Ten-year study comparing enzyme-linked immunosorbent assay with the immunofluorescent antibody test for detection of feline leukemia virus infection in cats

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Summary: *Immunodetection tests for feline retroviruses are powerful tools used in modern veterinary practice. Veterinarians must fully understand the characteristics—strengths and weaknesses—of the FeLV tests so that the information gained from them can be used properly. Any FeLV ELISA or immunofluorescent antibody (IFA) test is a method for detection of FeLV infection (the virus) and is not a diagnostic test for leukemia or other feline disease.*

From previous studies, it was determined that the most accurate test for detection of persistent FeLV infection is the IFA test, which detects FeLV antigens in cytoplasm of leukocytes in the blood of infected cats. In the study reported here, 1,142,600 FeLV IFA tests were performed between June 1972 and December 1990. During this period 19.8% of the IFA test results were positive and 78% were negative. Evaluation was not possible for the remaining 2.2% of the tests because of lack of enough leukocytes in the smears to evaluate, or nonspecific staining reactions.

In 1979, 7 years after introduction of the IFA test, in-hospital FeLV ELISA were introduced, which enabled veterinarians to test for FeLV in their hospitals. Ever since that time, continual discrepancies have been reported between results of FeLV ELISA and IFA tests, particularly between positive ELISA results and their IFA test confirmation. A 10-year comparison was made between practitioner-performed in-hospital FeLV ELISA (n = 20, 240 tests) results and FeLV IFA test performed by a commercial laboratory. All samples tested by ELISA were submitted (for confirmation of results) by veterinarians from the United States, Canada, Europe, Japan, and Australia. There was 86.9% agreement between negative ELISA results and the IFA test result, but only 46.3% agreement between positive ELISA results and the IFA test result. Overall agreement was 49.0% for all confirmatory IFA tests. However, there was bias toward confirmation of positive ELISA results because 93% of confirmatory tests requested were of ELISA-positive samples.

It is apparent from this study that between 26 and 69% of in-hospital positive ELISA results and 13% of in-hospital negative ELISA results are incorrect. On the basis of these observations, we recommend that veterinarians immediately confirm all FeLV positive ELISA results by IFA testing. In addition, negative ELISA results in cats that the veterinarian suspects have FeLV infection or FeLV-induced disease should also be confirmed by IFA testing. We also recommend that all cats be vaccinated for FeLV and that they be tested for FeLV and the feline immunodeficiency virus at time of first vaccine dose.

Retroviruses are 1 of the most important family of viruses that infect animals. Retroviral infections are often life-long chronic infections; healthy animals can carry these viruses for long periods and spread the viruses to other animals before they develop disease. Immunodetection tests for animal retroviruses are powerful tools in modern veterinary medicine and such tests are available for the following retroviruses: FeLV, feline immunodeficiency virus (FIV), avian leukosis virus, bovine leukemia virus, and equine infectious anemia virus.

In 1972, we introduced the first routinely available test for any retrovirus, the immunofluorescent antibody (IFA) test for detection of FeLV infection in pet cats.[1] This test has been used to elucidate all FeLV-induced diseases, the biology of FeLV, and the pathogenesis of FeLV infection.[1-19] Since then, several FeLV ELISA , in various formats, have been introduced to veterinary medicine.[20-29] Currently, some of the ELISA can be performed in <20 minutes and contain disposable receptacles. Several of the ELISA kits were compared with the IFA test by the manufacturers and others, with claims of good agreement between the tests.[20-23,26-28]

Almost as soon as the first FeLV ELISA kit was introduced in 1979, discordant FeLV ELISA and IFA test results were obtained, especially between positive-ELISA results and confirmatory IFA test results.[18,20-23,26-27] Considerable controversy and confusion have arisen relating to discordant ELISA-

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Table 1—Summary of FeLV immunofluorescent antibody (IFA) test results over 19 years*

Year	Results (%)		
	Positive	Negative	No evaluation
1972	32.9	66.5	0.6
1973	32.9	66.5	0.6
1974	32.8	64.8	2.4
1975	32.9	64.3	2.8
1976	29.6	67.6	2.8
1977	28.5	69.2	2.3
1978	26.4	71.3	2.3
1979	23.8	72.6	3.6
1980	23.5	73.4	2.8
1981	21.8	70.6	3.1
1982	21.5	76.0	2.5
1983	20.5	77.4	2.1
1984	19.3	79.2	1.5
1985	9.7	89.0	1.3
1986	11.4	87.0	1.6
1987	11.9	86.4	1.7
1988	13.1	84.6	2.3
1989	14.8	83.4	1.8
1990	14.9	83.2	1.9
Total tests 1,142,600	19.8	78.0	2.2

*Performed by laboratory A.

