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P.O. Box 239, 1Tice Road
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877-NVL-LABS (877-685-5227)
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NEWSLETTER

Feline Leukemia Virus- FeLV

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

Evelyn E. Zuckerman, Editor **Fall 2002** **Vol. 1, Number 4**

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.

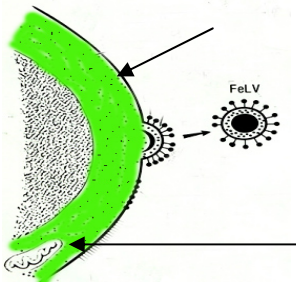
Sequence analysis of the genomes of the three subgroups of exogenous infectious FeLVs (FeLV-A, -B and -C) has shown that FeLV-B and FeLV-C arise through recombination of contagiously transmitted FeLV-A with endogenous *env* –B and –C sequences to form envelope (*env*) recombinant FeLVs. These *de novo* 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 pl5 (matrix protein, MA), pl2 (unknown) function, p27 (capsid protein, CP) and pl0 (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 pl5E (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

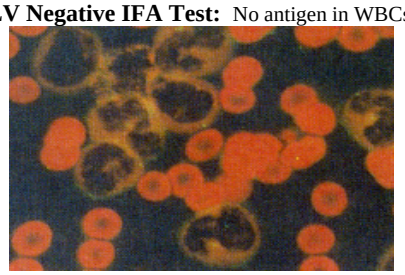


Cytoplasmic FeLV antigens

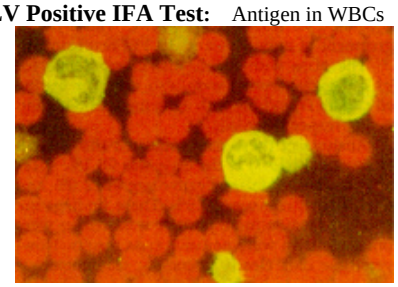
FeLV Tests:

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

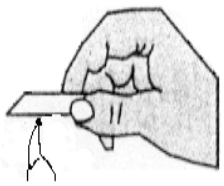
Years	Number ELISA + Cats	FeLeuk® IFA Positive	% Disagreement
1979-89	18,908	8,761	53.7%
1996-00	3,792	2,724	28.2%

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 x 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.

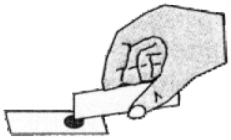
In cats that are unable to reject the virus in this early stage, FeLV spreads to the bone marrow where it replicates to high titers in all nucleated myeloid and erythroid cells. The virus spreads throughout the cat's body in infected leukocytes and platelets released from the infected bone marrow, or 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

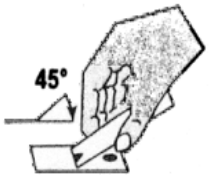


1. Clip the cat's nail to obtain a drop of blood. Touch the drop of blood from the cat's nail to the slide. If you obtain blood by syringe, place a **SMALL** drop of fresh blood or blood from an EDTA tube near one end of a slide. The drop should be no larger than the eraser on a pencil.

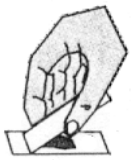
Blood drop size template= O



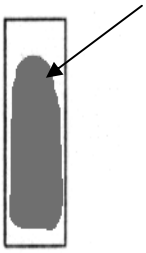
2. If you place an overly large drop of blood by mistake you can correct this by dipping one corner of a second slide (top slide) into the large drop of blood and placing the blood on the corner of the slide further up on the bottom slide. This small drop can then be spread properly



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.

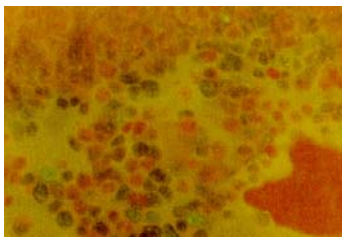


Feathered-edge

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**.**

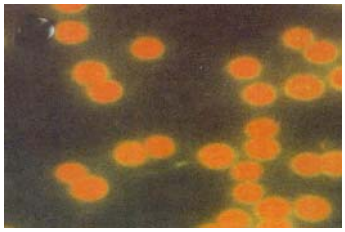
SUBMIT 2 BLOOD SMEARS

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.

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NEWSLETTER

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

Evelyn E. Zuckerman, Editor **Fall 2006** **Vol. 5, Number 4**

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 finding 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.

