Update on FeLV and FIV:
Testing...Diagnosing...Preventing

Richard B. Ford, DVM, MS
Diplomate ACVIM, Diplomate (Hon)ACVPM
North Carolina State University

The feline retroviruses, FeLV and FIV, today are well recognized for their ability to cause profound immune-suppressive disease in cats throughout the world. Clearly among the most complex infections affecting the cat, a retroviral infection demands an immune response that is robust and sustained if the infected cat is to survive long-term. Both innate immune responses (neutrophils, macrophages, and antigen presenting cells [APCs]) as well as adaptive immunity (B-lymphocytes and T-lymphocytes) are critical factors…all of which makes the outcome of exposure difficult to predict…even in vaccinated cats.

Generally, FeLV causes the most significant clinical infection, compared to FIV. FeLV is an oncogenic retrovirus and, unlike FIV, is capable of transforming infected cells into malignant cells (integrational mutagenesis). In the past, FeLV was considered the most common cause of death from infectious disease. Today, however, the incidence of FeLV-related disease has clearly declined significantly from what it was just 20 years ago. While vaccination accounts for some of that decline, the ability to perform rapid, accurate retrovirus testing of cats is recognized to be the principle reason behind the global decrease in FeLV incidence. It’s important to note that susceptibility to FeLV infection is greatest during the first 6 months of a cat’s life. After that, “age-related resistance” makes clinical infection (viremia, immunosuppression, and viral shedding) unlikely…this has been attributed to maturation of the immune system in an adult cat and production of one of the interleukins as well as decreased numbers of FeLV binding sites on adult cat monocytes. In the immunologically naïve kitten, exposure to FeLV seems most likely to result in infection and development of progressive disease.

NEW: recent advances in diagnostic technology (for FeLV proviral DNA) have revealed significantly new and important information concerning FeLV infection: while a POSITIVE FeLV test (SNAP or IFA) correlates strongly with active infection…a NEGATIVE test does NOT necessarily indicate the cat is free of infection…read on.

Susceptibility to FIV infection, on the other hand, does not change with a cat’s age. Risk among adults is similar to that in kittens. Furthermore, FIV infection is not associated with the same spectrum of clinical consequences seen in FeLV-infected cats. Although FIV does cause an acquired immunodeficiency syndrome, one that shares many similarities with HIV infection in humans, the prognosis of FIV infection is generally going to be better than that of FeLV, especially when level of medical care the cat receives during the course of infection is high. Opportunistic infections are common, but FIV-infected cats tend to live longer (given the opportunity to do so) than FeLV-infected cats. Many FIV-infected cats are known to die of age-related causes not directly linked to their retroviral infection. And…as such, FIV has not had the impact on the feline population that FeLV has.

FeLV and FIV: The Diagnostic Paradigm Shift

Advances in scientific technology are wonderful things…they can also be frustrating. And such is the case with FeLV diagnostic testing. As those of us who have practiced veterinary medicine for a while know, one of the most significant diagnostic technologic advances introduced over the last 20 years has been the ENZYME-LINKED IMMUNO-
SORBENT ASSAY, or ELISA, eg, the “SNAP Test” or commercial microwell test kit. The ability to perform in-hospital, simultaneous testing (and do it in about 8-minutes) for feline leukemia virus (FeLV) antigen and feline immunodeficiency virus (FIV) antibody, is clearly among the most important value-added laboratory services offered by veterinarians today. For FeLV...a POSITIVE Antigen (p27) test result is (appropriately) interpreted as an “active infection”; while a NEGATIVE test result meant “NOT infected”. However, all is not what it appears...advanced technology is changing our understanding of FeLV pathogenesis, which may change our recommendations for cats with a NEGATIVE test result, and, may even impact vaccination strategies for cats deemed to be at risk.

Here’s what’s going on...the “advanced technology” of which I speak centers around several studies that have employed molecular diagnostic technology (PCR-based) to gain greater “resolution” into the consequences of FeLV exposure in cats. In particular, the ability to detect FeLV proviral DNA\(^1\) has allowed the opportunity to look for infection in places not “visible” to IFA or conventional ELISA (eg, SNAP) test technology. Historically, it has been stated that most cats, following FeLV exposure and infection, mount a robust immune response and eliminate the virus (NEGATIVE SNAP test result); a smaller percentage would develop persistent infection, develop clinical illness, and frequently die from complications linked to profound immune suppression (POSTIVE SNAP test result).