Table 2—Summary of a ten-year comparison of FeLV ELISA and IFA tests: Sept 1979 through Aug 1989

In-hospital ELISA* results	IFA			
	No. tested	Test results†		Percentage agreement (%)
		Negative	Positive	
Negative	1,332	1,158	174	86.9
Positive	18,908	10,147	8,761	46.3
Total	20,240	Overall agreement		49.0

*ELISA were performed by veterinary practitioners (n = 18,828 tests) or by veterinary diagnostic laboratories (n = 1,412 tests). Submissions are biased toward ELISA-positive result confirmations. †Performed by laboratory A on blood smears submitted by veterinary practitioners or local veterinary laboratories.

positive, IFA-negative test results.[24-43] Because of the continual problem of discrepant test results, we conducted a 10-year comparative study of 20,240 samples, tested by ELISA, results of which were confirmed or compared with results of our FeLV IFA test.[a] The purpose of the study reported here was to ascertain the degree of concordance between in-hospital performed FeLV ELISA and the IFA test to formulate algorithms and recommendations for use of FeLV tests and management of ELISA-positive cats.[44,45] The IFA test is considered the reference standard (ie, the test against which the accuracy of other tests for infection with a specific agent is judged) for FeLV infection.

Materials and Methods

Immunofluorescent antibody test procedure—Most of the IFA FeLV testing services were performed at laboratory A[a] between June 1, 1972 and December 31, 1990 (Table 1) on slides submitted by veterinarians from the United States, Canada, Mexico, Europe, Japan, and Australia. Comparative IFA confirmatory tests were conducted for 10 years between September 1, 1979 and August 31, 1989. Other comparative research IFA tests were performed at laboratory B.[b]

[a]National Veterinary Laboratory Inc, Franklin Lakes, NJ.

Table 3—Overall ten-year comparison of FeLV ELISA and IFA test by individual ELISA kit: September 1979 through August 1989*

ELISA test kit	ELISA result	No. tested	IFA result		Percentage agreement (%)
			Negative	Positive	
Kit A	Negative	627	545	82	86.9
	Positive	8,492	5,210	3,282	38.7
Kit B	Negative	248	222	26	89.5
	Positive	2,326	612	1,714	73.7
Kit C	Negative	54	50	4	92.6
	Positive	1,280	792	488	38.1
Kit D (saliva)	Negative	25	21	4	84.0
	Positive	328	226	102	31.1
Kit E	Negative	89	77	12	86.5
	Positive	1,747	816	931	53.3
Unknown ELISA	Negative	51	46	5	90.2
Local laboratories	Positive	1,361	634	727	53.4
In-hospital	Negative	169	134	35	79.3
	Positive	1,968	1,030	938	47.7
	Negative	1,332	1,158	174	86.9
Total	Positive	18,908	10,147	8,761	46.3
		20,240	11,305	8,935	49.0

Overall agreement: Results for 9,919 of 20,240 (49.0%) total tests agreed

IFA tests performed by laboratory A.
This study was biased toward positive ELISA results confirmation.

*Table 4—Comparison of discordant ELISA and IFA results with isolation of FeLV in tissue culture**

Discordant FeLV results		Tissue culture isolation		
		No. Tested	Results	
ELISA	IFA		Positive	Negative
Positive	Negative	3	3	0
Negative	Positive	1	0	1

*100% agreement between IFA and tissue culture isolation results.

The IFA procedure was performed as described on blood smears mailed to the laboratory.[1,2,18,44-45] Good quality, thin feathered-edge, blood smears were fixed for 7 minutes in absolute methanol at 20 to 22 C (room temperature). Reaction wells (1.5-cm diameter) were drawn, using a china marker pencil, on the leading edge of the blood smears. Rabbit anti-FeLV serum, at dilution of 1:60, was placed in the reactive wells and slides were tested as described.[1,45] Each FeLV test was performed with 2 positive-control blood smears included.

ELISA procedure—All comparative FeLV ELISA were performed by veterinarians in their hospitals or by local veterinary diagnostic laboratories, using all of the commercially available ELISA kits (Tables 2 and 3). Blood smears from the 20,240 cats that were tested by ELISA were submitted by veterinarians to laboratory A for IFA test confirmation between September 1, 1979 and August 31, 1989 (Table 3).

