Feline Leukemia Virus Antigen Test Kit

ASSURE®/FeLV

For the Detection of Feline Leukemia Virus

DIRECTION INSERT

I. INTRODUCTION

Feline Leukemia Virus (FeLV) is a highly contagious oncogenic RNA virus that causes both neoplastic and non-neoplastic diseases in cats. Diseases caused by FeLV include lymphosarcoma, myelogenous leukemia, thymic atrophy, nonregenerative anemia and panleukopenia-like disease. Because FeLV is immunosuppressive, it predisposes infected cats to a variety of secondary diseases.

Rapid identification of infected animals allows them to be separated from non-infected cats to prevent the spread of FeLV. ASSURE®/FeLV uses highly specific antibodies to quickly identify FeLV-infected cats. The group specific antigen, p27, is found in high levels in infected cats and its presence is diagnostically definitive for FeLV infections. ASSURE®/FeLV is an immunoenzymatic assay using antibodies that specifically recognize p27 in cat blood and saliva.

Following exposure, a cat may test positive within 14 days. Persistent infections will remain positive while transient infections may turn negative within 1 to 2 months. A saliva test will not be strongly positive until the virus is amplified in the bone marrow, released into the circulation, and finally has infected the epithelial tissues. This could take a period from 1 to 2 months following exposure and would indicate a persistent infection.

Since infection may be transient in cats that develop immunity, antigen-positive animals should be retested in 1 to 2 months. A positive second test indicates persistent infection. A negative test indicates clearance of the virus.

II. TEST PRINCIPLES

The bulbous ends of the plastic wands are coated with an antibody directed against a specific FeLV antigen, p27. A second antibody directed against the p27 antigen is conjugated to the enzyme horseradish peroxidase. The sample is incubated in a single step with both the antibody-coated wand and enzyme-labeled antibodies. If antigen is present, it is captured by the wand. The enzyme-labeled antibodies are in turn captured by the antigen on the wand. The unbound enzyme labeled antibody is washed away and the wand placed into a chromogenic substrate.

The development of a distinct blue color in the solution indicates the presence of FeLV antigen. In the absence of FeLV, no color will develop.

When a **saliva** sample is used, ASSURE®/FeLV can detect antigen in cats actively shedding virus within 15-20 minutes.

When **whole blood, serum** or **plasma** samples are used, ASSURE®/FeLV can detect circulating antigen in infected cats within 10-15 minutes.

III. COMMON LEUKEMIA DIAGNOSTIC QUESTIONS AND ANSWERS

How soon after exposure/infection can antigen be detected?

As early as 4 days, but 14 days is average. If transmitted in-utero, antigen could be present and detected at birth.

Will FeLV vaccination cause the test to be positive?

No. ASSURE®/FeLV tests for FeLV p27 antigen in blood or saliva. Vaccination stimulates antibody production with a minute amount of p27; insufficient for detection. FeLV p27 antigen can only be found in detectable amounts when a cat is infected with the virus.

Is blood better to use for testing than saliva?

A positive result with a blood sample could indicate a persistent or transient infection. A positive result with a saliva sample is indicative of a persistent viremia. Positive blood samples can be repeated using saliva to determine the state/stage of the disease.

What does an ELISA positive, IFA negative test imply?

The infection is not yet established (i.e. amplified in the bone marrow) and may become transient, latent or persistant. Retesting in 1 to 2 months is recommended.

REFERENCES

1. Hardy, W.D., Jr., 1974 *Veterinary Clinics of North America* 4:133-146.
2. Hardy, W.D., Jr. McClelland, A.J., Zuckerman, E.E., Hess, P.W., Essex, M., Cotter, S.M., MacEwan, E.G., and Hayes, A.A., 1976, *Comps. Leuk. Research* (Karger, Basel) 43:511-514
3. Lewis, M.G., Wright, K.A., Lafrado, L.J., Shanker, P.U., Palumbo, N.E., Lemoine, E.D., and Olsen, R.G., 1987, *J. Clin. Micro.* 25:1320-1322.
4. Ettinger, S.J.: *Textbook of Veterinary Internal Medicine.* Philadelphia: W.B. Saunders Co., 1959, pp. 328-329.

