The retroviruses feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) and the filarial nematode *Dirofilaria immitis* (heartworm) are pathogens of cats that can cause chronic, severe, and sometimes fatal diseases. FeLV and FIV infections generally lead to immunodeficiency, and affected cats have an increased potential of developing opportunistic infections with varied clinical presentations. As atypical hosts, cats infected with heartworms are often asymptomatic or have subclinical disease; however, chronically infected cats may become lethargic, may cough, vomit, and may experience difficulty breathing. In-clinic identification of cats infected with these retroviral and filarial agents may be achieved by detection of FeLV p27 antigen, FIV antibodies, and heartworm antigen in anti-coagulated whole blood, serum, and plasma by immunoassay. The WITNESS® FFH Test Kit from Zoetis Diagnostics is an accurate and reliable Rapid Immuno-Migration (RIM™) test designed to aid in running both a retroviral screening that simultaneously assesses the two most common causes of immunodeficiency in sick cats as well as adult stage heartworm antigen. As a point of care screening diagnostic, WITNESS® FFH requires only 50 µL of sample for each test and approximately 10 minutes to produce results. This technical bulletin reviews key details about FeLV, FIV, and heartworm infections, examines RIM™ technology, highlights how the WITNESS® FFH Test Kit works, and summarizes supporting study data demonstrating test sensitivity and specificity.

**Section 1: Overviews of FeLV Infection, FIV Infection, and Feline Heartworm Disease**

**Section 2: WITNESS® and RIM™**

**Section 3: WITNESS® FFH Supporting Data**

With the WITNESS® FFH Test Kit, a positive result for FeLV p27 antigen, FIV antibodies, or adult heartworm antigen in a healthy or sick cat indicates that the cat may be infected and that a confirmatory test should be performed. Negative results indicate no detectable FeLV antigen, FIV antibodies, or heartworm antigen is present in the test sample. Negative results from healthy cats from environments where there is little suspicion of infection likely indicate the cats are not infected. Negative results from healthy cats from environments where the risk of recent exposure is high and from sick cats should be validated with repeat testing after waiting a minimum interval from last potential exposure, or with an appropriate confirmatory test.
Feline Practitioners (AAFP) recommends testing for FeLV infection when cats are newly acquired, sick, prior to vaccination against FeLV or FIV, or believed to have been exposed to potentially infected cats. The basis of in-clinic test kits used with serum, plasma, and whole blood is detection of the circulating FeLV soluble antigen p27, which is usually present in the blood of cats persistently infected with the virus within 30 days of exposure.

If the results of either ELISA or immunochromatographic screening methods are negative, but recent exposure to FeLV positive cats cannot be ruled out, the in-clinic test should be repeated a minimum of 30 days after the day of the last potential exposure. Follow-up laboratory testing of negative in-clinic screening results usually is not recommended because of the high negative predictive value of the ELISA and immunochromatographic tests. Confirmatory laboratory testing, however, is recommended if an unexpected positive (e.g., low-risk, healthy cat) result is obtained with an in-clinic screening test. For confirmation of a positive FeLV screening test result, a veterinary diagnostic or commercial laboratory may be able to provide the following options:

- Immunofluorescent assay (IFA)
- Virus isolation
- PCR for detection of provirus (DNA PCR)
- PCR for detection of viral RNA

Long-term prognosis for persistently infected cats is very guarded, but with proper care these cats can live years with a good quality of life. Research conducted in the 1970s noted that 70% to 90% of these cats typically died within 18 months to three years. Therefore, a decision for treatment or euthanasia should not be based solely on the presence of an FeLV infection. Furthermore, because of the importance of this decision, it should not be made based upon only a single positive test result.
FIV Infection