Comparison of ELISA and IFA discordant results with tissue culture isolation of FeLV—Three discordant ELISA-positive, IFA-negative cat sera and 1 dis-

[b]Laboratory of Veterinary Oncology, Memorial Sloan Kettering Cancer Center, New York, NY.

Table 5—Retesting of 48 FeLV ELISA-IFA discordant cats

Initial FeLV test results		Retests of discordant cats			
		No change of results—Retest Results		Change of results Retest results:	
ELISA + IFA −	ELISA − IFA +	ELISA + IFA −	ELISA − IFA +	ELISA + to − IFA − to −	ELISA + to +IFA − to +
47	1	13 (27.1%)	1 (2.1%)	34 (70.8%)	0 (0%)
48					

Table 6—Comparative sensitivities of FeLV tests

Dilution of blood* in saline solution	Comparative tests		
	Immunodiffusion	IFA†	ELISA‡
Undiluted	+	+	+
1:10	−	+	+w
1:100	−	+	−
1:1,000	−	+w	−

*Blood from 2 FeLV-positive cats. †Performed by laboratory A. ‡Kit B. + = positive; − = negative; w = weak reaction.

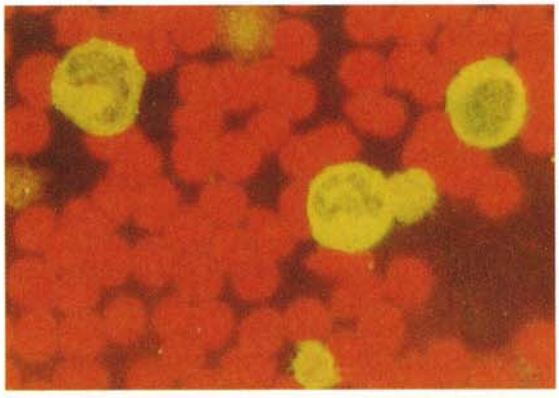

Figure 1—Positive immunofluorescent antibody (IFA) test result on a blood smear from a cat. The FeLV antigens are in neutrophils, lymphocytes, and platelets.

cordant ELISA-negative, IFA-positive cat serum were tested for FeLV by tissue culture isolation (TCI) (Table 4). Sera were obtained fresh and were inoculated within 3 hours onto feline embryo fibroblast (FLF-3) cell cultures.[45]

Retests of ELISA-IFA discordant cats—A group of 48 ELISA-IFA discordant cats were retested by ELISA and IFA within 1 week after obtaining original ELISA-IFA discordant test results (Table 5). The same ELISA kit[c] (kit A) that was used for the original ELISA was used to retest each cat, and blood smears were prepared at the same time for confirmatory IFA retesting.

Comparative sensitivities of FeLV tests—Relative sensitivities of various FeLV immunodetection tests were evaluated by obtaining sera and blood smears from 2 FeLV-infected cats. Each serum was tested by immunodiffusion (ID)[45-46] and ELISA[d] (kit B) undiluted and at various dilutions in phosphate-buffered saline solution (PBSS): 1:10, 1:100, and 1:1,000 (Table 6). Blood smears were prepared and tested by IFA after blood samples were diluted in PBSS.

[c]Leukassay-F, Pitman-Moore, Inc, Mundelein, Ill.
[d]CITE, Idexx Corp, Portland, Me.

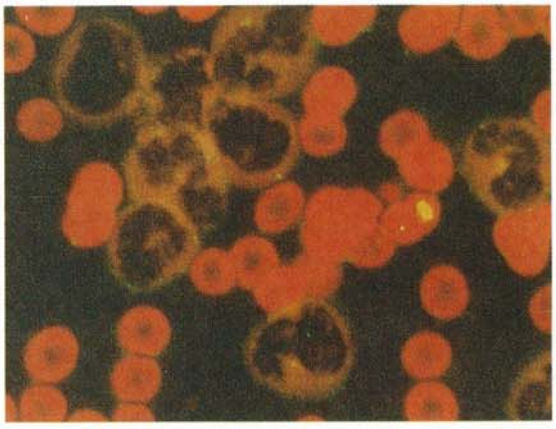

Figure 2—Negative IFA test result on a blood smear from a cat. No FeLV antigens are in the neutrophils and lymphocytes.

