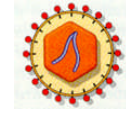




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NEWSLETTER

Feline Leukemia Virus- FeLV[®]

We have performed more than 1,280,000 FeLeuk[®] FeLV IFA tests.

Fall 2002

Vol. 1, Number 4

Evelyn E. Zuckerman, Editor

In This Issue:

In the fall issue of the NVL Newsletter we will review the feline leukemia virus (FeLV). FeLV is one of the most important infectious agents of pet cats. We will discuss the biology of FeLV and the test methods for detection of infected cats. In 1972 we developed the first FeLV test, the IFA test, and subsequently used the test to define many of the FeLV-induced diseases.

Feline Leukemia Virus

William D. Hardy, Jr., V.M.D.

Three subfamilies of *Retrovirinae* exist in pet cats: 1) *Oncovirinae*, 2) *Lentivirinae*, and 3) *Spumavirinae*. Cats have more retroviruses than any other species.

FELINE RETROVIRUSES

1. Subfamily *Oncovirinae*:

A. Endogenous Viruses: Genetically transmitted

- 1) FeLV related full length and shorter DNA sequences. Cannot be induced to replicate
- 2) Recombines with exogenous FeLV-A DNA to form FeLV-B and FeLV-C

B. Exogenous Viruses: Spread contagiously

Chronic transforming (leukemia) retroviruses:

- 1) FeLVs:
 - Subgroup A-** Found in all infected cats. Only subgroup transmitted contagiously
 - Subgroup B-** Found in 50% of infected cats
 - Subgroup C-** Found less than 1%

2) FeLV-A-FAIDS

Experimentally induces FAIDS

Acute transforming retroviruses:

- 3) FeSVs: (Feline Sarcoma Viruses) 11 isolates. Recombinants- FeLV and cellular oncogenes

2. Subfamily *Lentivirinae*:

A. FIV (Feline Immunodeficiency Virus)

Induces FAIDS

3. Subfamily *Spumavirinae*:

A. FeSFV (feline syncytium-forming virus)

Causes no known disease

Background:

Domestic cats are infected with members of all 3 retrovirus subfamilies. The feline leukemia virus (FeLV) was first isolated from a cat in Scotland in 1964. At that time all retroviruses were thought to be endogenous viruses that were only transmitted genetically (vertically). However, using the FeLV IFA test in pet cats, we demonstrated that FeLV is an exogenous retrovirus that is transmitted contagiously amongst cats.¹ This observation was the first conclusive proof that any retrovirus was transmissible by contagious means, and this finding changed the prevailing concepts on these viruses. Before the introduction of the FeLV vaccines, about 2%, or more than 1 million of the estimated 60 million pet cats in the United States, were infected with FeLV.²⁻⁸ The incidence of FeLV-infected cats has not been studied after the introduction of the FeLV vaccines.

Endogenous FeLV-Related Sequences:

Healthy FeLV-uninfected domestic cats possess cellular DNA sequences that are partially homologous to the RNA of exogenous contagiously transmitted FeLVs. Only the cellular DNA of rodents, and in particular rats, contains related retrovirus gene sequences. The presence of related sequences in rodents suggests that endogenous FeLVs were acquired by cats via trans-species infection with a rodent retrovirus.

Sequence analysis of the genomes of the three subgroups of exogenous infectious FeLVs (FeLV-A, -B and -C) has shown that FeLV-B and FeLV-C arise through recombination of contagiously transmitted FeLV-A with endogenous *env* -B and -C sequences to form envelope (*env*) recombinant FeLVs. These *de novo* generated subgroups are not transmitted contagiously and are far more pathogenic than the contagiously transmitted FeLV-A.