Feline immunodeficiency virus (FIV) is a retrovirus of the genus Lentivirus that can cause an acquired immunodeficiency syndrome (AIDS) in domestic cats. Although the morphology, biochemistry, and pathogenesis pattern of the FIV and the human immunodeficiency virus (HIV) are similar, the viruses are antigenically distinct. Convenience testing of cats in veterinary clinics, shelters, and spay-neuter programs shows that the prevalence of FIV is highly variable, depending upon factors such as age, sex, lifestyle, physical condition, and geographic location. In a survey of 18,038 cats tested in North American veterinary clinics and animal shelters, FIV prevalence was 2.5% of cats overall.\(^3\) In other studies, seroprevalence estimates ranged from 1% to 14% in cats with no clinical signs and up to 44% in sick cats.\(^{17,18}\) The saliva of FIV-infected cats contains high concentrations of the virus, which is transmitted primarily by aggressive biting behavior. Older, male outdoor cats with clinical signs of disease are most commonly found to be infected. Transplacental and perinatal transmission occurs from infected queens to kittens, but the percentage of kittens becoming infected depends upon the queen’s viral load during pregnancy and birth.\(^{19-21}\) During the first few weeks to months after FIV infection, mild fever, lethargy, and peripheral lymphadenopathy lasting from a few days to a few weeks may be evident but frequently go unnoticed by cat owners. Thereafter, cats enter a subclinical latent period of infection of variable duration.\(^1\) During the immunodeficiency stage of disease, cats may be presented with non-specific signs such as anorexia, weight loss, and depression, or abnormalities associated with specific organ systems. Even when a clinical syndrome is diagnosed in an FIV-seropositive cat, the workup should include diagnostic tests for other possible causes.\(^9\)

Concurrent viral, bacterial, fungal, and protozoal infections have been reported in FIV-infected cats.\(^{20,22,23}\) as have tumors, and neoplastic conditions (B-cell lymphosarcomas, myeloproliferative disease, and squamous cell carcinoma).\(^{22-25}\) Chronic gingivostomatitis is a common presenting sign.\(^{26,27}\) Additionally, neurological signs have been associated with FIV infection in both experimental and natural infections.\(^{19-21,28}\)

Reproductive failure that has occurred in infected cats has been associated with PCR-positive placental and fetal tissues.\(^{29}\) Infectious co-factors profoundly influence the course of clinical disease in infected cats.\(^{19}\)

FIV infection can be reliably identified by virus isolation, but the method is laborious and not routinely used. PCR assays that detect proviral DNA are also available to diagnose FIV infection, but these tests vary in performance, with sensitivities ranging from 41% to 93% and specificities from 81% to 100%.\(^{30}\) Additionally, although current PCR assays often detect clade A viruses, more variability occurs in detecting the other clades.\(^{31}\) Field isolates of FIV are divided into several phylogenetic subtypes (clades) based on sequencing of the envelope (env) gene. Properties of this env gene are clinically important because they determine cell tropism, influence pathogenicity, and affect the immune response.\(^{20}\) Some laboratories also sequence the gag gene to help classify FIV into different clades.

Because FIV produces a persistent, lifelong infection, detection of antibodies to FIV in peripheral blood is regarded as sufficient for routine diagnostic screening in adult cats not vaccinated against FIV.\(^{32}\) Immunochromatographic and ELISA tests often detect antibodies to the transmembrane peptide, gp40, and/or the capsid protein, gp24.

\(^{3}\)
Usually cats produce antibodies to FIV within 60 days of exposure; however, detectable antibodies can be delayed in some cats. For this reason when the results of antibody testing are negative, but recent infection cannot be ruled out, testing should be repeated a minimum of 60 days after the last potential exposure. Cats vaccinated against FIV also produce FIV antibodies, which were once thought to be indistinguishable by existing serological methods from antibodies induced by FIV infection. In recent studies, however, two point of care tests (Anigen Rapid FIV/FeLV; WITNESS® FeLV/FIV) had excellent overall sensitivity and specificity and were shown to discern the true FIV infection status of cats, regardless of the cats’ FIV vaccination status. Investigators noted such results could change the way FIV screening is conducted in situations where FIV vaccination is practiced, and in animal shelters where the vaccination history is often unknown.

In general, negative antibody screening results are highly reliable because of the high specificity of the tests and the low prevalence of infection in most populations. However, when positive immunochromatographic or ELISA results are obtained in healthy or low-risk cats where the possibility of a false-positive result is higher, confirmatory testing with Western blot immunoblot assay (the test detects antibody in cat serum to several FIV proteins) or IFA can be performed. In one study, however, these methods were found to be less sensitive and specific than the in-clinic screening tests. Alternatively, a second soluble antibody test could be performed, preferably using a test from a different manufacturer. Because kittens can have detectable, colostrum-derived antibodies for several months, FIV-seropositive kittens younger than 6 months of age should be tested every 60 days until the result is negative. If antibodies are still evident at 6 months of age, the kitten is likely infected.