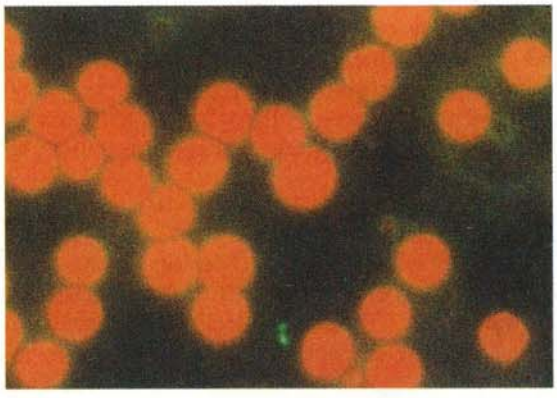

Figure 3—An FeLV IFA test on blood smears from a cat with severe leukopenia. Leukocytes are not in the smear, and thus, evaluation is not possible.

Results

IFA testing—The IFA test for FeLV detects FeLV antigens as apple-green punctate fluorescent granules in the cytoplasm of neutrophils, eosinophils, lymphocytes, and platelets in blood smears (Fig 1).[1,7,45] All leukocytes are FeLV antigen-positive in most IFA-positive cats. Occasionally however, only 10 to 90% of the leukocytes will be positive for FeLV antigens. In this instance, the IFA test is positive for FeLV infection and the veterinarian is advised to recommend isolation of the cat and to retest the cat in 1 month. The 1-month retest is suggested to determine whether the cat's leukocytes will become 100% antigen-positive or 100% antigen-negative. Cats with <100% IFA antigen-positive leukocytes are either in the early stages of infection or are in the act of rejecting FeLV infection and becoming immune. The ELISA are not able to identify this type of early infection or rejection of the virus.

No fluorescence is seen in noninfected cat leukocytes, which appear red because of the Evans blue counter stain (Fig 2). The IFA test is difficult to

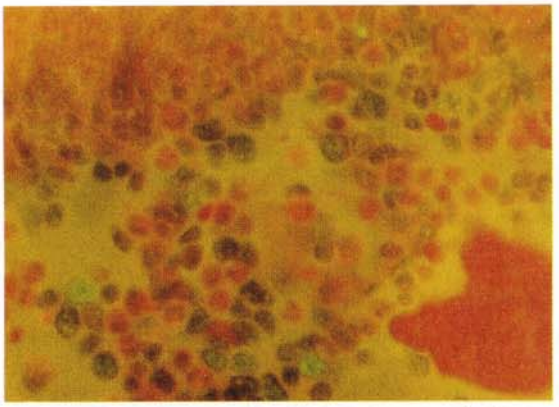

Figure 4—An IFA test on a blood smear that is too thick. This causes a no-evaluation result because of nonspecific reaction.

interpret in cats that are leukopenic because of low numbers or lack of leukocytes (Fig 3). Blood smears that are too thick are also difficult to interpret because of lack of visible leukocytes within the heavy cell layer and because of the nonspecific reaction attributable to the trapping of the IFA reagents (Fig 4).

Between June 1, 1972 and December 31, 1990, laboratory A performed 1,142,600 IFA tests (Table 1), of which 19.8% had positive results and, 78.0% had negative results; in 2.2%, evaluation was not possible because of lack of leukocytes in the smears or nonspecific staining reaction. A higher percentage of positive results was obtained from 1972 through 1978 than in more recent years, from 1979 through 1990.

IFA and ELISA comparative testing—For 10 years, between September 1, 1979 and August 31, 1989, laboratory A performed IFA confirmatory testing of samples from 20,240 cats that had been tested for FeLV by in-hospital ELISA (Tables 2 and 3). All 6 commercially available ELISA kits were compared with the IFA test.

As can be seen from the data in Table 2, the overall concordance of ELISA and IFA tests was only 49%. However, it should be noted that most (93%) of the confirmatory tests that were requested by veterinarians were for positive ELISA results, which introduced bias toward confirmation of positive ELISA results. There was 46.3% agreement between positive ELISA results and positive IFA results. The concordance for negative ELISA results was higher, with 86.9% agreement. However, this indicates that 13.1% of negative ELISA results were positive by IFA testing.

Concordance between ELISA results of each manufacturer's ELISA kit and the IFA test was calculated (Table 3). The ELISA kit with the highest concordance for FeLV-positive results was kit B[d] with 73.7% agreement, whereas the ELISA kit with the highest concordance for FeLV-negative results was kit C[e] with 92.6% agreement. The ELISA with the lowest concordance for FeLV-positive results were those used for testing saliva (kits C[e] and D[f]) with agreement of only 31.1 and 38.1%, respectively.

