AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
SMALL ANIMALS

REVIEW

Diagnosing feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infection: an update for clinicians

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With the commercial release in Australia in 2004 of a vaccine against feline immunodeficiency virus (FIV; Fel-O-Vax FIV®), the landscape for FIV diagnostics shifted substantially. Point-of-care (PoC) antibody detection kits, which had been the mainstay for diagnosing FIV infection since the early 1990s, were no longer considered accurate to use in FIV-vaccinated cats, because of the production of vaccine-induced antibodies that were considered indistinguishable from those produced in natural FIV infections. Consequently, attention shifted to alternative diagnostic methods such as nucleic acid detection. However, over the past 5 years we have published a series of studies emphasising that FIV PoC test kits vary in their methodology, resulting in differing accuracy in FIV-vaccinated cats. Importantly, we demonstrated that two commercially available FIV antibody test kits (Witness™ and Anigen Rapid™) were able to accurately distinguish between FIV-vaccinated and FIV-infected cats, concluding that testing with either kit offers an alternative to PCR testing. This review summarises pertinent findings from our work published in a variety of peer-reviewed research journals to inform veterinarians (particularly veterinarians in Australia, New Zealand and Japan, where the FIV vaccine is currently commercially available) about how the approach to the diagnosis of FIV infection has shifted. Included in this review is our work investigating the performance of three commercially available FIV PoC test kits in FIV-vaccinated cats and our recommendations for the diagnosis of FIV infection; the effect of primary FIV vaccination (three FIV vaccines, 4 weeks apart) on PoC test kit performance; our recommendations regarding annual testing of FIV-vaccinated cats to detect 'vaccine breakthroughs'; and the potential off-label use of saliva for the diagnosis of FIV infection using some FIV PoC test kits. We also investigated the accuracy of the same three brands of test kits for feline leukaemia virus (FeLV) diagnosis, using both blood and saliva as diagnostic specimens. Based on these results, we discuss our recommendations for confirmatory testing when veterinarians are presented with a positive FeLV PoC test kit result. Finally, we conclude with our results from the largest and most recent FIV and FeLV seroprevalence study conducted in Australia to date.

Keywords feline immunodeficiency virus; feline leukaemia virus; point-of-care diagnosis; vaccination

Abbreviations FeLV, feline leukaemia virus; FIV, feline immunodeficiency virus; PoC, point-of-care

Aust Vet J 2019;97:47–55 doi: 10.1111/avj.12781

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Structure of FIV and FeLV

Both feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) are retroviruses with a similar three-layered structure (Figure 1). The innermost layer consists of the genome–nucleoprotein complex, which contains the viral genetic material (two copies of single-stranded RNA), enzymes essential for viral activity (including integrase, reverse transcriptase and protease) and nucleocapsid protein; the middle layer consists of capsid protein surrounding the genome–nucleoprotein complex, which in turn is surrounded by a matrix protein shell; and the outer layer is the envelope from which glycoprotein 'spikes' project.[1–5]

The envelope spikes, which are composed of transmembrane and surface glycoproteins, are important for the binding of virus to cell surface receptors and thereby largely determine cell tropism, and also represent an important target for the host immune response. Detection of a humoral response to FIV transmembrane glycoprotein (gp40) with point-of-care (PoC) test kits is commonly used to diagnose FIV infection (e.g. Witness™ and Anigen Rapid™), although a humoral response to epitopes of the FIV matrix and capsid proteins (p15 and p24, respectively) also occurs and is the target for some commercially available FIV antibody detection test kits (e.g. SNAP Combo™). Detection of antibodies to FeLV is unreliable for the diagnosis of FeLV infection, because of the variable antibody response in cats and the potential for 'abortive infections', so all currently available FeLV PoC test kits detect FeLV capsid antigen (p27) (Table 1).

Pathogenesis

FIV infection

Infection with FIV results in integration of a DNA copy of the viral RNA (called provirus) into the cat's genome, resulting in lifelong infection. Three 'classic' phases of FIV infection are recognised (Figure 2).[6] The first phase is primary infection, during which the animal is viraemic and may display malaise (typically mild, but variable and occasionally severe) or present with peripheral lymphadenopathy (duration weeks to months). The second, and longest phase, is asymptomatic infection, during which viral replication is very limited and the animal is clinically healthy (duration many years; some suggest for certain cats this phase is indefinite). The third, and final, phase is secondary (terminal) infection, during which viral replication increases and clinical disease becomes apparent, in part due to a $CD4^+$ lymphocytopenia.

