I. INTRODUCTION

Feline infectious peritonitis (FIP) is currently the leading infectious cause of death among young cats 3 months to 3 years of age and also in cats older than 10 years, especially those originating from purebred catteries and other large multiple-cat households. Feline enteric coronavirus (FeCV) is a highly infectious virus and is endemic in multiple-cat households. FeCV poses a considerable health risk to cats as it is generally thought to be the precursor of FIP. The ultimate control of FIP in catteries is linked to the control and prevention of FeCV infection. FeCV is included in the broad classification of Feline Coronavirus (FCoV). The presence of antibodies to FCoV indicates the possibility of FeCV and/or FIP infection. A positive titer does not necessarily mean that the cat has FeCV and/or FIP; however, a positive FCoV serology should be considered in conjunction with other clinical findings and symptomology to support a diagnosis of FeCV and/or FIP. A negative antibody titer strongly suggests lack of clinical disease due to FeCV and/or FIP.

II. TEST PRINCIPLES

The plastic wells are coated with purified coronavirus antigen. Protein A derived from *Staphylococcus aureus* is conjugated to HRP. The serum or plasma sample is incubated in the coated wells followed by incubation with the Protein A conjugate. Antibodies to FCoV, if present in the feline sample, are bound to the well and in turn bind the Protein A conjugate. The free enzyme-linked Protein A is washed away and a chromogenic substrate is added. The development of a distinctly blue color indicates the presence of antibody to FCoV. In the absence of FCoV antibody, no color change will be observed.

ViraCHEK®/CV is highly specific, sensitive and simple to perform. Test results can be obtained in 30 minutes. The diagnostic kit contains a positive control and a negative control which should be included each time the assay is performed. Visual comparison of the color of samples to the negative control will allow accurate detection of the presence of FCoV antibody in the sample.

III. SAMPLE INFORMATION

One microliter (1 µl) of serum or plasma is required. Use only feline samples for test specimens. Samples may be stored at 2°-7°C up to seven days. If longer storage is desired, samples may be stored at -20°C. Severely hemolyzed or lipemic serum may produce background color. When in doubt, obtain a better quality sample.

IV. PREPARATION OF WASH SOLUTION

Allow wash concentrate to come to room temperature. Mix gently by inversion. Dilute wash concentrate 10-fold using distilled or deionized water (1 part concentrate to 9 parts dH_2O) in a squirt bottle. Diluted wash solution may be stored at 2°-7°C.

V. RESULTS

1. For the test to be valid, the fluid in the positive control well must be distinctly blue, while that in the negative control well should remain clear.
2. A color change in the test sample well of greater intensity than the negative control well indicates the animal has previously been exposed to, or currently has an active infection with FCoV and may be capable of transmitting virus by shedding of coronavirus in fecal material. It is important that a positive diagnosis of FIP be supported by other clinical data and findings.
3. No color development in the test sample well strongly suggests a lack of clinical disease due to FIP.

VI. CONTENTS OF ViraCHEK®/CV KIT

The following items are packaged in each kit:

	24 test	48 test	96 test
Antigen coated wells	2x12 wells	4x12 wells	8x12 wells
Vial A–Positive Control Serum	1.0 mL	1.0 mL	1.5 mL
Vial B–Negative Control/Sample Diluent	3.0 mL	3.0 mL	7.0 mL
Vial C–Protein A Hrp Conjugate	3.0 mL	3.0 mL	5.0 mL
Vial D–TMB Chromogen	3.0 mL	3.0 mL	7.0 mL
Vial E–Substrate buffer	3.0 mL	3.0 mL	7.0 mL
Vial F–10X Wash Concentrate	100.0 mL	100.0 mL	100.0mL

Additional material provided:
- Disposable Sample Loops
- Holder for microwells
- Direction insert with instructions for conducting the test

Additional material required:
- Deionized or distilled water
- Squirt bottles (2)
- Timer

VII. PRECAUTIONS

1. Allow kit to come to room temperature (21°-25°C; 70°-78°F) prior to use.
2. Use separate sample loop for each sample.
3. Do not expose kit to direct sunlight.
4. Do not use expired reagents or mix from different kit lots.
5. Follow instructions exactly. Improper washing or contamination of reagents may produce nonspecific color development.
6. FOR VETERINARY USE ONLY.