Many cats naturally infected with FIV do not develop a severe clinical disease directly related to that infection and can live many years with a high quality of life. This suggests that cats should not be euthanized solely on the basis of an FIV-positive test result, and this is especially the case if the cat has a history of FIV vaccination. FIV-infected cats, however, have a higher risk of developing clinical signs associated with secondary infections, immune-mediated diseases, or neoplasia.

## Feline Heartworm Disease

Heartworm infection (dirofilariasis) is caused by the filarial nematode *Dirofilaria immitis*, which is transmitted between animals via infected mosquitoes that deposit larval heartworms as they feed (Figure 1). Heartworm disease is reported worldwide, and has been diagnosed in all 50 states in the U.S. Heartworm persists within the animal population of the contiguous 48 states, Hawaii, Puerto Rico, U.S. Virgin Islands, and Guam. Heartworm infections have been reported in domestic dogs, domestic cats, wolves, foxes, coyotes, ferrets, muskrats, sea lions, nondomestic cats, coatimundi, and, rarely, humans.
Cats are susceptible hosts for heartworm infections (Table 1). *Dirofilaria immitis* infects cats the same way as in dogs, although the time to develop adult worms is longer, and worms are less likely to reach full maturity. Clinical signs can result as a consequence to damage caused by dying immature worms. This has been identified as a condition known as Heartworm Associated Respiratory Disease (HARD). For cats with adult heartworms, many are asymptomatic or have subclinical disease, but the sudden death of mature heartworms can result in collapse, difficult breathing, convulsions, vomiting, diarrhea, blindness, and, in rare cases, death. Chronic signs of a heartworm infection include coughing, vomiting, difficult breathing, and lethargy.

Currently, no products are approved in the U.S. for the treatment of feline heartworm infections. Once diagnosed, infected cats should be monitored for complications and provided with supportive care as needed. Some cats with mild signs may resolve the infection spontaneously with little to no intervention. Surgical extraction of adult worms has been performed for some heavily infected cats and for those cats with obstruction of blood flow returning to the heart and liver (caval syndrome).

**Diagnosing Heartworm Infection in Cats**

In cats, a comprehensive approach to heartworm testing may be required because:

- Infected cats have both lower worm burdens and lower antigen levels
- Immature/larval infections have increased importance in this species

**Table 1.**

<table>
<thead>
<tr>
<th>FELINE HEARTWORM INFECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cat</strong>:</td>
</tr>
<tr>
<td>- Atypical hosts. Many fewer cats than dogs develop adult heartworms when exposed to infected mosquitoes.</td>
</tr>
<tr>
<td>- Likely, many immature heartworms in the lungs of cats fail to mature to adult heartworms.</td>
</tr>
<tr>
<td>- Infected cats usually harbor fewer than 6 adult heartworms (commonly 1–3 worms).</td>
</tr>
<tr>
<td>- Only 50% of cats develop circulating microfilariae. Microfilaraemia persists for just one to two months.</td>
</tr>
<tr>
<td>- Feline heartworm disease affects the lungs much more often than the heart.</td>
</tr>
<tr>
<td>- Generally, only symptomatic treatment is practiced in cats. Approved adulticides are lacking; use of canine adulticides is not recommended.</td>
</tr>
</tbody>
</table>

Adapted from Blagburn BL and Dillon AR.38

---

*Figure 1—Heartworm life cycle in dogs and cats. Adapted from the American Heartworm Society (www.heartwormsociety.org/pet-owner-resources/heartworm.html. Accessed September 9, 2016).*
Both antigen and antibody testing are routinely used to evaluate cats for heartworm infections. A positive antigen test confirms the presence of an infection with adult worms. Adult heartworm antigen in circulating blood can be identified in cats at approximately 5½ to 8 months after infection. Antibody testing can be used to confirm heartworm exposure and development of heartworm larvae as early as 8 weeks after infection. The positive result of a heartworm antibody test could signal that a cat has an adult infection, or it could be at risk for HARD or future infection, but it does not confirm the presence of adult worms. Heartworm antigen and antibody tests are useful tools that can complement one another and assist in making an appropriate diagnostic decision. Testing for both antibody and antigen improves sensitivity compared with running either test alone. Microfilariae are rarely observed in infected cats, and, when they do develop, generally they are detectable only for one to two months; as such, this limits the utility of microfilaria testing.

