NATIONAL VETERINARY LABORATORY

P.O. Box 239, 1Tice Road
Franklin Lakes, NJ 07417
877-NVL-LABS (877-685-5227)
www.natvetlab.com

NEWSLETTER

The 3 Common Feline Pathogens- 53 Years of Data©

Evelyn E. Zuckerman, Editor | Fall 2025 | Vol. 24, Number 4

In This Issue:

In the Fall 2025 issue of the NVL Newsletter we will again discuss our test data for the 3 common cat pathogens, FeLV, FIV and *Bartonellae*. Our laboratory is a specialty lab that developed the FeLeuk® FeLV IFA test and the *Bartonella* WB Fe*Bart*® screening tests and brought them to routine testing for veterinarians for the past 53 years. After the advent of the in-hospital FeLV and FIV ELISA test kits, we have been committed to our confirmatory testing service for veterinarians and, also very importantly, we have always been interested in obtaining new data and practical information about controlling these important pathogens. It is with a sad heart that I, Dr. W.D. Hardy, Jr., after 59 years in the veterinary profession, wish to pursue my other interests in writing, ecology, conservation and photography. My expert scientific collaborator of more than 50 years, Evelyn Zuckerman, has prepared all our viral and bacterial reagents that we use in our tests, which has been a very critical and time-consuming endeavor over the years. My wonderful competent, dedicated staff, who have been together for more than 40 years, are all looking forward to following their interests.

We often get questions about how our tests compare with the tests offered by other veterinary diagnostic laboratories. We find that some veterinarians do not know the mechanisms of the various tests and each test's strengths and weaknesses. It is very important to know each test's parameters and use the results properly to manage each case.

Introduction:

We are updating the data on our testing for the 3 common cat pathogens from our Winter 2016, Newsletter, which is available on our website, which we will continue to maintain.

General Detection Methods:

There are 2 general test categories for detection of any pathogen: direct and indirect methods.

Direct Methods:

- Visualization in tissue- staining
- Isolation in culture
- Detection of pathogen nucleic acid
- Detection of pathogen antigens

Indirect Methods:

- Serology- antibody detection:
  - ELISA
  - IFA
  - WB

Direct visualization methods include FeLeuk® FeLV IFA detection of the viral antigens in the cytoplasm of infected cells.[1] Similarly, *Bartonella* can be stained with a silver-based stain in inflamed tissues. FeLV and *Bartonella* can be isolated in culture, though this method is not practical or economical for either pathogen. FIV can neither be visualized nor isolated as a practical method of diagnosis. Detection of pathogen nucleic acids can be performed for all three but again this method has its practical limitations. Detection of antigens can be used for FeLV but not for FIV or *Bartonella*. Serologic detection methods can be used effectively for both FIV and *Bartonella*, but not for FeLV, and in fact are the preferred methods.

The most accurate and sensitive serologic assay method is the WB. Here a virus or bacterium is isolated, purified, broken up into constituent proteins and the proteins are separated by size via electrophoresis, transferred to membranes where the individual proteins can be detected by the antibodies produced by infected animals. The specificity of the WB comes in the power of visualizing the antibody profile, or "fingerprint," for each specific pathogen. The FIV pattern consists of 4 or 5 proteins and the *Bartonella* pattern can be as many as 14 different, but specific, immunogenic proteins.[2] No other bacterium has the same protein profile as *Bartonella* and cross-reacting antibodies, common between *Chlamydia* and *Bartonella,* can be discounted as they only amount to 1 or 2 shared protein epitopes of the 14 *Bartonella* proteins. By comparison, ELISA or IFA tests for antibody against FIV or *Bartonella* give only a color change, which in some cases, may be due to cross-reacting antibodies (Figure 1).

Figure 1 ELISA Tests



Color change as a positive ELISA result

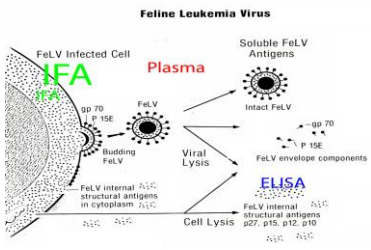
Thus, the FIV and *Bartonella* WB tests are the confirmatory serologic tests for each agent as are the WB tests, in human medicine, for HIV, Lyme, and even *Bartonella*.

Feline Leukemia Virus (FeLV):

FeLeuk® FeLV IFA Test:

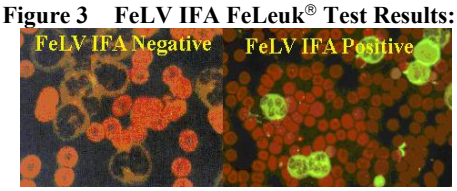
The IFA test was the first FeLV test and visualizes FeLV proteins in leukocytes and platelets of blood smears.[1] ELISA tests react with the same FeLV soluble proteins in the plasma of infected cats (Figure 2).