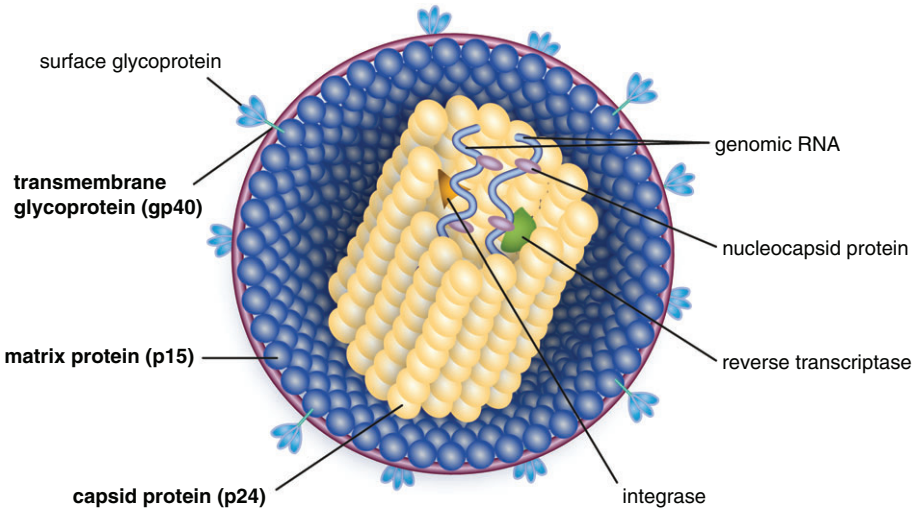

Figure 1. The basic structure of feline immunodeficiency virus. Reproduced from The Journal of Comparative Immunology, Microbiology and Infectious Diseases (Elsevier) with permission[11].

FeLV infection

Currently, three main outcomes are defined for cats following FeLV challenge.[7,8] Firstly, some cats mount a timely and appropriate immune response and eliminate the virus before it progresses beyond local replication in the oropharyngeal tissue ('abortive infections'; estimated 20–30% of exposed cats; a more likely outcome in cats exposed to FeLV at an older age). Secondly, some cats become persistently viraemic ('progressive infections'; estimated 30–40% of exposed cats; more common in kittens and younger cats). Thirdly, some cats are viraemic before mounting a partial immune response to eliminate the transient viraemia after 2–16 weeks, but not before a latent infection is established as DNA provirus in haematopoietic precursor cells in the bone marrow ('regressive infections'; estimated 30–40% of exposed cats; more common in older cats).

The estimates given regarding the rates of abortive, progressive and regressive infections in FeLV-exposed cats are based on research done under laboratory conditions using specific pathogen-free (SPF) cats and may not represent what occurs under natural field conditions, where co-infections with other pathogens and various other stressors are operating.

Unlike FIV infection, which is lifelong, some cats infected with FeLV are able to completely clear the infection (abortive infections) or partially clear the infection (regressive infections) because of effective humoral and cell-mediated immune responses working together.

Table 1. Core FIV and FeLV proteins

Protein size (kDa)	FIV	FeLV
Nucleocapsid	p10	p10
Capsid	p24*	p27*
Matrix	p15*	p15
Transmembrane glycoprotein	gp40*	p15E
Surface unit (receptor binding) glycoprotein	gp120	gp70

* Proteins important for the diagnosis of FIV infection (antibody testing) and FeLV infection (antigen testing). FeLV, feline leukaemia virus; FIV, feline immunodeficiency virus.

However, because many cats seroconvert following FeLV challenge, including some progressively infected cats, antibody testing is unable to discriminate between viraemic cats (i.e. progressive infections) and immune cats (i.e. abortive and regressive infections).[9,10] Consequently, FeLV antibody testing is currently only used in the research setting.[9,10]

FIV diagnosis using whole blood and PoC antibody test kits

The following section reviews the diagnosis of FIV infection by antibody detection using rapid PoC test kits, focusing on our own results[11] that have advanced earlier research performed by other groups.[12–14] More details of other diagnostic methods of FIV detection, including virus isolation, western blotting and PCR testing are published elsewhere.[15–26]

Prior to the commercial release of the dual subtype (A & D), inactivated whole virus FIV vaccine (Fel-O-Vax FIV®, Boehringer

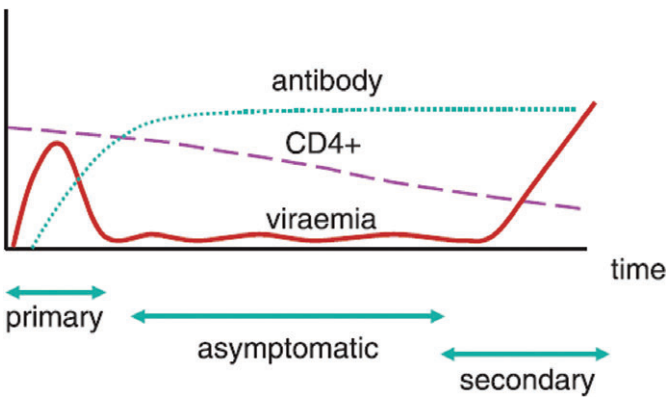

Figure 2. The three phases of feline immunodeficiency virus (FIV) infection.[6] Because of a strong and consistent host antibody response (dotted blue line) which begins during the primary phase and persists for the duration of infection, FIV antibody detection is the mainstay of all current PoC FIV diagnostic test kits. $CD4^+$ lymphocyte counts, which decline over time in FIV-infected cats, are currently not routinely measured.

