

Evaluation of a new in-clinic test system to detect Feline Immunodeficiency Virus(FIV) and Feline Leukemia Virus(FeLV) infection

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Key Words

FIV, FeLV, diagnosis, ELISA, immunochromatography

ABSTRACT

Background

Many diagnostic in-house tests for identification of Feline Immunodeficiency Virus(FIV) and Feline Leukemia Virus (FeLV) infection are licensed for use in veterinary practice. A new test with unknown performance has recently appeared on the market.

Objectives

The aim of this study was to define the efficacy of a new in-clinic test system, Anigen Rapid FIV Ab/FeLV Ag Test Kit¹, and to compare it to the market leader.

Methods

Western blot was chosen as the gold standard for verification of FIV infection and polymerase chain reaction (PCR) for FeLV infection. Three-hundred serum samples from randomly selected cats were tested. Sensitivity, diagnostic specificity, and positive and negative predictive values were evaluated.

Results

The presence of antibodies against FIV was confirmed by Western blot in nine of the 300 samples (prevalence 3.0%). FeLV DNA was detected by PCR in 15 of the 300 samples (prevalence 5.0%). The Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ test reached a sensitivity of 88.9% for FIV and of 40.0% for FeLV, a specificity of 99.7% for FIV and of 100.0% for FeLV, a positive predictive value of 88.9% for FIV and for FeLV of 100.0%, and a negative predictive value for FIV of 99.7% and of 96.9% for FeLV.

Conclusion

It can be concluded that the new test performed very good and can be recommended for use in veterinary practice. The results were similar to those of the SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3}.

INTRODUCTION

FeLV and FIV are both members of the retroviridae family of RNA viruses⁴. FIV is a member of the lentivirus genus, FeLV belongs to the subfamily orthoretrovirinae, genus gammaretrovirus. Both cause severe, often fatal diseases in domestic cats. Both infections occur worldwide with prevalences varying by location⁵. The clinical signs are mainly caused by the cats' immunodeficiency and the resulting secondary infections, tumours, and haematological abnormalities. This is the reason why underlying retrovirus infection is often overlooked. All cats should be tested for FIV and FeLV, especially those that are sick with signs that might be related to FeLV and/or FIV infection or before being introduced into a multiple-cat household⁶. Testing to identify infected cats is the most important method for preventing viral transmission. Accurate and simple diagnostic tests for in-clinic use are therefore indispensable. A variety of different testing methods are available, including fast screening tests for FeLV antigens and FIV antibodies in plasma, serum, or whole blood. In recent years, many rapid test systems have been licensed for use in veterinary practice and have been compared in previous studies. The preferred initial tests are fast-performing in-house tests, such as ELISA or immunochromatographic tests⁷. They offer the advantage of speed and convenience. These tests detect the presence of soluble FeLV p27 antigen and the presence of antibodies against FIV proteins (usually against p24, core protein and/or gp40 transmembrane protein). However, the sensitivity and specificity of each test format and the prevalence of the infectious agent in question must be considered when interpreting test results⁸.

A new test system, Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ is now on the European market in Austria, Spain, and Germany. The aim of this study was to define the strength and weakness of this new in-clinic test, and to assess its sensitivity and specificity, and predictive value of positive and negative test results. In addition, this test was compared to the SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3}, most commonly used in Europe today. Both tests are immunoassays for simultaneous detection of antibodies against FIV and FeLV antigen in feline serum, plasma, or whole blood.

MATERIALS and METHODS

Blood samples were obtained from 300 randomly selected cats. These cats were presented for a variety of diseases or tested routinely before vaccination against FeLV at the Clinic of Small Animal Medicine, College of Veterinary Medicine, Ludwig Maximilian University, Munich, Germany. Serum was separated shortly after blood sampling, was frozen at -20 °C, and thawed directly before use according to the manufacturers' instructions. The samples were tested with the Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ and the SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3}. Both tests are designed for use in practice and combine detection of FIV antibodies and FeLV antigen in one test system. Both evaluated test systems detect FeLV p27 antigen and specific antibodies against FIV. The Snap Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} contains FIV p24 and gp40 antigens and monoclonal anti-FeLV p27 antibodies. The Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ uses the same antibodies to detect FeLV infection but only gp40 for FIV antibody detection. Both tests contain a positive control, and the IDEXX test offers a negative control spot in addition. The SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} has one result window for FIV antibody, FeLV antigen, and positive and negative control spots. The Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ has two result windows, each of them with a positive control band.

