Comparison of different new tests for feline immunodeficiency virus and feline leukemia virus infection.

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Introduction

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are two retroviruses that are associated with the illness and death of more cats than any other infectious agent (1). Although FeLV and FIV infections occur worldwide, prevalence varies by location (2, 3). Infections with these viruses can be difficult to diagnose by clinical signs alone, and are sometimes clinically unapparent for months after initial exposure (4).
The most effective way to guard against infection is to prevent exposure to FeLV- and FIV-infected cats. Testing to identify infected cats is the mainstay of preventing transmission of the viruses. Neither FeLV nor FIV vaccination is a substitute for testing cats (5). General retrovirus testing principles that were prepared by the American Association of Feline Practitioners in 2001 are shown in Table 1.

The most preferred initial tests for **FeLV testing** are soluble antigen tests such as enzyme-linked immunosorbent assay (ELISA) and other immunochromatographic tests (in which color is generated as a result of an immunologic reaction) that detect free FeLV antigen (p27) in fluid (4). Positive results may be reflective of transient or persistent viremia (4). In experimental settings, most cats will have positive results with soluble antigen tests within 28 days after exposure (6). Indirect immunofluorescent antibody (IFA) tests detect cell-associated antigen, and positive results are highly likely to be reflective of persistent viremia (2, 7, 8).
Polymerase chain reaction (PCR) detects viral RNA or DNA and offers a promising approach to FeLV diagnosis. However, current reagents and testing protocols are neither standardized nor validated (9).

Currently available tests for **FIV testing** include Western blot, ELISA and other immunochromatographic tests. They detect antibodies directed against the virus (3). Cats that seroconvert to FIV following infection remain infected with FIV, resulting in a high correlation between antibodies to FIV and infection (10). Thus, up to now presence of antibodies to the virus indicated that a cat was infected with FIV (4). Since the introduction of the new FIV vaccine, however, it is not possible to distinguish between infection-induced and vaccine-induced antibodies. PCR-based assays offer a promising approach to FIV testing. As is the case with FeLV testing, however, current reagents and testing protocols are neither standardized nor validated (9).

Serological tests (ELISA or immunochromatographic tests), which are the most common tests for FeLV and FIV testing, are available in several formats for use in veterinary practices and diagnostic laboratories. However, the abilities and limitations of each type of test and the prevalence of the infectious agent in question must be considered when interpreting a test result (4).

In recent years, many new feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) rapid tests for use in veterinary practice have been introduced to the market. The question of relative merits of each kit has prompted comparative studies. This study was designed to define the strengths and weaknesses of 11 commercial tests and to assess sensitivity and specificity of the tests, as well as the predictive values of positive and negative test results.

Materials and Methods

The study was performed at the Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, USA. Different commercially available test systems for the detection of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) were evaluated using 535 serum samples. The test systems were either enzyme-linked immunosorbent assay (ELISA) or based on an immunochromatographic principle.

The eight FeLV antigen tests included **SNAP® Combo Plus** (IDEXX Laboratories Inc., USA), **PetChek®** (IDEXX Laboratories Inc., USA), **DUO Speed®** (BIO VETO TEST, France), **FASTest®** (MegaCor, Austria), **Witness®** (Synbiotics Corporation, USA), **Virachek®** (Synbiotics Corporation, USA), **Mapic®** (Sinovus Biotech Inc., USA) and **ONE-Step®** (EVL, The Netherlands). The seven different FIV test systems included **SNAP® Combo Plus** (IDEXX Laboratories Inc., USA), **PetChek® Plus Anti-FIV** (IDEXX Laboratories Inc., USA), **DUO Speed®** (BIO VETO TEST, France), **FASTest®** (MegaCor, Austria), **Witness®** (Synbiotics Corporation, USA), **Virachek®** (Synbiotics Corporation, USA), and **Mapic®** (Sinovus Biotech Inc., USA).

Samples reacting FIV-positive in at least one of the tests were confirmed by Western blot. Samples reacting FeLV-positive were confirmed by virus isolation. In addition, 100 negative samples were tested by Western blot using a protocol described by Egberink and coworkers (1991) (13), and 81 negative samples were tested by virus isolation.