Table 2. Combined results of three feline leukaemia virus (FIV) point-of-care antibody test kits and FIV RealPCR™ testing in a population of FIV-vaccinated cats (n = 119) and FIV-unvaccinated cats (n = 239), using whole blood[11]

	SNAP FIV Combo™	Witness™ FIV	Anigen Rapid™ FIV	FIV RealPCR™
Specificity (%)	64	98	98	99
Sensitivity (%)	100	100	100	92

Ingelheim, IA, USA), diagnosis of FIV infection using PoC antibody test kits was simple, inexpensive and with diagnostic accuracy comparable to western blotting analysis.[12,13] Following the release of the FIV vaccine, it was reported that FIV-vaccinated cats would test positive with FIV PoC test kits irrespective of their FIV infection status, making FIV antibody testing allegedly useless in FIV-vaccinated cats.[27,28] This diagnostic dilemma was particularly problematic in animal shelters and pounds that test for FIV infection during the adoption process, because a complete vaccination history is often unavailable for such cats. As stray and surrendered cats that test FIV-positive are often euthanased, the inability of PoC kits to discriminate between FIV-vaccinated and FIV-infected may lead to FIV-vaccinated/FIV-uninfected cats being unnecessarily killed.[15]

Our group investigated the accuracy of three commercially available FIV PoC antibody test kits and the FIV RealPCR™ assay (IDEXX Laboratories) in 119 FIV-vaccinated cats (of which 109 cats had received three or more annual FIV vaccinations before being sampled) and 239 FIV-unvaccinated cats (i.e. 358 cats in total), recruited from 12 veterinary clinics located in four states of Australia. We used a complex algorithm for the final assignment of FIV infection status, which involved consideration of all three antibody test results, the FIV RealPCR™ result (performed by IDEXX Laboratories in Brisbane, Australia) and, in rare discordant cases, virus isolation performed at the University of Florida or the University of Glasgow (when there were two positive and two negative results).[11] The three FIV PoC test kits we used were SNAP Combo™ (manufactured by IDEXX Laboratories; detects antibodies to p15 & p24); Witness™ (manufactured by Zoetis; detects antibodies to gp40); and Anigen