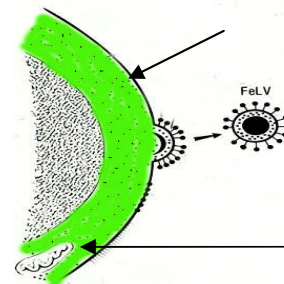
FeLV Proteins:

Nine proteins are encoded by the FeLV genome and include: 1) the *gag* gene internal viral structural proteins p15 (matrix protein, MA), p12 (unknown) function, p27 (capsid protein, CP) and p10 (nucleocapsid protein, NC); 2) the *pol* gene enzymes: p14 (protease, PR), p80 (reverse transcriptase, RT), p46

(integration protein, IN) and; 3) the *env* gene envelope proteins gp70 (surface protein, SU) and p15E (transmembrane protein, TM).

The FeLV structural proteins are produced in great excess in the cell membrane and the cytoplasm of infected cells and free viral proteins are released into the plasma and tissue fluids of infected cats after the cells die.

FeLV Infected Leukocyte

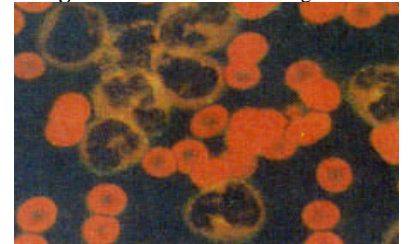


Cytoplasmic FeLV antigens

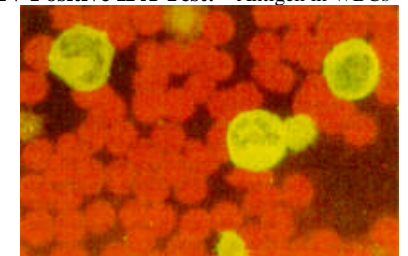
FeLV Tests:

The study of the occurrence and control of FeLV in pet cats has been accomplished by detection of FeLV antigens in the cytoplasm of peripheral blood leukocytes by indirect immunofluorescent antibody (IFA) tests or by detection of soluble antigens in the plasma by enzyme linked immunosorbent assays (ELISA). All of the FeLV biology and control

FeLV Negative IFA Test: No antigen in WBCs



FeLV Positive IFA Test: Antigen in WBCs



methods were elucidated using the IFA test for FeLV during the 1970s. A positive IFA test correlates 98% of the time with the ability to isolate FeLV from the blood and indicates persistent infection, usually life long (in 91% of IFA positive cats), viremia and shedding of the virus in the saliva. However, as many as 25% of positive FeLV ELISA tests cannot be confirmed by IFA and thus represent false positive tests.²⁻⁷

Comparison of FeLV Test Methods:

Comparison of IFA Test and FeLV Isolation

IFA Test Result	Number Tested	FeLV Isolated	% Agreement
Positive	176	173	98.3%
Negative	172	3	98.3%
Total:	348	176	98.3%

Comparison of FeLV ELISA Positive Tests with the FeLV IFA Test

Years	Number ELISA + Cats	FeLeuk® IFA Positive	% Disagreement
1979-89	18,908	8,761	53.7%
1996-00	3,792	2,724	28.2%

AVMA Expert FeLV Panel Recommendation:

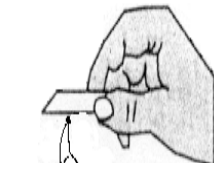
In 1991 the AVMA Expert FeLV Panel recommended that all FeLV positive ELISA tests be immediately confirmed by an IFA test.⁸

Pathogenesis of FeLV infection in cats:

The saliva of naturally infected pet cats has as many as 2×10^6 infectious FeLV per ml. The virus is mainly transmitted contagiously by intimate prolonged direct contact through the saliva to the mucous membranes of the head of uninfected cats. The pathogenesis of the stages of FeLV infection has been elucidated by use of the IFA test in experimentally inoculated SPF cats. After contact infection, the virus replicates initially in lymphocytes of the local lymph nodes of the head and neck. Most infected cats reject the virus at this early stage, become virus free, and immune. Studies of the spread of FeLV demonstrated that 28% of unvaccinated cats, exposed to FeLV, become persistently infected, 42% become immune, whereas the remaining 30% become neither immune nor infected.

