

XI. LIMITATIONS OF THE KIT

The Canine Distemper, Parvovirus and Adenovirus (type2) ImmunoComb® antibody test kit is subject to limitations inherent in any serologic assay, including false negative or false positive results. A false negative result occurs when the specific antibody is above the level detected by the test. This is most likely to happen when dogs are tested shortly after the onset of infection/vaccination.

As is the case for other serologic methods, Distemper Virus, Parvovirus and Adenovirus Type 2 serum IgG antibodies are not detectable by the ImmunoComb® assay until one to two weeks post virus exposure. A false positive result can occur in the ImmunoComb® assay if substantial amount of antibodies in a specimen bind non-specifically to an antigen spot for reasons that are not clearly understood. False positive as well as non-specific (NS) results may occur because of poor quality due to improper sample collection, preparation and/or storage. It is strongly recommended that NS and questionable results be corroborated by retesting specimens with the ImmunoComb® test and alternate diagnostic methods.

XIII. REFERENCES

- AAHA. Vaccine Task Force. (2006). *JAAHA*, **42**, 80-89.
Dudley et al. (1998). *JAVMA*, **213(1)**, 72-75.
Pollock & Carmichael. (1982). *JAVMA*, **180(1)**, 37-42.
Waner et al. (2006). *J. Vet. Dia. Invest.*, **18(3)**, 267-270.

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XII. STORAGE AND HANDLING

- Store the kit under normal refrigeration: 2°– 8° C (36° – 46° F). **Do not freeze the kit.**
- Before conducting the test maintain all kit elements and specimen at room temperature – preferably for 60 – 120 minutes or incubate plate for 22 minutes at 37°C (98.6° F). Perform assay at room temperature 20° – 25° C (68° – 77° F).
- Avoid spillage and cross-contamination of solutions.
- Mix reagents by inverting the developing plate several times prior to use.
- **Do not mix reagents from different kits or from different compartments of the same kit.**
- **Do not touch the teeth of the ImmunoComb® card.**
- When using developing plate, pierce the cover of each compartment according to the test procedure instructions. **Do not remove the aluminum cover of entire developing plate all at once.**
- The ImmunoComb® kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

CANINE DISTEMPER - ADENOVIRUS TYPE 2 - PARVOVIRUS ANTIBODY TEST KIT, DOT BLOT

ImmunoComb®
Canine VacciCheck

INSTRUCTION MANUAL

June 27, 2010

In Vitro Use Only



Produced by: Biogal Galed Labs. - Galed, 19240 - Israel

Produced for: Modern Veterinary Therapeutics, LLC
Coral Gables, Florida 33146 - USA
U.S. Permit No. 544-A

I. INTENDED USE OF THE KIT

This kit is used for the detection of antibodies to Infectious Canine Hepatitis (ICH), Canine Parvovirus (CPV) and Canine Distemper Virus (CDV) in dog whole blood or serum.

II. GENERAL INFORMATION

Infectious Canine Hepatitis (ICH), Canine Parvovirus (CPV) and Canine Distemper Virus (CDV) are recognized as important causes of illness and death in dogs. Puppies are most susceptible to ICH, 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, ICH, CPV and CDV continue to be of great clinical concern among veterinarians worldwide and still present a diagnostic challenge.

III. DESCRIPTION OF DISEASES

ICH

Infectious Canine Hepatitis is a disease that is caused by canine adenovirus (CAV). Transmission occurs by direct contact with infected dogs or virus contaminated areas. The first signs are coughing that may progress to pneumonia. Later, when the virus enters the bloodstream, liver, kidney and/or other body organs it may cause clinical signs such as: "blue eye", vomiting, diarrhea, increased thirst and seizures. Puppies have the highest mortality rate.

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). Fatalities are common in puppies.

CDV

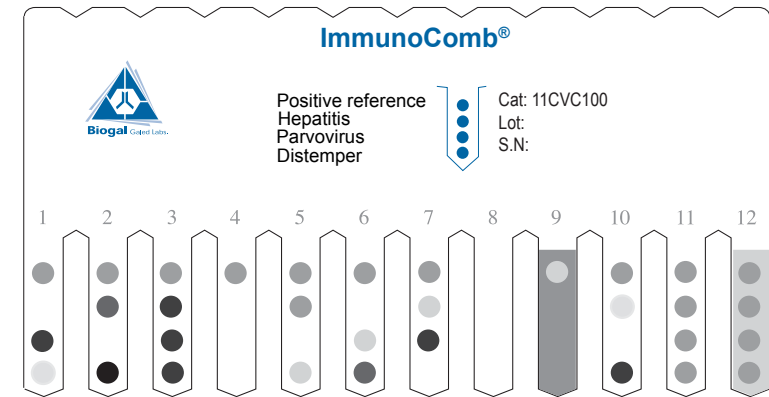
Canine Distemper Virus is naturally transmitted from dog to dog by aerosol route. Natural CDV infection may cause transient fever that can pass unnoticed. In some cases, when illness develops it is characterized by intermittent fever, depression, oculo-nasal discharge and anorexia. Respiratory and/or 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 most known CNS signs are ataxia, paresis and seizures.

Veterinarians typically make a presumptive diagnosis of Infectious Canine Hepatitis, Parvovirus and Distemper infections 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.

IV. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® is a modified ELISA, which can be described as an enzyme labeled "dot blot assay", that detects antibodies in serum or whole blood. The kit contains all the necessary reagents for developing the test. Results for the Canine VacciCheck test are obtained within 21 minutes.