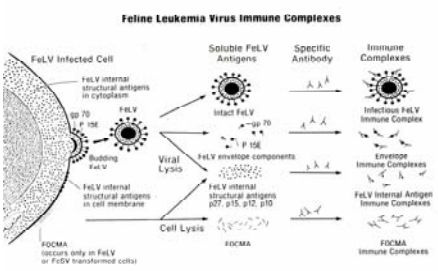
Scientific Explanation for the Discrepancy Between In-Hospital FeLV ELISA Positive Tests and IFA Negative Confirmatory Tests:

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 recommendations.[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 that 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:

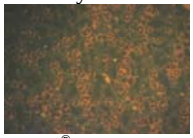
1. "The preferred initial tests are soluble antigen tests, such as ELISA…." Editor's note: The panel never mentioned that the IFA test was used for years as the sole FeLV test and all the biology of FeLV was elucidated using the IFA test.
2. "Indirect immunofluorescent antibody (IFA) tests detect cell-associated 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.

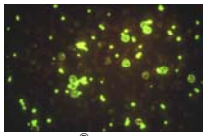
4. "If results of a soluble antigen test are positive and results of an IFA test are negative, both tests should be performed again in 60 days and then annually until results of both tests are in agreement." Editor's note: This is the most unscientific recommendation in the report. There are no scientific 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



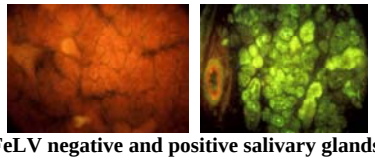
FeLeuk® Positive IFA

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:

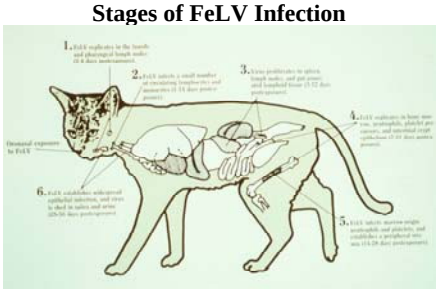
In order to understand the correct use of FeLV tests, it is important to understand the pathogenesis of FeLV infection in cats. The pathogenesis was elucidated by Dr. Jennifer Rojko, Dr. Edward Hoover and colleagues.[14]

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.

References:

1. Hardy, WD, Jr. & Zuckerman, EE. Ten-year study comparing enzyme-linked immunosorbent assays with the immunofluorescent antibody test for detection of FeLV infection in cats. JAVMA, 199, 1365, 1991.
2. Jarrett, O, Golder, MC, & Weijer, K. A comparison of three methods of feline leukaemia virus diagnosis. Vet Rec. 110: 325, 1982.
3. Hardy, WD, Jr., Old, LJ, Hess, PW, Essex, M & Cotter, S.: Horizontal transmission of feline leukemia virus. Nature 244: 266, 1973.
4. Hardy, WD, Jr., Hirshaut, Y & Hess, P: Detection of the feline leukemia virus and other mammalian oncornaviruses by immunofluorescence. in Unifying Concepts of Leukemia, 778, 1973.
5. Hardy, WD, Jr., & Zuckerman, EE. Development of the immunofluorescent antibody test for detection of feline leukemia virus infection in cats. JAVMA 199, 1327, 1991.
6. Hardy, WD, Jr. General principles of retrovirus immunodetection tests. JAVMA, 199: 1282, 1991.
7. Hardy, WD, Jr., McClelland, AJ, Zuckerman, EE, Hess, PW, Essex, M, Cotter, SM, MacEwen, EG, & Hayes, AA: Prevention of the contagious spread of feline leukemia virus and the development of leukemia in pet cats. Nature 263: 326, 1976.
8. Scott, FW, Hancock, B, Hardy, WD, Jr, Hoover, EA, Horzinek, MC, Jacobson, RH, Pedersen, NC & Saidla, JE. Panel report on the colloquium on feline leukemia virus/feline immunodeficiency virus: tests and vaccination. JAVMA 199: 1273, 1991.
9. 2001 Report of the American Association of Feline Practitioner and Academy of Feline Medicine Advisory panel on feline retrovirus testing and management.
10. Torres, AN, O'Halloran, KP, Larson, L, Schultz, RD & Hoover, EA. Insight into FeLV: host relationship using real-time DNA and RNA qPCR. 8th Inter. Feline Retrovirus Res. Symp., Wash DC, Oct., 2006.
11. Torres, AN, Mathiason, CK & Hoover, EA. Re-examination of feline leukemia virus: host relationships using real-time PCR. Virology 332: 272, 2005.
12. Jarrett, WFH., Crawford, EM, Martin, WB & Davie, F: A virus-like particle associated with leukemia (lymphosarcoma). Nature 202: 567, 1964.
13 Laird, H, Jarrett, O, Crighton, GW, Jarrett, WFH & Hay, D. Replication of leukemogenic type virus in cats inoculated with feline lymphosarcoma extracts. J Natl Cancer Inst 41: 879, 1968.
14. Rojko, JL, Hoover, EA, Mathes, LE, Olsen, RG & Schaller, JP: Pathogenesis experimental feline leukemia virus infections. J. Nat. Cancer Inst. 63: 759, 1979.