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Diagnostic performances of two rapid tests for detection of feline leukemia virus antigen in sera of experimentally feline leukemia virus-infected cats

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Abstract

Objectives The objective of this study was to compare the diagnostic sensitivities and specificities of WITNESS FeLV-FIV (Zoetis) and SNAP FIV/FeLV Combo Test (IDEXX) for the detection of FeLV p27 antigen in the sera of experimentally feline leukemia virus (FeLV)-infected cats.

Methods Diagnostic sensitivities of WITNESS and SNAP were determined through testing of 47 serum samples collected from cats day 56 post-experimental infection with a virulent FeLV Rickard strain. Successful experimental infection was confirmed based on observation of FeLV antigen and proviral DNA in anti-coagulated (EDTA) whole-blood samples by immunofluorescent antibody (IFA) test and PCR, respectively. Diagnostic specificities of both tests were determined through testing of sera of 92 laboratory-housed, non-FeLV-exposed specific pathogen-free (SPF) cats.

Results Forty-one of 47 blood samples were IFA positive, whereas all 47 samples were PCR positive. All 92 non-FeLV-infected SPF cats were IFA and PCR negative. In comparison to IFA as the reference method, both WITNESS and SNAP tests yielded equivalent sensitivities and specificities of 100% and 97.8%, respectively. In comparison to PCR as the reference method, both WITNESS and SNAP tests likewise performed equivalently, with sensitivities and specificities of 91.5% and 100%, respectively.

Conclusions and relevance Sensitivity and specificity of WITNESS FeLV-FIV for identifying FeLV p27 antigen in the sera of these experimentally FeLV-infected and non-FeLV-exposed SPF cats equaled those of the SNAP FIV/FeLV Combo Test. However, all positive results, regardless of the point-of-care test used, should be confirmed before making clinical decisions such as segregation from other cats or euthanasia.

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Introduction

Feline leukemia virus (FeLV) of the Retroviridae family is an important infectious agent of cats. The virus is often shed in saliva, nasal discharges, urine, feces and milk of persistently infected cats.[1–5] Transmission to susceptible cats not only occurs via the oronasal route during mutual grooming, but can also occur through bites.[3,4] Although seroprevalence of FeLV p27 antigen varies, a seroepidemiologic survey in North America using >18,000 client-owned and sheltered cats indicated a seroprevalence rate of 2.3%.[6] Failure to identify FeLV-infected cats may lead to inadvertent exposure and transmission to uninfected cats, and misdiagnosis of infection in uninfected

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cats may lead to unnecessary segregation, requirement of adoption into a single-cat household or euthanasia.[3] Veterinarians and pet owners may opt to euthanize FeLV p27 antigen-positive cats over the concern that the cats may have progressive infections and therefore will likely die within 18–36 months of diagnosis.[3,4] Although positive antigen test results should ideally be confirmed, confirmatory testing may not be feasible for some shelter organizations because of increased cost and difficulty with the interpretation of possible discordant test results.[3] Therefore, accurate diagnostic tests are a prerequisite to identifying and treating infected cats and to preventing transmission.

Virus isolation is the gold standard for the diagnosis of FeLV infection.[3] However, it is laborious and impractical for clinical application; isolation may require up to 10 days and many laboratories do not have expertise for such lengthy stays.[5] Most of the serological tests detect the presence or absence of either p27 core viral antigen by an ELISA or viral antigens in the white blood cells by an immunofluorescence antibody (IFA) test.[3,5,7] The p27 antigen is produced during the early primary viremic stage, typically within 14–30 days of infection and throughout all stages of the infection in progressively infected cats.[3,8,9] Commercial reference laboratories commonly utilize microwell-based ELISAs, IFA tests and PCR to detect the proviral (FeLV DNA) load or viral (FeLV RNA) load for both screening and confirmatory testing. However, because of the laborious and/or cost-intensive character of most of these methods, they are better used as confirmatory tests. Rapid immunochromatography and ELISA are common test formats for FeLV testing at the point of care (POC).

