white paper

Heska trūRapid FIV/FeLV Test Validation

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Key Topics

- Feline immunodeficiency virus and feline leukemia virus are two life-threatening diseases commonly afflicting cats across the globe.
- Regular and accurate screening for both feline immunodeficiency virus and feline leukemia virus is critical for the prevention, diagnosis, and segregation/treatment of infected animals.
- Heska's trūRapid FIV/FeLV test uses lateral flow immunoassay technology to bring convenient and accurate feline viral screening to veterinary practitioners.
- Heska's trūRapid FIV/FeLV test shows excellent sensitivity and specificity in the diagnosis of feline immunodeficiency virus and feline leukemia virus infections.
- Heska's trūRapid FIV test shows exceptional ability at differentiating between uninfected FIV-vaccinated and FIV-infected cats.

Introduction

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are significant infectious diseases present in cat populations throughout the world. Both viruses are in the Retroviridae family, a group of enveloped viruses that integrate into host DNA in order to replicate. These viruses frequently cause serious, life-threatening diseases in felids. Heska's trūRapid FIV/FeLV is a combination test that measures FIV antibodies and FeLV antigen via lateral flow immunoassay technology.

Clinical Significance

Feline Immunodeficiency Virus

Feline immunodeficiency virus is a retrovirus of the genus Lentivirus that causes life-long infections in cats. FIV disease is common worldwide, with a higher prevalence in regions with larger numbers of free-roaming cats. A large study performed in 2010 showed a prevalence of 3.6% for diagnosed FIV infection in the United States and Canada. For cats that are already sick or at high risk of infection, the prevalence can be as high as 15%.

Following infection, FIV typically results in a long asymptomatic phase where the virus causes progressive immune system dysfunction. After the asymptomatic phase, cats are subject to recurrent and/or chronic secondary infections and illnesses, including cancer. In fact, it has been shown that neoplasia is five times more common in FIV-infected cats when compared to non-infected cats.

Currently, vaccines for FIV are only available in select countries and are not available in the United States. However, previously vaccinated cats may still be present in countries where no vaccine is available due to previous availability of the vaccine or relocation. Additionally, a study in Australia of FIV-vaccinated cats found that the vaccine may not produce broadly neutralizing antibodies, bringing forth concerns of its effectiveness against some strains of FIV. These points illustrate the importance of a test that can differentiate FIV-infected from FIV-vaccinated cats. Because FIV vaccines are not widely available, nor are they completely protective in all circumstances, identification and segregation remains the most important method for control of spread.

At present, there is no definitive cure for FIV. Treatment consists of reducing risks for secondary infections and treating illnesses as they arise. Because the consequence of a positive screening test is significant, secondary testing is recommended to confirm the positive result. For confirmatory testing, the American Association of Feline Practitioners (AAFP) recommends FIV PCR, Western blot or another point-of-care antibody test from another manufacturer.

Cats do not recover from FIV infection and will produce high levels of FIV-specific antibodies throughout their lifetime. The majority of cats will produce detectable levels of antibodies within 60 days of infection. Therefore, serologic testing that is selective for FIV-infected versus FIV-vaccinated cats is advantageous for disease detection and is generally indicative of FIV infection. Many serological techniques exist as aids in the diagnosis of FIV with varying levels of technical expertise required. But not all of them have the ability to differentiate FIV-infected from uninfected FIV-vaccinated cats.

Feline Leukemia Virus

Feline leukemia virus is a retrovirus of the genus Gammaretrovirus and is a common disease in cats worldwide. A large study in 2010 showed FeLV antigen prevalence was approximately 3.1% in the United States and Canada.

FeLV is a common cause of cancer and can cause various blood disorders that may lead to significant immune system suppression increasing susceptibility to secondary infections.

When a cat becomes infected with FeLV, it will result in one of three outcomes – the cat will completely clear the infection (abortive infections), partially clear the infection (regressive infections), or become persistently viremic (progressive infection). Abortive and regressive infections are more common in older adult cats, while progressive infections are more prevalent in kittens and young cats. Cats with progressive infections will be infected for the remainder of their life and will continue to shed the virus and develop FeLV-associated diseases.

The rate of FeLV infections has decreased significantly since the development of several successful vaccines and accurate testing procedures. Although largely effective, vaccination against FeLV does not always prevent proviral DNA integration if the cat is exposed. If a cat's infection status is unknown prior to vaccination and is later clinical for FeLV infection, failure of the vaccine would be suspected. It is common for cats to be non clinical in early stages of the disease, making them a risk for spread to other cats. Thus, identification and segregation of infected cats remains an important tactic for reducing spread of this disease.

The AAFP recommends screening all cats for infection when they are first acquired, prior to initial vaccination, following potential exposure or if clinical signs of illness are observed. Because the consequence of a positive screening test is significant, secondary testing is recommended to confirm the positive result. For confirmatory testing, AAFP recommends FeLV PCR, referral laboratory microwell plate ELISA for antigen, or IFA test.

