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Comparative evaluation of nine lateral flow assays for FIV antibody detection using an in-house ELISA as a reference method

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Abstract

Feline immunodeficiency virus (FIV) induces immunosuppression in affected cats, increasing susceptibility to chronic and secondary infections. Rapid and accurate detection of FIV-specific antibodies is essential for effective clinical management and epidemiological monitoring. This study conducted a comparative evaluation of nine commercially available lateral flow assays (LFAs) for detecting FIV antibodies in whole blood, serum, or plasma, using a newly developed in-house enzyme-linked immunosorbent assay (ELISA) as a reference method. All tested LFAs demonstrated 100% specificity. While Vet Expert new and VetFor achieved 100% across all metrics indicating the best performance, formal statistical comparison did not reveal significant differences between the evaluated kits. Overall, the results confirm that all tested LFAs offer comparable reliability. Importantly, our in-house ELISA exhibited 100% concordance for positive samples with the commercial ELISA treated as the reference standard, confirming its reliability as a comparator. These findings emphasize the importance of selecting high-performing diagnostic tools to ensure reliable FIV detection and effective disease control strategies.

Keywords Feline immunodeficiency virus, Rapid test, Lateral flow assay, Antibodies, Antigens, Enzyme-linked immunosorbent assay

Introduction

FIV is a globally distributed retrovirus that compromises the feline immune system, rendering infected cats more susceptible to opportunistic infections and chronic diseases [1]. Prevalence rates vary by region, with estimates ranging from 2.5–5% among healthy cats in North America, while European data remain incomplete [1–3]. Although no curative treatment exists, supportive care and management strategies can significantly improve the quality of life and longevity of infected animals. These strategies emphasize immune support, prevention of secondary infections, and overall health maintenance.

Effective control of FIV requires both prevention of transmission and early, accurate diagnosis. Rapid, cost-effective, and user-friendly diagnostic tools are essential for both pet owners and veterinary professionals. Diagnosis of FIV infection is primarily based on the detection of antiviral antibodies rather than viral antigen or nucleic acid. Following initial infection, a brief period of viremia occurs, during which viral antigen or RNA may be detectable; however, FIV subsequently establishes a latent infection within host lymphocytes, leading to very low levels of circulating virus. Antibodies typically appear

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4–8 weeks post-infection and, in most cases, remain detectable for life. Consequently, serological assays provide a more reliable and practical diagnostic approach [4–6]. These include lateral flow assays (LFAs), enzyme-linked immunosorbent assays (ELISAs). Moreover, Western blotting as a highly specific method, is typically used to confirm results obtained through other diagnostic approaches. Each method has inherent advantages and limitations, particularly in terms of sensitivity, specificity, and practicality in clinical or field settings [7–9].

A major challenge in serological diagnostics lies in the variability of the host immune response to viral antigens. Considerable research has been devoted to characterizing feline antibody responses to FIV proteins, particularly those identified as immunodominant antigens [10–12]. Diagnostic accuracy is further complicated by factors such as vaccine-induced antibodies, cross-reactivity with other pathogens, low antibody titers in early infection, and the timing of sample collection post-infection [9, 13–15].

Among the viral proteins, the envelope glycoprotein gp40 has emerged as the most reliable antigen for serological detection of FIV [4, 16]. Its strong immunogenicity and consistent antibody-binding properties make it the cornerstone of most rapid diagnostic tests. While other antigens such as p15 and p24 are also utilized, they are generally considered supplementary, contributing to enhanced sensitivity or confirmatory testing rather than primary detection [11].

ELISA remains a useful tool for confirming FIV seropositivity due to its high sensitivity and specificity, often exceeding 95% in validated systems. Unlike rapid tests, ELISA platforms are less prone to false positives and offer robust, laboratory-based validation [8]. However, commercial ELISA kits are often prohibitively expensive for large-scale screening or research applications, and their reliance on proprietary reagents limits flexibility.

To address the limitations outlined above, our objective was to develop an in-house ELISA FIV Ab platform tailored for research and development applications, and to conduct a comparative evaluation of nine commercially available lateral flow assay (LFA) devices. The comparison focused on their diagnostic performance in detecting anti-FIV antibodies, and concordance with ELISA results.

Materials and methods

Sample collection and preparation

Feline blood samples were sourced from veterinary laboratories and animal shelters located across various regions of Poland. These samples represented surplus material remaining after routine diagnostic procedures performed as part of standard clinical care. No samples were collected specifically for this study, and no additional interventions were carried out on the animals. Consequently, no informed consent was required. All participating institutions provided formal approval for the use of these samples in the research. Blood was drawn into EDTA tubes and stored at 4 °C for a maximum of 24 h. For longer storage, samples were centrifuged at 1000 × g for 10 min at 4 °C to separate plasma, which was then stored at – 20 °C until analysis.