In clinical situations where there is an antemortem suspicion of heartworm infection, a comprehensive approach to diagnosing heartworm disease could include: antigen testing, antibody testing, thoracic radiographs, and echocardiography. In cases of unexplained death, necropsy results may be useful in identifying some infected animals.

For up-to-date recommendations on heartworm disease, prevention, and treatment, refer to the American Heartworm Society’s (AHS) Current Canine and Feline Guidelines at:

AHS Guidelines
https://heartwormsociety.org/veterinaryresources/americanheartworm-society-guidelines
(Accessed September 16, 2016)
WITNESS® AND RIM™

The first WITNESS® brand point of care diagnostic line utilizing Rapid Immuno-Migration, or RIM™, technology was introduced in 1997. RIM™ is a test format that uses tagged colloidal gold particles as a color signal rather than an enzyme-catalyzed color change reaction as in ELISA to allow combined detection of feline leukemia virus (FeLV) antigen, feline immunodeficiency virus (FIV) antibodies, and adult heartworm antigen in feline whole blood, serum, or plasma (Figure 2).

Colloidal gold is used as the signal because its inherent stability allows the WITNESS® FFH Test Kit to be stored without refrigeration for the duration of the kit’s shelf life. The WITNESS® FFH Test Kit provides patient-side FeLV, FIV, and heartworm results in approximately 10 minutes.

WITNESS® FFH: How It Works

- One drop, 0.05 mL (50 µL) of sample (Table 2) is added directly to each sample well from the provided pipette (Figure 3). After the samples are absorbed, two drops of buffer are added to each sample well.

<table>
<thead>
<tr>
<th>Table 2. APPROPRIATE SAMPLE TYPES FOR THE WITNESS® FFH TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anti-coagulated whole blood (EDTA/heparin)</td>
</tr>
<tr>
<td>2. Serum</td>
</tr>
<tr>
<td>3. Plasma</td>
</tr>
</tbody>
</table>

- The samples and buffer flow through their respective sample pads where blood cells and cellular debris are filtered, allowing the test samples to flow onto and across their respective conjugate pads and nitrocellulose membrane test strips.

- In all cases, sensitized colloidal gold particles form a complex with either FeLV antigen, FIV antibodies, or heartworm antigen present in the samples. The formed complexes migrate laterally across their respective strips.

- The complexes are then captured on a sensitized result line where their accumulation causes the formation of a clearly visible pink to purple line to develop.

- Sensitized colloidal gold particles continue to flow across the membrane towards the absorption (wicking) pad and are bound at the control line. A visible color line (pink to purple) verifies that the test is working properly.

---

Figure 2. The WITNESS® FFH test uses sensitized colloidal gold particles to form a complex with FeLV antigen, FIV antibodies, or heartworm antigen.

For the WITNESS® FeLV and heartworm tests, the colloidal gold is complexed to an associated antibody (as shown above); for the WITNESS® FIV test, the colloidal gold is complexed to Protein A (not shown), a surface protein derived from Staphylococcus aureus that binds antibodies (immunoglobulins). In cats with circulating FeLV antigen, FIV antibodies, or heartworm antigen, the formed complexes migrate along their respective test strips. The complexes are then captured on a sensitized reaction line where their accumulation causes formation of a pink to purple color change.
Figure 3  WITNESS® FFH Test Kit for cats.
Guidelines for Optimizing Test Outcomes

Various factors have the potential to affect the accuracy and overall performance of all diagnostic testing. Compliance with the following list of guidelines helps increase confidence that the results obtained with the WITNESS® FFH Test Kit are valid and can be relied upon to help in managing the care and well-being of feline patients:

- Always use the appropriate sample specified in the test kit insert. For example, WITNESS® FFH has only been validated with anti-coagulated whole blood, serum, and plasma. Failure to use the appropriate sample type can result in sensitivity and/or specificity errors. A common user error is not adding anticoagulant when required or using an inappropriate ratio of anticoagulant to whole blood. This is often the result of the desire to apply blood directly from a syringe, which introduces another error, inadequate or excess sample volume (see below). With too little or no anticoagulant, the sample may have migration issues. With too much, the sample itself may be diluted and sensitivity affected. Using syringes with or without the needle can adversely affect sample size, absorption, migration, and test accuracy.