The ELISA with the lowest concordance for FeLV-negative results were also used for testing saliva (kits D[f] and E[g]) with agreement of 84.0 and 86.5%, respectively.

Analysis of these data indicated that overall sensitivity (Table 7) of the ELISA was 98.1%, specificity was 10.2%, false-negative rate was 2.0%, and false-positive rate was 89.8%. These findings are distorted because more positive ELISA results were confirmed than were negative ELISA results. However these data clearly document that numerous false-positive in-hospital FeLV ELISA results are common.

Comparison of ELISA and IFA discordant results with TCI—Results of FeLV TCI agreed with IFA results in all 4 of the ELISA and IFA discordant cats tested (Table 4).

Retesting of ELISA/IFA discordant cats—Forty-eight ELISA/IFA discordant cats were retested by veterinarians within 1 week after results were initially obtained (Table 5). Of the 48 cats, 47 were initially ELISA-positive, IFA-negative, whereas 1 was initially ELISA-negative, IFA-positive. The ELISA and IFA retest results of 13 of the 47 initially ELISA-positive, IFA-negative cats and the 1 initially ELISA-negative, IFA-positive cat did not change. However, the ELISA results of retesting of 34 of the 47 (70.8%) initially ELISA-positive, IFA-negative discordant cats changed to ELISA-negative, whereas the IFA results remained unchanged and were still negative. These comparative retest results indicate that 70.8% of the initial ELISA results for cats in the ELISA/IFA discordant group were incorrect.

*Table 7—Calculation of the sensitivity and specificity of in-hospital ELISA**

ELISA results	Reference Standard results	
	Positive	Negative
Positive	a = 8,761	b = 10,147
Negative	c = 174	d = 1,158

Let a = IFA/ELISA-positive; b = IFA-negative, ELISA-positive; c = IFA-positive/ELISA-negative; d = IFA/ELISA-negative results; Total tested = a + c + b + d = 20,240 Overall agreement = 49.0%

*Data biased toward confirmation of positive ELISA results; thus, these specificity data do not represent true unselected values.

Calculations:

$$\text{ELISA Sensitivity} = \frac{a}{a+c} \times 100 = \frac{8{,}761}{8{,}761 + 174} \times 100 = 98.1\%$$

$$\text{ELISA Specificity} = \frac{d}{b+d} \times 100 + \frac{1{,}158}{10{,}147 + 1{,}158} \times 100 = 10.2\%$$

$$\text{ELISA false negative rate} = \frac{c}{a+c} \times 100 = \frac{174}{8{,}761 + 174} \times 100 = 2.0\%$$

$$\text{ELISA false positive rate} = \frac{b}{b+d} \times 100 = \frac{10{,}147}{10{,}147 + 1{,}158} \times 100 = 89.8\%$$

[e]Virachek, Synbiotics Inc, San Diego, Calif.

[f]ClinEase, Norden Laboratories, Lincoln, Neb.

[g]Diasystems, TechAmerica Veterinary Products, Kansas City, Mo.

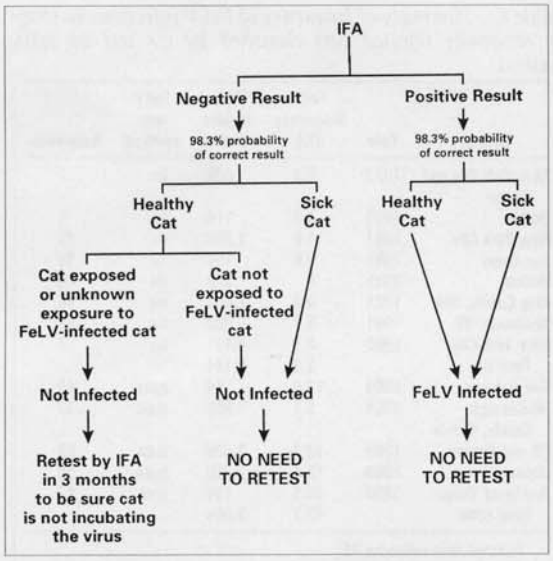

Figure 5—Algorithm for management of cats tested for FeLV infection by the immunofluorescent antibody (IFA) test.

