

ImmunoComb

CANINE PARVOVIRUS & DISTEMPER IgM ANTIBODY TEST KIT

INSTRUCTION MANUAL
Sufficient for 12/120 assays
21 OCT 2020



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I. INTENDED USE OF THE KIT

The ImmunoComb Canine Parvovirus and Distemper IgM Antibody Test Kit is designed to determine dog serum IgM antibody titer to Canine Parvovirus (CPV) and Canine Distemper Virus (CDV).

II. GENERAL INFORMATION

Canine Parvovirus (CPV) and Canine Distemper Virus (CDV) are recognized as important causes of illness and death in dogs. Puppies are most susceptible to CPV and CDV, especially after weaning when protective maternally derived antibody (MDA) levels decrease. Sometimes MDA may actually interfere with vaccinations that are given for immunization.

In many countries, vaccination programs have significantly curtailed, but not eliminated the incidence of these diseases. Thus, CPV and CDV continue to be of great clinical concern among veterinarians worldwide and still present a diagnostic challenge.

III. WHAT IS THE IMMUNOCOMB ASSAY?

The ImmunoComb test is a modified ELISA, which can be described as an enzyme labeled "dot assay", that detects antibody levels in serum, plasma or whole blood.

The kit contains all the necessary reagents for developing the test. Results for the IgM CPV and CDV tests are obtained within 23 minutes.

IV. HOW DOES THE IMMUNOCOMB WORK?

■ The ImmunoComb Kit contains 2 main components: a comb shaped plastic card, hereafter referred to as the Comb and a multi compartment developing plate.

- The Comb has 12 teeth – sufficient for 12 tests. Each tooth will be developed in a corresponding column of wells in the developing plate. Individual or multiple tests are processed by breaking off the desired number of teeth from the Comb.
- Test spots of CPV and CDV are attached to each tooth on the Comb. The upper most spot is a Positive Reference. Purified CPV antigen is attached at the middle spot and purified CDV antigen is attached at the lowest of the 3 spots (see figure in section X).
- The first step of the test is to deposit a serum, plasma or whole blood specimen in a well in row A of the multi-compartment developing plate.
- Next, the Comb is inserted into the well(s) with the sample(s) and transferred to the remaining wells (B-F) at timed intervals, according to the step by step instructions (see section VII). Specific IgM antibodies from the specimen, if present, bind to the antigen at the test spots.
- The Comb is transferred to the next well (row B) where non-bound antibodies are washed off.
- The Comb is inserted into the following well (row C) which contains an enzyme labeled anti-dog IgM antibody which will bind to the antigen-antibody complexes at the test spots.
- After 2 more washes (rows D & E) the Comb is moved to the next well (row F), where a color result develops via an enzymatic reaction.
- The intensity of the color result corresponds directly to the antibody level in the test specimen. Results are scored using the Positive Reference spot and the CombScale (see section IX).

V. DESCRIPTION OF DISEASE

CPV: Canine Parvovirus spread of infection can occur via exposure to contaminated surroundings. The clinical signs of CPV include lethargy, depression, inappetence, fever, vomiting and diarrhea (sometimes with blood). Mortality is high in puppies.

CDV: Canine Distemper Virus is naturally transmitted from dog to dog by aerosol route. Natural CDV infection may cause transient fever that can pass unnoticed. In some cases, when illness develops it is characterized by intermittent fever, depression, oculo-nasal discharge and anorexia. Respiratory and/or gastrointestinal signs may follow. In dogs that survive the acute stage of the disease, many (but not all) will develop central nervous system (CNS) signs, including optic neuritis and retinal lesions. The best known CNS signs are ataxia, paresis and seizures.

VI. DIAGNOSIS:

Veterinarians typically make a presumptive diagnosis of CPV and CDV based on clinical signs which range in severity from mild to severe.

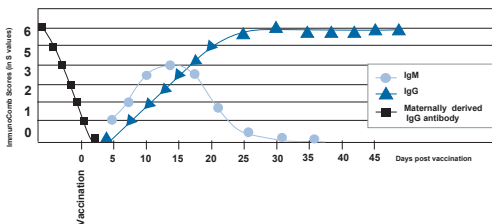
Laboratory tests can be helpful for confirming the diagnosis. In addition to hematology and blood chemistry, serology is becoming a more widely accepted diagnostic tool.

Serology provides a broader picture of the dog's immunologic status. The humoral immune response is largely composed of 2 classes of immunoglobulins (antibodies), IgM and IgG. In the initial days following infection or vaccination, IgM antibodies

are produced in large amounts. Later, IgM titers decline while IgG levels increase. Therefore, in dogs that are capable of mounting an immune response, elevated levels of IgM indicate recent infection or recent first vaccination.