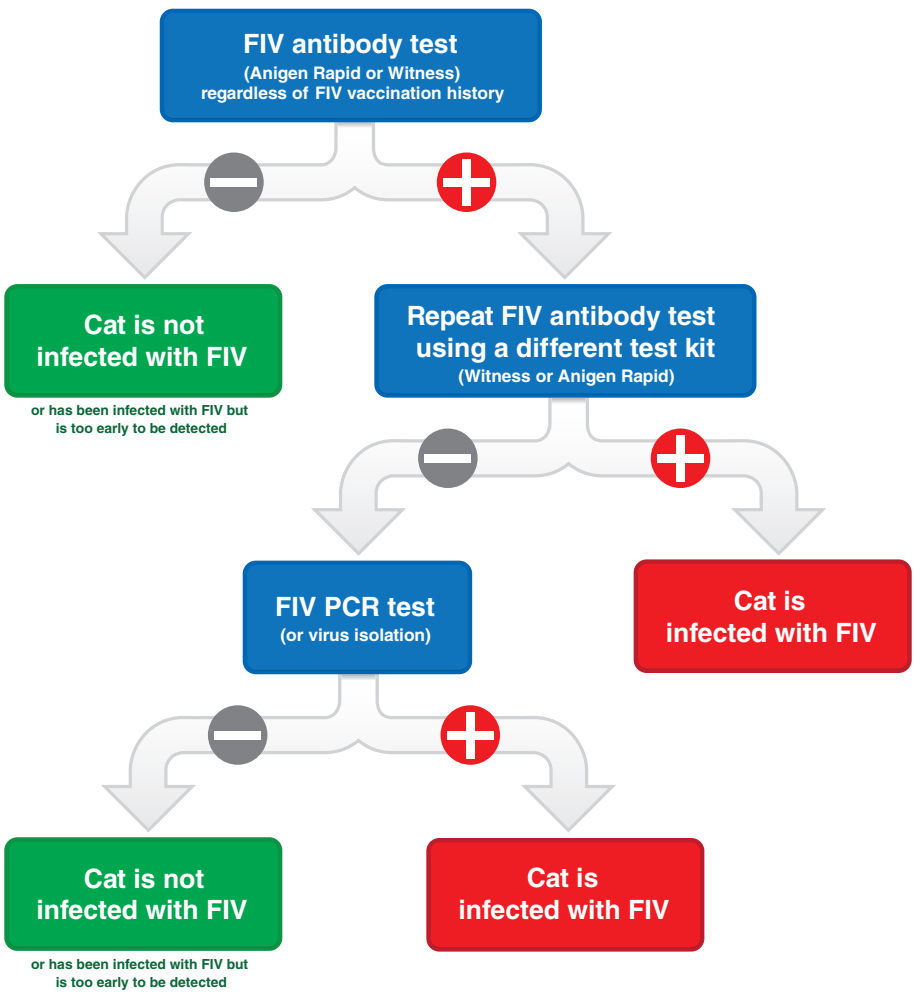

Figure 3. Suggested algorithm for diagnosis of feline immunodeficiency virus (FIV) infection.[11] If there is a possibility of recent FIV infection, re-testing is recommended because of the delay in seroconversion. Repeat testing of negative cats should be performed at least 8 weeks later for antibody testing and 4 weeks later for PCR testing, following last potential exposure.[16] Currently, virus isolation is not available for diagnostic samples in Australia.

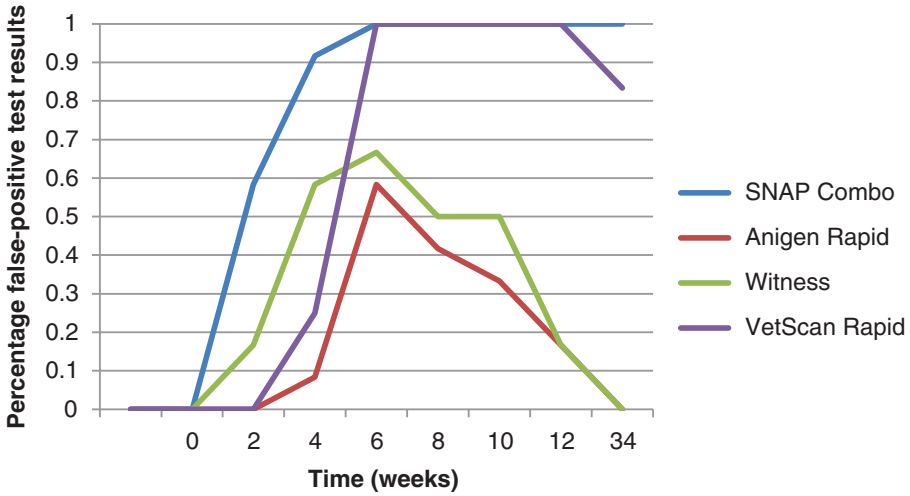

Figure 4. Summary of FIV antibody test results from 12 FIV-uninfected kittens and cats recruited for primary FIV vaccination. Fel-O-Vax FIV® was administered at 0, 4 and 8 weeks.[31] FIV, feline immunodeficiency virus.

Rapid™ (manufactured by BioNote; detects antibodies to gp40). SNAP Combo™ sold in Europe contains a third capture antigen (gp40) and we erroneously reported the Anigen Rapid™ test kit as detecting antibodies to p24 & gp40 following misinformation given by the manufacturer.[11]

Table 2 summarises our specificity and sensitivity results for each FIV PoC kit and for the FIV RealPCR™ assay. Most importantly, SNAP Combo™ could not differentiate FIV-vaccinated and FIV-infected cats and had a low overall specificity, whereas Witness™, Anigen Rapid™ and FIV RealPCR™ could differentiate in most cases. Anigen Rapid™ did not record any false-positive results in FIV-vaccinated/FIV-uninfected cats, but Witness™ recorded six false-positive results, and FIV RealPCR™ recorded four false-positive results. Repeated FIV RealPCR™ testing was required in two FIV-infected cats to ascertain their true FIV infection status, giving it a slightly lower sensitivity than testing with Witness™ and Anigen Rapid™. Consequently, we concluded that FIV antibody testing using Witness™ and Anigen Rapid™ was more accurate than FIV RealPCR™ testing and suggested it should be used as first-line FIV testing in all cats, with known or unknown FIV vaccination history, and FIV RealPCR™ testing only be used in rare cases of discordant results (Figure 3).[11] Our findings have since been reproduced overseas[29] and contributed to the upgrading of the FIV vaccine classification from 'not recommended' to 'non-core' in the most recent WSAVA Vaccination Guidelines (2015).[30]

In cats recently administered a primary course of FIV vaccination

We undertook further research to investigate if recent primary FIV vaccination (as opposed to annual FIV vaccination) would affect the results of FIV PoC antibody testing.[31] We recruited 12 kittens/cats, administered a primary course of three FIV vaccines subcutaneously 4 weeks apart (as per the manufacturer's instructions), and tested fortnightly with the same three FIV PoC antibody test kits. At 2 weeks after the second primary FIV vaccination had been administered, 67% (8/12) and 58% (7/12) of Witness™ and Anigen Rapid™ FIV results, respectively, tested false-positive because of the presence of vaccine-induced antibodies. By 6 months after the third primary FIV vaccination (week 34), none of the 12 FIV-vaccinated cats tested positive using either Witness™ or Anigen Rapid™ (Figure 4).[31] We concluded that care needs to be taken when interpreting positive Witness™ and Anigen Rapid™ FIV antibody results obtained from cats recently administered a primary course of FIV vaccination (i.e. vaccinated within the previous 6 months), and a positive test result should be followed by confirmatory FIV PCR testing or by antibody re-testing 6 months after the third primary FIV vaccination.

