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NEWSLETTER

Current Feline Leukemia Virus Research Supports: Confirm All In-Hospital FeLV ELISA Positive Tests by IFA[®]

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In This Issue:

The fall 2006 issue of the NVL Newsletter will review feline leukemia virus testing and summarize and interpret new exciting research presented at the recent 8th International Feline Retrovirus Research Symposium: Cat Genomics and Infectious Diseases in the 21st Century held in Washington DC. Dr. Hardy was honored to present the meeting's Banquet Keynote Lecture on October 10, 2006. Of practical importance was the scientific evidence for some of the discrepancies (~32%) between in-hospital ELISA positive FeLV tests and negative confirmatory IFA tests that we have reported.^{1,2} **These findings indicate that FeLV positive ELISA tests should be confirmed by an IFA test.**

The Feline Retrovirus Symposium:

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

The 8th International Feline Retrovirus Research Symposium was expanded to include genomics of the cat and other infectious disease microorganisms such as avian influenza, SARS, feline coronaviruses, *Helicobacter spp.*, *Bartonella*, and feline retroviruses: FeLV, FIV and Foamy viruses. Dr. Albert Osterhaus gave the Keynote lecture entitled: *Newly Emerging Infections*, Dr. Oswald Jarrett from the University of Glasgow gave a wonderful review of the FeLV field entitled: *How FeLV Changed the World*. My lecture was entitled: *Lessons Learned From Time Spent in Cat Houses: An Historical Overview of the Feline Leukemia Virus and Other Pathogens*. I outlined the biological observations of the discovery of the infectious transmission of FeLV, the development of the first FeLV blood test, the IFA test, and the prevention methods that were developed.^{3,4,5,6,7} I also described our findings of the veterinary and public health importance of *Bartonella*. All of our work over the past 37 years was carried out in "cat houses" (households) with the clinical observations of thousands of veterinary practitioners around the country.

Scientific Explanation for the Discrepancy Between In-Hospital FeLV ELISA Positive Tests and IFA Negative Confirmatory Tests:

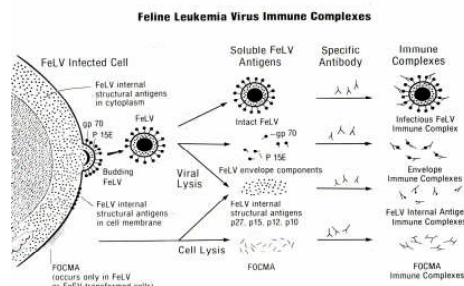
Ever since the introduction of the in-hospital FeLV ELISA tests, there have been reports of

discrepancies between ELISA positive results and confirmatory IFA tests.^{1,2} The problem was significant enough to convene an AVMA Expert Panel on FeLV in 1991 to address the problem.⁸ The panel met for 2 days and wrote a recommendation published in the JAVMA which recommended that all FeLV ELISA positive test results be immediately confirmed by an IFA test.⁸ The AVMA has not changed these recommendations but the American Association of Feline Practitioners (AAFP) has disregarded the Panel's main recommendation.⁹

An excellent paper presented by Dr. Andrea N. Torres in Dr. Edward Hoover's laboratory at Colorado State University presented evidence that some experimentally FeLV-infected cats will have circulating viral antigens in the blood for long periods after they clear the viral infection.^{10,11} These cats tested positive in the in-hospital ELISA test but had no infectious virus in the blood by isolation, were not shedding FeLV in their saliva, and remained healthy. Although IFA tests were not performed, these cats probably represent those pet cats that are ELISA positive but IFA confirmatory test negative. Antigen positive/virus-negative cats should not be managed as if they were infected cats. It has been our recommendation for more than 30 years, that any FeLV test should only detect persistently infected cats that will be shedding virus in their saliva. It is these cats that should be isolated from all other cats in the Test and Removal Program that was developed 30 years ago.⁷ It is these cats that the AVMA panel, in their recommendations in 1991, wanted to ensure are the only cats that test positive.