IV. CONTENTS OF ASSURE®/FeLV TEST KIT

Anti-FeLV Antibody Coated Wands	25 ea
Transfer Pipettes	25 ea
Predispensed HRP-Monoclonal Antibody Conjugate (A Tubes)	25 ea
Predispensed Substrate Buffer (B Tubes)	25 ea
Bottle C-Chromogen (White Cap)	3.0 ml
Bottle D-S.E.R. (Blue Cap)	3.0 ml

Materials required, but not provided:

- Marking Pen
- Timer
- Wash Bottle
- Distilled/deionized water or normal saline

V. PRECAUTIONS

1. Allow kit to come to room temperature (21°-25° C; 70°-78° F) prior to use; approximately one hour.
2. Do not expose kit to direct sunlight.
3. Do not use expired reagents or mix from different kit serials.
4. Hold reagent vials vertically for proper drop volume.
5. Dispose of potentially infected specimens appropriately.
6. FOR VETERINARY USE ONLY.

VI. SAMPLE INFORMATION

Saliva or 100 µl (0.10 ml) of whole blood, plasma or serum is required. Whole blood and plasma must contain an anticoagulant. Serum and plasma samples may be stored at 2°-7°C (36°-45°F) for up to 7 days; 24 hours for whole blood. For longer periods, serum and plasma (not whole blood) may be stored a -20°C. Samples may be run cold. Hemolyzed or lipemic samples can be used, however, severely hemolyzed or lipemic samples may produce background color. When in doubt, obtain a better quality sample. Saliva samples cannot be stored or preserved. Saliva cell debris or abrasions on the wand's surface will not affect test results. Do not chemically induce salivation.

VII. STORAGE AND STABILITY

Store the test kit at 2°-7°C (38°-45°F). Do not freeze. Reagents are stable until expiration date provided they have been stored properly.

FOR TECHNICAL ASSISTANCE:
1-800-228-4305

SYNBIOTICS CORPORATION
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U.S Veterinary License Number 312

ASSURE®/FeLV Test Procedure

NOTE: *Use WHOLE BLOOD, SERUM, PLASMA or SALIVA samples.*
Prior to use, allow components to come to room temperature (70˚ to 78˚F; 21˚ to 25˚C).

For each sample you will need:

- Anti-FeLV Antibody Coated Wand. Label with cat's I.D.
- Transfer Pipette (one use only) 3 drops = 100 µl (Not required for saliva samples.)
- Predispensed HRP-Monoclonal Antibody Conjugate A Tube. (A)
- Predispensed Substrate Buffer B Tube. (B)
- Workstation with Reagents.
- Squirt Bottle with Distilled or Deionized Water or Saline.

A. SAMPLE COLLECTION

1A. WHOLE BLOOD/SERUM/ PLASMA PROCEDURE:

- Pipette **3 drops** sample to **A Tube**.
- Tap to mix.

- Proceed to Step 2.

OR

1B. SALIVA PROCEDURE:

- Add **3 drops Bottle D** (Blue Cap) to **A Tube**
- Tap to mix.
- Set in workstation.

- Sample Collection:
  - Obtain labeled Wand.
  - Place bulbous end of Wand between cheek and gum in the back of the mouth.
  - Gently rotate for 5-10 seconds.

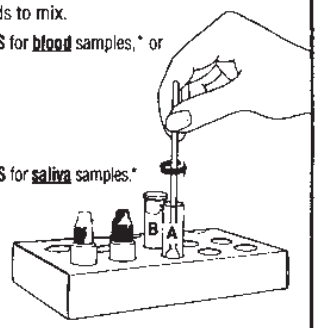
- Proceed to Step 2.

B. CONJUGATE INCUBATION

2.

- Place bulbous end of labeled **Wand** in **A Tube**.
- Twirl 1-3 seconds to mix.
- **WAIT 5 MINUTES** for **blood** samples,* or

- **WAIT 10 MINUTES** for **saliva** samples.*



*Or up to 20 minutes.

C. PREPARE B TUBE

3.