Sensitivity and specificity, as well as the positive and negative predictive value of each test, was assessed.
Possible test combinations were compared and evaluated for use in veterinary practice.

Results

Characteristics of the FIV test system are shown in Table 2. Evaluation of FeLV test systems was more difficult. Seventeen samples showed a positive test result in each test kit, but were not confirmed on virus isolation. Two different evaluations were performed. The

first one counted only the samples as true positives that were positive on virus isolation. The sensitivity and positive predictive value for each test system were very low in this case and not comparable with previous studies (Table 3). In the second evaluation, all 17 "not confirmable" samples that were positive in each test kit but negative on virus isolation were counted as true positives as well (Table 4).

A comparison of results of in-clinic FIV and FeLV test systems from two different studies (1997 vs. 2001) (12) is shown in Tables 5 and 6. Tables 7 and 8 show a ranking of the FIV and FeLV test systems from the studies in 1997 and 2001. Rankings were scored by best performing (1) through worst performing (5) for each category: invalid test result, difficult to interpret, sensitivity, specificity, positive and negative predictive value. Scoring for FIV and FeLV performance on each product is based on a nonweighted average. The final ranking is based on overall score, 1 being best and 5 being worst. Table 9 demonstrates the overall performance of each test for FIV and FeLV.

Tables 10 and 11 show the positive predictive values when combining two FeLV or FIV test systems. Positive predictive values (PPV) change with the prevalence (P) of infection, as well as the sensitivity (Se) and specificity (Sp) of the test. The values can be calculated by the Bayes formula: $PPV = P * Se / [P * Se + (1 - P) * (1 - Sp)]$. If the prevalence increases from 1% to 10%, positive predictive values improve significantly (especially in tests that have considerably lower values). Figure 1 demonstrates the dependence of positive predictive values on the prevalence of FeLV infection.

Conclusions

The results of each FIV test system, with the exception of Mapic®, were easy to interpret and the percentages of invalid results were low. About 23% of the Mapic® tests had to be discharged and 11% of the remaining tests were difficult to interpret. The FeLV test systems Mapic® and One-Step® showed a high percentage of invalid test results. About 13% of the One-Step® and 30% of the Mapic® tests had to be discharged. These two test systems, as well as the Witness® FeLV, also had a high percentage of results that were difficult to interpret. Therefore, Mapic® FIV and FeLV cannot be recommended for use in veterinary practice.

The positive predictive values of all FIV tests were between 91% and 100%. No significant differences were found between the positive and negative predictive values of each test system. The best value was assessed for PetChek® Plus Anti-FIV. The negative predictive values were between 96.1% and 100%. SNAP® Combo Plus was the test with the best negative predictive value. The FIV test systems DUO Speed®, FASTest®, Witness®, and SNAP® Combo Plus can all be recommended for use in veterinary practice. When comparing the improvements in performance of the in-clinic tests from the 1997 study (12) to the 2001 study, it was found that the SNAP product improved markedly concerning FIV detection, moving from the fourth position to the best performing test for FIV. This is due to improvements made by IDEXX, in which antibody responses to additional FIV-specific proteins are now detected in the SNAP® Combo Plus test kit.

Evaluating the FeLV results of this study, one has to consider the problem that occurred with the virus isolation. Seventeen serum samples, which showed a positive test result in each test system, were not confirmed with virus isolation. The sensitivity and positive predictive value of all test systems were low in this case and not comparable to previous studies. Therefore, two different evaluations were considered. The first one counted only the samples as "true positives" that were confirmed by virus isolation (Table 3). In the second evaluation, the 17 samples with "not confirmable results" were counted as "true positives" as well (Table 4).

