NATIONAL VETERINARY LABORATORY

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

NEWSLETTER

Diagnostic Tests for "The 3" Common Feline Pathogens©

Evelyn E. Zuckerman, Editor **Winter 2016** **Vol. 15, Number 1**

In This Issue:

In the winter 2016 issue of the NVL Newsletter we will discuss the diagnostic tests used to detect and prevent infection with "The 3" common feline pathogens: FeLV, FIV and *Bartonella*. 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 have tested 8,972 healthy cats and found a low prevalence of FeLV and FIV but a very high prevalence of the zoonotic pathogen *Bartonella* (see Table 1).

Table 1

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%**

All tests were performed by NVL.

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
Western Blot (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.

Many practitioners are under the impression that the FeLV ELISA and FeLeuk® IFA tests detect different antigens and thus have different biological interpretations. This is not true as both methods detect the same FeLV proteins, IFA in the cell cytoplasm and ELISA as soluble antigens in the plasma (Figure 2). FeLeuk® IFA positive cats are viremic as are most true ELISA positive cats. However, there is evidence that some ELISA positive, but IFA negative, cats have soluble FeLV antigens remaining after rejecting the virus. These cats will most likely become immune and the practitioner should not want to detect them.

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 single color change, which is some cases, may be due to cross-reacting antibodies.

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*.

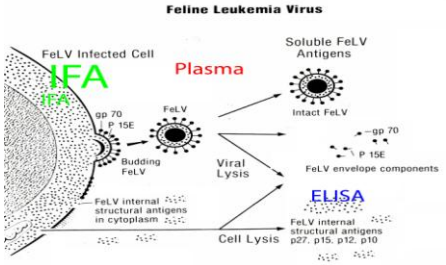
Understanding the biological principals and limitations of each test method will enable the practitioner to utilize the tests properly. In this regard, we have heard of numerous incidents where cats have been euthanized based on a FeLV ELISA positive result without the benefit of a confirmatory FeLV IFA test. Similarly, some practitioners choose not to treat cats that are culture negative but WB positive for *Bartonella* because they do not understand that taking blood for culture when there is no bacteremia may give a false negative result.

Feline Leukemia Virus (FeLV):

FeLeuk® FeLV IFA Test:

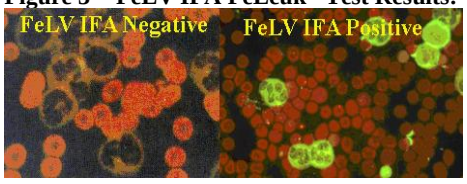
The IFA test was the first FeLV test and visualizes FeLV proteins in leukocytes and platelets of blood smears.[1] ELISA tests react with the same FeLV soluble proteins in the plasma of infected cats (Figure 2).

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 rabbit hyperimmune serum, attached to infected leukocytes and platelets, is captured by an anti-cat IgG labeled with fluorescent dye, and the slides are read with a fluorescent microscope. The advantage of the FeLeuk® IFA test is it generates a vibrant positive reaction in leukocytes and platelets where biologically the virus should be found (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:



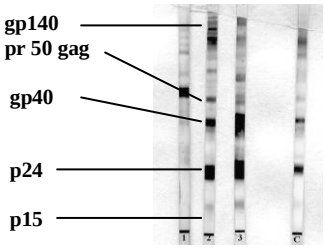
FeLV ELISA Test:

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.[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.

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 *Bartonella* often are only intermittently present in the peripheral blood (bacteremia) of infected animals with long periods without bacteremia as illustrated in the Table 2 below.** This study also illustrates the chronic nature of the untreated *Bartonella* infection which lasted almost 1½ years.

Table 2

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 *Bartonella* only circulate intermittently.[6] Isolation is proof of infection (bacteremia) whereas a negative culture may simply have been taken at a time when the organism was not circulating. Cultures can take as long as 6 weeks and are more expensive than serology. Positive cultures must be confirmed to be *Bartonella* by PCR or antigen analysis which increases the cost significantly. The same limitations apply for direct PCR tests of blood.