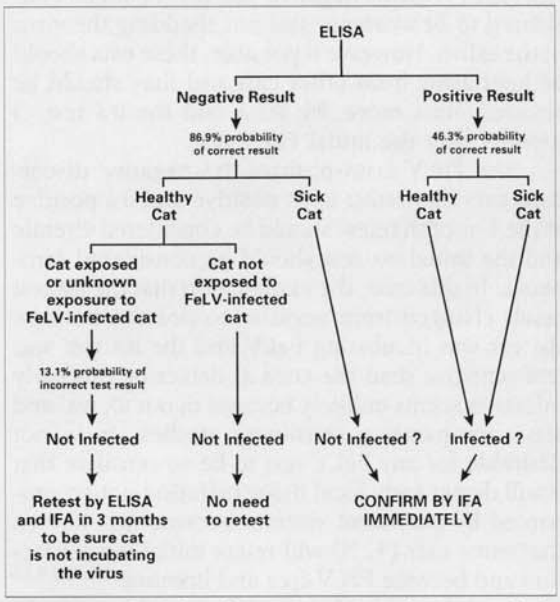

Figure 6—Algorithm for management of cats tested for FeLV infection by ELISA.

Comparative sensitivities of FeLV tests—Sera and blood from 2 FeLV-positive cats were diluted and tested by ID, IFA, and ELISA[d] (Table 6). The ID test result was positive only when undiluted sera were tested, the ELISA result was weakly positive for sera diluted 1:10, and the IFA test result was weakly positive for blood diluted 1:1,000. In other experiments (not described here), it was possible to consistently detect, by use of the IFA test, 1 FeLV-infected leukocyte mixed with 100,000 noninfected leukocytes.

Discussion

This study was initiated by veterinarians who submitted samples for IFA testing for confirmation

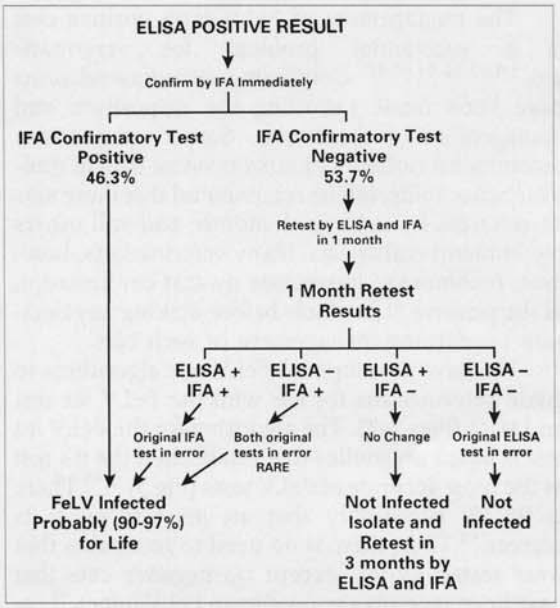

Figure 7—Algorithm for management of cats tested for FeLV infection by ELISA and confirmed by IFA testing.

of results of ELISA performed in their hospitals or at veterinary diagnostic laboratories. During the 10 years of this study, 20,240 confirmatory IFA tests were performed. Agreement between in-hospital performed FeLV ELISA and the IFA test was 86.9% for negative ELISA results and was 46.3% for positive ELISA results. It should be noted that 93% of all of samples submitted by veterinarians for confirmation had positive ELISA results, which introduced bias in the test selection. Practitioners apparently believed that their negative ELISA results were more reliable than their positive ELISA results. This assumption was correct, although 13.1% of all negative ELISA results were incorrect. These findings indicate that veterinarians should be more aware that even their negative ELISA results may be incorrect.

When the 4 discordant ELISA/IFA results were compared with TCI of the virus, all 4 TCI results agreed with the IFA test result. When samples with ELISA/IFA discordant results were immediately retested by ELISA and the IFA test, 70.8% of the original ELISA results changed and, thus, had been incorrect. In this retesting program, none of the IFA test results changed. The sensitivity of the IFA test was found to be higher than that of the ELISA when studied in dilution experiments. Results indicate that the IFA test is still the most accurate FeLV test for detection of persistent FeLV infection.

The biology of the virus and pathogenesis of FeLV infection have been elucidated by use of the IFA test.[1-19] In contrast, the biology of FeLV in ELISA-positive pet cats has not been extensively studied and the outcome of ELISA-positive, IFA-negative discordant cats is not known.[26-31] However, the biology of FeLV in ELISA (true)–positive cats should be identical to that of FeLV in IFA-positive cats.

The management of FeLV ELISA-positive cats is a substantial problem for veterinarians.[3,4,13,14,44,45,47] Conflicting recommendations have been made regarding the disposition and management of these cats. Some veterinarians recommend isolation of ELISA-positive cats in multicat households, others recommend that these cats be retested, by ELISA, in 1 month, and still others recommend euthanasia. Many veterinarians, however, recommend immediate IFA test confirmation of the positive ELISA result before making any decision concerning management of such cats.