- Always use test kit components before their expiration dates.

- Store test kits at label-specified temperatures. WITNESS® tests are made to be stored and used at room temperature (20°–25°C; 68°–77°F. Do not freeze). If the test is refrigerated, it will still perform accurately; however, if either the sample or the WITNESS® test is not at room temperature at the time of use, migration along the test strips might be affected.

- Use the test kit immediately after opening the sealed pouch. The WITNESS® FFH test must be run within 10 minutes of its removal from the foil pouch.

- Avoid touching or damaging membranes with any delivery device including the supplied pipette at the sample well, and the test (T) and control (C) windows, as erroneous results may be produced. Keeping the pipette and buffer bottle vertical delivers a sample drop by way of gravity and avoids interference with the membrane below the sample well and windows.

- Use the pipettes supplied with the test kit to help ensure that the correct volume (50 µL) of sample is dispensed. Too little of any sample type may lead to a weak positive or false negative result. Too much of any sample type may lead to too much of the sample migrating along the membrane, making test results difficult to interpret (e.g., pink/purple lines at the T and C windows may not be visible if anti-coagulated blood obscures them).

- Use a separate pipette for each sample.

- Place the WITNESS® cassette on a flat, horizontal surface while the test is being performed to ensure proper flow along the test strips.
SECTION 3.
WITNESS® FFH
SUPPORTING DATA

Summary Supporting Data

Heartworm Study

**Design**
Serum samples were collected from cats on day 224 or day 238 (n = 16) after experimental infection with *Dirofilaria immitis* L3, and additional serum samples (n = 16) were collected from cats experimentally infected through transplantation of adult heartworms. Success of experimental heartworm infection/transplantation was confirmed by necropsy examination. Tested as negative controls was a third set of serum samples (n = 100) from uninfected, laboratory-housed specific pathogen free (SPF) cats. Sensitivity and specificity results and 95% confidence intervals for the heartworm test strip of the WITNESS® FFH test kit were determined on the basis of comparison with results obtained using the enzyme-linked immunosorbent assay (ELISA) DiroCHEK® Heartworm Antigen Test Kit.

**Results**
In this study, the diagnostic sensitivity and specificity of the WITNESS® FFH heartworm test strip, as compared to the DiroCHEK® test, were determined to be 96.3% (95% CI: 84.0%–99.6%) and 100% (95% CI: 97.6%–100%), respectively (Table 3).

Feline Leukemia Virus Study

**Design**
Serum samples collected from experimentally infected, ViraCHEK® FeLV characterized infected (n = 43), and non-FeLV infected, laboratory-housed specific pathogen free (SPF) cats (n = 90) were tested using the FeLV strip of the WITNESS® FFH test for detection of FeLV antigen. Sensitivity and specificity results and 95% confidence intervals for the FeLV test strip were determined on the basis of comparison with results obtained using the ViraCHEK® FeLV antigen ELISA.

<table>
<thead>
<tr>
<th>Test</th>
<th>DiroCHEK® HW Positive</th>
<th>DiroCHEK® HW Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITNESS® FFH Positive</td>
<td>26</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>WITNESS® FFH Negative</td>
<td>1</td>
<td>105</td>
<td>106</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>105</td>
<td>132</td>
</tr>
</tbody>
</table>

Sensitivity: 96.3% (95% CI: 84.0%–99.6%)
Specificity: 100% (95% CI: 97.6%–100%)

<table>
<thead>
<tr>
<th>Test</th>
<th>ViraCHEK® FeLV Positive</th>
<th>ViraCHEK® FeLV Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITNESS® FFH Positive</td>
<td>42</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>WITNESS® FFH Negative</td>
<td>1</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>90</td>
<td>133</td>
</tr>
</tbody>
</table>

