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Evaluation of rapid diagnostic test kits for feline leukemia virus infection using samples from naturally infected cats

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Abstract

Objectives Feline leukemia virus (FeLV) is a potentially life-threatening oncogenic retrovirus. The p27 viral core protein is produced by the virus in infected feline cells, is found in the cytoplasm in several blood cells and can be free in the serum and plasma. ELISA or particle-based immunoassay are commonly used to detect the presence of the p27 core protein in samples obtained from blood. The objective of this study was to compare the performance of several in-clinic tests: the SNAP Feline Triple Test (IDEXX Laboratories), the WITNESS FeLV-FIV Test (Zoetis) and the VetScan Feline FeLV/FIV Rapid Test (Abaxis).

Methods The sample population (100 positive, 105 negative samples) consisted of serum and plasma samples submitted to IDEXX's worldwide reference laboratory for feline retrovirus testing. Virus isolation and reverse transcriptase PCR results were not available and so samples were judged to be positive or negative based on the results of the ViraCHEK FeLV (Zoetis) microtiter plate assay.

Results The percentage of samples positive and negative for FeLV p27 antigen using the three in-clinic tests compared with the ViraCHEK method were as follows: IDEXX Feline Triple (positive 98.0%, negative 100%); Zoetis WITNESS (positive 79.0%, negative 97.1%); Abaxis VetScan (positive 73.0%, negative 97.1%).

Conclusions and relevance The SNAP Feline Triple Test demonstrated a high level of agreement for FeLV-positive and FeLV-negative samples when assessed in this model. Results of FeLV assays can vary among tests.

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Introduction

Feline leukemia virus (FeLV) is a highly contagious virus that is readily spread among cats in casual close contact, which can include sharing food and water, as well as mutual grooming.[1] Approximately one-third of cats exposed to FeLV develop progressive viremia and most die of FeLV-related diseases within 3 years. The diseases associated with chronic FeLV infection include lymphomas, leukemias, anemia and infectious diseases that can be potentiated by immunosuppressive effects of the virus. Different clinical courses, stages and outcomes of FeLV infection are possible in individual cats depending primarily on the immune status, genetic makeup, age of the cat and the pathogenicity and infectious dose of the virus.[1–3]

The widespread use of in-clinic tests for circulating FeLV antigen along with the introduction of effective vaccines are generally assumed to be responsible for the reported reduction in the prevalence of FeLV during the past 20 years.[1] There are several commercial vaccines that have been shown to protect cats from

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FeLV-associated disease and prolong life.[1,4–6] Nonetheless, testing and removal of infected cats is the mainstay of preventing transmission and vaccination should not be considered as a substitute for testing.[1] The American Association of Feline Practitioners' Feline Retrovirus Management Guidelines state that the identification and segregation of infected cats is the single most effective method for preventing infections with FeLV.[1] The FeLV infection status of all cats, including vaccinated cats, should be determined. Cats should be tested for FeLV infection at the time of acquisition, following exposure to an infected cat or a cat of unknown infection status, prior to vaccination, prior to entering group housing and when becoming ill.[1]

The p27 core viral antigen is the target used for in-clinic diagnostic testing for FeLV.[2,7] The antigen is produced during the early primary viremia stage typically within 30 days of infection and throughout all stages of the infection in progressively infected cats. Reference laboratories and veterinary clinics use anti-FeLV antibodies and ELISA or colloidal particle-based assays to detect the presence of FeLV antigen. In general, reference laboratories utilize microtiter well format ELISAs to accommodate a large number of samples, while clinics prefer single-use delivery formats.[8] As accurate diagnosis of FeLV-infected cats is essential in the management and control of FeLV, the use of sensitive assays is paramount. Test sensitivity can be important for detection of low antigen levels which are possible during the course of different stages of infection and following different infection outcomes.[1–3] Failure to identify infected cats could delay supportive care and result in the transmission of the virus to causal-contact cats. However, as FeLV-positive cats are also euthanized in some situations, specificity of diagnostic assays is of equal importance.