In-house ELISA FIV Ab development and validation

Plate Preparation

Clear round-bottom 96-well Maxisorp plates (Thermo Scientific, Roskilde, Denmark) were coated and sealed overnight at room temperature with 50 µL per well of gp40 antigen (Rekom Biotech, Granada, Spain) diluted in PBS (Merck, Darmstadt, Germany) to a final concentration of 1 µg/mL. Plates were washed once with PBS-T (0.05% Tween-20 (Merck, Darmstadt, Germany) in PBS) and once with PBS alone. Blocking was performed for 2 hours at 37°C using 200 µL of a solution containing 1% CaseiNSblock (Merck, Darmstadt, Germany) and 1% BSA (Merck Darmstadt, Germany) in PBS-T. Plates were washed three times with PBS, stabilized with 60 µL per well of sucrose solution in PBS for 1 hour at 37°C, dried for 1.5 hours at 37°C, sealed, and stored at –20°C.

Assay procedure

Plates were washed three times with PBS. Samples (whole blood, serum or plasma) were diluted 1:2000 in PBS-T/BSA (0.5% BSA and 0.05% Tween-20 in PBS), and 50 µL of each dilution was added in duplicate. After 1 h incubation at 37 °C, plates were washed three times with PBS-T. Then, 50 µL of Goat Anti-Cat HRP-conjugated antibody (Jackson ImmunoResearch, West Grove, PA, USA) was added and incubated for 30 min at 37 °C. After another washing step, 50 µL of TMB substrate (Thermo Scientific, Stockholm, Sweden) was added and incubated for 5 min at room temperature. The reaction was stopped with 50 µL of acidic stop solution (Thermo Scientific, Stockholm, Sweden), and absorbance was measured at 450 nm using a Victor Nivo (Perkin Elmer, Waltham, Massachusetts, USA) microplate reader. Optimization and validation of in-house ELISA FIV Ab was performed according to available protocols [17–19].

Cut-off determination

To establish the positive cut-off for the in-house ELISA FIV Ab, 123 known negative, randomly chosen, serum and plasma samples—confirmed as FIV-negative by submitting diagnostic laboratories—were tested in duplicate. The mean optical density (OD) and standard deviation (SD) were calculated from these measurements. The cut-off value was defined using the formula described elsewhere [20, 21]:

$$Cut-off = \overline{X}_{negativesamples} + (3 \times SD)$$

where $\bar{X}$ is the arithmetic mean and SD is the standard deviation of the readings obtained for the negative samples.

This method accounts for background variability and reduces the likelihood of false-positive results. Samples with OD values equal to or greater than this cut-off were classified as positive for FIV antibodies.

Diagnostic parameter calculation

A total of 164 randomly chosen serum and plasma samples with known FIV status, were initially screened using the Agrolabo FIV Ab ELISA kit (Agrolabo, Torino, Italy), following the manufacturer's instructions. This assay was selected because it is a commercially available, validated diagnostic tool with documented high sensitivity and specificity in independent studies [17]. Its role in this study was limited to confirming the diagnostic performance of the in-house ELISA, not as a universal reference method for all analyses. Based on the optical density (OD) values obtained and protocol provided with the kit, samples were classified as positive, negative, or indeterminate. Indeterminate samples—those with OD values falling between the defined cut-off thresholds—were excluded from further analysis.

To assess the diagnostic performance of the in-house ELISA FIV Ab, results from the same set of samples were compared with those obtained using the Agrolabo ELISA FIV Ab kit, which served as the reference and method for in-house ELISA validation. Concordance between the two assays was evaluated by categorizing each sample according to agreement: samples positive in both assays were considered true positives (TP), while those negative in both were classified as true negatives (TN). Discrepant results were designated as false positives (FP) when the in-house ELISA FIV Ab was positive but the reference assay was negative, and as false negatives (FN) when the in-house ELISA FIV Ab was negative but the reference assay was positive.

Diagnostic performance metrics were calculated as follows: sensitivity (TP ÷ [TP + FN]), representing the proportion of infected individuals correctly identified; specificity (TN ÷ [TN + FP]), indicating the proportion of non-infected individuals correctly classified; and overall accuracy (ACC = [TP + TN] ÷ total samples tested).