It’s the cat with the NEGATIVE (by IFA or ELISA) test result that’s at issue here. With the ability to now look into cells, particularly bone marrow lymphocytes, it is apparent that cats having a NEGATIVE IFA or ELISA test may, in fact be POSITIVE. Based on the ability to detect FeLV proviral DNA of challenged, IFA and ELISA Negative cats, latent FeLV-infected cats can now be identified...the so-called “hidden virus” residing in healthy appearing cats. And it happens more often that we realized in the pre-Proviral DNA days.

As will be described in the lecture, investigations into FeLV pathogenesis using PCR technology have revealed better understanding on the consequences of FeLV exposure. Generally (subtle variations occur), exposure takes on 1 of 4 defined pathways:

1) **abortive** infection; the immune response rapidly neutralizes virus and eliminates the infection (these appear to be rare; it’s quite unlikely you would ever detect this cat in practice);

2) **progressive** infection; most often occurring in young cats/kittens, progressive infection is associated with persistent viremia (beyond 16 weeks), rapid onset of clinical signs, immune suppression and frequently death (this is the sick cat with a POSTIVE IFA or SNAP test result).

3) **regressive** infection; this is now what appears to happen most often after FeLV exposure. Following a short period of viremia (generally 3 to 6 weeks but less than 16 weeks), Virus will 'infect' cells (especially bone marrow lymphocytes)...during that process, the viral RNA is released into the cytosol of the cell where the unique enzyme (characteristic of retroviruses), called Reverse Transcriptase, allows single-stranded RNA to transform into complementary DNA...this is **proviral DNA**. And...this is this the origin of the latently infected cat. as proviral DNA then enters the nucleus of the cell and actually integrates into the cat's genome...where it

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\(^1\) Proviral DNA: feline retroviruses are RNA viruses. The use of the term “DNA” in reference to FeLV may be confusing. However, during retrovirus infection, through production of the unique enzyme reverse transcriptase, FeLV is able to duplicate its own single-stranded viral RNA making a double-stranded provirus...refered to as “proviral DNA”.
can either sit...for years...or begin to produce new FeLV RNA (detectable by PCR as viral RNA). Conventional IFA and SNAP tests do not detect proviral DNA or viral RNA. ...and

4) focal infection: although believed to be uncommon, FeLV may sequester, latently, within selected tissues/organs (eg, kidney, GI tract, bone marrow). Testing blood by IFA or SNAP test will be negative.

It’s the apparent incidence of regressive FeLV infection that will continue to challenge all of us... ie, what are the clinical consequences of latency in a SNAP negative, healthy cat. Based on information available today, the odds favor the cat...there is a good chance the cat will remain healthy, may eventually clear the proviral DNA, and they are NOT shedding FeLV as long as the virus remains as proviral DNA (latent). Some, however, don’t do as well...a small number of regressive infections will re-activate...this is the adult cat...with a history of having been healthy and FeLV negative for some time (years even). And despite the fact they may have never encountered another cat throughout life...they appear to develop disease spontaneously and may become progressive (IFA or SNAP positive, sick cat)...or...they may develop complications of their infection, including solid tumors (FeLV is an oncogenic retrovirus)...and may become IFA or SNAP negative!

In accordance with current retrovirus management guidelines, it is still recommended that the FeLV and FIV status of all cats seen by the practice be established and that all cats be tested (and determined to be NEGATIVE) prior to vaccination. The point here is that there’s no value in administering vaccine to an FeLV (SNAP) positive cat. However, the commitment to perform routine screening of cats for retroviral infection raises important issues pertaining to interpretation of test results and follow-up actions needed to further manage those households with confirmed FeLV and/or FIV positive cats. Current testing recommendations outlined by the AAFP’s Advisory Panel on Retrovirus Management have recently been updated and be reviewed/downloaded at:

www.catvets.com

Fundamental to the proper use of the SNAP (ELISA-based) testing for the diagnosis of FeLV and FIV infected cats is an understanding that FeLV tests are designed to detect circulating (not intracellular) p27 antigen while FIV tests detect the presence of FIV antibody.