VIII. STORAGE AND STABILITY

Store the test kit and unused diluted wash solution at 2°-7°C (36°-45°F). Do not freeze. Reagents should be stable until expiration date provided they have been stored properly.

FOR TECHNICAL ASSISTANCE:
1-800-228-4305

REFERENCES

1. Foley, Poland, Carlson, Pedersen; Risk Factors for feline infectious peritonitis among cats in multiple-cat environments with endemic feline enteric coronavirus; Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments; pp. 1313-1318, *JAVMA*, Vol 210, No. 9, May 1, 1997
2. Foley, Poland, Carlson, Pedersen; Patterns of feline coronavirus infection and fecal shedding from cats in multiple-cat environments; pp. 1307-1312, *JAVMA*, Vol 210, No. 9, May 1, 1997
3. McReynolds, Macy; Feline Infectious Peritonitis. Part I. Etiology and Diagnosis; pp. 1007-1014, *The Compendium*, September 1997

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03-1200-1098

Feline Infectious Peritonitis Antibody Test Kit

ViraCHEK®/CV

988

For the Detection of Antibodies to Feline Coronavirus

DIRECTION INSERT

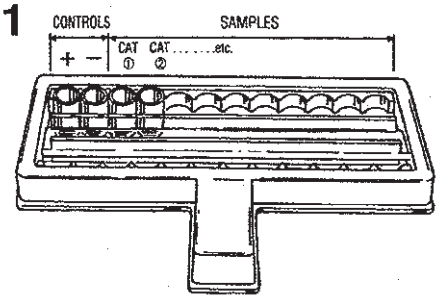
ViraCHEK®/CV Test Procedure

For use with Serum or Plasma only. (No Whole Blood.)

***NOTE:** Prior to use, allow kit components to come to room temperature (21°-25°C; 70°-78°F)*

A. SET UP AND SAMPLE INCUBATION

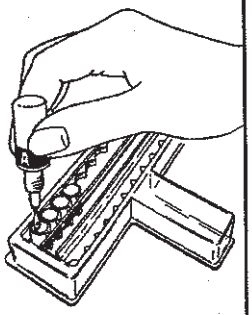
1



Remove and place in holder one well for Positive (+) Control, one well for Negative (–) Control/Sample Diluent, and one well for each sample. Leave the wells attached to each other.

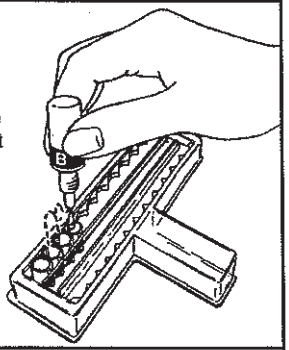
2

Add **1 drop** of Positive Control **(Bottle A – Red Cap)** into the first well.



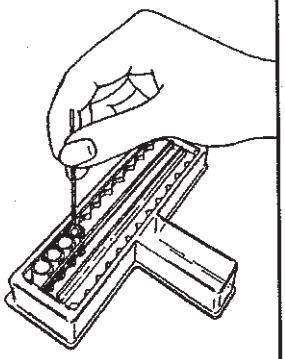
3

Add **1 drop** of Negative Control/Sample Diluent **(Bottle B – Gray Cap)** into the second well **and to each test sample well**.



4

Using a separate sample loop for each sample, add 1 loopful (1 µL) of each Sample to appropriate wells (well 3, 4, etc.) and swirl. Gently tap the holder for 10-15 seconds being careful not to splash from well to well.

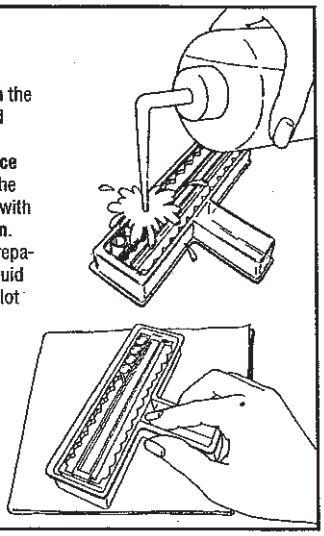


10 minutes Incubate for 10 minutes at room temperature (21°-25°C; 70°-78°F).
If several specimens are run simultaneously, only one set of controls is needed.

B. BLOT AND WASH

5

Discard the fluid from the wells by inverting and blotting onto a paper towel. Wash wells **once** by vigorously filling the wells to **overflowing** with **diluted wash solution.** (See section IV for preparation.) Discard the fluid from the wells, and blot after wash.