Sensitivity: 97.7% (95% CI: 89.6%–99.7%)
Specificity: 100% (95% CI: 97.3%–100%)
Results
In this study, the diagnostic sensitivity and specificity of the FeLV test strip of WITNESS® FFH, as compared to the ViraCHEK® FeLV antigen ELISA, were determined to be 97.7% (95% CI: 89.6%–99.7%) and 100% (95% CI: 97.3%–100%), respectively (Table 4).48

Feline Immunodeficiency Virus Studies

Design: Study 1
In the first of two FIV antibody detection studies, serum samples collected from experimentally infected (n = 6) and non-FIV infected, laboratory-housed SPF cats (n = 48) were tested on the FIV strip of the WITNESS® FFH test for detection of FIV antibody. Sensitivity and specificity results and 95% confidence intervals for the FIV test strip were determined on the basis of comparison with results obtained using the ViraCHEK® FIV antibody ELISA.

Results
In this study, the diagnostic sensitivity and specificity of the FIV strip of WITNESS® FFH, as compared to the ViraCHEK® FIV antibody ELISA, were determined to be 100% (95% CI: 67.0%–100%) and 100% (95% CI: 94.9%–100%), respectively (Table 5).

Sensitivity and specificity results of the WITNESS® FFH FIV antibody test strip and the ViraCHEK® FIV antibody ELISA

<table>
<thead>
<tr>
<th>Test</th>
<th>ViraCHEK® FIV Positive</th>
<th>ViraCHEK® FIV Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WITNESS® FFH Positive</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>WITNESS® FFH Negative</td>
<td>0</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>48</td>
<td>54</td>
</tr>
</tbody>
</table>

Sensitivity: 100% (95% CI: 67.0%–100%)
Specificity: 100% (95% CI: 94.9%–100%)

Sensitivity and specificity results of the WITNESS® FFH FIV antibody test strip, the ViraCHEK® FIV antibody ELISA, and the immunofluorescent antibody test

<table>
<thead>
<tr>
<th>Serum Samples</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Sensitivity 95% Lower Limit</th>
<th>Specificity 95% Lower Limit</th>
<th>Sensitivity 95% Upper Limit</th>
<th>Specificity 95% Upper Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum samples from Veterinary Diagnostic Laboratory A</td>
<td>95.2%</td>
<td>99.0%</td>
<td>98.2%</td>
<td>92.0%</td>
<td>99.8%</td>
<td></td>
</tr>
<tr>
<td>WITNESS® FFH vs ViraCHEK® FIV (reference)</td>
<td>95.2%</td>
<td>98.2%</td>
<td>92.0%</td>
<td>99.8%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum samples from Veterinary Diagnostic Laboratory B</td>
<td>100%</td>
<td>91.7%</td>
<td>67.2%</td>
<td>99.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WITNESS® FFH vs ViraCHEK® FIV (reference)</td>
<td>100%</td>
<td>91.7%</td>
<td>67.2%</td>
<td>99.1%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WITNESS® FFH vs IFA FIV (reference)</td>
<td>92.3%</td>
<td>99.2%</td>
<td>100%</td>
<td>78.3%</td>
<td>100%</td>
<td></td>
</tr>
</tbody>
</table>
100%) and 100% (95% CI: 94.9%–100%), respectively (Table 5, see page 11).49

**Design: Study 2**
In the second study, serum samples from cats naturally infected with FIV and from non-infected cats obtained from a collection of diagnostic accessions submitted to two veterinary diagnostic laboratories were tested on the FIV strip of WITNESS® FFH. The reference test for comparing sensitivity, specificity, and 95% confidence intervals on the 98 samples obtained from laboratory A was the ViraCHEK® FIV antibody ELISA; for the 23 samples from laboratory B, the reference tests were the ViraCHEK® FIV antibody ELISA and the immuno-fluorescent antibody (IFA) test.