In clinical practice, these facts have important implications. For example, the FeLV test can detect FeLV virus in the blood (serum or plasma) of kittens, even as young as 1-day of age; a positive test is consistent with infection. It is important to note that FeLV tests designed to detect the presence of virus in tears and/or saliva are also ELISA-based tests.

Saliva and tear tests should not be used for routine screening of individual cats. It should also be noted that neither maternal antibody nor prior FeLV vaccination interferes with the SNAP or IFA test results. Figure 1 will be used during the lecture to explain some of the fundamental issues behind interpreting test results in cats at various stages in the course of FeLV infection as well as provide an update on new information concerning pathogenesis of the infection and the meaning of a Negative test result.

In contrast to FeLV, ELISA-based FIV tests are not considered to be reliable in kittens less than 6 months of age for at least 2 reasons:
1) Since antibody response to FIV infection requires weeks or months to become detectable, a **negative** test result could occur in an exposed, infected kitten that has not seroconverted...

2) also, uninfected kittens from FIV-infected queens may test **positive** as a result of having acquired maternally-derived (colostrum) FIV antibody; detectable levels of maternal FIV antibody can persist until around 6 months of age. **NOTE:** vaccinated queens that become seropositive are also able to transfer maternally derived antibody to healthy, non-infected kittens resulting in a FALSE POSITIVE test result.

   Among healthy cats with a positive ELISA test for either FeLV or FIV, follow-up testing is recommended. The clinician should repeat the **ELISA test in 1 to 3 months**. As noted in **Figure 1**, re-testing 2-3 months following a POSITIVE test result is justified in healthy cats considering the possibility that the infection may become regressive and the cat will subsequently develop a NEGATIVE test result. (The consequences here are that the cat may still be infected...i.e., with latent proviral DNA hidden away somewhere).

   Among the retrovirologists in veterinary medicine, it is agreed today that the FeLV Ag test (**NOTE:** FeLV Ab titer is not a reliable diagnostic test), as performed on the SNAP test, is a reliable (highly specific test) for infection. While at one time it was said that the indirect fluorescent antibody (IFA) test was necessary to “confirm” FeLV infection, that is no longer the case. Today, the IFA merely **corroborates** positive results on the SNAP test.

   On the other hand, the SNAP Test for FIV antibody is **not** a confirmatory test. Confirmation of infection is predicated on a positive Western blot assay. In contrast to FeLV, the FIV-infected cat may live for years with its infection; early detection and treatment of associated illness will enhance longevity and quality of life.

   **POINT OF FACT:** any cat having an FeLV POSITIVE test (SNAP) result is considered to be shedding virus, regardless of its health status. A cat that has an FeLV NEGATIVE test (SNAP) is not viremic and is not considered to be shedding virus. A cat having a “positive” test result for FIV, and confirmed by Western Blot, still must be assessed for prior FIV vaccination history before it is possible to confirm the diagnosis and establish risk. At this time, there is NO TEST that is considered to be consistently reliable in distinguishing **infected** from **vaccinated** cats.

   **Here’s the problem**...all cats vaccinated with the current killed FIV vaccine are expected to develop FIV antibodies following administration of the first dose. Antibodies are known to persist for at least 1 year (much longer, actually). Vaccine-induced antibodies interfere with all commercially available FIV Ab tests in the North America, UK, and Europe.

   - SNAP® FeLV Antigen/FIV Antibody Combo (IDEXX Laboratories)
   - PetCHEK® FIV (IDEXX Laboratories)
   - All commercial Western Blot immunoassays

   **NOTE:** Generally, a **NEGATIVE** FIV Antibody test result may still be reliably interpreted as negative for exposure and active infection.
FeLV Vaccination

Today, there is good evidence to support the fact that the prevalence of FeLV within the US cat population has declined over the last decade. Two factors are most likely to have played a major role in this decrease: vaccination and (especially) testing for FeLV antigen (allowing identification/ removal/isolation of infected cats). However, results of a recent survey of >18,000 cats in the US suggest that the prevalence of FeLV is still relatively high, around 3% of all cats (feral and non-feral). Clearly the need for routine testing and vaccination of susceptible cats is justified.