Evaluation of rapid lateral flow assays

Nine commercially avaiable lateral flow assays (LFAs) were evaluated using 50 feline samples with FIV status determined by the in-house ELISA FIV Ab (15 of which was whole blood, 30 plasma and 5 serum samples). Given the low global seroprevalence of FIV, an enriched sampling design was used, consisting of selecting samples based on predefined criteria to increase the proportion of positive cases, without altering the specimens themselves [22]. This pragmatic approach aligns with established methodological frameworks for diagnostic test evaluation. Prevalence-dependent predictive values were therefore recalculated for realistic prevalence scenarios (1%, 3%, and 5%) [8], enabling a rigorous head-to-head comparison of nine diagnostic assays within a feasible study size. The selected LFAs represented diverse geographic and market coverage, including SNAP® (global standard), Fassisi (global standard), FASTest (DACH region), Vet Expert (global standard), Vet Expert new (new version of the test), UranoVet (Southern Europe and South America), AllTest (Mainland China and Southeast Asia), Anigen (global standard) and VetFor (Turkey, Greece, Middle East). Each assay was performed according to the manufacturer's instructions. Results were compared with the in-house ELISA FIV Ab classification, and samples were categorized as TP, TN, FP or FN. Diagnostic performance metrics—sensitivity, specificity, and overall accuracy—were then calculated for each test To formally assess whether differences between the test kits were statistically significant, pairwise comparisons of sensitivity and specificity were performed using McNemar's test, and overall comparisons across all kits were evaluated using Cochran's Q test.

Results

In-house ELISA evaluation

The diagnostic performance of the in-house ELISA FIV Ab was evaluated by comparing its results with those obtained using the commercial Agrolabo FIV Ab ELISA kit, which served as the reference and validation method. Summary outcomes are presented in Fig. 1 and Table 1, while detailed data—including sample identifiers, OD values, classification status and sensitivity, specificity as well as accuracy values—are provided in Additional File 1.

Based on the Agrolabo ELISA FIV Ab kit, 140 samples (85.4%) were classified as negative and 22 (13.4%) as positive. Two additional samples (1.2%) were excluded from further analysis because their OD values were inconclusive according to the manufacturer's interpretation protocol. Both samples OD results were in between positive and negative cut-off (i.e. Sample 13—OD value = 0.287, negative cut-off OD ≤ 0.249 and positive cut-off: OD ≥ 0.329; Sample 106—OD value = 0.255, negative cut-off OD ≤ 0.253 and positive cut-off: OD ≥ 0.333) calculated according to protocol.

The same set of samples, excluding the two with indeterminate results, was tested using the in-house ELISA FIV Ab platform. Classification was based on a cut-off established during assay optimization (see Additional File 2 for detailed information about cut-off determination).

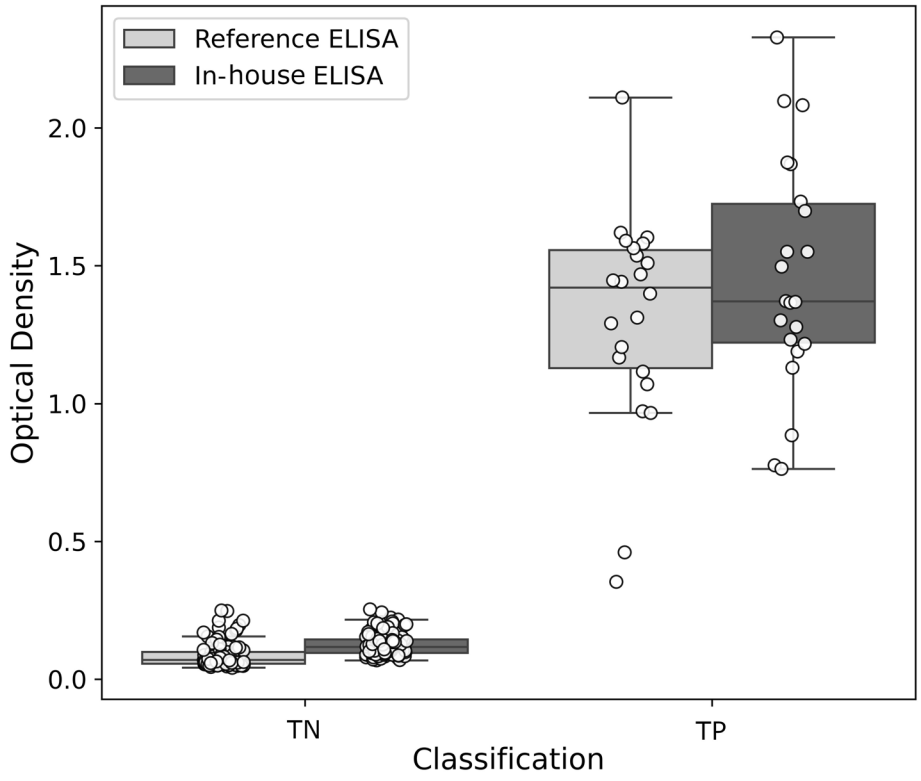


Fig. 1 Comparison of optical density values obtained with the reference ELISA and the in-house ELISA FIV Ab for samples classified as true negatives (TN) and true positives (TP). Measurements were performed at a wavelength of 450 nm. The boxes illustrate the central tendency of the OD values, showing median and the interquartile range (IQR). Whiskers extend to data points within 1.5 times the IQR from the lower and upper quartiles, indicating the spread of the data. Outliers are plotted as individual points and represent values that fall outside this range