Choosing the appropriate gold standard for FeLV confirmation is a critical point. There is no generally accepted gold standard and the opinions about using virus isolation, IFA, or PCR as confirmation tests differ from author to author. In this study, virus isolation was chosen as the confirmation test. A possible disadvantage of virus isolation, which can lead to false-negative results, is that virus might be inactivated during the period of storage and transportation to the laboratory (14). Furthermore, presence of virus is not identical to presence of antigen. It is known that there are cats (e.g., with a so-called "atypical infection") in which virus replication is incompletely contained and virus-expressing cells remain in sequestered sites leading to low-degree or intermittent antigenemia and only occasionally shed virus (15). Thus, in these cats, antigen can sometimes be detected in the blood while virus isolation is not possible. For these reasons, some true positive results could be misinterpreted as false positive because of the limitations of the confirmation method leading to a lower sensitivity and positive predictive value of each test system.

Relying on the second evaluation of FeLV test systems (Table 4), the positive predictive values of all the FeLV tests were between 62% and 90%. Significant differences were found between the better test systems DUO Speed®, Virachek®, PetChek®, FASTest® and the test systems Witness®, SNAP® Combo Plus and One-Step®. The last three systems had a positive predictive value lower than 77%. The best value was achieved by the test system DUO Speed®. The positive predictive values for the three test systems Virachek®, PetChek® and FASTest® were about 85%. No significant differences were found between the negative predictive values that ranked between 99.4% and 99.7%. The DUO Speed® FeLV and FASTest® FeLV are reliable FeLV test systems and can be used as diagnostic tools in veterinary practice.

Due to the low prevalence of both infections, positive results always have to be interpreted carefully, and a confirmatory test should be performed in every positive sample. The gold standard for confirmation of FIV is the Western blot. If Western blot is not available, the best option to confirm FIV positive results, as shown in Table 10, is the PetChek® Plus Anti-FIV. The recommendation for the best test to confirm a positive FeLV test result is not as clear as for FIV.

Also, One-Step® FeLV cannot be recommended as a screening test, due to its low positive predictive value.
However, it was the best choice for combination with another test system according to Table 11, and, therefore,
can be used as a confirmatory option for FeLV positive samples.

Table 1: General Retrovirus Testing Principles (16)

- All cats should be tested for FeLV and FIV infection.
- Cats infected with FeLV or FIV may live for many years. A decision for euthanasia should never be made solely on the basis of whether a cat is infected.
- A confirmed positive test result should be considered only an indication of retrovirus infection, not clinical disease.
- Diseases in cats infected with FeLV or FIV may not necessarily be a result of retrovirus infection.
- No test is 100 % accurate at all times and under all conditions; therefore, all test results should be interpreted in light of the patient's health and prior likelihood of infection.

Table 2: Comparison of seven different FIV test systems
(n.e. = not examined; WB = Western blot; green = best result; red = worst result)

	Witness®	SNAP® Combo Plus	FASTest®	DUO Speed®	Virachek®	PetChek® Plus Anti-FIV	Mapic®
number of samples (n)	535	535	535	535	535	535	402
invalid	2 0.4%	6 1.1%	3 0.6%	6 1.1%	3 0.6%	1 0.2%	93 23.1%
calculated n	533	529	532	529	532	534	309
difficult interpretation	4 0.8%	3 0.6%	3 0.6%	4 0.8%	8 1.5%	0	n.e.
number of positive confirmed samples (WB)	55	52	55	54	53	55	n.e.
prevalence	10.3%	9.8%	10.3%	10.2%	10.0%	10.3%	n.e.
number of positive test results	55 10.3%	55 10.4%	57 10.7%	57 10.8%	51 9.6%	52 9.7%	n.e.
number of negative test results	478 89.7%	474 89.6%	475 89.3%	472 89.2%	481 90.4%	482 90.3%	n.e.
number of true positives	52 94.5%	52 94.5%	53 93.0%	52 91.2%	50 98.0%	52 100%	n.e.
number of false positives	3 5.5%	3 5.5%	4 7.0%	5 8.8%	1 2%	0	n.e.
number of true negatives	475 99.4%	474 100%	473 99.6%	470 99.6%	477 99.2%	479 99.4%	n.e.
number of false negatives	3 0.6%	0	2 0.4%	2 0.4%	4 0.8%	3 0.6%	n.e.
specificity	99.4%	99.4%	99.2%	99.0%	99.8%	100%	n.e.
sensitivity	94.5%	100%	96.4%	96.3%	92.6%	94.5%	n.e.
pos. predictive value	94.5%	94.5%	93.0%	91.2%	96.1%	100%	n.e.
neg. predictive value	99.4%	100%	99.6%	99.6%	99.2%	99.4%	n.e.