Based on the results from our first study, in which 109/119 recruited cats had received at least three annual FIV vaccinations,[11] we believe the transient false-positive antibody test results encountered in some cats with Witness™ and Anigen Rapid™ following a primary course of FIV vaccination do not occur following annual booster FIV vaccination and thus represent only a fraction of the lifetime of a FIV-vaccinated cat. However, more research is required to confirm this observation.

False-positive antibody results with Witness™ and Anigen Rapid™ for up to 6 months following primary FIV vaccination are not relevant when testing cats immediately prior to an annual FIV vaccination booster, because 12 months will have elapsed since the previous FIV vaccine was administered. This information is useful because it enables clinicians to quickly and easily identify FIV vaccine 'breakthroughs' prior to annual FIV vaccine administration and we know from our work that the FIV vaccine is not 100% effective in the field.[11] Our group conducted the first field study into the performance of Fel-O-Vax FIV® anywhere in the world by recruiting 301 client-owned Australian cats (89 FIV-vaccinated cases and 212 FIV-unvaccinated controls) and determining FIV infection status using the same diagnostic algorithm we used previously.[11] A protective rate (effectiveness) for the FIV vaccine of 56% was calculated and five vaccine breakthroughs were documented, including complete sequencing of the envelope gene for each breakthrough virus.[32] All five breakthrough isolates were FIV subtype-A, the most common FIV subtype found in Australian cats.[33–35]

In response to our finding that Fel-O-Vax FIV® does not provide complete protection against all FIV isolates in Australia, we recommended that cats vaccinated against FIV should undergo annual testing prior to FIV vaccination using an Anigen Rapid™ or

Witness™ FIV antibody kit, to check FIV infection has not occurred in the preceding year.[32] This is because ongoing field evaluation of the effectiveness of all vaccines across different geographic regions is good practice and, in effect, a form of pharmacovigilance. Additionally, in the absence of proven benefits for FIV-infected cats to continue to receive the FIV vaccine (e.g. suppressed viral load, delayed progression to clinical disease or protection against co-infection with other FIV subtypes), annual FIV vaccination should not be undertaken in FIV-infected cats.

FeLV diagnosis using whole blood and PoC antigen test kits

Progressively infected FeLV-positive cats behave the opposite to FIV-infected cats, in that they have a persistently high circulating viral load and an unreliable antibody response. Consequently, in-clinic diagnosis of FeLV infection relies on antigen testing rather than antibody testing. All currently available FeLV PoC test kits detect FeLV viral capsid protein (p27) and FeLV antibody testing is only performed in the research setting to provide a more complete picture of a cat's possible exposure to FeLV. Evaluation of FeLV PoC test kit performance is challenging because of the absence of a clearly defined 'gold standard' for diagnosing FeLV infection.[12,13,36] In our work to date, we have evaluated PoC test results against proviral PCR testing,[37,38] as has also been done by other researchers.[14,39] For more detail about other diagnostic methods of FeLV detection, including virus isolation, immunofluorescence for detection of FeLV cell-associated p27 antigen in circulating leukocytes and platelets, and most recently a laboratory-based ELISA to detect p27 antigen (PetChek FeLV 15™), we refer to studies published elsewhere.[7,8,16,21,40–42]

FeLV PCR testing is not without its limitations, with false-positives caused by DNA contamination during PCR set up and false-negatives caused by mutations in the targeted region of the virus being possible. However, one important advantage of PCR testing over all other currently available diagnostic methodologies is its ability to detect regressive FeLV infections. PCR testing identified regressive infections in 5–10% of Swiss cats tested,[43,44] 10% of UK cats tested,[38] 3% of Australian cats tested[45] and 1% of German cats tested.[46] The effect of regressive FeLV infections on the health of cats is largely unknown, especially in relation to infections in the field, although a possible association between regressive FeLV infections and the later development of lymphoma has been suggested.[47,48] Alarmingly, a recent study demonstrated the ability of regressively infected cats to cause progressive infections in naïve cats following a blood transfusion, highlighting the need to screen potential blood donors for regressive FeLV infection with PCR testing prior to transfusion instead of relying solely performing PoC testing.[49] Although immunofluorescence testing is still recommended as the confirmatory test of choice for FeLV diagnosis in North America,[16] PCR testing is now recommended as the confirmatory test of choice for FeLV diagnosis in Europe[7] and studies evaluating the performance of FeLV PoC test kits using PCR as the confirmatory test are becoming more common.[14,39]