During waiting period:

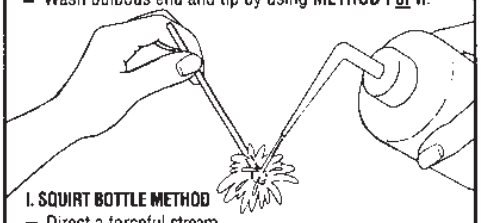
- Remove stopper from **B Tube**.
- Add **3 drops Bottle C** (White Cap) to **B Tube**.
- Tap to mix.
- Set aside for use in Step 5.

D. WASH STEP.

4. NORMAL SALINE MUST BE USED WITH WHOLE BLOOD SAMPLES.

Deionized/distilled water or normal saline can be used with serum, plasma or saliva samples.

- Remove Wand from **A Tube**.
- Wash bulbous end and tip by using **METHOD I or II**:

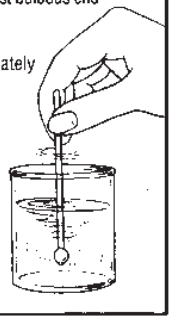


I. SQUIRT BOTTLE METHOD

- Direct a forceful stream of the *appropriate washing agent* against bulbous end and tip of Wand and work up handle.
- Shake off excess water.
- Repeat 5-7 times totaling approximately 8 oz. of *appropriate washing agent.*

II. CUP METHOD

- Perform initial rinse of Wand with a forceful stream of *appropriate washing agent.*
- Swirl/Swish vigorously in 100 ml of *appropriate washing agent* for a minimum of 30 seconds.
- Shake off excess liquid.
- Replenish liquid between Wands.



E. COLOR DEVELOPMENT

5.

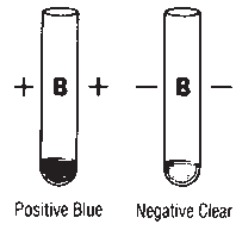
- Place washed Wand in **B Tube**.
- Twirl 1-3 seconds to mix.
- **WAIT 5 MINUTES.** (Weak positives may be verified by waiting up to 10 minutes).

- Remove Wand.
- **READ RESULTS.**

F. INTERPRETATION OF RESULTS

6.

- Observe solution against workstation window or a white background for blue color.

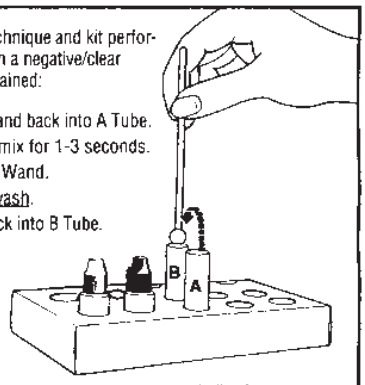


NOTE: Color intensity may vary with level of FeLV antigen present.

OPTIONAL PROCEDURAL CONTROL

To verify technique and kit performance when a negative/clear result is obtained:

- Place Wand back into A Tube.
- Twirl to mix for 1-3 seconds.
- Remove Wand.
- Do not wash.
- Place back into B Tube.



Blue color will develop within 1 minute indicating reagents were added correctly and kit is performing properly. If color does not develop, repeat the test. (This is a procedure and reagent check only. FeLV antigen is not present).

GOOD TECHNIQUES = ACCURATE RESULTS

- Whole blood and plasma samples must contain anticoagulant.
- Hemolyzed and lipemic samples may be used, however, normal saline should be used in place of distilled or deionized water in step 4. Severely hemolyzed and lipemic samples may produce background color. When in doubt, obtain a better quality sample.
- **Washing is the most important step. Wands cannot be overwashed.** Underwashing will result in non-specific blue color development in the B Tube.
- Prolonged incubation for more than 10 minutes in step 5 may result in non-specific blue color development. Read results at 5 minutes. If no color is seen at 5 minutes, the sample is negative. Weak or suspect positives at 5 minutes may be verified by waiting up to 10 minutes.
- Do not use the test kit past the expiration date and do not intermix components from different serial numbers.
- Store kit at 2˚ to 7˚C (36˚ to 45˚F). Allow kit to come to room temperature before use.

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