Table 1 Summary of the classification of feline samples tested for FIV antibodies using two ELISA methods: a commercial Agrolabo ELISA FIV Ab kit and an in-house ELISA FIV Ab. Samples were categorized as positive, negative, or excluded, based on the measured OD value. For each category, the mean optical density (Mean OD) and standard deviation (SD) are reported. Excluded samples showed intermediate OD values that did not clearly fall within the defined cut-off ranges and were therefore not included in the final analysis. The percentage of concordantly classified samples between the two assays was 100% for both positive and negative categories, indicating complete agreement in classification

Classification of samples	Agrolabo ELISA FIV Ab			In-house ELISA FIV Ab			Percentage of concordantly classified samples
	Number of samples	Mean OD	SD	Number of samples	Mean OD	SD	
Positive	22	1.308	0.381	22	1.461	0.410	100%
Negative	140	0.084	0.041	140	0.125	0.038	100%
Excluded	2	0.271	*NA*	*NA*	*NA*	*NA*	*NA*

Agrolabo ELISA FIV Ab positive cut-off: OD ≥ 0.329–0.333

Agrolabo ELISA FIV Ab negative cut-off: OD ≤ 0.249–0.253

In-house ELISA FIV Ab positive cut-off: OD > 0.263

The in-house assay categorized 22 samples as positive and 140 as negative, achieving 100% concordance with the reference method. The calculated cut-off value of 0.263 proved to be an effective threshold for distinguishing positive from negative samples.

Diagnostic performance of LFAs

Results indicate that all evaluated tests (i.e.: SNAP®, Fassisi, FASTest, Vet Expert together with a new version of Vet Expert test, UranoVet, AllTest, Anigen and VetFor) correctly identified negative samples ($n = 11$), as no false-positive results were observed. Among positive samples ($n = 39$), only two tests—Vet Expert new and

VetFor— detected all positives in this set. UranoVet and AllTest identified antibodies in 38 positive samples, each yielding one false negative (Sample ID 12). The remaining tests detected 37 positive samples, resulting in two false negatives (Sample IDs 12 and 29) (Table 2).

Based on these results, diagnostic parameters—including sensitivity, specificity, and overall accuracy—were calculated for each LFA (Table 3). All tests demonstrated 100% specificity; however, only Vet Expert new and VetFor achieved 100% sensitivity, resulting in perfect accuracy. AllTest and UranoVet showed slightly lower performance, with sensitivity of 97.44% and overall accuracy of 98%. SNAP, Fassisi, FastTest, Vet Expert, and Anigen exhibited sensitivity of 94.87%, corresponding to an accuracy of 96%. These differences are minor and statistically insignificant, reflecting the overall high and comparable performance of all kits (sensitivity ranging from 94.87% to 100%, specificity consistently at 100%).

Predictive values were calculated for hypothetical FIV prevalence scenarios designed to resemble natural populations. The negative predictive value (NPV) represents the probability that a subject with a negative test result is truly free of disease. As shown in Table 4, higher FIV seroprevalence is associated with a reduced likelihood that a negative result accurately reflects the absence of antibodies. Across all prevalence scenarios, the PPV, representing the probability that a subject with a positive test result truly has the disease, remained consistently at 100%. A new version of Vet Expert test and VetFor maintained also 100% NPV. In contrast, other LFAs exhibited a gradual decline in NPV with increasing prevalence. For UranoVet and AllTest, NPV decreased from 99.99% at 1% prevalence to 99.86% at 5%, while SNAP, Fassisi, FastTest, Vet Expert, and Anigen showed a reduction from 99.99% to 99.73% over the same range.

Discussion

Selecting the appropriate diagnostic method is not merely a technical decision, it is a vital component of feline healthcare, influencing early detection, disease management, and long-term well-being. In both clinical and research settings, the reliability of diagnostic tools directly impacts treatment decisions, epidemiological understanding, and the maintenance of animal health. It is even more important when taking into consideration a local prevalence of tested pathogen.