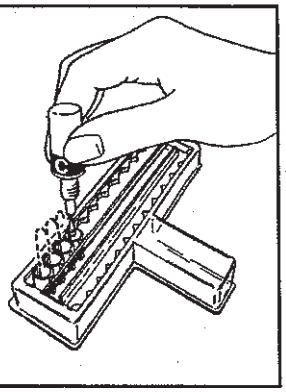


C. CONJUGATE

6

Add 1 drop of Protein A conjugate **(Bottle C – Blue Cap)** into each well. Gently tap the holder for 10 - 15 seconds and incubate 10 minutes at room temperature (21°-25°C; 70°-78°F).

10 minutes

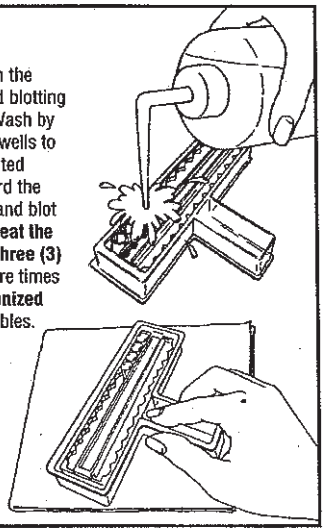


D. BLOT AND WASH

7

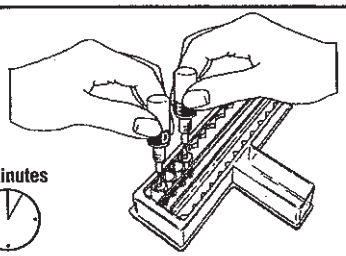
Discard the fluid from the wells by inverting and blotting onto a paper towel. Wash by vigorously filling the wells to **overflowing** with diluted wash solution. Discard the fluid from the wells, and blot after each wash. **Repeat the washing procedure three (3) times.** Wash two more times with **distilled or deionized water** to remove bubbles.

Blot dry on a paper towel.



E. DEVELOP

8

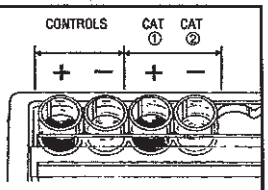


5 minutes

Place **1 drop** of Chromogen **(Bottle D – Green Cap)** into each well, followed by 1 drop of Substrate Buffer **(Bottle E – White Cap)**. Mix by gently tapping the holder several times. **Incubate 5 minutes.** After incubating, gently tap holder for 5 seconds and **read results immediately.** See Interpretation of Results section.

F. INTERPRETATION OF RESULTS

9



A Positive result (antibody to feline coronavirus is present) is indicated by any degree of blue color in the sample well when read immediately after the 5 minute incubation period.

A Negative result (antibody to feline coronavirus is absent) is indicated by no blue color development in the sample well. A Negative test result indicates that the animal is free of the virus or has not yet sero-converted after exposure. Cats may sero-convert in as little as 4 weeks or as long as twelve weeks after becoming infected with Feline Coronavirus. Care must be taken when interpreting positive test results in cats under six months of age as maternal FCoV antibodies may be present.

For the test to be valid, the fluid in the Positive Control well must be distinctly blue, while that in the Negative Control well must be clear.

Results should be interpreted immediately after the five minute incubation period. Prolonged incubation may result in non-specific color development. Wells can be detached and compared alongside the negative control well against a white background for easier visual inspection.

GOOD TECHNIQUES = ACCURATE RESULTS

- Serum or plasma must be used as a sample.
- Hemolyzed and lipemic serum samples may be used however, severely hemolyzed and lipemic samples may produce background color. When in doubt, obtain a better quality sample.
- **Washing is the most important step.** Wells cannot be overwashed. Underwashing will result in color development in the negative control and negative sample wells.
- Prolonged incubation for more than 5 minutes in step 8 may result in non-specific color development. If no color is seen after 5 minutes, the sample is negative.
- Always compare results to the negative control. The kit positive is engineered to be a moderate antibody level and is used to verify proper addition of reagents and good washing technique. It should not be used to differentiate positive from negative results.
- Do not use the test kit past the expiration date and do not intermix components from different serial numbers.
- Store kit at 2°-7°C (36°-45° F). Allow kit to come to room temperature before use.

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