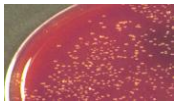


Figure 5 *Bartonella* isolated (tan rounded colonies) from the blood of a 6-month-old healthy kitten. >1,000 *Bartonella*/ml were isolated after 35 days.

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. Since the introduction of our Fe*Bart*® *Bartonella* WB test, various groups and laboratories have recommended different tests. Some state that culture is the "Gold Standard" test while others recommend PCR. Any test has its strengths and weaknesses. Several veterinary laboratories now offer IFA and ELISA antibody and PCR tests. 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

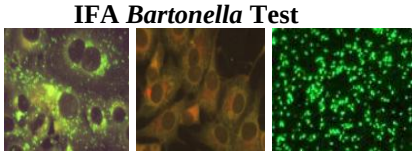


Figure 6 The IFA *Bartonella* test developed in our laboratory was not as accurate, or as reproducible, as our western blot test for detecting *Bartonella* infected cats. Left panel: antibody-positive in infected cells. Middle panel: antibody-negative. Right panel: positive.

Western Blot:

WB detection of a profile of at least 7 antibodies against *Bartonella* proteins (Figure 7) insures the specificity of the technique.[2] We are able to detect infection with all 6 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. WB tests are accurate, reproducible, economical, and require 24 hours.

Fe*Bart*® Western Blot (WB) Test

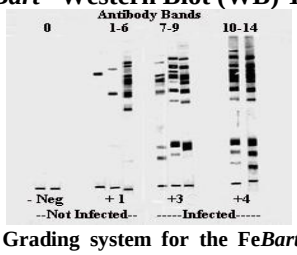


Figure 7 Grading system for the Fe*Bart*® Western Blot Test. – & +1: not infected, and +3 & +4: infected.[2]

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 the 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 drop in titer indicates elimination of infection.[7]

Conclusion:

Veterinarians must become familiar with the methods, interpretations, and limitations of the tests they use to detect the 3 common feline pathogens. Used properly, these tests can prevent the spread of all the pathogens and, with *Bartonella*, can be used to select appropriate antibiotics to treat *Bartonella* induced diseases and to prevent the zoonotic spread to cat owners.

Comparative Test Fees- March 2016:

Our FeLeuk@ FeLV IFA test fee is $20, the FIV WB fee is $35 and the Fe*Bart*® *Bartonella* WB fee is $35. A combo of all 3 tests cost $45 while the FeLV/FIV combo test cost $38, and the *Bartonella* therapy titration test cost $75. Fees for these tests at Antech and Idexx laboratories cost 2 to 3 time more than our comparable tests.

References:

1. Hardy, WD, Jr., Hirshaut, Y & Hess, P: Detection of the feline leukemia virus and other mammalian oncornaviruses by immunofluorescence. in Unifying Concepts of Leukemia, Karger, Basel, 778, 1973
2. Hardy, W.D., Jr., Zuckerman, E.E., Gold, J.W.M., *et al*. Immunogenic proteins of *Bartonella henselae* defined by western immunoblots with naturally infected cat sera. ASM, Washington, D.C., May 21-25, 1995.
3. Jarrett, O, Golder, MC, & Weijer, K. A comparison of three methods of feline leukaemia virus diagnosis. Vet Rec. 110: 325, 1982.
4. 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.
5. Scott, FW, Hancock, B, Hardy, WD, et al. Panel report on the colloquium on feline leukemia virus/feline immunodeficiency virus: tests and vaccination. JAVMA 199: 1273, 1991.
6. Kordick D.L. *et al*. Clinical and Pathologic Evaluation of Chronic *Bartonella henselae* or *Bartonella clarridgeiae* Infection in Cats. J Clin Microbiol 37:1536-1547, 1999.
7. Hardy, WD, Jr., Zuckerman, EE, Corbishley, J, Gold, JWM, Baron, P, Polsky, B, Gilhuley, K, Kiehn, TE, and Armstrong, DA. Efficacy of High Dose, Long Duration Doxycycline or Azithromycin Treatment for *Bartonella* Infections in Pet Cats. International Conference of the American Society for Rickettsiology, Big Sky, Montana, August,2001.

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

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