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.

In general terms, FeLV causes the most significant clinical infection, compared to FIV. In the past, FeLV was considered the most common cause of death from infectious disease. Today, however, the incidence of disease has clearly declined 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 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, “natural resistance” develops…which is attributed to maturation of the immune system in an adult cat and production of one of the interleukins. In the immunologically naïve kitten, exposure to FeLV is likely to result in life-long infection. With FeLV, ‘life-long’ may be months or it may be years…it’s a disease that is very difficult to prognose due to the wide range of clinical outcomes possible once the virus reaches the infected cat’s bone marrow.

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 linked to their retrovirus infection. And…as such, FIV has not had the impact on the feline population that FeLV has.

**FeLV and FIV: DiagnosticTesting**

One of the most significant technologic advances introduced into clinical practice within the last 20 years has been the ENZYME-LINKED IMMUNOSORBENT ASSAY, or **ELISA**. The ability to perform in-hospital, same-day testing for feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), canine parvovirus (CPV), along with several others, are among the most important value-added laboratory services offered by veterinarians today.

In accordance with current guidelines, it is 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. 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 Testing 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 the p27 antigen (viral core antigen) while FIV tests detect the presence of 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. However…these tests are significantly less sensitive/specific (ie, large numbers of false-positive and false-negative results) than tests utilizing blood, serum, or plasma.

Saliva and tear tests should not be used for routine screening of individual cats. It should also be noted that neither maternal antibody nor recent FeLV vaccination interferes with the ELISA-based FeLV test. 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.

On the other hand, ELISA-based FIV tests are not reliable in kittens less than 6 months of age for a couple of 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, and…

2) On the other hand, uninfected kittens from FIV infected queens may test positive as a result of having acquired maternal FIV antibody; detectable levels of maternal FIV antibody can persist until around 6 months of age. NOTE: vaccinated queens that become seropositive are 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, waiting 1-3 months is justified in healthy cats with a positive FeLV test considering the possibility that the infection is early (transient) and a protective, neutralizing antibody response can completely eliminate virus.

Among the retrovirologists in veterinary medicine, it is agreed today that the FeLV Ag test (remember…there is no reliable FeLV Ab 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 findings on the SNAP test. Put another way, a ‘sick’ cat with a positive test result for FeLV on a SNAP test is considered FeLV infected.
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 either an FeLV “positive test” result is considered to be shedding virus. As such, strong precautions are recommended to prevent unnecessary exposure to healthy, susceptible cats, especially kittens. 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.

Here’s the problem…all cats vaccinated with the 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. Vaccine-induced antibodies interfere with all antibody tests commercially available in the US and Europe:
- SNAP® FeLV Antigen/FIV Antibody Combo (IDEXX Laboratories)
- PetCHEK® FIV (IDEXX Laboratories)
- All commercial Western Blot tests

In addition, kittens of vaccinated queens can have a positive test result due to passively acquired vaccine-induced FIV antibody. Negative test results for antibody may still be interpreted as negative for exposure and infection. Attempts to distinguish an FIV INFECTED cat vs. an FIV VACCINATED cat by rtPCR technology has not been consistently reliable.

Alternative Testing

Virus isolation (VI) has been suggested as possible means of distinguishing vaccinated cats from infected cats. However, virus stability during transport, availability, and cost are such significant limiting factors that VI is not a reasonable consideration for veterinarians in clinical practice. Isolating FIV from infected cats is well suited to experimental laboratories where the sample collection and virus isolation methods can be highly controlled.

On the other hand, commercially available (now) polymerase chain reaction (PCR)-based tests for identification of RNA and proviral DNA1 have received considerable attention subsequent to the release of the killed FIV vaccine. PCR testing for FIV has been marketed as “the” alternative test for detecting infected cats…whether or not they’ve received prior vaccination. While it is possible to identify FIV, in both vaccinated and unvaccinated cats, using PCR technology, the ability to provide consistently reliable results on samples from cats seen in clinical practice has not yet been accomplished. In the long run, this may prove to be quite problematic given the nature of PCR technology.