We have developed 3 FeLV test algorithms to guide veterinarians for use with the FeLV IFA test and ELISA (Figs 5-7). The algorithm for the FeLV IFA test is based on studies that established the IFA test as the most accurate of FeLV tests (Fig 5).[1,45] There is 98.3% probability that an IFA test result is correct.[45] Thus, there is no need to retest cats that were tested by IFA, except IFA-negative cats that have been recently exposed to an FeLV infected cat or cats that have unknown exposure, such as stray cats. These cats should be retested 3 months after the first test to be sure they were not incubating the virus at that time.

The algorithm for FeLV ELISA also was developed (Fig 6). There is 86.9% probability that a negative ELISA result is correct. If the negative ELISA result is in a healthy cat exposed to an FeLV-infected cat, the former cat should be retested in 3 months by ELISA and the IFA test to be sure the cat was not incubating the virus. If the negative ELISA result is in a healthy cat that was not exposed to an FeLV-infected cat, there is no need for FeLV retesting. All sick cats with negative ELISA results, should have those results confirmed by the IFA test immediately if the veterinarian suspects FeLV-induced disease. There is only 46.3% probability that a positive ELISA result is correct. Thus, we recommend that positive ELISA results in all healthy and sick cats be immediately confirmed by IFA testing.

An algorithm for ELISA-positive IFA confirmatory testing was developed (Fig 7). In-hospital positive ELISA results are confirmed to be positive by IFA 46.3% of the time. Cats with such results are infected and 90 to 97% of them will remain infected for life. However, 53.7% of the positive ELISA results are not confirmed by IFA testing and thus, are negative. Cats with such results should be retested 1 month later by ELISA and IFA testing. If the IFA confirmatory test result is positive, the cat should be considered viremic and most likely will remain infected for life. However if the IFA confirmatory test result is negative, the veterinarian should inform the cat's owner of the discordant results and recommend retesting of the cat, using both tests, in 1 month. If the 1-month retest ELISA result changes from positive to negative but the IFA test result remains negative, the cat should be considered free of FeLV infection and the initial ELISA result should be considered erroneous. If the 1-month ELISA result is again positive and the IFA test result remains negative, the cat should be considered to be aviremic and not shedding the virus in the saliva. However if possible, these cats should be kept away from other cats and they should be retested once more, by ELISA and the IFA test, 3 months after the initial FeLV test.

Table 8—Summary of frequency of FeLV infections in stray or randomly selected cats classified by IFA test OR ELISA method

Region	Year	FeLV frequency (%)	No. of cats tested	FeLV test method	Reference
New York City and Boston	1973	0.3	638	IFA	2
Boston	1975	1.8	114	IFA	5
New York City	1981	1.0	1,290	IFA	18
San Diego	1981	0.6	158	IFA	18
Boston	1981	0	120	IFA	18
King County, WA	1981	4.9	1,451	IFA	54
Northwest US	1981	5.2	232	IFA	54
New York City	1990	2.1	4,191	IFA	*
Total IFA		2.3	8,194		
San Antonio	1984	13.6	44	ELISA	43
Hillsborough County, Florida	1984	9.4	555	ELISA	33
US and Canada	1989	13.3	1,609	ELISA	55
Japan	1989	12.4	700	ELISA	56
Southeast Texas	1990	18.0	156	ELISA	57
Total ELISA		12.7	3,064		

Adapted from reference 33.
*Data from National Veterinary Laboratory, Inc.

The FeLV ELISA-positive, IFA-negative discordant cats that retest ELISA positive and IFA positive at the 1-month retest should be considered viremic and the initial IFA test should be considered erroneous. In this case, the explanation that the IFA test result changed from negative to positive because the cat was incubating FeLV and the IFA test was less sensitive than the ELISA at detecting this early infection seems unlikely because of our ID, IFA, and ELISA comparative sensitivity studies. It is not desirable for any FeLV test to be so sensitive that it will detect early local tissue infection not accompanied by persistent viremia because it is known that some cats (42%) will reject initial local infection and become FeLV-free and immune.[10-12,15,18] Cats with latent FeLV infection do not have replicating FeLV in their tissues, and thus, these cats will not test positive by the IFA test or ELISA.[48,49,50] A practical and useful FeLV test is one that will only detect persistent viremia in cats that do not reject initial infection.