All 300 serum samples were tested with both fast tests according to manufacturers' instructions. The SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} was stored at 2 – 7 °C and used at room temperature. The Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ can be stored at room temperature.

Parameters used to compare the quality and usability of the two test systems were the percentage of diagnostic sensitivity (proportion of positive test results of infected patients), diagnostic specificity (proportion of negative test results in uninfected animals), positive predictive value (probability that a test-positive animal is infected), and the negative predictive value (probability that a test-negative animal is uninfected). The evaluation was based on a total verification, and the gold standards (Western blot for FIV detection and TaqMan real-time PCR for FeLV detection) were performed on each of the 300 samples.

Western blot as described by Egberink⁹, Lutz and Hornizek, was used to confirm FIV infection. The blots contained purified proteins of FIV and envelope protein fragments expressed by *Escherichia coli*¹⁰. These FIV-specific proteins were separated by electrophoresis in 12.5% polyacrylamide gels in the presence of sodium dodecyl sulphate (sds page), then electrophoretically transferred to nitrocellulose filters, and cut into blot strips. The nitrocellulose strips were blocked for 2 hours at room temperature using a gelatine buffer, and subsequently incubated with a 1:10 dilution of feline serum followed by incubation with a horseradish peroxidase- (HRP-) conjugated anti-cat IgG. The antigen-antibody reaction was visualised using 4-chloro-1-naphtol as chromogen. The proteins that can be visualised on the blot strips are structural proteins of the purified strain CrFK-113, CA (capsid protein, gag), KSU3 (surface protein, envelope), and GTM2 (transmembrane, envelope). The samples were regarded FIV positive in Western blot if two or more of the described bands were visible.

TaqMan real-time PCR used to verify FeLV infection was developed for the detection of proviral DNA in infected cats^{11,12}. Probe and Primers were designed to amplify all 3 subtypes of FeLV within the unique region (U3) of the long terminal repeat (LTR)¹³.

RESULTS

The presence of antibodies against FIV was detected by Western blot in nine of the 300 examined samples (prevalence 3.0%). FeLV DNA was detected by TaqMan real-time PCR in 15 of the 300 samples (prevalence 5.0%). Both FIV antibodies and FeLV antigens were present in two samples. Results obtained for the 300 samples, including the percentage, the sensitivity, specificity, and the positive and negative predictive values of both test kits, are summarized in table 1 and 2.

DISCUSSION

FIV and FeLV infections occur worldwide, with prevalences varying by location⁵. Identification and segregation of infected cats are considered to be the most effective methods for preventing new infections¹⁴. For that reason, accurate diagnosis is important for both uninfected and infected cats. Misdiagnosis of infection can lead to inappropriate changes in lifestyle or even euthanasia of cats.

Today, testing of FIV and FeLV in private practice is usually performed using rapid diagnostic test kits. Many of these tests have already been compared in previous studies. The aim of this study was to compare a new test system with the test most commonly used in Europe and the United States, which is the SNAP Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3}.

Valid test evaluation studies should fulfil certain criteria¹⁵. Test systems should be compared to a reference gold standard performed by an independent person. In addition, samples should be representative for patients on which the test systems are applied in private practices. In this study, Western blot was chosen as gold standard for FIV infection. Western blot is the accepted gold standard for FIV diagnosis^{9,16} at least in countries in which no FIV vaccine is available, although it has some shortcomings. The number of bands on the blot strips required to definitively identify a positive result, is matter of discussion. For a positive result, e.g., Hosie and Jarrett¹⁷ consider only the presence of antibodies against at least three core proteins as positive, while Barr¹⁸ and Reid¹⁹ suggest that the presence of two virus specific bands is adequate to indicate a positive result, and reactivity against a single viral protein is considered equivocal⁸.