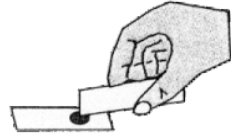
In cats that are unable to reject the virus in this early stage, FeLV spreads to the bone marrow where it replicates to high titers in all nucleated myeloid and erythroid cells. The virus spreads throughout the cat's body in infected leukocytes and platelets released from the infected bone marrow, or as whole virus in the plasma (10^5 infectious FeLV per ml). Within 6 to 8 weeks the virus infects cells of the salivary glands, oral mucosa, and respiratory epithelium from where it is shed. FeLV is also transmitted *in utero* to unborn fetuses and through the milk of infected queens. The period of time from FeLV infection to disease development is highly variable but 83% of infected healthy cats die within 3.5 years from FeLV-induced diseases. Most cats (91%) that have widespread replication of FeLV in their bone marrow remain persistently infected and only 9% can reject FeLV infection and rid themselves of all virus-replicating cells.

Preparation of Thin, Feathered-Edge, Blood Smears for the FeLeuk® IFA Test for FeLV

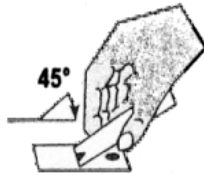


1. Clip the cat's nail to obtain a drop of blood. Touch the drop of blood from the cat's nail to the slide. If you obtain blood by syringe, place a **SMALL** drop of fresh blood or blood from an EDTA tube near one end of a slide. The drop should be no larger than the eraser on a pencil.

Blood drop size template= O



2. If you place an overly large drop of blood by mistake you can correct this by dipping one corner of a second slide (top slide) into the large drop of blood and placing the blood on the corner of the slide further up on the bottom slide. This small drop can then be spread properly.



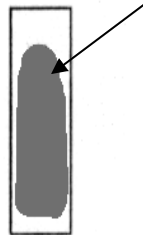
3. Take a second slide and hold the edge at a 45° angle on the slide containing the drop of blood. Pull the top slide back into the drop of blood and allow the blood to completely spread along its edge.



4. While holding the top slide at the same angle, rapidly and smoothly push the slide forward to spread the blood into a "feathered-edge" smear. Wave the slide in the air, or blow on the slide, to ensure the smear dries rapidly in order to preserve the WBC morphology.

DO NOT FIX THE BLOOD SMEARS.

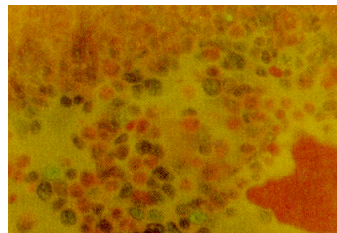
Feathered-edge



5. Write the name of the owner on the slide with a marker pen and store at room temperature. The WBCs with FeLV antigens are stable at least 1 month at room temperature. **DO NOT STORE SLIDES IN THE REFRIGERATOR** as WBCs will lyse when removed from the refrigerator due to condensation.

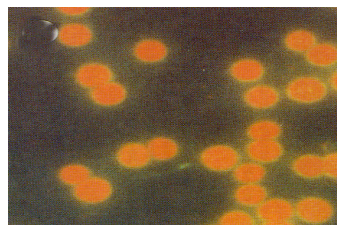
SUBMIT 2 BLOOD SMEARS

There is no need to submit bone marrow smears for the FeLV IFA test since all cat peripheral blood WBCs are replaced twice daily from the bone marrow pool.



IFA result: No evaluation due to non-specific reaction.

Non-specific reaction due to blood smears that were too thick. The leukocytes (WBCs) cannot be seen clearly and are non-specifically stained.



IFA result: No evaluation due to lack of WBCs.

No WBCs were found in this smear due to a severe leukopenia or because the smears were made after the blood had coagulated on the slide.

References: 1. Nature 244: 266, 1973; 2. JAVMA 199: 1327, 1991; 3. JAVMA 199: 1365, 1991; 4. JAAHA 17: 941, 1981; 5. JAAHA 17: 951, 1982; 6. Vet Rec. 110: 325, 1982; 7. Vet Rec. 110: 225, 1982; 8. JAVMA 199: 1273, 1991.