All lateral flow assays evaluated in this study performed well, achieving 100% specificity, highlighting their robust ability to correctly identify negative samples. Sensitivity was slightly more variable, ranging from 94.87% to 100%, yet these values remain highly satisfactory. These findings are consistent with the results reported by Levy et al. and Pawelczak et al., who found that commercially available FIV tests exhibit high sensitivity and specificity, whereas performance of FeLV tests was more variable [8, 23]. The observed discrepancies were confined to two samples that showed weakly positive signals in ELISA, indicating that detection of low-level positives may be more challenging and could occasionally result in false-negative outcomes. Notably, in the context of LFA testing, a new version of Vet Expert (Vet Expert new) test achieved both 100% sensitivity and specificity, correctly classifying all samples, whereas the previous version (Vet Expert) failed to detect the two aforementioned samples, reflecting a marked improvement in assay performance.

It should be noted that this study was conducted on a limited sample set using an enrichment strategy with positive specimens, an approach that has been previously employed in similar studies [8, 23]. While such enrichment does not reflect routine clinical practice, it enables robust evaluation of sensitivity and specificity. Predictive values were subsequently recalculated for several plausible prevalence scenarios (1%, 3%, 5%) to provide meaningful insights into test performance in real-world populations [24]. The negative predictive value is particularly relevant when ruling out disease, as it expresses the likelihood that a negative result corresponds to the absence of infection. As shown in Table 4, NPV decreases as seroprevalence rises, meaning that in populations where FIV is more common, a negative result becomes less certain. Conversely, the positive predictive value remains consistently high across all scenarios, indicating that positive results are highly reliable regardless of prevalence. This pattern underscores the importance of considering population-level prevalence when interpreting diagnostic outcomes and highlights why predictive values should complement sensitivity and specificity in evaluating test performance [25–27].

As highlighted by Ben-Haim & Dacso (2024), PPV and NPV are strongly dependent on disease prevalence. In this study, an enriched sample set was used to calculate predictive values across hypothetical prevalence scenarios; however, because current data on FIV prevalence remain incomplete, these estimates may not accurately reflect performance in real-world populations [28]. Therefore, further studies are needed to determine FIV prevalence across different populations. In large-scale epidemiological or screening studies, ELISA is commonly used, and the assay described in this study appears well-suited for such research applications, providing a reliable tool for assessing infection status across diverse settings.

However, clinical interpretation of serological results remains challenging due to several confounding factors, including cross-reactivity with other pathogens, low antibody titers during early stages of infection – particularly when testing too soon after exposure – which may result in an undetectable immune response [15, 29]. Tested material – blood, serum or plasma, may also influence

Table 2 Summary of in-house ELISA FIV Ab test results for feline samples submitted for subsequent LFA analysis, including optical density measurements and classification outcomes

Sample ID	OD Value	ELISA Classification	SNAP	Fassisi	FastTest	Vet Expert	Vet Expert new	UranoVet	AllTest	Anigen	VetFor
1	1.821	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
2	1.585	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
3	1.502	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
4	1.627	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
5	1.507	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
6	1.557	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
7	0.097	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
8	0.106	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
9	1.224	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
10	2.063	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
11	1.779	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
12	0.326	P	FN	FN	FN	FN	TP	TP	TP	FN	TP
13	1.427	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
14	1.595	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
15	0.268	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
16	0.129	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
17	2.194	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
18	1.594	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
19	1.481	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
20	1.857	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
21	1.447	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
22	1.503	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
23	1.664	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
24	2.090	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
25	1.685	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
26	1.657	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
27	1.679	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
28	2.028	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
29	0.297	P	FN	FN	FN	FN	TP	FN	FN	FN	TP
30	2.000	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
31	1.253	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
32	1.726	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
33	1.977	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
34	1.865	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
35	1.702	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
36	1.263	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
37	1.454	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
38	1.617	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
39	1.819	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
40	1.822	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
41	0.084	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
42	0.108	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
43	0.198	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
44	0.086	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
45	0.939	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
46	1.057	P	TP	TP	TP	TP	TP	TP	TP	TP	TP
47	0.090	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
48	0.104	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
49	0.102	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
50	0.106	N	TN	TN	TN	TN	TN	TN	TN	TN	TN
ELISA positive cut-off: OD > 0.263											
Sum of TP			37	37	37	37	39	38	38	37	39

Table 2 (continued)

Sample ID	OD Value	ELISA Classification	SNAP	Fassisi	FastTest	Vet Expert	Vet Expert new	UranoVet	AllTest	Anigen	VetFor
Sum of TN			11	11	11	11	11	11	11	11	11
Sum of FP			0	0	0	0	0	0	0	0	0
Sum of FN			2	2	2	2	0	1	1	2	0