PCR also has been promoted as a potential reference test for FIV infection²⁰ and is at the moment the only test available to distinguish between vaccinated and infected cats (in countries where vaccines are used)²¹. PCR can diagnose FIV infection in cats by identifying virus-infected cells in blood using primers to detect specific DNA sequences in a target region of the virus genome. FIV is not an ideal organism to be detected reliably by PCR, since gene sequences differ by as much as 20.0% among field isolates²². PCR can detect 1 to 10 copies of viral DNA in one sample and is therefore much more sensitive than any other test methods²³. But the high sensitivity may lead to false positive results due to contamination during collection, storage or processing of samples^{24,25}. In a recent study of Levy et al (2008)²¹ sensitivity and specificity of three FIV PCR assays in different commercial laboratories in the United States and Canada ranged from 51 to 93% and from 44 to 95% respectively, and 7 to 49% of infected cats were not detected.

Performing virus culture from peripheral blood mononuclear cells offers the possibility to verify the infection status of FIV-infected, uninfected or vaccinated cats^{26,27}. However, virus isolation is a method with high technical demands and is expensive, time consuming, and not widely available. For all these reasons, Western Blot was chosen as an appropriate gold standard for FIV detection in this study.

The choice of an appropriate reference test to verify FeLV infection is even more difficult. No generally accepted gold standard for FeLV detection exists. A variety of methods, such as immunofluorescence assay (IFA) and virus isolation, have been used in the past^{28,29}. In previous studies, mostly virus isolation was used as a reference test, although there are many limitations^{30,31}. Virus isolation detects the presence of replication-competent virus while the evaluated test detects the soluble antigen in blood. Presence of the virus, however, is not always consistent with the presence of soluble antigen in blood. In addition, transport and storage of samples can lead to false negative virus isolation results due to destruction of the virus. The technique furthermore is technically demanding, expensive, and time consuming. Virus isolation as gold standard can lead to misinterpretation of true positive results as false positive, because of its limitations. Other methods to detect FeLV are also not ideal, including the IFA that produces a high number of false positive and negative results^{32,33}. In this present study, TaqMan real-time PCR therefore was used as gold standard for FeLV infection. The PCR used is an improved real-time PCR assay which was developed for the detection of FeLV proviral DNA in infected cats^{11,12}. Sensitivity is higher than that of ELISA and virus isolation in the early phase of infection. In addition, this assay allows the identification of provirus carriers that have overcome antigenaemia. This was shown in a previous study by Hofmann-Lehmann et al (2001)¹¹, in which naturally infected cats were identified as FeLV provirus positive but p27 ELISA negative. Key benefits of this method are the possibility to quantify the input template over a broad linear range, low sample consumption, the rapid investigation of large sample quantities, and low risk of contamination³⁴⁻³⁶. Thus, Tandon et al (2005)¹³ recommends TaqMan real-time PCR as most effective test for diagnosing FeLV infection. The PCR used in the present study was designed to amplify all three subtypes of FeLV, (FeV-A, FeLV-B, and FeLV-C) but not endogenous FeLV-like sequences, because not all naturally infected cats could be detected with a FeLV-subtype A-based real time PCR assay¹¹. Due to these advantages and as it is considered to be the most sensitive assay, PCR was used as gold standard.