In accordance with AAFP Feline Vaccination Advisory Panel (2012), FeLV vaccination is highly recommended in kittens/young cats (because of the high degree of susceptibility among kittens vs. adults). A reasonable vaccination schedule would be:

Two initial doses (required): eg, at 12 weeks and 16 weeks of age
One dose (booster): 1 year later.

Subsequent vaccination would depend on the assessment of risk for the individual cat:

- Annual (or bi-annual) vaccination for “High Risk” cats, eg, those that spend significant time outside, unsupervised…versus…
- No vaccination requirement for No to “Low Risk” adult cats, eg, exclusive indoor cats.

Of the various FeLV vaccines available today, 2 are killed, whole-virus, vaccines (Merck and Boehringer-Ingelheim), one is a subunit vaccine (Zoetis), and, one is a recombinant (rFeLV) vaccine (Merial). All killed and sub-unit vaccines do contain an adjuvant and are licensed for parenteral administration as a 1.0 mL dose. A non-adjuvanted, recombinant FeLV( rFeLV) vaccine, licensed for parenteral administration, was introduced into the US market in January 2012 and replaces the transdermal (VetJet) vaccine. None of the licensed vaccines interfere with the either the IFA or ELISA (SNAP) test platforms.\(^2\)

All FeLV vaccines, however, are NOT the same. The killed and subunit vaccines contain adjuvant\(^3\) and require a 1.0 ml dose administered parenterally. The immunity conferred by these products centers on antibody production. The new, parenteral recombinant FeLV vaccine is a 1.0 mL dose of a non-adjuvanted, canarypox vectored vaccine administered subcutaneously. The recombinant FeLV vaccine immunizes by its ability to deliver 2 discrete FeLV genes that express 2 immunogenic proteins: p27 (gag) and gp70 (env). Furthermore, the gp70 gene in the vaccine has been genetically modified such that both innate (NK cells) and adaptive (CD8+ and T-cell) immune responses are enhanced. While none of the FeLV vaccines on the market have demonstrated “sterile” immunity, the rFeLV vaccine has been shown to enhance a cat’s cell mediated immune response to virulent FeLV. (Cats vaccinated with the rFeLV vaccine containing modified gp70 produce more INF\(_\gamma\) but less IL10, hence the enhanced CMI to FeLV following vaccination.)

The canarypox virus is a widely recognized “vector-virus” for vaccines licensed for horses cats, dogs, and ferrets (a canarypox virus vectored vaccine is currently being studied in an HIV vaccine clinical trial in humans). The canarypox virus does not multiply (replicate) in the

\(^2\) FeLV-FIV Combo Test, Idexx Laboratories, Westbrook, ME (USA)
\(^3\) Adjuvanted FeLV vaccines have been implicated as a cause of vaccine-associated fibrosarcoma in cats.
vaccinated animal (or person). Because the virus does not replicate, there is no risk of post-vaccinal canarypox virus shedding.

**FIV Vaccination**

Infection by FIV is characterized by a long latent period; infected cats gradually experience deterioration of immune function associated with declining numbers of T helper lymphocytes (CD4+). [REF: Levy, 2000] The consequences are manifest as a wide spectrum of vague clinical features, none of which are diagnostically distinctive. Complicating the clinical picture is the fact that infected (presumably shedding) cats can appear to be quite healthy as reported by both the owner and subsequent to examination by a veterinarian.

The principle serological test for FIV infection used throughout the world is the determination of FIV antibody in serum. There is no reliable ‘antigen’ test. The enzyme-linked immunosorbent assay (ELISA) and immunoblot (Western Blot) methods used to detect FIV antibody have become the mainstay for diagnosing infected cats and conducting surveys among populations of cats at risk for infection. Although PCR (polymerase chain reaction) assays are commercially available for both viral RNA and proviral DNA, independent studies have cited poor sensitivity and specificity when using these tests.

