Validation the scil v-RetroFel assay as combined assay for detection of FeLV antigen, FeLV antibody and FIV antibody

Abstract:

The aim of this study is to show that the diagnosis of feline leukaemia virus (FeLV) infection through a combination of antigen and additional antibody detection is feasible and useful. The addition of FeLV antibody detection now for the first time provides the user with a valuable tool to diagnose FeLV infection, even replacing PCR in some cases. The background to this study is the validation of the new FeLV antibody detection in feline serum. Because FeLV antibody detection as sole test strip for FeLV diagnosis is not conclusive, FeLV antibody detection in combination with FeLV antigen detection and FIV antibody detection is validated and designated as scil v-RetroFel test in this study. Different feline sera from routine diagnostic tests were evaluated with the FeLV antigen, FeLV antibody and FIV antibody test strips (scil v-RetroFel) as part of validating the innovative FeLV antibody test strip. ELISA is used as comparative method. As an innovative new lateral flow detection within 10 minutes, the FeLV antibody detection already achieved in this validation a total test performance (TTP) of 97.18% with a sensitivity of 94.44% and a specificity of 99.99%. The FeLV antigen detection achieved a 98% TTP and the FIV antibody detection could attain a 94% total test performance.

This study confirms that antibody detection, with its highly specific structure in the test strip, is promising for serological diagnosis and allows improved FeLV diagnosis.

General information on FeLV antigen and FIV antibody detection

Like the feline immunodeficiency virus (FIV), the feline leukaemia virus (FeLV) belongs to the class of retroviruses. FeLV belongs to the oncovirus subfamily and FIV belongs to the lentivirus subfamily. Infections with feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are common viral diseases that damage the immune system of cats.

Although its significance is assessed differently in many studies, infection with the feline leukaemia virus (FeLV) and the feline immunodeficiency virus (FIV) is always of veterinary relevance. The clinical presentation of FeLV and FIV infection is diverse. They can go without symptoms for a long time. The course of the disease depends on many factors, such as the virus subtype, the age and general condition of the cat.

The diagnosis of FeLV infection is mainly based on the detection of viruses or viral antigens in the plasma, serum or whole blood. The most common serological tests detect the presence of both p27 antigens by an enzyme-linked immunosorbent assay (ELISA), nanogold-coupled antibodies in the lateral flow test, or FeLV structural antigens in the cytoplasm of infected leukocytes and platelets by an immunofluorescent antibody test (IFA). In addition, the Western blot analysis detects the presence of specific FeLV antibodies. Alternatively, virus isolation or PCR can be used to detect provirus (FeLV-DNA) load or viral (FeLV-RNA) load for diagnosis. These alternative methods are usually not suitable for clinical use, as they are very labour and cost-intensive.

For the diagnosis of FIV infection, antibody detection is used to detect specific antiviral antibodies that circulate in the blood. These anti-FIV antibodies are produced during the normal immune response to the viral infection.

Why has no antibody detection been used for FeLV diagnostics until now?

It is well known that infected cats produce antibodies against the feline leukaemia virus. However, the theory is steadfastly held that vaccinated cats react positively in a FeLV antibody test, which means the test cannot be used in vaccinated cats.

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Test performance study

In addition, there are three other scientific reasons why the detection of antibodies against FeLV may have limited relevance:

1. Antibody detection alone cannot predict FeLV infection.
2. The suitability of the antibodies to be detected must be proven in practice.
3. It would be necessary to distinguish between endogenous and exogenous FeLV (PCR).

For these reasons, the detection of FeLV antibodies has not been considered a useful diagnostic parameter.
Several publications have identified antibodies against p15E that are indicative of FeLV infection but may have little involvement in virus neutralisation. Based on these results, the hypothesis was confirmed in the 2014 study (Boenzli E, Hadorn M, Hartnack S, Huder J, Hofmann-Lehmann R, Lutz H.) that FeLV trans-membrane (TM) envelope protein p15E and other viral proteins have the potential to be used as a useful diagnostic tool in serology and to enable FeLV antibody detection. These findings and the antibody detection described in this study are used in the third test strip of the scil v-RetroFel test to provide FeLV antibody detection for routine diagnostics for the first time.

Ocurrence of FeLV antibodies
Meaningful integration of FeLV antibody detection in FeLV diagnosis requires that one has to be aware that the level of FeLV antibodies is determined by the immune status and age of the infected cat, the virulence of the virus and the infectious dose.

Antibodies initially develop after an infection. Many cats (so-called "regressor cats") succeed in eliminating the virus even before the onset of viraemia through an effective immune system response. **Regressor cats** usually have large amounts of antibodies and also excrete the virus during this time (antigen negative, antibody positive). If the immune system cannot eliminate the virus, the virus gains against the immune system (also positive antigen detection). The virus then reaches the bone marrow after about three weeks. But if the cats are still able to build up an effective immune response and stop the viraemia from penetrating the bone marrow, the virus is completely eliminated and the cat becomes a regressor cat (antigen negative, antibody positive).
However, if the bone marrow is infected, the virus genome can no longer be completely eliminated and **latent infection** occurs. Virus-neutralising antibodies prevent the virus from replicating. As there is no free virus, the cats test negative in antigen detection (antigen negative, antibody positive).

This additional determination of the FeLV antibody makes the scil v-RetroFel test a novel and effective tool in veterinary practice. For the first time, this addition also allows the conclusive identification of regressor cats and latently infected cats in routine diagnostic tests.