The requirement that testing should be performed by an independent person was fulfilled. In valid studies to compare diagnostic tests, samples should be representative of those patients to whom the test will be applied in clinical practice¹⁵. The samples were collected from patients of the normal clinical population, including healthy and diseased cats, for which FIV and FeLV tests were requested by clinicians. The prevalence for FIV in the considered population was 3.0%, and the prevalence of FeLV infection was 5.0%. These prevalences are representative for the population examined at the Clinic of Small Animal Medicine, Ludwig Maximilian University, Munich, Germany. Prevalence studies in the past are generally based on testing of healthy cats, sick cats, feral cats in veterinary clinics, and cats from shelters. These studies have shown that the prevalence of FIV is highly variable and depends on the age, gender, lifestyle, physical condition, and most importantly geographic location. It was shown in the past that the prevalence of FeLV infection has decreased during the last 20 years, presumably as a result of implementation of testing programs and development of effective vaccines^{37,38}. In contrast, a vaccine for FIV has only recently been introduced and is not available in all countries. The antibody FIV prevalence ranges from less than 1.0% in healthy cats in North America to 44.0% in sick cats in Japan¹⁴. A recent prevalence study used a prospective cross-sectional survey to examine 18,038 cats at 345 veterinary clinics and 145 shelters in North America³⁹. The prevalence for FIV was 2.5%, the prevalence for FeLV was 2.3%³⁹. According to the results of the present study, the prevalence of FeLV was significantly higher. The higher prevalence for FeLV infection can be explained by the fact that in the present study, there was a high number of cases referred to the Veterinary Teaching Hospital with unclear diseases. Therefore, the number of FeLV-positive cats may be higher than expected in primary veterinary practices.

Compared to previous studies^{30,31} both test systems evaluated performed well and were comparably high in sensitivity and specificity as well as in the predictive values. For FIV infection, the sensitivity, specificity, and the positive and negative predictive values of both test systems were between 88.9 and 100.0%. These high percentages are due to the fact that both tests only showed one false negative result, and that the Snap Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} had no false positive, the Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ only one false positive result. Therefore, negative and positive predictive values for both tests are above 88.9%.

Comparing general performance of the FeLV test systems evaluated in this study, both the Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ and the Snap Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} showed good results. In comparison to the Anigen Rapid FIV Ab/FeLV Ag Test Kit¹ the Snap Kombi

Plus FeLV Antigen/FIV Antibody Test^{2,3}, however, had more invalid and difficult to interpret results due to a coloured background window. For both tests, specificity and positive predictive values were 100.0% and sensitivity was 40.0 and 53.3% respectively. Thus, the calculated sensitivity was rather low in both tests. This can be explained by the fact, that the antigen tests and PCR detect virus in different form and in different compartments. FeLV antigen is present during viremia, which occurs in the early phase of infection with or without bone marrow infection. In many cats, however, viremia is transient and thus, antigen tests are negative. However, latent FeLV infection may be present that is characterized by a persistence of the virus in the bone marrow in non-viremic cats. The PCR used in this study also may detect latent infections due to its sensitivity. Hofmann-Lehmann et al (2001)¹¹ detected 10.0% PCR-positive cats, that were antigen-negative. The fact that six of the antigen-negative PCR-positive cats tested negative in both antigen tests makes it likely that these cats are actually true antigen-negative cats in the stage of a latent infection.

It can be concluded that the performance of both test systems, the Snap Kombi Plus FeLV Antigen/FIV Antibody Test^{2,3} and the Anigen Rapid FIV Ab/FeLV Ag Test Kit¹, was very good and both tests can be recommended for use in veterinary practice. However, in situations in which lower prevalence of both infections are expected and due to the fact that a positive result leads to inconvenient changes for the owner and cat, all positive results should be interpreted carefully. According to the advisory panels of the American Association of Feline Practitioners on feline retrovirus infections⁴⁰, positive results should be confirmed by a second test⁶, preferably more sensitive test, especially in asymptomatic cats.