Depending on sources of samples and tests of reference, accuracy of POC tests for the detection of FeLV p27 antigen is generally excellent.[10] In late 2014 a dual analyte POC test – WITNESS FeLV-FIV (Zoetis) – was introduced in the USA for the detection of FeLV p27 antigen and of FIV gp40 antibodies within the serum, plasma and anticoagulated whole blood of cats. To date, variable data have been published regarding its sensitivity and specificity for the detection of p27 antigen vs those of other POC tests.[11–13] Therefore, the objective of this study was to compare the diagnostic sensitivities and specificities of two commonly used POC tests – WITNESS FeLV-FIV and SNAP FIV/FeLV Combo Test (IDEXX) – for detecting FeLV p27 antigen in the sera of experimentally FeLV-infected cats using IFA testing and PCR as references.

Materials and methods

Cats enrolled in a related study were concurrently enrolled in this study. Domestic shorthair cats were 7–8 weeks of age, consisted of both males (n = 24) and females (n = 24), and were housed in groups of 12 in four rooms. Cats were vaccinated subcutaneously with ULTRA Fel-o-Vax FVRCP (Boehringer Ingelheim) on days 0 and 21. All cats were sedated with Dexdomitor (Zoetis) administered according to manufacturer's recommendation (40 µg/kg IM) immediately prior to FeLV challenge. Challenge consisted of the administration of 2 ml virulent FeLV (Rickard strain) by the intranasal route (1 ml in each nostril) on days 33, 35, 38 and 40. The 2 ml inoculum contained $10^{6.5}$ virus particles. Thereafter, anticoagulated (EDTA) whole blood and sera were collected at weekly intervals. The Institutional Animal Care and Use Committee of Zoetis reviewed and approved this study.

Confirmation of FeLV infection in all cats was determined by IFA (National Veterinary Laboratory, Franklin Lakes, NJ, USA) and PCR testing for proviral DNA (Zoetis) using different sample types (bone marrow, buffy coats and sera) and purification method (QIAamp DNA Blood Mini Kit; Qiagen) than previously published.[14] Sera collected from cats (n = 47) at day 89 (56 days after the first challenge dose of FeLV) and also from non-FeLV-exposed, laboratory-housed, specific pathogen-free (SPF) cats (n = 92) were randomized to establish testing order and monitored for FeLV p27 antigen using the WITNESS FeLV-FIV and SNAP FIV/FeLV Combo tests as per the respective manufacturers' instructions. Day 89, 56 days post-administration of the first FeLV challenge dose, was selected for this study because day 89 was a predetermined time point for phlebotomy with the concurrent study, and 56 days after the first challenge was expected to be sufficient time for the establishment of FeLV infection. Technicians performing the diagnostic assays were masked to the true serological status of all serum samples and serum samples were tested in the same order in batches of up to 50 using the two tests to be evaluated. Samples were stored refrigerated until testing. Diagnostic sensitivity and specificity and Jeffrey's 95% confidence interval (CI) were calculated for both tests using IFA and PCR testing as reference methods (SAS version 9.4 software).

Results

Forty-seven of 48 cats were available for testing. One of the FeLV-infected cats was euthanized because of depression, dehydration and lameness approximately 1 week prior to scheduled phlebotomy on day 89, and therefore a sample from this cat was unavailable for testing. Forty-one of the remaining 47 anticoagulated whole-blood samples were IFA positive, and all 47 samples were PCR positive for proviral DNA, resulting in six discordant results (Table 1). Both the WITNESS and SNAP tests correctly identified all 41 IFA-positive samples as positive, yielding equivalent sensitivities of 100% (95% CI 94.1–100%). Both the WITNESS and SNAP tests correctly identified 43/47 PCR-positive samples, yielding equivalent sensitivities of 91.5% (95% CI 81.0–97.1%). Therefore, when compared with PCR, both tests identified 4/47

Table 1 Feline leukemia virus (FeLV) results for WITNESS FeLV-FIV and SNAP FIV/FeLV Combo tests for those samples collected from 6/47 cats experimentally infected with FeLV and yielding discordant results between immunofluorescence antibody (IFA; negative [NEG]) and PCR (positive [POS]) testing

