

I. INTRODUCTION

TiterCHEK® CDV/CPV is an ELISA based assay used for the determination of antibody levels to Canine Distemper Virus (CDV) and Canine Parvovirus (CPV) in canine serum or plasma samples.

II. TEST PRINCIPLES

Color coded plastic wells are coated with either purified canine distemper virus (CDV) antigen or canine parvovirus (CPV) antigen. Serum or plasma samples are incubated in the coated wells followed by incubation with polyclonal rabbit anti-dog IgG conjugated to Hrp. Antibodies to CDV and/or CPV, if present in the canine samples, are bound to the specific antigen coated wells and, in turn, bind the anti-dog IgG conjugate. The free, unbound enzyme-linked conjugate is washed away and a chromogenic substrate is added. For CDV, a positive test result is intended to indicate a serum neutralization titer of 1:16 or greater, and a negative test result should indicate a serum neutralization titer of less than 1:16. For CPV, a

positive test result is intended to indicate a hemagglutination titer of 1:80 or greater, and a negative test result should indicate a hemagglutination titer of less than 1:80.

TiterCHEK® CDV/CPV is highly specific, sensitive and simple to perform. Test results can be obtained in 15 minutes. The diagnostic kit contains a positive control and a negative control which must be included each time the assay is performed. Visual comparison of the color of samples to the positive control for each assay will allow accurate detection of the presence of CDV and/or CPV antibody in the sample.

III. RESEARCH RESULTS

In order to evaluate the sensitivity and specificity of TiterCHEK, the veterinary diagnostic laboratories of Kansas State University (KSU), University of Georgia (UGA), University of Wisconsin (UW) and Synbiotics Corporation each tested samples for which CDV serum neutralization or CPV hemagglutination titers had been determined in the respective laboratories. Results of these studies are summarized below.

CDV SENSITIVITY			
	Samples	Correct	% Identified
UGA	53	41	77%
KSU	62	56	90%
UW	54	46	85%
Synbiotics	99	97	98%

CDV SPECIFICITY			
	Samples	Correct	% Identified
UGA	8	7	88%
KSU	2	2	100%
UW	11	11	100%
Synbiotics	57	53	93%

CPV SENSITIVITY			
	Samples	Correct	% Identified
UGA	60	55	92%
KSU	64	61	95%
UW	51	38	75%
Synbiotics	103	91	88%

CPV SPECIFICITY			
	Samples	Correct	% Identified
UGA	1	1	100%
KSU	56	55	98%
UW	69	68	99%

IV. SAMPLE INFORMATION

One microliter (1 µL) of serum or plasma is required. Use only canine samples for test specimens. Samples may be stored at 2°-7°C (36°-45°F) up to seven days. If longer storage is desired, samples may be stored at -20°C or below. Severely hemolyzed or lipemic serum may produce background color. When in doubt, obtain a better quality sample.

V. PREPARATION OF WASH SOLUTION

Allow wash concentrate to come to room temperature. Mix gently by inversion. Dilute wash concentrate 10-fold with distilled or deionized water (1 part concentrate to 9 parts dH₂O) in a squirt bottle. Reconstituted wash solution may be stored at 2°-7°C (36°-45°F).

VI. RESULTS

1. For the test to be valid, the fluid in the positive control well must be moderately blue, while that in the negative control well should show no color change from the initial substrate color.
2. Development of a blue color in the sample well that is of equal or greater intensity than the color of the positive control is considered to be positive (CDV SN titer ≥ 1:16 or CPV HI titer ≥ 1:80). Development of a blue color in the sample well that is of less intensity than the color of the positive control is considered to be negative (CDV SN titer < 1:16 or CPV HI titer < 1:80).

VII. CONTENTS OF KIT

The following items are packaged in each kit:

	16 test	24 test	48 test
CDV Antigen Coated Wells	2 x 8	3 x 8	6 x 8
CPV Antigen Coated Wells	2 x 8	3 x 8	6 x 8
Bottle A - Positive Control (Red Cap)	1.0 mL	2.0 mL	3.0 mL
Bottle B - Negative Control / Sample Diluent (Gray Cap)	3.0 mL	5.0 mL	7.0 mL
Bottle C - Conjugate (Blue Cap)	2.0 mL	3.0 mL	5.0 mL
Bottle D - Chromogenic Substrate (Green Cap)	3.0 mL	5.0 mL	7.0 mL
Bottle E - 10X Wash Solution (Orange Cap)	100 mL	100 mL	100 mL
Direction Insert	1	1	1
Disposable Sample Loops	50	50	100
Well Holder	1	1	1