FeLV Antigens:

FeLV tests detect viral antigens in the leukocytes in the blood (IFA Test) or soluble viral antigens (ELISA Tests) released from cells into the blood.



FeLV ELISA Test Positive

Confirmation by IFA FeLeuk[®] Test:

During the past 3 years we have tested 2,821 in-hospital FeLV positive ELISA tests (most were Snap Tests, Idexx) by our IFA test. 32% were not confirmed by our IFA test and should not have been managed as if they were infected.

In Hospital ELISA (+) vs. FeLeuk[®] IFA Tests

ELISA Positive	# Tests	% Tests
IFA Positive	1,838	65%
IFA % Positive	51	2%
IFA Negative	898	32%
IFA Indeterm.	34	1%
Total:	2,821	100%

During the past 3 years we have also tested 527 in-hospital FeLV negative ELISA tests (most were Snap Tests, IDEXX) by our IFA test. 4.2% were not confirmed by our IFA test and should have been managed as if they were infected.

In Hospital ELISA (-) vs. FeLeuk[®] IFA Tests

ELISA Negative	# Tests	% Tests
IFA Positive	21	4%
IFA % Positive	1	0.2%
IFA Negative	496	94%
IFA Indeterm.	9	1.8%
Total:	527	100%

The finding of FeLV antigenemia in the absence of FeLV in the blood of some cats makes the recommendation to confirm all ELISA positive in-hospital FeLV tests more imperative.

AVMA FeLV Expert Panel Recommendations:

In 1991 the AVMA Expert FeLV Panel recommended that all FeLV positive ELISA tests be immediately confirmed by an IFA test.⁸ There is no recommendation to repeat the ELISA test again. ELISA positive but IFA negative results indicate the cat is not infected with FeLV. We now know that most of these cats may be antigenemic but have no infectious FeLV in their blood or saliva and should not be managed as infected cats.

AAFP FeLV Recommendations:

In 2001 (reprinted in 2005) the American Association of Feline Practitioners (AAFP) produced a report on Feline Retrovirus Testing and Management.⁹ None of the panel members had any experience developing or evaluating FeLV tests. The AAFP panel did not mention the AVMA FeLV Panel's recommendation, **that all ELISA positive tests should be immediately confirmed by an IFA test**, but rather modified the recommendations. "The work of the AAFP/AFM Advisory panel on Feline Retrovirus Testing and Management was made possible by an educational grant from IDEXX Laboratories, Inc." **Editor's note: IDEXX Laboratories is the maker of the predominant in-hospital FeLV ELISA test kit.**

The following statements were made:

1. "The preferred initial tests are soluble antigen tests, such as ELISA..." **Editor's note: The panel never mentioned that the IFA test was used for years as the sole FeLV test and all the biology of FeLV was elucidated using the IFA test.**

2. "Indirect immunofluorescent antibody (IFA) tests detect cell-associated antigens." **Editor's note: The IFA test detects FeLV antigens in cells that are replicating the virus. They do not mention that the AVMA Expert FeLV Panel recommends the IFA test as the confirmatory test for ELISA positive tests.**

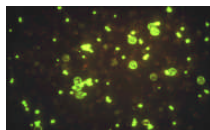
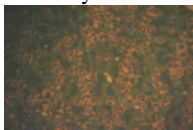
3. "In populations with a low prevalence of FeLV infection, more than half of cats for which test results are positive are likely to be uninfected." **Editor's note: They neglected to state that the study refers to ELISA tests and not IFA tests. This degree of false positive test results did not occur in studies that used the IFA test.**

Editor's note: Although the AAFP report does not recommend that ELISA positive tests be immediately confirmed by an IFA test, they did make recommendations on discrepant test results.