Animal ID	Infection status	Reference tests		Point-of-care tests	
		PCR	IFA	WITNESS	SNAP
16JJY3	FeLV infected	POS	NEG	POS	NEG
16CJM7	FeLV infected	POS	NEG	NEG	NEG
16CJN5	FeLV infected	POS	NEG	NEG	NEG
16CJH2	FeLV infected	POS	NEG	POS	POS
16JCN4	FeLV infected	POS	NEG	NEG	POS
16JJX4	FeLV infected	POS	NEG	NEG	NEG

samples as 'false' negative. WITNESS, SNAP and IFA testing yielded negative results for 3/6 discordant (IFA-negative/PCR-positive) samples. Of the remaining three discordant samples, one was WITNESS positive and SNAP negative, one was WITNESS negative and SNAP positive, and one was WITNESS and SNAP positive. Specificities of both tests when referenced to either IFA or PCR were equal at 97.8% (95% CI 93.2–99.5%) and 100% (95% CI 97.1–100%), respectively.

Discussion

In the present study, sensitivity and specificity of WITNESS FeLV-FIV for identifying FeLV p27 antigen in the sera of experimentally FeLV-infected and non-FeLV-exposed SPF cats equaled those of the SNAP FIV/FeLV Combo Test. However, these results differ from those recently reported in which sensitivity of WITNESS test was less than that of SNAP test.[11–13] However, differences between the current study and the earlier studies included sources of samples and types of reference standards used. In the two of the previous studies, microwell ELISAs were used as the reference, and in the current study results of both POC tests were compared to IFA and PCR. Virus isolation is the gold standard for FeLV detection, but this method is laborious and impractical for clinical application; therefore, it was not selected as the reference in this study nor previous studies.[3,4,10–13] Furthermore, estimates of sensitivity and specificity of a test may differ depending on the reference standard used for comparison.[15] PCR is considered more sensitive than IFA for confirmation of FeLV status.[8,9,13,14] Therefore, when the results of a test using a different methodology are compared with the results obtained with PCR, the sensitivity of the test may be lower than it would be have been had the test results been compared with those obtained with IFA.[13] When PCR was used as the reference test, sensitivities for both WITNESS FeLV-FIV and SNAP FIV/FeLV Combo tests were much lower than expected in a previous study,[13] and were lower than with IFA in the current study.

Forty-one of 47 samples from the experimentally infected cats were identified as IFA positive. The six IFA-negative, PCR-positive samples may have been misidentified as IFA negative (ie, false negative) (Table 1). Three of these samples are likely falsely IFA negative because both WITNESS and SNAP yielded positive results for one sample, and, singly, WITNESS and SNAP yielded positive results for two other samples. The other three of these six samples yielded negative results for both WITNESS and SNAP; therefore, the three cats from which these samples were obtained may have had regressive infections such that circulating p27 antigen was not present. A regressive infection is one in which an infected cat is only transiently viremic (antigenemic); the cat mounts an effective immune response to clear circulating virus yet the cat continues to harbor proviral DNA within its bone marrow and yields a positive PCR result.[3,4,9,16,17] Regressive infection is the probable reason for reported decreased sensitivity of both tests as compared with PCR. At day 56 post-experimental infection, FeLV p27 antigenemia may have waned and therefore was no longer detectable. Viremia may end 2–16 weeks post-infection.[4,8] In a survey of cats in southern Germany, occurrence of regressive infections – p27 antigen negative and proviral DNA positive – was reported at 1.2%.[17]

Two of the 48 FeLV-infected cats developed clinical signs through day 201, the termination of the concurrent study for which these cats were enrolled. In addition to the cat mentioned above that was euthanized approximately 1 week prior to scheduled day 89 phlebotomy, a second cat was euthanized approximately 1 month after day 89 phlebotomy. This cat was anorexic, dehydrated and lethargic. Blood and serum collected from this cat yielded positive results for all tests – PCR, IFA, WITNESS and SNAP – confirming a progressive infection.[3,4,9,16,17]

Conclusions

In this study, the sensitivity and specificity of WITNESS FeLV-FIV for identifying FeLV p27 antigen in the sera of FeLV experimentally infected and non-FeLV-exposed

SPF cats are equivalent to those of SNAP FIV/FeLV Combo Test when results were compared to IFA and PCR. Because the consequences of a positive test result are significant (eg, unnecessary segregation from other cats, euthanasia), all positive results, regardless of the POC test used, should be confirmed before veterinarians make clinical decisions.

Conflict of interest All authors are employed by Zoetis and WITNESS FeLV-FIV is a product of the company with a business and/or financial interest.

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