Abbreviations: *P* Positive, *N* Negative, *TP* True Positive, *TN* True Negative, *FP* False Positive, *FN* False Negative

Table 3 Diagnostic performance of nine commercial lateral flow assays (LFAs) for FIV antibody detection in feline samples ($n = 50$), including sensitivity, specificity, and overall accuracy

	SNAP	Fassisi	FastTest	Vet Expert	Vet Expert new	UranoVet	AllTest	Anigen	VetFor
Sensitivity (%)	94.87	94.87	94.87	94.87	100.00	97.44	97.44	94.87	100.00
Specificity (%)	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Accuracy (%)	96.00	96.00	96.00	96.00	100.00	98.00	98.00	96.00	100.00

Table 4 Positive predictive value (PPV) and negative predictive value (NPV) for nine FIV antibody tests across three assumed seroprevalence scenarios (1%, 3%, and 5%)

Prevalence	Diagnostic measure	SNAP	Fassisi	FastTest	Vet Expert	Vet Expert new	UranoVet	AllTest	Anigen	VetFor
1%	PPV	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	NPV	99.99	99.99	99.99	99.99	100.00	99.99	99.99	99.99	100.00
3%	PPV	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	NPV	99.84	99.84	99.84	99.84	100.00	99.92	99.92	99.84	100.00
5%	PPV	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	NPV	99.73	99.73	99.73	99.73	100.00	99.86	99.86	99.73	100.00

obtained results [30]. Furthermore, some studies suggest that vaccination can lead to false-positive results [13]; however, researchers indicate that it is possible to distinguish antibodies induced by vaccination from those generated by natural infection [31–33].

It is important to emphasize that the interpretation of any diagnostic test result should always be made in the context of the individual patient and clinical presentation. In fact, most manufacturers explicitly recommend that test results be interpreted alongside clinical findings and other relevant information available to the attending veterinarian, who is best positioned to integrate test results with clinical judgment and patient history. They also emphasize that in cases where clinical symptoms persist despite a negative result, further diagnostic testing should be considered, as a negative result does not definitively rule out infection, which is particularly relevant in this study, where not all LFAs tests detected all positive samples.

Although most commercially available LFAs may not offer ideal diagnostic accuracy, their ease of use, relatively low cost, and rapid result delivery provide veterinarians and pet owners with a valuable opportunity to assess an animal's health status during a single visit. This represents a significant improvement over waiting for results from reference methods such as ELISA, Western blot, or PCR, which are typically conducted in diagnostic laboratories and require sample transportation—thereby extending the time to diagnosis by several days. Consequently, LFA tools are widely used in most veterinary clinics and animal shelters [34, 35].

Despite the strengths of this study, several limitations warrant consideration. Our analysis was conducted on a relatively small, enriched sample set of 50 positive and negative specimens, which, while sufficient to allow meaningful head-to-head comparison, may limit extrapolation to broader populations. Detailed information on sample characteristics—including vaccination status, geographic origin, and viral lineage—was not available, restricting evaluation of test performance across diverse settings or in early-infection scenarios with low antibody titers. Furthermore, the number of samples is modest compared with larger validation studies; however, the comprehensive comparison of nine distinct diagnostic tests provides a unique and valuable perspective on relative test performance. These considerations highlight opportunities for future multi-site studies with larger and more diverse cohorts to confirm and expand upon our findings, while the current work delivers an important, robust foundation for informed selection of FIV diagnostic assays.

Our findings indicate that LFAs designed to detect FIV antibodies in whole blood, serum, or plasma are generally reliable diagnostic tools. However, slight differences in performance exist among available products. While Vet Expert new and VetFor achieved 100% across all metrics indicating the best performance, formal statistical comparison did not reveal significant differences between the evaluated kits. Overall, the results confirm that all tested

LFAs offer comparable reliability, even where slight differences in favor of Vet Expert new and VetFor were observed.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12917-026-05303-3.

Supplementary Material 1.

Supplementary Material 2.

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Not applicable.

Authors' contributions
A.L.-M. and P.P. designed in-house ELISA FIV Ab. A.L.-M. and P.P. developed in-house ELISA FIV Ab. A.L.-M., P.P. and A.B designed a new version of Vet Expert FIV Ab LFA. A.L.-M. and P.P. developed a new version of Vet Expert FIV Ab LFA. A.L.-M. and P.P. performed the experiments. A.L.-M., P.P., M.J., A.B., M.S. analyzed the data and interpreted the results. A.L.-M. and P.P. prepared the figure, tables and additional files. A.L.-M., P.P., M.J., A.B., M.S. drafted, edited, and revised the manuscript. All authors reviewed and approved the final version of the manuscript. M.J. obtained funding. M.J. and A.B. supervised the project.