The absence of IgM antibodies with an elevated IgG titer suggests that exposure to the virus occurred earlier, and the dog is currently immune. Elevated IgG levels are typically found in dogs that survive the acute phase of infection, or following vaccination (Fig. 1).

Fig. 1 - Post-Vaccination Antibody Levels to ICH, CPV & CDV in Healthy Dogs



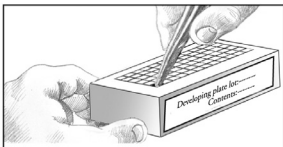
VII. STEP BY STEP WITH IMMUNOCOMB

Before conducting the test, bring the developing plate to room temperature by removing all kit components from the kit carton and place them on the work bench for 60-120 minutes or incubate only the plate at 37°C/98.6°F for 25 minutes.

Perform assay at room temperature 20° – 25° C / 68° – 77° F.

(1) Obtain blood sample from dog. When testing whole blood, collect sample in EDTA or heparin anticoagulant tube.

(2) Mix reagents by gently shaking the developing plate several times prior to use. Use the tweezers to pierce the protective aluminum cover of row A. One well for each sample/specimen.



Do not open any wells of row A or other rows which you do not intend to use.

Do not remove aluminum cover of developing plate all at once.

(3) Deposit a sample into a well in row A.

For testing serum or plasma use 5 μ l.

For testing whole blood use 10 μ l*.

Raise and lower pipette plunger several times to achieve mixing. (See Pipetting Technique section). Avoid spillage and cross-contamination of solutions.

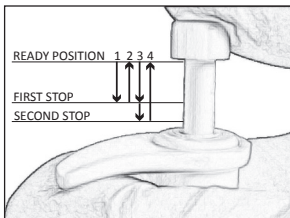
***For whole blood only: If dispensing the sample with a fix pipette provided with kits catalogue number 55CPD301 , use the same tip to deposit twice 5 μ l into the same well in row A.**

Pipetting Technique

Forward Pippeting

1- Press the operating button to the first stop.

2- Dip the tip attached to the pipette into the sample to a depth of about 1 cm and slowly release the operating button.



Wait for a while, then withdraw it from the liquid touching it against the edge of the reservoir to remove excess liquid adhering to the outer surface of the tip.

3- Dispense the sample into a well in row A by gently pressing the operating button to the first stop. After a second, press the operating button to the second stop. This will empty the tip completely. Remove the pipette from the well.

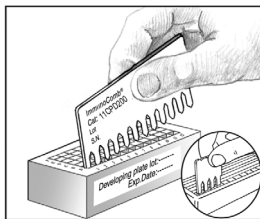
4- Release the operating button to the ready position.

(4) Remove the Comb from its protective envelope. Do not touch the teeth of ImmunoComb card.

For testing less than 12 samples, cut or break the Comb by folding in allocated notches for the number of tests required.

Note: Mixing during incubation according to instructions is critical for valid results.

****To improve mixing, move the Comb up and down 3-4 times. During incubation, repeat the same mixing process 2-3 times.**



Avoid scratching the front active side of the Comb by leaning it to the back while mixing.

Gently shake off excess liquid from Comb teeth onto a tissue before moving it to the next row.

■ Insert the Comb into the open well(s) in row A (printed side facing you) and incubate for 5 minutes. Mix as described above.**

■ Use tweezers to pierce the foil of the next well(s) in row B.

Shake off excess liquid and insert Comb for 2 minutes. Mix as described above.**

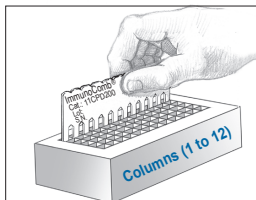
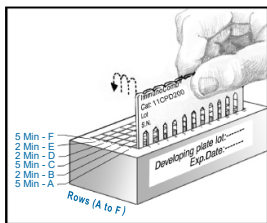
■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 5 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row D. Shake off excess liquid and insert the Comb for 2 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row E. Shake off excess liquid and insert the Comb for 2 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row F. Shake off excess liquid and insert the Comb for 5 minutes. Mix as described above.**

■ Upon completion of the color development in row F, move the Comb back to row E for 2 minutes for color fixation. Take the Comb out and let it dry for 5 minutes before reading the results.