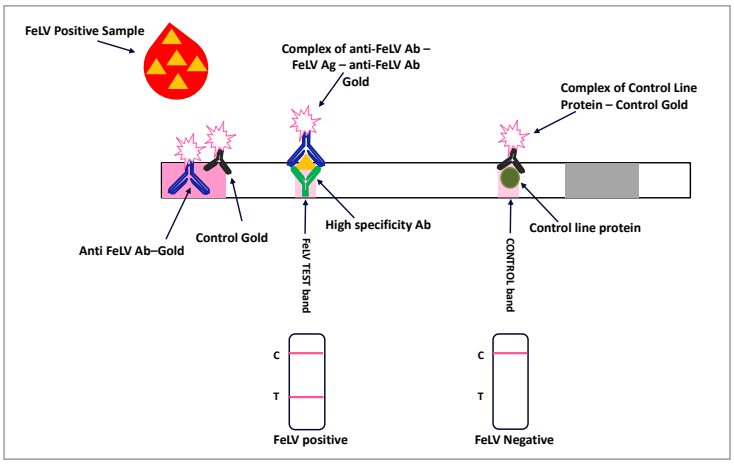
Most cats will have high enough circulating blood antigen for detection within 30 days of exposure. Cats with regressive infection are typically antigen-positive during the early phase of transient viremia and any time there is reactivation of the infection. A test performed when the cat is not currently viremic may produce a false negative result. Progressively infected cats have a persistently high circulating viral antigen load, but have an unreliable antibody response. Therefore, serological testing for soluble FeLV antigen is an excellent diagnostic tool and is currently the detection method of choice for initial disease screening. Many serological techniques exist as aids in the diagnosis of FeLV with varying levels of technical expertise required.

Technology and Development

Heska trūRapid FeLV and FIV point-of-care test strips are sandwich-immunoassays. The FeLV test strip detects feline leukemia virus antigen in anticoagulated whole blood, plasma and serum. The FIV test strip detects feline immunodeficiency virus antibodies in anticoagulated whole blood, plasma and serum.

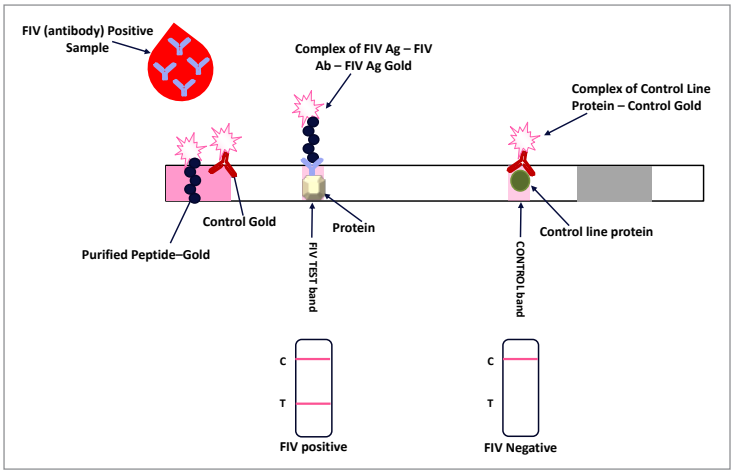
The FeLV test strip is set up as follows: two monoclonal antibodies are used in the test strip for the detection of the FeLV p27 antigens. One antibody is coupled on gold nanoparticles and a second highly specific antibody is immobilized in the T-line area. The control gold nanoparticles bind to the protein immobilized in the C-line area, forming a control line.

Figure 1. FeLV Test Methodology



The FIV test strip is set up as follows: a combination of a protein and a highly purified peptide are used in the test strip for the detection of the FIV antibodies. The purified peptide is coupled on gold nanoparticles and the protein is immobilized in the T-line area. The control gold nanoparticles bind to the antigen, a protein immobilized in the C-line area, forming a control line.

Figure 2. FIV Test Methodology



When the sample, followed by the buffer, is put into the sample wells, it will be absorbed into the absorbing pad of the test strip. The fluid mixes with the gold labelled antibodies in the conjugate pad. Then due to capillary action of the test strip, the fluid runs over the test line and control line regions. The control line should always appear, signifying correct functioning of the test.

If the patient sample contains the FIV antibody or FeLV antigen, a line will appear in the test line region for each respective test strip. This happens by building a sandwich between the gold labelled peptides/antibodies from the conjugate pad, the FIV antibody/FeLV antigen from the sample, and the immobilized antibody/protein of the test line region.

If no FIV antibody/FeLV antigen is in the sample, the gold labeled peptides/antibodies cannot connect to the immobilized antibodies/protein in the test line region and therefore no test line appears. In this case, only the control line will be visible and the test result is negative.