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Data availability
All data generated or analyzed during this study are included in this published article and its additional information files.

Declarations

Ethics approval and consent to participate
Not applicable. This study did not involve live animals, and all samples were surplus material obtained after routine clinical procedures.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Akhtar N, et al. Feline immunodeficiency virus: current insights into pathogenesis, clinical impact, and advances in treatment and vaccine development. Front Vet Sci. 2025;12:1665999. https://doi.org/10.3389/FVETS.2025.1665999.
2. M. Nehring, E. M. Dickmann, K. Billington, and S. VandeWoude, "Study of feline immunodeficiency virus prevalence and expert opinions on standards of care," J Feline Med Surg. 2024;26(7). https://doi.org/10.1177/1098612X241245046.
3. H. J. Munro, L. Berghuis, A. S. Lang, L. Rogers, and H. Whitney, "Seroprevalence of feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) in shelter cats on the island of Newfoundland, Canada," Canadian Journal of Veterinary Research. 2025;78(2):140, 2014. Accessed: Oct. 28, 2025. [Online]. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC3962277/
4. Little S, et al. 2020 AAFP feline retrovirus testing and management guidelines. J Feline Med Surg. 2020;22(1):5–30. https://doi.org/10.1177/1098612X19895940.
5. D. Bienzle, F. Reggeti, X. Wen, S. Little, J. Hobson, and S. Kruth, "The variability of serological and molecular diagnosis of feline immunodeficiency virus infection," The Canadian Veterinary Journal. 2004;45(9):753. Accessed: Oct. 28, 2025. [Online]. Available: https://pmc.ncbi.nlm.nih.gov/articles/PMC545975/
6. Hartmann K. Feline immunodeficiency virus infection: an overview. Vet J. 1998;155(2):123–37. https://doi.org/10.1016/S1090-0233(98)80008-7.
7. Levy JK, et al. Differentiation of feline immunodeficiency virus vaccination, infection, or vaccination and infection in cats. J Vet Intern Med. 2008;22(2):330–4. https://doi.org/10.1111/J.1939-1676.2008.0078.X.
8. Levy JK, Crawford PC, Tucker SJ. Performance of 4 point-of-care screening tests for feline leukemia virus and feline immunodeficiency virus. J Vet Intern Med. 2017;31(2):521–6. https://doi.org/10.1111/JVIM.14648.
9. Frankenfeld J, et al. Decreased sensitivity of the serological detection of feline immunodeficiency virus infection potentially due to imported genetic variants. Viruses. 2019;11(8):697. https://doi.org/10.3390/V11080697.
10. Osborne R, Rigby M, Siebelink K, Neil JC, Jarrett O. Virus neutralization reveals antigenic variation among feline immunodeficiency virus isolates. J Gen Virol. 1994;75(Pt 12):3641–5. https://doi.org/10.1099/0022-1317-75-12-3641.
11. Singh S, et al. Diagnostic accuracy of a point-of-care immunoassay for feline immunodeficiency virus antibodies, feline leukemia virus antigen, and *Dirofilaria immitis* antigen. Viruses. 2023;15(10):2117. https://doi.org/10.3390/V15102117.
12. Bęczkowski PM, Beatty JA. Feline immunodeficiency virus: current knowledge and future directions. Advances in Small Animal Care. 2022;3(1):145–59. https://doi.org/10.1016/J.YASA.2022.05.007.
13. Levy JK, Crawford PC, Slater MR. Effect of vaccination against feline immunodeficiency virus on results of serologic testing in cats. J Am Vet Med Assoc. 2004;225(10):1558–61. https://doi.org/10.2460/JAVMA.2004.225.1558.
14. Miller C, et al. Fiv vaccine with receptor epitopes results in neutralizing antibodies but does not confer resistance to challenge. NPJ Vaccines. 2018;3(1):1–12. https://doi.org/10.1038/S41541-018-0051-Y.
15. Moskaluk A, Nehring M, Vande Woude S. Serum samples from co-infected and domestic cat field isolates nonspecifically bind FIV and other antigens in enzyme-linked immunosorbent assays. Pathogens. 2021;10(6):665. https://doi.org/10.3390/PATHOGENS10060665.
16. Hartmann K, et al. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. J Feline Med Surg. 2007;9(6):439–45. https://doi.org/10.1016/J.JFMS.2007.04.003.
17. I. Ferrero, P. Poletti, E. Giachino, J. Filipe, and P. Dall'Ara, "A New Rapid Indirect ELISA Test for Serological Diagnosis of Feline Immunodeficiency," Vet Sci. 2025;12(2):89. https://doi.org/10.3390/VETSCI12020089/S1.
18. Tabatabaei MS, Ahmed M. Enzyme-Linked Immunosorbent Assay (ELISA). Methods Mol Biol. 2022;2508:115–34. https://doi.org/10.1007/978-1-0716-2376-3_10.
19. Lequin RM. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem. 2005;51(12):2415–8. https://doi.org/10.1373/CLINCHEM.2005.051532.
20. Classen DC, Morningstar JM, Shanley JD. Detection of antibody to murine cytomegalovirus by enzyme-linked immunosorbent and indirect immunofluorescence assays. J Clin Microbiol. 1987;25(4):600. https://doi.org/10.1128/JCM.25.4.600-604.1987.
21. Lardeux F, Torrico G, Aliaga C. Calculation of the ELISA's cut-off based on the change-point analysis method for detection of *Trypanosoma cruzi* infection in Bolivian dogs in the absence of controls. Mem Inst Oswaldo Cruz. 2016;111(8):501. https://doi.org/10.1590/0074-02760160119.
22. Mercaldo ND, et al. Enrichment sampling for a multi-site patient survey using electronic health records and census data. J Am Med Inform Assoc. 2019;26(3):219–27. https://doi.org/10.1093/JAMIA/OCY164.
23. Pawelczak P, Laska-Modzelewska A, Jank M, Skupińska M, Belter A. A novel and superior diagnostic test for Feline Leukemia Virus: enhanced precision in rapid detecting viral antigen in blood, plasma and serum. Virol J. 2026;4. https://doi.org/10.1186/s12985-025-03015-y.
24. Aliu O, Chung KC. Assessing strength of evidence in diagnostic tests. Plast Reconstr Surg. 2012;129(6):989e. https://doi.org/10.1097/PRS.0B013E31824ECD61.
25. Habibzadeh F. Diagnostic tests performance indices: an overview. Biochem Med Zagreb. 2025;35(1):512. https://doi.org/10.11613/BM.2025.010101.
26. Vetter TR, Schober P, Mascha EJ. Diagnostic testing and decision-making: beauty is not just in the eye of the beholder. Anesth Analg. 2018;127(4):1085. https://doi.org/10.1213/ANE.0000000000003698.