Table 3: Comparison of 8 different FeLV test systems considering the 17 "not confirmable samples" as true negatives
(n.e. = not examined; VI = virus isolation; pos. = positive; neg. = negative; green = best value; red = worst value)

	Witness®	Snap® Combo Plus	FASTest®	DUO Speed®	Virachek®	PetChek	Mapic®	One-Step®
number of samples (n)	528	528	528	528	528	528	378	517
non valid	7 1.3%	3 0.6%	1 0.2%	10 1.9%	1 0.2%	2 0.4%	115 30.4%	69 13.3%
calculated n	521	525	527	518	527	526	263	448
difficult interpretation	72 13.8%	2 0.4%	7 1.3%	8 1.5%	8 1.5%	6 1.4%	n.e.	37 8.4%
number of positive confirmed samples (VI)	22	22	22	21	22	22	n.e.	18
prevalence	4.2%	4.2%	4.2%	4.1%	4.2%	4.2%	n.e.	4.0%
number of positive test results	47 9.0%	49 9.3%	42 8.0%	40 7.7%	45 8.5%	41 7.8%	n.e.	50 11.2%
number of negative test results.	474 91.0%	476 90.7%	485 92.0%	478 92.3%	482 91.5%	485 92.2%	n.e.	398 8.8%
number of true positives.	19 40.4%	19 38.8%	20 47.6%	19 47.5%	20 44.4%	19 46.3%	n.e.	17 34.0%
number of false positives	28 59.6%	30 61.2%	22 52.4%	21 52.5%	25 55.6%	22 53.7%	n.e.	33 66.0%
number of true negatives	471 99.4%	473 99.4%	483 99.6%	476 99.6%	480 99.6%	482 99.4%	n.e.	397 99.7%
number of false negatives.	3 0.6%	3 0.6%	2 0.4%	2 0.4%	2 0.4%	3 0.6%	n.e	1 0.3%
sensitivity	86.4%	86.4%	91.0%	90.5%	91.0%	86.4%	n.e.	94.4%
specificity	94.4%	94.0%	95.6%	96.8%	95.0%	95.6%	n.e.	92.3%
pos. predictive value	40.4%	38.8%	47.6%	47.5%	44.5%	46.3%	n.e.	34.0%
neg. predictive value	99.4%	99.4%	99.6%	99.6%	99.6%	99.4%	n.e.	99.7%

Table 4: Comparison of eight different FeLV test systems considering the 17 "not confirmable samples" as true positives
(n.e. = not examined; VI = virus isolation; pos. = positive; neg. = negative; green = best value; red = worst value)

	Witness®	SNAP® Combo Plus	FASTest®	DUO Speed®	Virachek®	PetChek®	Mapic®	One-Step®
number of samples (n)	528	528	528	528	528	528	378	517
non valid	7 1.3 %	3 0.6%	1 0.2%	10 1.9%	1 0.2%	2 0.4%	115 30.4%	69 13.3%
calculated n	521	525	527	518	527	526	263	448
difficult interpretation	72 13.8%	2 0.4%	7 1.3%	8 1.5%	8 1.5%	6 1.4%	n.e.	37 8.4%
number of positive confirmed samples (VI)	22	22	22	21	22	22	n.e.	18
number of samples that are positive in all ELISA kits	16	17	16	17	17	16	n.e.	14
prevalence	7.3	7.4	7.2	7.3	7.4	7.2	n.e.	7.1
number of positive test results	47 9.0%	49 9.3%	42 8.0%	40 7.7%	45 8.5%	41 7.8%	n.e.	50 11.2%
number of negative test results	474 91.0%	476 90.7%	485 92.0%	478 92.3%	482 91.5%	485 92.2%	n.e.	398 8.8%
number of true positives	35 74.5%	36 73.5%	36 85.8%	36 90.0%	37 82.2%	35 85.4%	n.e.	31 62.0%
number of false positives	12 25.5%	13 26.5%	6 14.2%	4 10.0%	8 17.8%	6 14.6%	n.e.	19 38.0%
number of true negatives	471 99.4%	473 99.4%	483 99.6%	476 99.6%	480 99.6%	482 99.4%	n.e.	397 99.7%
number of false negatives	3 0.6%	3 0.6%	2 0.4%	2 0.4%	2 0.4%	3 0.6%	n.e.	1 0.3%
sensitivity	92.1%	92.3%	94.7%	94.7%	94.9%	92.1%	n.e.	96.8%
specificity	97.5%	97.3%	98.8%	99.2%	98.4%	99.4%	n.e.	95.4%
pos. predictive value	74.5%	73.5%	85.7%	90.0%	82.2%	85.4%	n.e.	62.0%
neg. predictive value	99.4%	99.4%	99.6%	99.6%	99.6%	99.4%	n.e.	99.7%