Epidemiological studies using FIV antibody and Western blot analysis provided good evidence for horizontal transmission of FIV among cats and have identified adult male cats living outdoors as those at greatest risk of infection. Since the virus can be recovered from the saliva of infected cats, bite wounds sustained during fighting are believed to be a principle means of virus transmission. On the other hand, casual contact among infected and non-infected cats is an unlikely means of transmission. Although it appears possible that FIV can be sexually transmitted, as the virus has been recovered from the semen of infected cats, this mode of transmission appears to be uncommon in nature. Likewise, transmission from infected queen to fetus (vertical transmission) is possible, but rare. On the other hand, it is more likely that infected queens will transfer FIV antibody, rather than virus, via colostrum to nursing kittens. Since maternal FIV antibody may persist in kittens for several months, it is customary to disregard a “positive” FIV antibody test result in healthy kittens under 6 months of age. Follow-up testing is indicated once a cat reaches maturity.

The introduction and use of the killed FIV vaccine (2002, Fort Dodge Animal Health; now sold by In July 2002, a Feline Immunodeficiency Virus (FIV) vaccine was introduced (Fort Dodge, now Boehringer Ingleheim Vet Medica). Initially, the FIV vaccine changed the approach clinicians use to assess potentially infected cats. Concerns over the occurrence of False POSITIVE Test result among vaccinated cats quickly surfaced. Vaccination is known to be associated with development of FIV antibody that:

1) causes FALSE POSITIVE test results with all FIV tests on the market today;
2) appears to cause False POSITIVE interference for years; and,
3) today, there is NO KNOWN TEST (including PCR) that can reliably and consistently distinguish between an INFECTED cat and a VACCINATED cat.

In addition, it has recently been demonstrated that a vaccinated, seropositive queen will pass antibody to kittens (presumably through colostrum). FIV testing of kittens that nursed from FIV seropositive cats is likely to result in a false positive test. Until an alternative, reliable, and accessible laboratory test for FIV infection is made available, or an alternative (recombinant)
vaccine is introduced, veterinarians have lost the ability to distinguish between a vaccinated cat and an infected cat. Administration of the FIV vaccine should be accompanied by some means of identifying the cat (tattoo or microchip) in the event the cat becomes lost.

**Most authors today do not recommend use of the FIV vaccine in cats.** Considering the high risk for FALSE Positive test results following vaccination and the lack of evidence supporting an immunoprotective role of this vaccine, there is little to no indication for use of this product in routine vaccination protocols.

**Treatment of FeLV/FIV**

Over the past decade, a number of studies have been published that address a wide variety of treatment modalities for retrovirus-infected cats targeting cancer (FeLV-related), hematologic disorders (anemia, leucopenia), antiviral chemotherapy, immunomodulator therapy, and antibody therapy. While it is possible to manage lymphoid tumors and hematologic disorders, the outcomes are expected to be transient as complications associated with the persistently infected and viremic cats unfold. Despite several attempts to treat the viremia, no modality has demonstrated sustained beneficial effects. Reference 6 (below) details several options for the treatment of FeLV and FIV.

**Prognosis**

Overall, when supportive care is provided, FeLV-infected, viremic cats have a poorer prognosis than FIV-infected cats. That said, it is not unreasonable for some clientele to maintain FeLV + cats (several in the same household) and report long-term survivals despite evidence of active viremia and shedding. A positive test (IFA or ELISA) for FeLV is not necessarily associated with impending death or poor quality life.

**Additional Reading**


*Updated: June 2015*
Figure 1: FeLV Testing in the Clinical Setting

ORSNASAL INFECTION usually via saliva

Viremic Phase
...typically lasts 3 to 6 weeks; not more than 16 weeks.

Infection is ‘ABORTED’.
NO VIREMIA (uncommon)

> Local lymphoid tissue
> Circulating monocytes
> Systemic lymphoid tissue
> Bone marrow

Infection is ‘PROGRESSIVE’ (Less Common)

Viremic Phase
IF extended beyond 16 weeks
Persistent Viremia is Expected

Infection is ‘REGRESSIVE’ (Common)

Virus is contained in Bone Marrow lymphocytes (Latent Infection)

REACTIVATION of VIREMIA is possible… risk may decline with age.

Well Cat

Not so ‘well’ Cat