References

1. Animal Genetics Inc., Anigen Rapid FIV Ab/FeLV Ag Test Kit, 404-5, Kyunggi-do, Korea. January 2008.
2. IDEXX GmbH, Snap Combi Plus FeLV Antigen/FIV Antibody Test, 71636 Ludwigsburg, Germany. 2008.
3. Lutz H, Pedersen NC, Theilen GH. Course of feline leukemia virus infection and its detection by enzyme-linked immunosorbent assay and monoclonal antibodies. *Am J Vet Res* 1983;44:2054-2059.
4. Pedersen NC, Ho EW, Brown ML, et al. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 1987;235:790-793.
5. Hartmann K. Feline immunodeficiency virus infection: an overview. *Vet J* 1998;155:123-137.
6. Levy J, Richards J, Edwards D., et al. Report of the American Association of Feline Practitioners and Academy of Feline Medicine Advisory Panel on feline retrovirus testing and management. *J Feline Med Surg* 2003;3-10.
7. Levy J, Richards J, Edwards D., et al. Feline retrovirus testing and management. . *Compendium on Continuing Education for the Practicing Veterinarian* 2001;23:652-657.
8. Barr MC. FIV, FeLV, and FIPV: interpretation and misinterpretation of serological test results. *Semin Vet Med Surg (Small Anim)* 1996;11:144-153.
9. Egberink HF, Lutz H, Horzinek MC. Use of western blot and radioimmunoprecipitation for diagnosis of feline leukemia and feline immunodeficiency virus infections. *J Am Vet Med Assoc* 1991;199:1339-1342.
10. De Ronde A, Stam, J.G., Boers, P., Langedijk, H., Melen, R., Hesselink, W., Keldermans, L.C.E.J.M., van Vliet, A., Verschoor, E.J., Horzinek, M.C., Egberink, H. F. Antibody response in cats to the envelope proteins of feline immunodeficiency virus: identification of an immunodominant neutralisation domain. *Virology* 1994;198:257-264.
11. Hofmann-Lehmann R, Huder JB, Gruber S, et al. Feline leukaemia provirus load during the course of experimental infection and in naturally infected cats. *J Gen Virol* 2001;82:1589-1596.
12. Torres AN, Mathiason CK, Hoover EA. Re-examination of feline leukemia virus: host relationships using real-time PCR. *Virology* 2005;332:272-283.
13. Tandon R, Cattori V, Gomes-Keller MA, et al. Quantitation of feline leukaemia virus viral and proviral loads by TaqMan real-time polymerase chain reaction. *J Virol Methods* 2005;130:124-132.
14. Crawford PC, Levy JK. New challenges for the diagnosis of feline immunodeficiency virus infection. *Vet Clin North Am Small Anim Pract* 2007;37:335-350, vii.
15. Polzin DJ, Lund, E., Walter, P, Klausner, J. From journal to medicine: evidence bases medicine. In Kirks Current Veterinary Therapy XIII Small Animal Practice. Ed J. D. Bonagura. Philadelphia, W.B. Saunders Company. . 1999:2-8.
16. Lutz H, Arnold P, Hubscher U, et al. Specificity assessment of feline T-lymphotropic lentivirus serology. *Zentralbl Veterinarmed B* 1988;35:773-778.
17. Hosie MJ, Jarrett O. Serological responses of cats to feline immunodeficiency virus. *AIDS* 1990;4:215-220.
18. Barr MC, Pough MB, Jacobson RH, et al. Comparison and interpretation of diagnostic tests for feline immunodeficiency virus infection. *J Am Vet Med Assoc* 1991;199:1377-1381.
19. Reid RW, Barr MC, Scott FW. Retrospective serologic survey for the presence of feline immunodeficiency virus antibody: a comparison of ELISA and IFA techniques. *Cornell Vet* 1992;82:359-369.