Objectives of This Study

Table 1. Objectives to reach.

Parameter	Value	Comments
Detection of Feline Leukemia Virus (FeLV) antigens and Feline Immunodeficiency Virus (FIV) antibodies	Positive (test and control line) Negative (only control line)	Positive: the test specific antigen/antibody was detected Negative: no test specific antigen/antibody was detected
Study: Validation study of the Heska trūRapid FIV/FeLV (Detection of FIV antibody and FeLV antigen)	Expected results sensitivity and specificity compared to ELISA and IFA: Sensitivity > 90 % Specificity > 90 %	Each Lot is tested with determined field samples and standards.

Materials and Methods

Heska trūRapid FIV and FeLV test strips were tested in this validation study. A total of 239 FIV and 235 FeLV serum/plasma samples were collected from naturally infected cats and were evaluated with the FIV antibody and FeLV antigen test strips. As comparison, indirect fluorescent antibody assay (IFA) for FIV and enzyme linked immunosorbent assay (ELISA) for FeLV results were used as reference tests.

Results

Sensitivity and Specificity

The sensitivity and specificity of the test kit were evaluated by testing a total of 239 FIV and 235 FeLV serum samples with trūRapid FIV/FeLV tests. Sensitivity and specificity were determined using enzyme linked immunosorbent assay for FeLV infections and indirect fluorescent antibody assay for FIV infections, as shown with a 2x2 analysis table (Table 2). All data is from naturally infected animals.

Table 2. Sensitivity and specificity of trūRapid FIV and FeLV compared to IFA and ELISA respectively.

	ELISA	
	Positive	Negative
trūRapid FeLV Pos	99	0
trūRapid FeLV Neg	1	135
Sensitivity	99.0% (95% CI: 94.6-100%)	
Specificity	100.00% (95% CI: 97.3-100%)	
	IFA	
	Positive	Negative
trūRapid FIV Pos	102	2
trūRapid FIV Neg	1	134
Sensitivity	99.0% (95% CI: 94.7-100%)	
Specificity	98.5% (95% CI: 94.8-99.8%)	

FIV-Vaccinated Versus FIV-Infected Cats

Heska's trūRapid FIV test shows exceptional ability at differentiating between FIV-infected and uninfected FIV-vaccinated cats when tested at least three months post-vaccination. Samples taken three months after a primary vaccination series (which included 1 initial and 2 booster vaccinations), the trūRapid FIV test detected only one positive result out of 17 uninfected vaccinated samples.

Table 3. Number of confirmed FIV-negative cats testing FIV-positive at 3 months post-vaccination.

	Disease Negative Cats 3 Months Post-Vaccination*
Heska trūRapid FIV	1/17 FIV-Positive

*Primary vaccination series which included 2 booster vaccinations

Conclusions

FIV and FeLV are serious, often fatal diseases of felids. Early identification and segregation of infected animals is a crucial strategy for control of spread for both diseases. AAFP recommends screening all cats for infection at the time they are first acquired, prior to initial vaccination against FeLV or FIV, regularly in cats that spend any time outdoors, following potential exposure to infected cats, or if clinical signs of illness are displayed.

This study shows Heska trūRapid FIV/FeLV test is intuitive and has excellent accuracy. Heska trūRapid FIV conveys a sensitivity and specificity of 99% and 98.5% respectively for detection of FIV antibodies when compared to IFA results. Heska trūRapid FeLV shows a sensitivity and specificity of 99% and 100% respectively for detection of FeLV antigen when compared to ELISA testing results. Additionally, trūRapid FIV shows excellent ability at differentiating FIV-infected versus uninfected FIV-vaccinated cats, correctly diagnosing 94% of the cats as FIV-negative. This allows for more confidence that a positive result indicates actual infection. It is important to note that although less common, false positives can occur with FIV-vaccinated uninfected cats. Therefore, medical history, vaccination history and clinical judgement should always be considered when interpreting results.

While rare, false negatives and false positives can occur with all available FIV and FeLV point-of-care tests. False positive results may occur because the cat was tested too soon after vaccination for FIV, from improperly executed tests, or test failure. False negatives are sometimes seen when the cat is in the early phase of infection, before FeLV antigens are at detectible levels or FIV antibodies have developed. Due to the potential clinical consequences of a positive screening test result, especially when testing cats with a history of FIV-vaccination, the AAFP recommends additional confirmatory testing for all positive screening results and for negative results when there is high suspicion for infection.

Early diagnosis is vital for both treatment and control of these retroviruses. Given the exemplary accuracy displayed in this validation study, Heska's trūRapid FIV/FeLV point-of-care test provides veterinary professionals with an efficient and reliable diagnostic tool for FIV and FeLV infection and disease.

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