1:**<br>Acute phase<br>Initial Stage | Lymphadenopathy<br>Neutropenia accompanied by fever<br>Appetite and weight loss<br>Rarely diarrhea<br>Rarely respiratory symptoms<br>Symptoms usually moderate | weeks - months |
| **Stage 2:**<br>Asymptomatic phase<br>Latent phase | Without pathological findings | months - years |
| **Stage 3:**<br>Non specific symptomes | Recurrent fever<br>Massive generalized lymphadenopathy (often persistent)<br>Leukopenia<br>Anemia<br>Anorexia,<br>Weight loss,<br>Chronic stomatitis<br>Apathy and behavioral changes | months – 1 year |
| **Stage 4:**<br>Terminal phase | Immunodeficiency<br>Symptoms of 3rd phase<br>Opportunistic infections<br>Myeloproliferative disorders<br>Neoplasia<br>Neurological symptoms | months |

FIV infection can be diagnosed by:

- Serology : ELISA, IFA, Westernblot

# 2 Intended Use

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

# 3 Principle of the Assay

The qualitative immunoenzymatic determination of antibodies against FIV is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with FIV 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 FIV 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 FIV 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 Immunodeficiency 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 | 0,747 | 3,6 |
| 2 | 24 | 0,510 | 2,6 |
| 3 | 24 | 0,794 | 3,8 |

Table 2: Between-Run Precision

| Sample | n | Mean (NTU) | CV [%] |
|---|---|---|---|
| 1 | 12 | 13,8 | 3,0 |
| 2 | 12 | 8,8 | 8,8 |
| 3 | 12 | 6,4 | 4,2 |
| 4 | 12 | 2,5 | 5,4 |
| 5 | 12 | 2,0 | 8,7 |
| 6 | 12 | 7,0 | 9,2 |

### 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

A panel of 29 specimens from patients with confirmed diseases other than FIV was tested to establish the analytical specificity of the NovaTec Feline Immunodeficiency Virus ELISA.

The specimens were from mammals infected with pathogens that may cause similar signs and symptoms to those observed for FIV or from individuals with diseases or conditions that have the potential for cross-reactivity.

**Material**

| | | | |
|---|---|---|---|
| NovaTec VetLine Feline Immunodeficiency Virus ELISA | | Lot: | FIVVT-002 |
| Production date: | 2015-06 | Expiry date: | 2016-06-30 |

29 potentially cross-reactive samples

## Results

Table 3: Cross-Reactivity

| Disease Type | Sample | NTU | Evaluation |
|---|---|---|---|
| FeLV | 1 | 8,1 | Neg |
| | 2 | 8,6 | Neg |
| | 3 | 8,7 | Neg |
| | 4 | 7,9 | Neg |
| | 5 | 8,2 | Neg |
| | 6 | 8,2 | Neg |
| Parvovirus | 1 | 3,1 | Neg |
| | 2 | 2,9 | Neg |
| | 3 | 6,2 | Neg |
| | 4 | 5,9 | Neg |
| | 5 | 6,5 | Neg |
| | 6 | 7,6 | Neg |
| | 7 | 2,8 | Neg |
| Herpesvirus | 25 | 2,0 | Neg |
| | 61 | 1,7 | Neg |
| | 76 | 8,7 | Neg |
| | 82 | 1,8 | Neg |
| | 83 | 1,4 | Neg |
| | 28 | 1,8 | Neg |
| | 52 | 2,2 | Neg |
| | 85 | 2,8 | Neg |
| | 20 | 1,3 | Neg |
| | 21 | 8,6 | Neg |
| | 50 | 1,9 | Neg |
| | 80 | 8,9 | Neg |
| Calcivirus | 1 | 4,7 | Neg |
| | 7 | 5,7 | Neg |
| | 19 | 1,9 | Neg |
| | 14558 | 2,0 | Neg |

## Summary of the Results

Table 4: Summary

| Pathogen/Disease Type | Total Specimens | Positive Result |
|---|---|---|
| FeLV | 6 | 0/6 |
| Parvovirus | 7 | 0/7 |
| Herpesvirus | 12 | 0/12 |
| Calcivirus | 4 | 0/4 |
| | **29** | **0/29** |

## Conclusion

The investigation of a specimen panel with antibody activities to potentially cross-reacting parameters (antibodies to several infectious agents) did not reveal significant evidence of false-positive results due to cross-reactions. However 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.

### Materials

NovaTec VetLine Feline Immunodeficiency Virus ELISA Lot: FIVVT-001

Production date: 2015-04 Expiry date: 2016-04-30

44 positive samples

80 negative samples

### Results

Total number of samples: 124

Table **5**: Diagnostic Sensitivity and Specificity

| | | Demand | | |
|---|---|---|---|---|
| | | positive | negative | **Σ** |
| **VetLine Feline Immunodeficiency Virus ELISA** | positive | 42 | 3 | **45** |
| | negative | 2 | 77 | **79** |
| | **Σ** | **44** | **80** | **124** |

Diagnostic Sensitivity: 95,45 % (95 % confidence interval: 84,53 % - 99,44 %)

Diagnostic Specificity: 96,25 % (95 % confidence interval: 89,43 % - 99,22 %)

Agreement: 95,97 % (119/124)

### Conclusion

The diagnostic sensitivity was 95,5 % and the diagnostic specificity was 96,3 % (agreement: 96,0 %).