PCR technology must not be viewed as simply another “new and improved” means of detecting FIV antibody. In fact, it doesn’t detect antibody at all…but that’s just the beginning.

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1 Proviral DNA: feline retroviruses are RNA viruses. The use of the term “DNA” in reference to FIV may be confusing. However, during retrovirus infection, through production of the unique enzyme reverse transcriptase, FIV is able to duplicate its own single-stranded viral RNA making a double-stranded provirus. This is referred to as “proviral DNA”. Specific proviral DNA sequences are the target of the PCR test for FIV.
The “family” of feline immunodeficiency viruses, is varied and their expression, once they’ve infected a cat, is quite complex. That, combined with the inherent sensitivities of PCR-based test methods make turning a PCR test into the replacement test for FIV antibody, and doing so at the levels of reliability and consistency we have enjoyed, a major technological challenge.

Although most clinicians will not be interested in the technical issues pertaining to PCR testing, it is important to understand that PCR is an exceptionally sensitive method of “amplifying” DNA (or RNA) in a patient sample and detecting remarkably low amounts of antigen (virus). In effect, PCR has made it possible to identify unique sequences of DNA even when the sample size is miniscule. Obviously, such technology would be of considerable value in diagnosing infection, particularly viral infections, where the virus quantity can be quite small and the genetic features of the virus quite distinct. The ability of a novel quantitative polymerase chain reaction (qPCR) method to detect proviral DNA in FIV-infected cats was described in 1999. While using the Taqman® PCR to detect FIV provirus is a significant fact, reliably incorporating the technology into routine use in the clinical setting will take time.

Now that PCR testing for FIV has become commercially available, the clinician must understand and appreciate the fact that the incredible sensitivity of PCR methodology may, in fact, be one of its downfalls…at least for the clinician. For example, in-hospital PCR testing for FIV, or anything else, is simply not feasible today. An outside laboratory must analyze all specimens. In addition to contending with the risk of sample transport and contamination (with extraneous nucleic acid), it will become critical (at least it should be) for all laboratories offering PCR testing for FIV to use standardized, validated reagents and testing protocols. The process of validating PCR test methods is not in place today. Doing so will require documenting the accuracy of PCR against various FIV field strains (variants of virus subtypes) seen in the United States. Then, with all that said, it's a matter of price.

Conclusion

Over the next year, it is anticipated that commercial PCR testing for FIV will become increasingly more available to veterinarians in clinical practice. However, until significant refinements to conventional PCR-based tests can be assured, clinicians must appreciate the implications that FIV vaccination will have on our ability to survey the at-risk population of cats for FIV infection.

For the future, what may be even more important that developing PCR testing for commercial FIV testing purposes is the development and introduction of a recombinant FIV vaccine. Recombinant vaccine technology has already been introduced into veterinary medicine and promises to be an important, perhaps critical, contribution to vaccine safety and efficacy in the future. A recombinant FIV vaccine offers the prospect of inducing a protective, sustained cell-mediated immune response, but without antibody. Distinguishing infected cats from vaccinated would again be possible.

FeLV Vaccination

Today, it does appear that the prevalence of FeLV within the domestic cat population has declined over the last decade. Two factors are most likely to have played a major role in this decrease: vaccination and in-hospital testing for FeLV antigen in sick cats and vaccination. However, results of a recent survey of cats in the US suggest that the prevalence of FeLV is still as high, around 3% of all cats (feral and non-feral). Clearly the need for routine testing and
vaccination of susceptible cats is justified (…and it’s the recommendation of the AAFP Advisory Panel on Retrovirus Testing).