Figure 2



For the FeLeuk® IFA test, rabbits are immunized with purified FeLV proteins and their hyperimmune sera are extensively absorbed to remove any non-specific anti-cat antibodies. The rabbit serum is placed on cat blood smears to react with FeLV antigens, washed, and the rabbit hyperimmune serum, attached to infected leukocytes and platelets, is then reacted with an anti-rabbit IgG labeled with fluorescent dye, and the slides are read with a fluorescent microscope. The test takes about 3 hours to perform. The advantage of the FeLeuk® IFA test is it generates a vibrant positive reaction in leukocytes and platelets where biologically the virus is found in infected cats (Figure 3). The IFA test is 98.4% concordant with isolation of FeLV.[3] In addition, early infection, or cats rejecting their infections, can be determined when only a percentage of leukocytes are positive. Cats clearing their infections will retest negative, in all leukocytes, weeks later and, conversely, cats becoming infected will retest 100% positive.

Figure 3 FeLV IFA FeLeuk® Test Results:



FeLV ELISA Test:

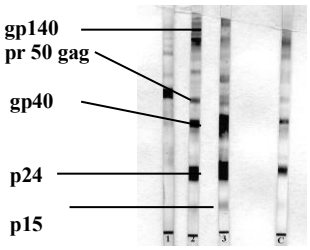
In FeLV ELISA tests, monoclonal anti-FeLV protein antibody is absorbed onto a membrane or cup surface and the cat test serum is applied. Following reaction, the antibody-antigen complex is visualized by an enzyme-linked antibody reactive to the captured FeLV protein. A positive reaction is indicated by a color change on the test membrane or in the plate well (Figure 1). A disadvantage of the FeLV ELISA method is that a positive reaction is an all or none color reaction and often is discordant with our FeLeuk® IFA test.[3,4] FeLV ELISA positive tests should be confirmed by IFA, as

recommended by the AVMA FeLV Expert Panel and the AAFP (Tables 2-4).[5]

Feline Immunodeficiency Virus: FIV

We developed an accurate confirmatory FIV WB (Figure 4) and offer it as a screening and confirmatory test for FIV. All FIV ELISA positive tests should be confirmed by WB (Tables 2-4).

Figure 4 1 2 3 C



Western immunoblot for FIV antibodies. Lane 1: ELISA+ WB–, Lanes 2 and 3: ELISA+ WB+, C= Control WB+.

Bartonella:

Presently there are 5 blood tests available to detect *Bartonella spp.*: Serology- 1-3) western blot, ELISA, and IFA tests, 4) PCR DNA detection, and 5) culture. **However, it must be emphasized that *Bartonellae* often are only intermittently present in the peripheral blood (bacteremia) of infected animals with long periods without bacteremia as illustrated in Table 1.** This study also illustrates the chronic nature of the untreated *Bartonella* infection which lasted almost 1½ years.

Table 1

Intermittent *Bartonella* Bacteremia- Cat 17[6]

Days- Post Infection	Bacteremia	Antibody
4-46	+	+
67-93	--	+
108-129	+	+
149-213	--	+
253-276	+	+
317-388	--	+
409-454	+	+

Culture from Blood:

Bartonella are very slow growing and fastidious, requiring special media for growth (Figure 5). Some recommend blood culture as the most reliable test but state that several consecutive cultures are needed since *Bartonellae* only circulate intermittently.[6]



Figure 5 *Bartonella* isolated from the blood of a 6-month-old healthy kitten. >1,000 *Bartonella*/ml were isolated after 35 days.

Serology:

Serologic methods for detection of antibodies directed against *Bartonella* have been employed more than any other technique in the literature for detection of *Bartonella* infections in cats, dogs and people. ELISA and IFA antibody tests result in an all or none color development (Figure 6) whereas WB tests result in a specific multiple antibody profile (fingerprint) (Figure 7).[2] Multiple studies have shown that the WB is the most specific and sensitive serologic assay. Our *Bartonella* ELISA and IFA tests were less accurate than our WB and thus we only offer the Fe*Bart*® *Bartonella* WB test.

IFA *Bartonella* Test

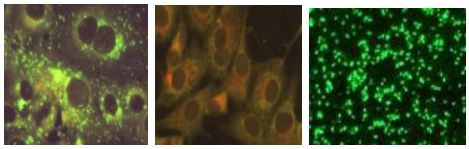


Figure 6 The IFA *Bartonella* test developed in our laboratory was not as accurate, or as reproducible, as our western blot test for detecting *Bartonella* infected cats. Left panel: antibody-positive in infected cells. Middle panel: antibody-negative. Right panel: positive.