We evaluated the performance of three FeLV PoC test kits commercially available in Australia (the same three brands used for FIV testing, i.e. SNAP Combo™, Witness™ and Anigen Rapid™) by recruiting 536 cats for FeLV testing (comprising 45 progressively FeLV-infected cats and 491 FeLV-uninfected cats) and using PCR testing as the reference gold standard.[50] Table 3 summarises our specificity and sensitivity results for these three FeLV PoC test kits. Regressively FeLV-infected cats (i.e. p27 antigen negative/PCR positive; n = 27) were excluded from analysis because, by definition, PoC test kits are only able to identify progressive infections (i.e. p27 antigen positive). Witness™ and Anigen Rapid™ out-performed SNAP Combo™, with both tests producing significantly less false-positive FeLV results than SNAP Combo™ (i.e. higher specificity; $P < 0.01$). Practically speaking, this meant 21–38% of positive FeLV PoC results in our study were false-positives. The rate of false-positives produced by PoC testing will vary depending on the FeLV kit used and the FeLV prevalence in the population[45,51] (which was 8% for our study because of a positive selection bias). The false-positives we recorded may have been caused by the presence of naturally occurring cat anti-mouse antibodies,[52] although all three manufacturers claim to have modified their kits to overcome this phenomenon.[50] SNAP Combo™ recorded fewer false-negatives than Witness™ and Anigen Rapid™ (i.e. higher sensitivity), although this difference was not significant ($P = 0.50$).[50]

Table 3. Results of testing using three feline leukaemia virus (FeLV) point-of-care antigen test kits commercially available in Australia using blood (n = 536), comprising 45 progressively FeLV-infected cats and 491 FeLV-uninfected cats

	SNAP FeLV Combo™	Witness™ FeLV	Anigen Rapid™ FeLV
Specificity (%)	94	98	98
Sensitivity (%)	100	91	91
PPV (%)	62	80	79
NPV (%)	100	99	99

PPV, positive predictive value; NPV, negative predictive value.[50]

Testing with two different FeLV antigen test kits (i.e. in parallel), preferably from different manufacturers, has been suggested as an option for confirmatory testing when virus isolation, immunofluorescence and ELISA testing are unavailable.[12,16,21] In our study, testing in parallel reduced, but did not completely eliminate, the occurrence of false-positives. Of 35 cats that recorded a false-positive p27 result with at least one of the PoC kits, 6 cats tested FeLV positive with two of the three kits, and 4 cats tested FeLV positive with all three kits. Of these 10 cats that tested false-positive with two or more PoC kits, 7 were displaying clinical signs consistent with FeLV-associated disease and 3 were clinically well cats.[50] We concluded that FeLV testing in parallel is not ideal for confirming infection nor is relying on the presence of clinical signs and/or haematological abnormalities consistent with progressive FeLV infection (e.g. severe anaemia and lymphadenopathy). Instead, PoC testing for FeLV infection should always be followed by confirmatory PCR testing.[7,50]

We recommend that clinicians also perform serial testing with PoC kits, because results from p27 antigen testing can change from

positive to testing negative in regressively infected cats following the 2–16-week period of transient viraemia and this has profound implications for prognosis.[7,8]

FIV and FeLV diagnosis using saliva and PoC test kits

FIV

We decided on a centrifugation technique (Figure 5), based on methodology described by another Australian research group.54 A sterile, individually packaged cotton swab mounted on a plastic rod (not a cotton or wooden rod) was rubbed against the buccal mucosa on each side of the cat's mouth, with the cheek pressed gently against the upper dental arcade while slowly twisting the swab, for approximately 10 s per side. The plastic rod was cut approximately 2 cm from the cotton tip, the tip transferred to a sterile microcentrifuge tube (with the plastic rod at the bottom of the tube and the tip facing upwards), 450 µL of sterile phosphatebuffered saline added and the tube shaken vigorously by hand for 10 s. The tube, still containing the cut cotton swab, was centrifuged for 30 s at 10,000 g. Next, the swab was removed from the tube using forceps and the supernatant was tested using the same three FIV/FeLV PoC kits used for testing with whole blood (i.e. SNAP Combo™, Witness™ and Anigen Rapid™). Testing was performed as per manufacturers' instructions except that an equivalent volume of saliva-containing supernatant was substituted for blood in the test protocol. None of the manufacturers endorses using saliva as a diagnostic specimen for their FIV/FeLV test kits.