Table 5: Comparing the results of in-clinic FIV test systems from two different studies (12) (1997 vs. 2001)
(PPV = positive predictive value; NPV = negative predictive value; green = best result; red = worst result)

	1997				2001			
FIV Ab Results	Witness *Synbiotics*	SNAP Combo *IDEXX*	FasTest *MegaCor*	DUOSpeed *BioVito Test*	Witness *Synbiotics*	SNAP Combo Plus *IDEXX*	FasTest *MegaCor*	DUO Speed *BioVito Test*
invalid test results	12.6%	1.7%	0.1%	0.3%	0.4%	0.1%	0.6%	1.1%
difficult to interpret	8.7%	14.4%	6.8%	6.4%	0.8%	0.6%	0.6%	0.8%
sensitivity (%)	95.5%	86.1%	90.3%	97.3%	94.5%	100.0%	96.4%	96.3%
specitivity (%)	99.7%	98.6%	99.0%	98.6%	99.4%	99.4%	99.2%	99.0%
positive predictive value (%)	97.0%	86.1%	90.3%	87.7%	94.5%	94.5%	93.0%	91.2%
negative predictive value (%)	99.5%	98.6%	99.0%	99.7%	99.4%	100.0%	99.6%	99.6%

Table 6: Comparing the results of in-clinic FeLV test systems from two different studies (12) (1997 vs. 2001)
(PPV = positive predictive value; NPV = negative predictive value; green = best result; red = worst result)

	1997					2001				
FeLV Ag Results	Witness *Synbiotics*	SNAP Combo *IDEXX*	FasTest *MegaCor*	DUOSpeed *BioVito Test*	One Step *EVL*	Witness *Synbiotics*	SNAP Combo Plus *IDEXX*	FasTest *MegaCor*	DUO Speed *BioVito Test*	One Step *EVL*
invalid test results	13.6%	1.7%	0.0%	0.3%	1.0%	1.3%	0.6%	0.2%	1.9%	13.3%
difficult to interpret	20.1%	14.4%	3.4%	4.0%	10.7%	13.8%	0.4%	1.3%	1.5%	8.4%
sensitivity (%)	66.6%	91.3%	85.5%	89.6%	88.4%	92.1%	92.3%	94.7%	94.7%	96.8%
specitivity (%)	98.7%	98.2%	98.2%	98.1%	91.8%	97.5%	97.3%	98.8%	99.2%	95.4%
positive predictive value (%)	83.3%	82.9%	81.9%	81.1%	50.8%	74.5%	73.5%	85.7%	90.0%	62.0%
negative predictive value (%)	96.9%	99.2%	98.6%	99.0%	98.8%	99.4%	99.4%	99.6%	99.6%	99.7%