Feline leukemia virus tests should be used in conjunction with FeLV vaccination to prevent spread of this deadly virus among cats.[51,52,53] The FeLV test-and-removal program has been successful worldwide in eliminating the spread of FeLV in multicat households even before introduction of the FeLV vaccines.[3,4,13,14] However, we now recommend that all cats be inoculated with an FeLV vaccine in conjunction with an FeLV and FIV prevaccination screening program. Cats should be tested for FeLV and FIV at the initial office visit. Blood should be drawn for the FeLV and FIV tests,

and the first dose of the FeLV vaccine should be given. If the FeLV test result is obtained after the office visit is completed, it should be forwarded to the owner. It seems more economical to begin vaccination of cats with the first dose even before their FeLV status is known, because only 2.3% of random healthy cats, on the basis of IFA test surveys (Table 8), are expected to be infected with the virus.[15,16,18] The ELISA incidence data of random healthy cats are much higher than those compiled for the IFA test (Table 8), and we believe they represent an overestimate based on false-positive ELISA results that were not confirmed by IFA testing.[2,5,18,33,43,54-56]

Any cat that tests positive for FeLV after being given the first dose of the FeLV vaccine should be isolated from other cats and, although the vaccine is not harmful to infected cats, there appears to be no benefit to continuing the vaccination program in such cats. Healthy cats that test negative for FeLV and positive for FIV should be vaccinated for FeLV but should be kept indoors to prevent the spread of FIV through fighting and biting.[57] Because FeLV infection is life-long in 90 to 97% of IFA-positive cats and effective anti-viral treatment is not available, it is important to identify healthy FeLV carrier cats so they can be removed from contact with other cats.[58] The FeLV test-and-removal program is an excellent example of an efficient and effective anti-retroviral preventive veterinary medicine program.[3,4,13,14]

The percentage of positive IFA test results apparently decreased from 1972 to the present. A higher percentage of positive FeLV test results was observed in the years 1972 through 1978, than in more recent years. One explanation is that in the early 1970s, veterinarians were mainly testing sick cats or cats known to have been exposed to FeLV-infected cats. Another possibility is that, with the introduction of the first vaccine for FeLV in 1985, many veterinarians began prevaccination FeLV screening programs of more healthy FeLV-nonexposed cats. A review of 1,417 IFA test requisition forms from 1990 indicates that more healthy cats are currently being tested than were tested in the early 1970s. Currently, it is difficult to determine whether FeLV vaccines have reduced prevalence of FeLV-infected cats in the general population.

In this study, analysis of the comparison of practitioner-performed in-hospital ELISA with the IFA test performed by a specialized retrovirus testing laboratory indicated that more than half of positive ELISA results were incorrect. Results of this study are biased toward confirmation of a large number (n = 18,908), of positive ELISA results and only a relatively small number (n = 1,332) of negative ELISA results. However, our results indicate that many in-hospital positive ELISA results are incorrect and support the recommendation that all positive ELISA results be confirmed by IFA testing.

During the past several years, better correlation has been reported between ELISA and other IFA and virus-isolation tests done in university research laboratories.[18,20-44] Unlike our study, none of those studies compared large numbers of in-hospital performed ELISA. The probable explanation for the differing results between those comparative studies and our study is the fact that all comparative tests, ELISA and confirmatory tests, were performed by skilled laboratory-trained technicians in the other studies and not by veterinary hospital personnel as was associated with our study. Our study encompassed more than 1,000 veterinary hospitals where there probably were differing degrees of technical laboratory competence.

Many clinically oriented reviews exist of the biology of FeLV and the diseases induced by this virus.[3,17,18,47,59,60] Veterinarians in small animal practice, where cats now comprise more than half of all patients, must familiarize themselves with this virus and the methods used to detect infected cats. Veterinarians must also fully understand the characteristics (strengths and weaknesses) of the FeLV tests they use, so that the information gained from these tests can be used properly. An FeLV test is for detection of the virus and is not a diagnostic test for leukemia or any other feline disease.[44,45]

Careful confirmation of all positive retroviral test results should be done to practice the highest standards of veterinary medicine. In this regard, results of all retroviral screening ELISA for the human immunodeficiency virus types 1 and 2, and the human T-lymphotropic retrovirus types I and II are confirmed by more specific western blot assays before any person is informed of positive retroviral status. A similar standard should be adopted in veterinary medicine where routine retroviral testing was introduced and practiced 10 years before similar tests were available in human medicine.

As a result of this study, we recommend that all FeLV ELISA-positive cats have such results immediately confirmed by IFA testing.

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