The objective of this study was to determine the extent of agreement of three in-clinic diagnostic tests: SNAP Feline Triple Test (IDEXX), WITNESS FeLV-FIV Test (Zoetis) and VetScan Feline FeLV/FIV Rapid Test (Abaxis) for FeLV antigen with respect to results from a microtiter-based format assay (ViraCHEK FeLV [Zoetis]).

Materials and methods

Test samples

A total of 100 positive and approximately the same number of negative samples were intended to be used in this study. Serum or plasma samples were sourced from an international network of IDEXX reference laboratories (IRL) by a single criterion that they were initially submitted by practicing veterinarians for feline retroviral testing. The requested diagnostic tests included the immunofluorescence assay, PCR or ELISA (PetChek FeLV); however, as noted below, the IRL test results were not taken into account for selection of the samples for this study. All samples that remained with sufficient volume after the requested diagnostic testing were collected and kept at −20°C or lower during transportation and storage. Samples were screened for the presence of FeLV p27 antigen using the microtiter plate ViraCHEK FeLV ELISA (Zoetis) according to manufacturer's instructions. A total of 100 positive samples were identified from this screening, along with a large number of negative samples. From the negative sample set, 105 samples were randomly selected for this study. Samples were then randomized and blind-labeled before testing with three rapid in-clinic test kits following instructions supplied with the test kits. There was no additional freeze and thaw cycle or sample handling differences between the ViraCHEK FeLV reference assay testing and evaluation by the three in-clinic test kits.

Data collection and analysis

The in-clinic test kits included the SNAP Feline Triple Test, the WITNESS FeLV-FIV Test and the VetScan Feline FeLV/FIV Rapid Test. Each test result was interpreted independently by three laboratory technicians without knowledge of the ViraCHEK reference assay results. Samples were judged to be positive or negative by majority agreement (two or more readers) of the three visual interpretations. Results of virus isolation, frequently used as the gold standard for determination of the sensitivity and specificity of FeLV p27 antigen tests, were not available for these samples. Results of reverse transcriptase PCR were available for only 11 samples. PetChek FeLV ELISA and SNAP Feline Triple use the same diagnostic reagents; therefore, results of the PetChek FeLV ELISA were not considered to avoid sample selection bias. Thus, we chose to use the ViraCHEK FeLV microtiter plate assay as a reference method as it has shown high sensitivity and specificity compared with virus isolation.[9] The percent positive (negative) agreement for each of the in-clinic assays was calculated as the number of samples read positive (negative) by the in-clinic test divided by those deemed positive (negative) by the ViraCHEK reference assay.

Statistical analysis was performed in SAS version 9.4. To determine if differences in percentage of samples found positive or negative by the in-office tests were statistically significant, McNemar's exact tests for paired comparisons were performed on 2×2 contingency tables of samples which were deemed positive (or negative) by the reference method. To adjust for multiple comparisons, we used the step-down Holm–Bonferroni method applied to the exact McNemar P values, which allowed us to control for the family-wise error rate in the strong sense without independence assumption. Clopper–Pearson (exact) confidence intervals for the percentage of samples positive and negative compared with the

ViraCHEK method were also calculated. The κ statistic and the corresponding confidence interval for each of the three tests were calculated. McNemar's tests, Clopper–Pearson confidence intervals, κ statistics and confidence levels were carried out using the FREQ procedure in SAS 9.4. The post-hoc power calculation was performed by simulating the experiment 1000 times using a positive agreement of 98% for the IDEXX test and 75% for the competitor tests. Paired analyses were assessed to determine the frequency of statistically significant differences using a probability significance cutoff of $P < 0.0167$, which was corrected for multiple comparisons using a Bonferroni adjustment.

Results

Each device was read by three readers who recorded the same result for 95.6% of the SNAP Feline Triple Tests, 91.7% of WITNESS FeLV-FIV Tests and 96.0% of VetScan Feline FeLV/FIV Rapid Tests. There were no invalid test results.