4. "If results of a soluble antigen test are positive and results of an IFA test are negative, both tests should be performed again in 60 days and then annually until results of both tests are in agreement." **Editor's note: This is the most unscientific recommendation in the report. There are no scientific publications that show the test results will eventually become concordant. In fact, the recent studies have elucidated the scientific explanation for ELISA positive but IFA negative FeLV test; that some cats can be antigenemic but have no infectious virus in the blood or saliva.**

History of FeLV:

FeLV was discovered in 1964 at the University of Glasgow by William Jarrett and his colleagues.¹² In 1968 Helen Laird, Oswald Jarrett and their colleagues reported that FeLV replicates in leukocytes and platelets in the blood.¹³ This was a "eureka moment," since I then knew that we could develop an IFA test for detection of FeLV and test cats. In 1973 we developed the test and used it to discover that FeLV was transmitted infectiously.^{3,4,5}



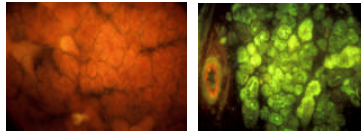
FeLeuk® Negative IFA FeLeuk® Positive IFA

Until then, all retroviruses were thought to be transmitted genetically from one generation of animals to the next. Using the IFA test, we and others found that FeLV caused numerous proliferative and degenerative diseases. The degenerative diseases are more common than leukemia and include immunosuppressive diseases and non-regenerative anemia.

Pathogenesis of FeLV Infection:

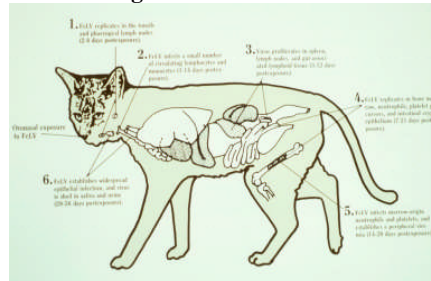
In order to understand the correct use of FeLV tests, it is important to understand the pathogenesis of FeLV infection in cats. The pathogenesis was elucidated by Dr. Jennifer Rojko, Dr. Edward Hoover and colleagues.¹⁴

After experimental infection of FeLV, which in nature usually occurs most often as oronasal exposure in the head area by mutual grooming, there are 6 stages in the pathogenesis leading to persistent infection. Stage 1: Days- 2-4 FeLV replicates in the tonsils and pharyngeal lymph nodes. Stage 2: Days 1-14 FeLV replicates in a small number of peripheral blood lymphocytes and monocytes. Stage 3: Days 3-12 FeLV replicates in spleen, lymph nodes, and gut associated lymphoid tissues. Stage 4: Days 7-21 FeLV replicates in bone marrow, neutrophils, platelet precursors and intestinal crypt epithelium. Stage 5: Days 14-28 FeLV infects bone marrow neutrophils and megakaryocytes-platelets and establishes a peripheral blood viremia. Stage 6: 28-56 days FeLV establishes epithelial infections in many tissues (nares, oropharynx and salivary glands) and is shed in the saliva and urine.



FeLV negative and positive salivary glands

Stages of FeLV Infection



Courtesy Dr. J. Rojko and Dr. E. Hoover

The long term survival of persistently infected pet cats was poor as 83% died by 3.5 years after we found them positive by the IFA test.⁷

Survival of FeLV Infected Pet Cats

FeLV Status	# Cats	# Cats Died	% Cats Died
Uninfected	512	82	17%
Infected	96	80	83%

Consequences of FeLV Exposure:

Not all pet cats exposed to an FeLV infected cat will become infected. In fact, more exposed cats became immune (42%) to the virus than develop persistent, life-long, infection (30%).

Consequences of Exposure to FeLV

Result of FeLV Exposure	Percent
Persistently infected	30%
Immune to FeLV	42%
Not infected nor immune	28%

It is our contention that the AAFP Panel's report on FeLV testing needs to be immediately revised to reflect the past and current pathogenesis data for FeLV infections. Many owners elect to remove FeLV infected cats from their households and presently do so without understanding that the veterinary profession (AVMA) recommends that all in-hospital positive ELISA tests should be immediately confirmed by an IFA test. 32% of FeLV ELISA positive tests were not confirmed by our IFA test. All in-hospital FeLV ELISA positive tests should be immediately confirmed by an IFA test. ELISA positive but IFA negative discordant cats should be considered FeLV uninfected.

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