VIII. READING AND INTERPRETING THE IgM

ANTIBODY RESULTS

■ The upper most spot is the Positive Reference spot and it should give a distinct purple-grey color. This is the same color tone that is generated by a high positive IgM response. This spot should be read as S3 on a scale of S0 to S6.

- The middle spot on the Comb gives the result of CPV IgM antibodies in the specimen.
- The bottom spot on the Comb gives the result of CDV IgM antibodies in the specimen.
- Compare the color tone of CPV and CDV test spots with the Positive Reference spot (separately).
- To evaluate the antibodies score use, the CombScale provided in the kit (see section IX).
- A clearly distinct color result that is equal or higher than S2 is considered positive .
- No color is considered a negative result.
- A faint color scored as $\leq S1$ is considered inconclusive.

IX. READING RESULTS WITH THE COMBSCALE

The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3.

When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.

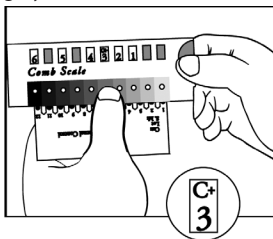
Find the tone of purple-grey on the CombScale that

most closely matches

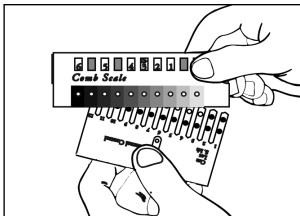
the **Positive Reference spot** (upper spot). Slide the yellow ruler until the C+ mark appears in the window above that color you just found.

Hold the ruler in this position during the entire reading. This step

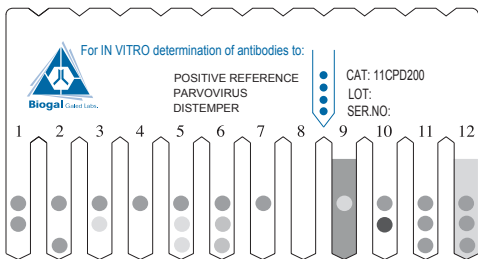
actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.



While holding the ruler, find the tone of purple-grey on the CombScale that most closely matches the desired **test result spot** (one of the lower spots). The number that appears in the window above is the CombScale score (S0-S6). Repeat this step with every test spot separately.



X. EXAMPLE OF A DEVELOPED COMB



Tooth N°	Results of CPV		Results of CDV	
	Result	Result	Result	Result
1	≥S3	Positive	S0	Negative
2	S0	Negative	≥S3	Positive
3	≥S1	Inconclusive	S0	Negative
4	S0	Negative	S0	Negative
5	≥S1	Inconclusive	≥S1	Inconclusive
6	S2	Positive	S2	Positive
7	S0	Negative	S0	Negative
8*		Invalid		Invalid
9**		Invalid		Invalid
10	≥S5	Positive	S0	Negative
11	≥S3	Positive	≥S3	Positive
12***	≥S3	Positive	≥S3	Positive

Remarks:

* No Positive Reference. Repeat test.

** High background. Repeat test.

*** High background with positive results.

XI. STORAGE & HANDLING

1. Store the kit under normal refrigeration (2° – 8° C / 36° – 46° F). **Do not freeze the kit.**
2. **Do not mix reagents from different kits or from different compartments of the same kit.**
3. The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

XII. SAMPLE HANDLING AND STORAGE

- Fresh samples are recommended for use.
- Store whole blood at 2-8°C if the test is to be run within 1 day of collection. Do not freeze whole blood samples.
- Store serum and plasma samples at 2-8°C if the test is to be run within 3 days of collection. If test is delayed more than 3 days, freeze samples to -20°C or colder.
- Bring samples to room temperature and mix well before testing.

XIII. KIT CONTENTS

Components	12 Test Kit (55CPD301)	12 Test Kit (55CPD401)	120 Test Kit (55CPD210)
A. ImmunoComb card (wrapped in aluminum foil)	1	1	10
B. Developing plate	1	1	10
C. Disposable tweezers	1	1	1
D. Calibrated CombScale	1	1	1
E. Junior fix pipette 5µl	1	-	-
F. 10 µl universal grad tip	15	-	-
Instruction manual	1	1	1



XIV. REFERENCES

- Harrus et al. (2002). *Jl of Small Animal Prac.*, **43**,27-31.
Waner T. (2002). *27th WSAVA Congress*, **Oct.**, Spain.
Waner et al. (2003). *Vet. Record*, **152**,588-591

For further assistance please contact your local Distributor, or Biogal Galed Laboratories directly by E-mail: info@biogal.com or by tel: 972-4-9898605 / fax: 972-4-9898690.