Western Blot:

WB detection of a profile of at least 7 antibodies against *Bartonella* proteins (Figure 7) ensures the specificity of the technique.[2] We are able to detect infection with all known species infecting cats and dogs since the sera of infected cats and dogs are cross-reactive for all infecting *Bartonella* species. In addition, cross-reacting antibodies to other bacteria are discounted by WB. As with other chronic pathogens such as FIV, HIV, and *Borrelia* (Lyme), antibodies to *Bartonella* co-exist with the chronic persistent infection and rarely are sterilizing of the infection.

Fe*Bart*® Western Blot (WB) Test

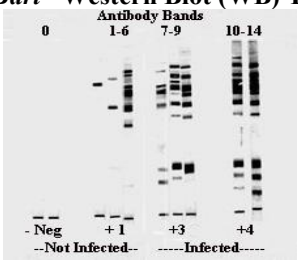


Figure 7 Grading system for the Fe*Bart*® Western Blot Test. – & +1: not infected, and +3 & +4: infected.[2]

There are 4 interpretations of a Fe*Bart*® *Bartonella* positive (+3 or +4) test result. 1) It indicates the cat is infected and the cat is a healthy carrier or *Bartonella* is the sole cause of the inflammatory disease such as uveitis, URI, gingivitis, etc. 2) the result may mean *Bartonella* is the partial cause, co-pathogen, with a virus or another bacterium: 3) the result may mean that *Bartonella* is in the cat (background) but some other pathogen is the sole cause of the inflammatory disease; and finally; 4) the result indicates a history of *Bartonella* infection and the animal has cleared the infection (not common). Appropriate and effective antibiotic therapy is available to eliminate *Bartonella* infections.[7]

Therapy Evaluation Test:

The comparative WB therapy titration test is our serologic test to determine if therapy has eliminated *Bartonella* infection. The titration test compares the titer of antibodies in the pre-therapy sample with the post-therapy sample taken: **6 MONTHS OR LONGER AFTER THE END OF THERAPY**. A decrease titer indicates elimination of infection.[7]

Results of 53 Years of Tests:

In 2016 we reported the incidence of the 3 cat pathogens in Table 2. We tested 8,972 healthy cats and found a low prevalence of FeLV and FIV but a very high prevalence of the zoonotic pathogen *Bartonella.*

Table 2

2016 Tests Results for "The 3" Common Feline Pathogens in 8,972 Healthy Cats

Pathogen	No. Positive	% Positive
FeLV (IFA)	98	1.1%
FIV (WB)	199	2.2%
Bartonella (WB)	3,125	**35%**

First FeLV Test done June 1972, all tests were performed by NVL.

Sadly, the incidence of these pathogens, based on our data over the last 16 years, has remained basically the same despite our efforts at "test and control." As of this writing on August 10, 2025, we have tested 1,352,443 cats for FeLV, 103,132 for FIV, and 475,407 for *Bartonellae* over the past 53 years. In collaboration with our 5,378 veterinary hospital clients and laboratories we certainly have prevented the spread of these 3 pathogens within pet households of our clients around the country and maybe even within geographic areas served by our clients, but not in the general households throughout the country.

Table 3

2025 Tests Results for "The 3" Common Feline Pathogens Over Last 16 Years in Healthy Cats*

Pathogen # Tested	No. Positive	% Positive
FeLV (IFA)* 24,746	3,501	14.2%
FIV (WB)* 8,640	1,388	16.1%
Bartonella (WB) 44,376	14,783	**33.3%**

***Biased results because many tests were sent in for confirmation of in-hospital FeLV & FIV test results.**

Table 4

2025 Confirmation of in-hospital Tests Results for FeLV and FIV Over Last 16 Years in Healthy Cats

Pathogen # Tested	# FeLeuk IFA & # FIV WB Negative	% Not-Confirmed
In-hospital FeLV ELISA kit Positive 5,948	2,729	**48.0%**
In-hospital FIV ELISA kit Positive 1,527	346	**22.7%**

Conclusion:

In-hospital FeLV ELISA positive tests should be confirmed by IFA as we found 48% are discordant.[3,4] This is the recommendation of the AVMA FeLV Expert Panel and the AAFP.[5] Our FIV in-hospital test comparisons also show that positive results should be confirmed by WB.

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***Bartonella* references can be obtained at:**
www.nlm.nih.gov/ or natvetlab.com

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