27. T. F. Monaghan et al., "Foundational Statistical Principles in Medical Research: Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value," Medicina. 2021;57(5)503. https://doi.org/10.3390/MEDICINA57050503.
28. Y. Ben-Haim and C. C. Dacso, "Interpreting PPV and NPV of Diagnostic Tests with Uncertain Prevalence," Rambam Maimonides Med J. 2024;15(3). https://doi.org/10.5041/RMMJ.10527.
29. Saejung W, et al. Detection of feline immunodeficiency virus by neutral red-based loop-mediated isothermal amplification assay. Vet World. 2024;17(1):72–81. https://doi.org/10.14202/VETWORLD.2024.72-81.
30. M. J. Beall, D. Moore, S. Turner, and J. K. Levy, "Feline leukemia virus lifetime study: whole blood samples increase detection of low positive cats with extended long-term survival," J Feline Med Surg. 2025;27(11). https://doi.org/10.1177/1098612X251379219.
31. Westman ME, Malik R, Hall E, Sheehy PA, Norris JM. Determining the feline immunodeficiency virus (FIV) status of FIV-vaccinated cats using point-of-care antibody kits. Comp Immunol Microbiol Infect Dis. 2015;42:43–52. https://doi.org/10.1016/j.cimid.2015.07.004.
32. Westman M, et al. Antibody responses in cats following primary and annual vaccination against feline immunodeficiency virus (FIV) with an inactivated whole-virus vaccine (Fel-O-Vax® FIV). Viruses. 2021;13(3):470. https://doi.org/10.3390/V13030470.
33. Cheang A, Westman ME, Green J. Evaluation of a Point-of-Care Feline Immunodeficiency Virus (FIV) Test Kit (RapidSTATUS™ FIV) to Determine the FIV Status of FIV-Vaccinated and FIV-Unvaccinated Pet Cats in Australia. Vet Sci. 2022. https://doi.org/10.3390/VETSCI9110618.
34. G. Li et al., "Lateral flow immunoassays for antigens, antibodies and haptens detection," Int J Biol Macromol. 2023;242(Pt 4). https://doi.org/10.1016/j.ijbiomac.2023.125186.
35. Velayudhan BT, Naikare HK. Point-of-care testing in companion and food animal disease diagnostics. Front Vet Sci. 2022;9:1056440. https://doi.org/10.3389/FVETS.2022.1056440/BIBTEX.

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