Of the various FeLV vaccines available today, 2 are vaccines are killed, whole-virus, vaccines (Schering-Plough and Fort Dodge [now owned by Berhinger]), 1 is a subunit vaccine (Pfizer), and, one is a recombinant (rFeLV) vaccine (Merial). All killed and sub-unit vaccines (also considered ‘killed’) are approved for parenteral administration as a 1.0 mL dose. All killed vaccines contain an adjuvant. Only available in the US, the rFeLV vaccine is a 0.25 mL, is the only non-adjuvanted product on the market; the vaccine is administered by the transdermal route using the VetJet (an air-pressure driven) administration system. We have recently completed a study at NCSU in which ALL 4 leukemia vaccines were tested in adult cats. At 21 days post vaccination, NONE of the vaccines caused false + test results for p27 antigen in cats tested by the ELISA method.²

All FeLV vaccines, however, are NOT the same. The killed and subunit vaccines contain adjuvant³ and require a 1.0 ml dose administered parenterally. The immunity conferred by these products is associated with antibody production only. The recombinant FeLV vaccine is a 1.0 mL dose of a non-adjuvanted, canarypox vectored vaccine. The recombinant FeLV vaccine immunizes by its ability to deliver 2 genes that express 2 important immunogenic proteins: p27 (gag) and gp70 (env). The canarypox virus is a widely recognized "vector-virus" for cats, dogs, and humans (canarypox virus is currently used in the only HIV vaccine still in clinical trials). The ‘vector-virus’ does not recognize mammalian tissues and therefore does not multiply (replicate) in the vaccinated animal (or person). Because the virus does not replicate, there is no risk of the canarypox virus being shed from vaccinated cats.

Vaccination of kittens should consist of 2 vaccine doses administered at a 2 to 4 week interval beginning as early as 8 or 9 weeks of age (typical recommendation is to administer the first dose at 12 weeks of age and the second at 15 or 16 weeks of age; a booster is recommended 1 year later for all cats). A 2-dose regimen is required at the time of administration of the first vaccine, regardless of the cat’s age. Cats presented for the second dose of vaccine at more than 4 weeks following the first dose should be given a third dose 2 to 3 weeks later. Administration of an annual booster is recommended. Regardless of the vaccine used during the initial 2-dose regimen, it is not necessary to use vaccine from the same manufacturer when administering subsequent annual boosters.

**FIV Vaccination**

In July 2002, the first (and currently, only) licensed vaccine (Fort Dodge, now owned by Berhinger) against Feline Immunodeficiency Virus (FIV) was introduced in the United States. Although few articles have addressed FIV in the clinical literature over the past few years, most clinicians would agree that the consequences of FIV infection in the individual cat can be significant and justify the need for an FIV vaccine. 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

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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.
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 immunsorbent 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 have 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, not 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.

The introduction and use of the killed FIV vaccine substantially changed the approach clinicians use to assess potentially infected cats. Of particular importance is the fact vaccination is known to be associated with development of FIV antibody that interferes with all FIV tests on the market today. In addition, it has recently been demonstrated that a vaccinated, seropositive queen will pass antibody to kittens (presumably through clostrum). FIV testing of kittens that nursed from FIV seropositive cats will cause a false positive test result. 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.

The FIV Vaccine

The current (and only) FIV vaccine is a killed, whole virus vaccine containing virus representing 2 subgroups, or clades, of FIV: one is from clade A (Petaluma strain) and another from clade D (Shizuoka strain). The manufacturer recommends a vaccination schedule that entails administration of 3 doses initially, 2-3 weeks apart, followed by annual revaccination. Each 1 ml dose is administered subcutaneously to cats 8 weeks of age or older. The vaccine is adjuvanted.

Additional Reading


*Updated January 2011*
Table 1: FeLV Testing in the Clinical Setting

ORONASAL INFECTION via saliva

Transient Infection is HIGHLY variable. 2 weeks to 6 months, average

1st: Local lymphoid tissue, esp. tonsils
2nd: Circulating lymphocytes/monocytes
3rd: Systemic lymphoid tissue
4th: Bone marrow...now it's “Persistently Infected”

Possible Latent Infection (15% or more?)

Regressive Infection (Most Likely)

Transient, self-limiting infection

FeLV Negative Neutralizing Ab + Healthy Cat

FeLV Positive + Neutralizing Ab Sick Cat

Progressive Infection (Least Likely)

Progressive immune suppression developing over months to years; approximately 20% will develop solid tumors.