We tested the performance of the three FIV PoC antibody test kits and the FIV RealPCR™ assay using saliva and the same cohort of FIV-vaccinated and FIV-unvaccinated cats as used for blood testing[11] (although unfortunately two FIV-vaccinated cats from the initial cohort were unavailable for saliva testing, leaving 356 cats for analysis instead of 358).[53] Saliva testing for FIV antibodies seemed a reasonable proposition, considering saliva contains some IgG (≈ 190–340-fold less IgG than plasma) owing to its gingival fluid content, which is derived from buccal mucosa capillaries.[55,56]

Our specificity and sensitivity results for each FIV PoC antibody test kit and for the FIV RealPCR™ assay using saliva instead of whole blood showed that the specificity of all FIV test methodologies was comparable (Table 4). SNAP Combo™ was the only test to record any false-positive results (six in FIV-vaccinated/FIV-uninfected cats and one in a FIV-unvaccinated/FIV-uninfected cat), but this finding did not reach statistical significance. The phenomenon of

Figure 5. Centrifugation method for feline immunodeficiency virus and feline leukaemia virus testing. (a) Swabbing for saliva sample. (b) Saliva-laden swab prior to cutting off the tip. (c) Placement of cut tips in centrifugation tubes.

Table 4. Combined results of three feline immunodeficiency virus (FIV) point-of-care antibody test kits and FIV RealPCR™ testing in a population of FIV-vaccinated cats (n = 117) and FIV-unvaccinated cats (n = 239), using saliva[53]

	SNAP FIV Combo™	Witness™ FIV	Anigen Rapid™ FIV	FIV RealPCR™
Specificity (%)	98	100	100	100
Sensitivity (%)	44	92	96	72

false-positive results in FIV-vaccinated cats using SNAP Combo™ was much less obvious when saliva was used instead of blood (6/113 false-positive results compared with 113/113). Presumably, this difference was related to the concentration of anti-FIV IgG in diagnostic specimens from FIV-vaccinated/FIV-uninfected cats falling below the detection threshold of the SNAP Combo™ kit because of dilution of gingival fluid by saliva. However, this inability of SNAP Combo™ to detect low levels of anti-FIV IgG in saliva resulted in a significantly lower sensitivity than Witness™ and Anigen Rapid™ ($P = 0.001$), with SNAP Combo™ misdiagnosing more than half (14/25) of the FIV-infected cats. Witness™ and Anigen Rapid™ recorded the highest sensitivity and FIV RealPCR™ recorded the third highest sensitivity, with SNAP Combo™ the least sensitive test ($P < 0.05$).[53]

We concluded that saliva can be used instead of whole blood to diagnose FIV infection, using Witness™ or Anigen Rapid™ and the centrifugation technique described. We recommended that SNAP Combo™ should not be used to diagnose FIV infection using saliva, irrespective of FIV vaccination status, because of its poor sensitivity. Based on our earlier recommendation to test FIV-vaccinated cats annually for FIV infection to identify vaccine breakthroughs, we are interested in the possible application of our research for the collection of saliva prior to annual FIV vaccination for FIV antibody testing with Witness™ or Anigen Rapid™. Presuming a cat presenting for annual FIV vaccination is clinically healthy and not in need of a geriatric blood profile, there is an obvious welfare benefit to collecting saliva instead of blood. Another possible scenario for saliva testing proving helpful is in animal shelters, pounds and catteries where large numbers of cats may need to be screened for FIV infection quickly and affordably, additional haematological tests are not indicated, FIV vaccination history is unknown and lay staff might be able to be trained to perform testing in situations when veterinarians are unavailable. However, before either of these recommendations can be endorsed, further investigation of test kit accuracy (particularly using a refined 'direct' testing technique that does not require sample centrifugation) and validation of FIV antibody test kits for saliva testing (as currently, saliva testing represents 'off-label' diagnostic testing), need to be pursued.

FeLV

We evaluated the performance of the same three FeLV antigen PoC test kits using saliva and the same cohort of progressively FeLV-infected and FeLV-uninfected cats as used for blood testing (although unfortunately 126 cats from the initial cohort were unavailable for saliva testing, leaving 410 cats for analysis instead of 536).[50] We were interested in the use of saliva as an alternative diagnostic specimen to blood because saliva contains on average 5-fold more FeLV per mL than plasma in FeLV-infected cats.[57,58]

Table 5. Results of testing using three feline leukaemia virus(FeLV) point-of-care antigen test kits commercially available in Australia using saliva (*n* = 410), comprising 17 progressively FeLV-infected cats and 393 FeLV-uninfected cats

	SNAP FeLV Combo™	Witness™ FeLV	Anigen Rapid™ FeLV
Specificity (%)	100	100	100
Sensitivity (%)	82	82	82
PPV (%)	100	100	100
NPV (%)	99	99	99

PPV, positive predictive value; NPV, negative predictive value.[50]

Table 5 summarises our specificity and sensitivity results for each FeLV PoC antigen kit using saliva instead of whole blood. Regressively FeLV-infected cats (i.e. p27 antigen negative/PCR positive; n = 9) were excluded from analysis because, by definition, PoC test kits are only able to identify progressive infections (i.e. p27 antigen-positive). The specificity and positive predictive value of the three PoC kits were identical when saliva was used (Sp = 100%) and no false-positive p27 results were recorded with any of the kits using saliva (unlike with blood testing). However, 28 progressively FeLV-infected cats were available only for whole blood testing and not for saliva testing, which may have explained this discrepant finding. The sensitivity of the three kits was also identical when saliva was used (Se = 82%), but of concern were 3/17 (18%) false-negative FeLV results that were recorded with each kit. Although the lower sensitivity of saliva testing compared with whole blood testing was not statistically significant, we suspect with a larger study that this difference in diagnostic sensitivity may have become more apparent.