Additional material required but not provided:
Deionized or distilled water
Squirt bottles (2)
Timer

VIII. 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.

IX. 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.

Canine Distemper-Parvovirus Antibody Test Kit

TiterCHEK® CDV/CPV

For the Determination of Canine Distemper Virus and Canine Parvovirus Antibody Levels

DIRECTION INSERT

FOR TECHNICAL ASSISTANCE:
1-800-228-4305

REFERENCES AND ADDITIONAL READING

- Olson et al. *AJVR* 49: 1460-1466, 1988; idem, *JVIM* 11:148, 1997
Schultz, *Proc San Diego Spring Vet Conf*:100-113; idem *Proc 1st IVVDC*: 47, 1997
Hoskins, *Canine Practice* 22(4):29-31, 1997
Twark L and Dodds W.J. *JAVMA* 217:1021-1024, 2000
Pollock RVM and Carmichael LE. *JAVMA* 180:37-42, 1982
McCaw DL et al. *JAVMA* 213:72-75, 1998
Tizard I and Ni Y. *JAVMA* 213:54-60, 1998

A. SET UP AND SAMPLE INCUBATION

1

Remove and place **CDV wells** (white rim) in top half of holder; one well for Positive Control, (**Bottle A - Red Cap**) one well for Negative Control (**Bottle B - Gray Cap**), and one well for each sample to be tested. Place **CPV wells** (red rim) in bottom half of holder; one well for Positive Control, one well for Negative Control and one well for each sample to be tested. Leave the required number of wells attached to each other.

2

Add **1 drop** of Positive Control (**Bottle A - Red Cap**) into the first CDV well and first CPV well.

3

Place **1 drop** of Negative Control/ Sample Diluent (**Bottle B - Gray Cap**) to the second CDV well and to each CDV test sample well. Place **1 drop** of Negative Control/ Sample Diluent to the second CPV well and to each CPV test sample well.

4

Using a separate sample loop for each sample, add **1 loopful** (1 μ L) of each sample to each of the CDV and CPV sample wells. Mix sample in diluent thoroughly by twisting the handle of the loop between the thumb and forefinger. Be careful not to splash from well to well. Incubate for 5 minutes at room temperature (21°-25°C; 70°-78°F).

5 minutes

If several samples are run simultaneously, only one set of CDV and CPV controls are needed.

B. BLOT AND WASH

5

Discard the fluid from wells into sink or appropriate container. Wash wells **once** by vigorously filling the wells to **overflowing** with **diluted wash solution**. (See section IV for preparation.)

Discard excess fluid into sink or appropriate container. Invert holder and blot firmly onto a paper towel to remove final drops.

C. CONJUGATE

6

Add **1 drop** of Conjugate (**Bottle C - Blue Cap**) into each well. Gently tap the holder for 10 - 15 seconds and incubate for 5 minutes at room temperature (21°-25°C; 70°-78°F).

5 minutes

D. BLOT AND WASH

7

Discard the fluid from wells into sink or appropriate container. 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.

Discard excess fluid into sink or appropriate container. Invert holder and blot firmly onto a paper towel to remove final drops.

E. DEVELOP

8

Place **2 drops** of Chromogenic Substrate (**Bottle D - Green Cap**) into each well. Mix by gently tapping the holder several times. Incubate 5 minutes.

5 minutes

After incubating, gently tap holder for 5 seconds and **read results immediately**. See Interpretation of Results section.

F. INTERPRETATION OF RESULTS

9

Compare each CDV sample well with the CDV positive and negative control wells.

Compare each CPV sample well with the CPV positive and negative control wells. Development of a blue color in the sample well that is of equal or greater intensity than the color of the Positive Control well is considered to be positive (CDV SN titer \geq 1:16 or CPV HI titer \geq 1:80).

No blue color in the sample well or color that is of less intensity than the color of the Positive Control Well is considered to be negative (CDV SN titer $<$ 1:16 or CPV HI titer $<$ 1:80).

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 show no color change from initial substrate color.

Results should be interpreted immediately after the 5 minute incubation period in Step 8. Wells can be detached and compared alongside the positive control well using 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 positive control. The kit negative control is used to verify 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.

FOR TECHNICAL ASSISTANCE: 1-800-228-4305