Table 1 shows the percent positive and negative agreement and the κ agreement statistic for the in-clinic FeLV antigen testing devices compared with the ViraCHEK reference assay. There were two samples that were positive in the ViraCHEK reference assay and negative in all three in-clinic tests. Among the remaining 98 ViraCHEK reference assay-positive samples, all were positive in the SNAP Feline Triple assay, 19 were negative in both the WITNESS FeLV-FIV assay and the VetScan FeLV/FIV Rapid Test and an additional six were negative in the VetScan FeLV/FIV Rapid Test alone. Among 105 ViraCHEK reference assay-negative samples, all were negative in SNAP Feline Triple, three were positive in the Witness FeLV-FIV assay, and three different samples were positive in the VetScan FeLV/FIV Rapid Assay.

SNAP Feline Triple had 98.0% agreement with the ViraCHEK reference assay for positive samples, which was significantly greater than the percent agreement for the WITNESS FeLV-FIV Test (79.0%) or the VetScan Feline FeLV/FIV Rapid Test (73.0%) (both raw McNemar's exact test and step-down Bonferroni adjusted two-sided $P < 0.00001$).

The SNAP Feline Triple showed strong overall agreement with the ViraCHEK reference assay (κ statistic = 0.981) and was greater than the WITNESS FeLV-FIV Test (κ statistic = 0.765) and the VetScan Feline FeLV/FIV Rapid Test (κ statistic = 0.706). A post-hoc power calculation was performed using experimental simulation as described in the 'Materials and methods'. A statistically significant difference was found in 995 of 1000 simulations, suggesting that the power of the study was approximately 99.5%.

Discussion

FeLV test sensitivity and specificity studies frequently use results of virus isolation as the reference test.[9] In this study, results of virus isolation and molecular assays were not available and is the major limitation of the study. All four assays used in this study detect p27 antigen. We defined the ViraCHEK FeLV microtiter plate assay as the reference test for two reasons. First, this assay had high sensitivity (94.9%) and specificity (98.4%) when compared with virus isolation in an independent study,[9] and, second, this assay detects the same circulating antigen as does each of the three in-clinic tests evaluated in this comparative study.

There were two samples that were positive in the ViraCHEK reference assay and negative in all three in-clinic tests. Given the previously reported specificity of 98.4%, these may represent ViraCHEK false-positives. Three of 105 ViraCHEK reference assay-negative cats had positive test results when evaluated with the WITNESS FeLV-FIV Test and three different cats were positive in the VetScan Feline FeLV/FIV Rapid Test. Each of these samples was negative in the ViraCHEK assay and in the two remaining in-clinic tests, suggesting that these may be false-positives. Thus, cats at low risk for FeLV infection or no evidence of FeLV-associated diseases with positive test results should have performance of confirmation tests considered before making significant clinical decisions like euthanasia.

Table 1 Percentage of samples positive and negative for feline leukemia virus (FeLV) p27 antigen and Kappa statistic for the three in-clinic tests compared with the ViraCHEK reference assay

Tests	SNAP Feline Triple (IDEXX)	WITNESS FeLV-FIV (Zoetis)	VetScan Feline FeLV/FIV (Abaxis)
Percent positive agreement	98	79	73
95% CI	93.0–99.8	69.7–86.5	63.2–81.4
Percent negative agreement	100	97.1	97.1
95% CI	96.4–100	91.9–99.4	91.9–99.4
Kappa statistic	0.981	0.765	0.706
95% CI	0.95–1.00	0.68–0.85	0.61–0.80

CI = confidence interval; FIV = feline immunodeficiency virus

Given the importance of identifying FeLV-infected cats, the use of a screening test with high diagnostic sensitivity is imperative.[1–3] In this study, among the three tests evaluated, SNAP Feline Triple had a significantly higher percent positive agreement compared with results of the ViraCHEK reference assay. These findings are significant because in a clinical setting test results can be the only mechanism to identify viremic cats and false-negative results could delay administration of supportive care and result in transmission to causal-contact cats.

Further studies will be required to determine the sensitivity and specificity of the current derivations of these assays when compared with a true gold standard.

Conclusions

The SNAP Feline Triple Test demonstrated a high level of agreement for FeLV-positive and FeLV-negative samples when compared with results of the ViraCHEK reference assay. The percent positive agreement was significantly greater than the WITNESS FeLV-FIV Test and the VetScan Feline FeLV/FIV Rapid Test. Results of different in-clinic FeLV assays can vary significantly among tests which can lead to clinical issues if false-positive or false-negatives occur.

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