**Results**
For the 98 samples obtained from laboratory A, the diagnostic sensitivity and specificity of the FIV strip of WITNESS® FFH, as compared to the ViraCHEK® FIV antibody ELISA, were 95.2% (95% CI: 85.6%–99.0%) and 98.2% (95% CI: 92.0%–99.8%), respectively (Table 6, see page 11). For the 23 samples obtained from laboratory B, the diagnostic sensitivity and specificity of the FIV strip of WITNESS® FFH, as compared to the ViraCHEK® FIV antibody ELISA, were 100% (95% CI: 80.0%–100%) and 91.7% (95% CI: 67.2%–99.1%), respectively; as compared with the IFA, 92.3% (95% CI: 69.3%–99.2%) and 100% (95% CI: 78.3%–100%) (Table 6).49

**CONCLUSIONS**
In the U.S., studies have shown a prevalence of FeLV and FIV infections ranging from 6% to 33% among high-risk cats and cats that are tested during illness.4,5,20 Similarly, surveys conducted by the American Heartworm Society (AHS) document the risk of heartworm disease in each of the contiguous United States, Hawaii, and in almost every county and parish.39 To control the spread of new FeLV and FIV infections, AAFP recommends identification and segregation of infected cats. AAFP guidelines specify that all cats be tested for FeLV and FIV based on individual risk assessments at the time of acquisition, following exposure to an infected cat or cat of unknown infection status, prior to FeLV or FIV vaccination, prior to entering group housing, and when cats become sick.9 To reduce the incidence of preventable feline heartworm disease, the AHS recommends comprehensive annual testing for heartworm, which includes testing for the presence of adult heartworm antigen, prior to administration of a heartworm preventive.42

Several diagnostic tools are sometimes necessary to diagnose or confirm FeLV and FIV infections. In-clinic FeLV screening generally is accomplished with either ELISA or immunochromatography test for soluble p27 antigen, a marker of FeLV infection but not necessarily of viremia. Confirmation of results may be obtained with immunofluorescent assay (IFA), virus isolation, or PCR for detection of FeLV provirus or FeLV RNA. All in-clinic FIV screening tests detect antibodies to various viral proteins, most commonly the transmembrane peptide (gp40) and/or the capsid protein p24, via immunochromatography or ELISA. Laboratory methods to confirm screening test results include a labor-intensive virus isolation procedure, IFA, PCR assays that vary in performance, and Western blot.

Because cats infected with heartworms typically have low heartworm burdens and lower antigen levels and because larval infections are more important to cats than dogs, a more comprehensive approach to heartworm testing—one that includes both antigen and antibody
testing—may be required. Whereas an antibody test can confirm heartworm exposure and development of larvae, a positive heartworm antigen test confirms the presence of an adult heartworm infection.

In a single test, the WITNESS® FFH Test Kit uses Rapid Immuno-Migration—RIM™—technology to detect the presence of FeLV antigen, FIV antibodies, and adult heartworm antigen in three different sample types. The entire test can be completed at the point of care in approximately 10 minutes.

Performance of the test was demonstrated in a series of studies:

• The feline heartworm strip of the WITNESS® FFH test reliably detects feline heartworm antigen in serum derived from infected cats and that test results with the WITNESS® FFH test were highly comparable to those achieved with the DiroCHEK® Heartworm Antigen Test, with sensitivity and specificity determined to be 96.3% and 100%, respectively.

• The FeLV strip of the WITNESS® FFH test is highly accurate in detecting FeLV p27 antigen in the serum of experimentally infected cats and test sensitivity and specificity were determined to be 97.7% and 100%, respectively, as compared with the ViraCHEK® FeLV antigen ELISA.

• The FIV strip of the WITNESS® FFH test was highly accurate in detecting FIV antibodies in a natural infection study. As compared to the ViraCHEK® FIV antibody ELISA in one evaluation of the study using 98 samples, the diagnostic sensitivity and specificity of the FIV strip of WITNESS® FFH were 95.2% and 98.2%, respectively. Additionally, in a second evaluation of 23 samples, the diagnostic sensitivity and specificity of the FIV strip, as compared to the ViraCHEK® FIV antibody ELISA, were 100% and 91.7%, respectively, and, as compared with the IFA test, 92.3% and 100%, respectively. Lastly, in a study of six experimentally infected cats and 48 negative purpose-bred cats, WITNESS® FFH was 100% sensitive and specific.

Overall results show that the new WITNESS® FFH Test Kit provides the sensitivity and specificity practitioners require to screen their feline patients for FeLV antigen, FIV antibodies, and adult heartworm antigen.
REFERENCES