We conclude that until further research is conducted, caution should be exercised about the usefulness of saliva for FeLV PoC testing and that screening large numbers of cats for FeLV infection (e.g. in shelters prior to rehoming) should continue to be done using FeLV PoC test kits and whole blood, with confirmatory FeLV PCR testing pursued for any positive results.[50]

Prevalence of FIV and FeLV infections in Australia

Testing for FIV and FeLV is routinely conducted in private practice and prior to adoption in animal shelters. We conducted the largest FIV and FeLV prevalence study in Australia to date, reporting rates of 15% (305/2,083) and 2% (32/2,032), respectively, in over 2000 pet cats older than 2 years of age with outdoor access (Table 6).[59] Prevalence rates were based on results from a single PoC test kit detecting FIV antibodies and FeLV antigen (SNAP FIV/FeLV Combo™, IDEXX Laboratories). We believe the FIV seroprevalence rate reported was accurate, because the FIV vaccination status of pet cats was known by the attending veterinarian. However, as discussed earlier, FeLV testing using a single test kit in a population with a low

Table 6. Prevalence of feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV) infections in Australia by State/Territory in a population of pet cats aged 2 years or older with outdoor access using results from a single FIV/FeLV PoC test kit (SNAP FIV/FeLV Combo™, IDEXX Laboratories)

State/Territory of Australia[a]	FIV prevalence	FeLV prevalence
New South Wales	13% (95/749)	1% (9/743)
Victoria	15% (46/312)	2% (7/310)
Queensland	16% (110/700)	1% (7/657)
South Australia	8% (3/38)	0% (0/38)
Western Australia	20% (47/239)	4% (9/239)
Australian Capital Territory	9% (4/45)	0% (0/45)
Total	15% (305/2083)	2% (32/2032)

For 51 cats, only a FIV result was recorded (i.e. no FeLV result), and for 25 cats their health status (i.e. 'sick' vs 'healthy') was not recorded, including one cat that had no FeLV result or health status recorded. [a]Cats living in the Northern Territory and Tasmania were not sampled as part of the study.[59]

overall FeLV prevalence (such as Australia) is not ideal because of the resulting low PPV, and we believe some false-positive FeLV results would almost certainly have been recorded. Consequently, it is likely the true FeLV prevalence rate is actually lower than we reported. Ideally, confirmation of all positive PoC results should have been performed, using FeLV PCR testing. In addition, it would have also been useful to perform FeLV PCR testing on all FeLV p27 antigen negative samples in order to identify regressive FeLV infections in this Australian population. Using the attending veterinarian's assessment of whether the cat subjected to testing was 'sick' or 'healthy', the prevalence of both FIV and FeLV infections were found to be significantly higher in sick cats than healthy cats ($P < 0.0001$ for both). The FIV prevalence was 20% in sick cats (176/864) versus 11% in healthy cats (129/1,194), with an odds ratio for FIV infection of 2.1. The FeLV prevalence was 3% in sick cats (25/857) versus 0.6% (7/1151) in healthy cats, with an odds ratio for FeLV infection of 4.9. Note that the health status was not reported for 25 cats, including one for which the FIV status result was recorded but not the FeLV result.[59]

Acknowledgments

Derek Brown, Senior Veterinary Laboratory Scientist for IDEXX Australia, was generous with his time and expertise processing samples for FIV RealPCR™ testing and also provided technical information about the assay. Yamamoto Laboratory (Florida, USA) and Veterinary Diagnostic Services (Glasgow, Scotland) kindly performed virus isolation. Kate Patterson from MediPics and Prose supplied the schematic for Figure 1. Phillip McDonagh provided helpful edits for the manuscript.

Conflicts of interest and sources of funding

The authors declare no conflicts of interest. Mark Westman was supported by an Australian Postgraduate Award (APA), the Neil and Allie Lesue Scholarship (University of Sydney) and an Endeavour Research Fellowship. Richard Malik was supported by the Valentine Charlton Bequest. Many of the studies referenced in this review were supported financially by the Australian Companion Animal Health Foundation, the Feline Health Research Fund and Boehringer Ingelheim, Australia. We are also grateful for the significant in-kind contribution of diagnostic kits by IDEXX Laboratories, Zoetis Animal Health and BioNote, without which many of the studies referenced would not have been possible.

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(Accepted for publication 23 October 2018)