Table 7: Ranking of in-clinic FIV test systems from 1997 and 2001

	1997				2001			
FIV Ab Ranking	Witness *Synbiotics*	SNAP Combo *IDEXX*	FasTest *MegaCor*	DUOSpeed *BioVito Test*	Witness *Synbiotics*	SNAP Combo Plus *IDEXX*	FasTest *MegaCor*	DUO Speed *BioVito Test*
invalid test results	4	3	1	2	2	1	3	4
difficult to interpret	3	4	2	1	2	1	1	2
sensitivity (%)	2	4	3	1	4	1	2	3
specitivity (%)	1	3	2	3	1	1	3	4
positive predictive value (%)	1	4	2	3	1	1	2	3
negative predictive value (%)	2	4	3	1	3	1	2	2
Overall Rank	**2.2**	**3.7**	**2.2**	**1.8**	**2.2**	**1.0**	**2.2**	**3.0**

Table 8: Ranking of in-clinic FeLV test systems from 1997 and 2001

	1997					2001				
FeLV Ag Ranking	Witness *Synbiotics*	SNAP Combo *IDEXX*	FasTest *MegaCor*	DUOSpeed *BioVito Test*	One Step *EVL*	Witness *Synbiotics*	SNAP Combo Plus *IDEXX*	FasTest *MegaCor*	DUO Speed *BioVito Test*	One Step *EVL*
invalid test results	5	4	1	2	3	3	2	1	4	5
difficult to interpret	5	4	1	2	3	5	1	2	3	4
sensitivity (%)	5	1	4	2	3	4	3	2	2	1
specitivity (%)	1	3	3	4	2	3	4	2	1	5
positive predictive value (%)	1	2	3	4	5	3	4	2	1	5
negative predictive value (%)	5	1	4	2	3	3	3	2	2	1
Overall Rank	**3.6**	**2.5**	**2.7**	**2.7**	**3.2**	**3.5**	**2.8**	**1.8**	**2.1**	**3.5**

Table 9: Overall ranking of in-clinic FIV and FeLV test systems for 2001

	2001				
	Witness *Synbtiotics*	SNAP Combo Plus *IDEXX*	FasTest *MegaCor*	DUO Speed *BioVito Test*	One Step *EVL*
FIV Rank	2.3	1.0	2.2	3.0	N/A
FeLV	3.5	2.8	1.8	2.1	3.5
Overall Rank	**2.9**	**1.9**	**2.0**	**2.6**	**N/A**

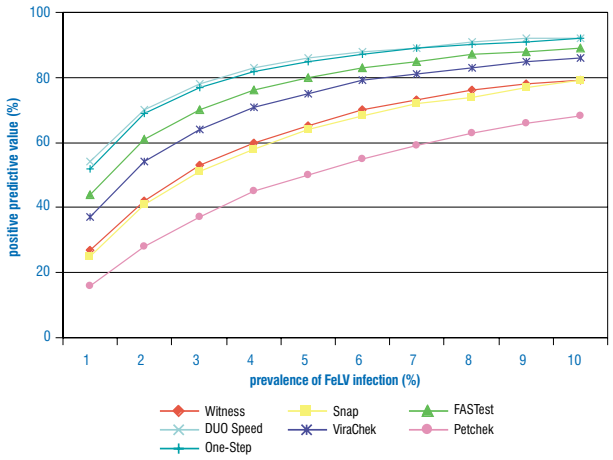
Table 10: Positive predictive value (%) in combination of two FIV test systems

	Witness®	SNAP®	FASTest®	DUO Speed®	Virachek®	PetChek®
Witness®		98.0	98.1	98.1	98.0	100
SNAP®			98.0	98.0	97.8	100
FASTest®				92.9	98.0	100
DUO Speed®					98.0	100
Virachek®						100
PetChek®						

Table 11: Positive predictive value (%) in combination of two FeLV test systems

	Witness®	SNAP®	FASTest®	DUO Speed®	Virachek®	PetChek®	OneStep®
Witness®		81.4	91.9	94.6	83.3	87.2	93.5
SNAP®			87.5	92.3	81.8	85.0	90.6
FASTest®				94.6	89.7	91.9	96.7
DUOSpeed®					92.3	92.1	94.0
Virachek®						85.4	90.6
PetChek®							90.3
OneStep®							

Figure 1: Example of the dependence of positive predictive values dependant on the prevalence of FeLV infection



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