20. Uhl EW, Heaton-Jones TG, Pu R, et al. FIV vaccine development and its importance to veterinary and human medicine: a review FIV vaccine 2002 update and review. *Vet Immunol Immunopathol* 2002;90:113-132.
21. Levy JK, Crawford PC, Kuschara H, et al. Differentiation of feline immunodeficiency virus vaccination, infection, or vaccination and infection in cats. *J Vet Intern Med* 2008;22:330-334.
22. Bachmann MH, Mathiason-Dubard C, Learn GH, et al. Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades. *J Virol* 1997;71:4241-4253.
23. Leutenegger CM, Klein D, Hofmann-Lehmann R, et al. Rapid feline immunodeficiency virus provirus quantitation by polymerase chain reaction using the TaqMan fluorogenic real-time detection system. *J Virol Methods* 1999;78:105-116.
24. Bastien P, Chabbert E, Lachaud L. Contamination management of broad-range or specific PCR: is there any difference? *J Clin Microbiol* 2003;41:2272; author reply 2272.
25. Steinrigl A, Klein D. Phylogenetic analysis of feline immunodeficiency virus in Central Europe: a prerequisite for vaccination and molecular diagnostics. *J Gen Virol* 2003;84:1301-1307.
26. 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;225:1558-1561.
27. Crawford PC, Slater MR, Levy JK. Accuracy of polymerase chain reaction assays for diagnosis of feline immunodeficiency virus infection in cats. *J Am Vet Med Assoc* 2005;226:1503-1507.
28. Hardy WD, Jr., Hirshaut Y, Hess P. Detection of the feline leukemia virus and other mammalian oncornaviruses by immunofluorescence. *Bibl Haematol* 1973;39:778-799.
29. Jarrett O, Golder MC, Weijer K. A comparison of three methods of feline leukaemia virus diagnosis. *Vet Rec* 1982;110:325-328.
30. Hartmann K, Griessmayr P, Schulz B, et al. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. *J Feline Med Surg* 2007;9:439-445.
31. Hartmann K, Werner RM, Egberink H, et al. Comparison of six in-house tests for the rapid diagnosis of feline immunodeficiency and feline leukaemia virus infections. *Vet Rec* 2001;149:317-320.
32. Jarrett O. Detection of FeLV antigen. *Vet Rec* 1995;137:127.
33. Floyd K, Suter PF, Lutz H. Granules of blood eosinophils are stained directly by anti-immunoglobulin fluorescein isothiocyanate conjugates. *Am J Vet Res* 1983;44:2060-2063.
34. Gibson UE, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996;6:995-1001.
35. Heid CA, Stevens J, Livak KJ, et al. Real time quantitative PCR. *Genome Res* 1996;6:986-994.
36. Livak KJ, Flood SJ, Marmaro J, et al. Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *PCR Methods Appl* 1995;4:357-362.
37. Levy JK, Crawford PC. Feline leukemia virus In: Ettinger SJ, Feldman, E.C., ed. *Textbook of veterinary internal medicine*. Philadelphia: WB Saunders Co, 2005;653-659.
38. Cotter SM. Feline viral neoplasia In: Greene CE, ed. *Infectious diseases of dog and cat*. Philadelphia: WB Saunders Co, 1998;71-84.
39. Levy JK, Scott HM, Lachtara JL, et al. Seroprevalence of feline leukemia virus and feline immunodeficiency virus infection among cats in North America and risk factors for seropositivity. *J Am Vet Med Assoc* 2006;228:371-376.
40. Levy J, Crawford C, Hartmann K, et al. 2008 American Association of Feline Practitioners' feline retrovirus management guidelines. *J Feline Med Surg* 2008;10:300-316.

Table 1: Comparison of two FIV test systems used on 300 samples with a prevalence of FIV infection of 3.0% (number of false negative results: IDEXX: 1, ANIGEN: 1).

Tests	Anigen Rapid FIV Ab/FeLV Ag Test Kit	SNAP Kombi Plus FeLV Antigen/FIV Antibody Test
Companies	Animal Genetics	IDEXX
Countries	Korea	Germany
sensitivity (%)	88.9%	88.9%
95% CI (sensitivity)	56.5-98.0%	56.5-98.0%
specificity (%)	99.7%	100.0%
95% CI (specificity)	98.1-99.9%	98.7-100.0%
positive predictive value (%)	99.7%	99.7%
Negative predictive value (%)	88.9%	100.0%
number of samples	300	300

Table 2: Comparison of two FeLV test systems used on 300 samples with a prevalence of infection of 5.0% (false negative results: IDEXX: 7, ANIGEN: 9).

Tests	Anigen Rapid FIV Ab/FeLV Ag Test Kit	SNAP Kombi Plus FeLV Antigen/FIV antibody test
Companies	Animal Genetics	IDEXX
Countries	Korea	Germany
sensitivity (%)	40.0%	53.3%
95% CI (sensitivity)	19.8-64.3%	30.1-75.2%
specificity (%)	100.0%	100.0%
95% CI (specificity)	98.7-100.0%	98.7-100.0%
positive predictive value (%)	96.9%	97.6%
negative predictive value (%)	100.0%	100.0%
number of samples	300	300