The scil v-RetroFel test setup of the test strips

The test strips come on a backing card that includes all other components of the lateral flow test. The test and control areas are applied to the membrane and dried. They contain the highly specific proteins/peptides or antibodies. The conjugate also contains antibodies or proteins bound to nanogold particles which are sprayed and dried on fibreglass plates. These specific combinations of the various components will be described again in detail below.

Test setup of the FeLV antibody test strip
Antibodies against p15E can be detected in whole blood, serum or plasma using the FeLV antibody test strip. This innovative test strip is the first lateral flow test that can detect FeLV antibodies directly in the field in just a few minutes. This is made possible by a highly specific transmembrane protein.

Test setup of the FeLV antigen test strip
The detection of specific proteins of the feline leukaemia virus (p27) in whole blood, serum or plasma enables highly specific monoclonal antibodies in the FeLV antigen test strip.

Test setup of the FIV antibody test strip
The FIV antibody test strip enables the detection of antibodies against the associated proteins p24 and gp41 of FIV in whole blood, serum or plasma using a highly specific peptide.

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Evaluation of the test strips (total test performance)

The FeLV antigen and the FIV antibody test strips were additionally retested in the 2017 validation study as part of the development of the new FeLV antibody lateral flow test. 94 FIV and 120 FeLV samples which had been identified by ELISA were available for this purpose. The test procedure was carried out according to the instructions. For this purpose, one drop of serum material and two drops of running buffer were added to the specimen well; the result was read visually after 10 minutes.

All three test strips confirm very high accuracy compared to the ELISA results in the evaluation. The total test performance is higher than 90% in all three cases. The FIV antibody test was carried out with a total of 94 feline serum samples. Of these 94 serum samples, 45 serum samples were defined negative with the ELISA and 49 positive. The FIV antibody test correctly identified all 45 negative samples. One serum sample was defined as false positive. There were some samples among the positively defined ELISA samples with a borderline antibody titer, i.e. a titer near the detection limit of the ELISA. Of these 6 borderline samples, the FIV antibody test correctly detected 2 samples as positive. The remaining 4 borderline samples were defined as negative. In summary, the FIV antibody test strip has a total test performance of 94.68%.

The test performance of the FeLV antigen test was determined from 120 feline serum samples. There were 83 samples among these that were defined as negative by ELISA and 37 as positive. The FeLV antigen test detected all the negative ones correctly. 2 samples were defined as negative and should therefore be considered a false negative. This results in a total test performance of 98%.

	FIV antibodies	FeLV antigen
Sensitivity	91.67%	94.59%
Specificity	97.83%	99.99%
TTP	94.68%	98.00%

Finally, another 71 sera were tested with the scil v-RetroFel test. In these samples, the respective FeLV antibody concentration was established by means of an ELISA and the test performance of the FeLV antibody test strip was determined. Of 36 positive serum samples, FeLV antibody detection identified 34 samples. Two samples were thus a false negative. Of the 35 negatively defined samples, all samples were correctly detected by the FeLV antibody test strip.

Overall, the following results can be recorded in the validation study of the FeLV antibody test:

FeLV antibodies, n = 71	
Sensitivity	94.44%
Specificity	99.99%
TTP	97.18%

This gives the FeLV antibody test strip a total test performance of 97.18% with defined serum samples from the University of Zurich. The last 20 serum samples from the FeLV antibody test validation (N = 71) with the total results of the scil v-RetroFel test are presented one more time. The following results were found:

FeLV-AB positive serum samples (ELISA def.):

No	FeLV-AG	FeLV-AB	FIV-AB
1	neg	pos	neg
2	neg	pos	neg
3	neg	pos	pos
4	neg	pos	neg
5	pos	pos	neg
6	neg	pos	neg
7	pos	pos	neg
8	pos	pos	neg
9	pos	pos	neg
10	pos	pos	Neg

FeLV-AB negative serum samples (ELISA def.):

No	FeLV-AG	FeLV-AB	FIV-AB
11	neg	neg	neg
12	neg	neg	neg
13	neg	neg	neg
14	neg	neg	neg
15	neg	neg	neg
16	neg	neg	neg
17	neg	neg	neg
18	neg	neg	neg
19	neg	neg	neg
20	neg	neg	neg

The positive FeLV antigen samples and FIV antibody samples occurring here were additionally checked with another antigen or antibody detection (ELISA) and correctly classified as positive. In this test, FeLV antibodies were also present in all cats with positive FeLV antigen detection. This relationship shall be examined even more intensively in further tests.

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Test performance study

Conclusion and field of application of the scil v-RetroFel test

In addition to the acute diagnosis (FeLV antigen and FIV antibody detection), an FeLV test should be carried out on each new cat in a cat population. It is especially in combination with FeLV antibody detection that the scil v-RetroFel test becomes a novel and effective tool in veterinary practice. For the first time, this addition also allows the conclusive identification of regressor cats and latently infected cats in routine diagnostic tests without having to carry out a PCR, which significantly saves cost and time.

In summary, it is clear that additional checking of the FeLV antibody in combination with FeLV antigen detection and FIV antibody detection provides a useful diagnostic parameter for routine diagnostic tests. Clinically symptomatic animals can be promptly tested for two parameters and combining the FeLV antigen and FeLV antibody tests significantly improves and simplifies the differentiated diagnosis and treatment control of feline leukaemia.

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