X. EXAMPLE OF A DEVELOPED COMB



Tooth No.	Results of Inf. Hepatitis V.	Results of Parvovirus	Results of Distemper V.	Remarks
1	S0 Negative	≥S5 High Positive	S0-S1 Negative	
2	S4 Positive	S0 Negative	≥S5 High Positive	
3	≥S5 High Positive	≥S5 High Positive	≥S5 High Positive	
4	S0 Negative	S0 Negative	S0 Negative	
5	≥S3 Positive	S0 Negative	S2 Inconclusive Result	
6	S0 Negative	S2 Inconclusive Result	S4 Positive	
7	S1-2 Inconclusive Result	≥S5 High Positive	S0 Negative	
8	Invalid	Invalid	Invalid	No Positive Reference. Repeat test.
9	Invalid	Invalid	Invalid	High Background. Repeat test.
10	S0-S1 Negative	S0 Negative	≥S5 High Positive	
11	≥S3 Positive	≥S3 Positive	≥S3 Positive	
12	≥S3 Positive	≥S3 Positive	≥S3 Positive	High Background with positive result.

VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

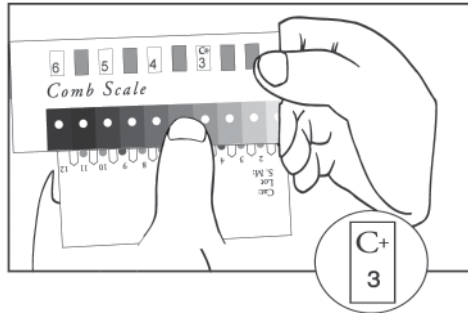
- The upper most spot should give a distinct purple-grey color. This is the same color tone that is generated by a positive response of anti ICH (CAV) antibodies at 1:16 V.N., anti CPV antibodies at 1:80 titer of H.I. test or of anti CDV antibodies equal to 1:32 V.N. When using the CombScale, this spot should be read as S3 (see section IX).
- The upper middle spot on the Comb gives the result of ICH IgG antibodies in the specimen.
- The lower middle spot on the Comb gives the result of CPV IgG antibodies in the specimen.
- The bottom spot on the Comb gives the result of CDV IgG antibodies in the specimen.
- Compare the color tone of ICH, CPV and CDV test spots with the Positive Reference spot (separately).
- A color tone equal or darker (S3-S6) than the reference spot is considered a positive response (VN titer $\geq 1:32$ for CDV, $\geq 1:16$ for ICH or a HI titer $\geq 1:80$ for CPV).
- A faint color tone of S1 or S0 is considered a negative result (VN titer $< 1:32$ for CDV, < 16 for ICH or a HI titer $< 1:80$ for CPV).
- A color tone that matches with S2 is considered an inconclusive result.

IX. READING RESULTS WITH THE COMBSCALE

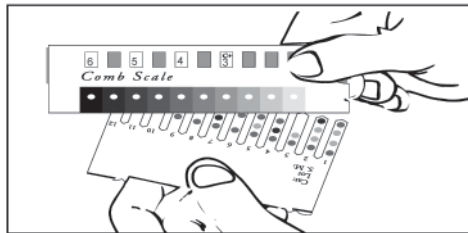
The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3.

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 slide 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.



Find the tone of purple-grey on the CombScale that most closely matches the test result spot (lower spots or each of the middle spots). The number that appears in the window above is the Comb score (S0-S6).



Another way to read the results is by using the CombScan. This is a software program that utilizes a computer and a TWAIN compatible scanner. When a Comb is placed on the scanner, the program translates the color results into numerical values. The CombScan assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

V. 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 ICH, CPV and CDV are attached to each tooth on the Comb. The upper most spot is a Positive Reference. Purified CAV antigen (for ICH testing) is attached to the upper middle spot, purified CPV antigen is attached at the lower middle spot and purified CDV antigen is attached at the lowest of the 4 spots (see figure in section X).

Specific antibodies, if present in serum or blood sample used in row A of the multicompartiment developing plate, react with the relevant antigen(s) on Comb test spots.

At the end of the developing process, described in section VII, positive results will be indicated by purple-grey colored spots developed via enzymatic reaction.

On each tooth of the developed Comb you should see the upper Positive reference spot. Test spots of ICH, CPV and/or CDV may appear, depending on the results.

Results are scored using the Positive Reference spot and CombScale (see section IX).

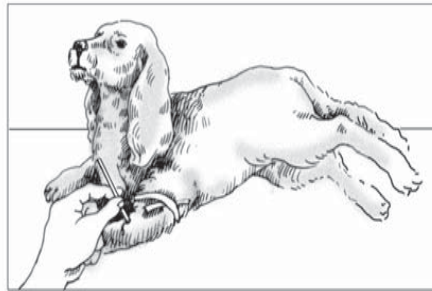
VI. KIT CONTENTS

- **The ImmunoComb® Card** - One Comb sufficient for 12 tests.
- **Developing Plate** - One plate, divided into compartments A-F, that are subdivided into 12 wells. The plate compartments are pre-filled with the reagent solutions.
- **Disposable Tweezers** - For piercing the foil cover of the developing plate compartments.
- **CombScale** - One calibrated CombScale color card for scoring reaction intensities.
- **Heparinized Capillary Tubes** - A vial with thirteen capillary tubes and a wire piston, intended for transferring whole blood or serum specimen into compartment A of the developing plate. The tubes are calibrated for dispensing 5 microliters serum (lower band), 10 microliters blood (upper band).
- **Documentation** - Instructions manual with detailed instructions.

VII. STEP BY STEP WITH IMMUNOCOMB®

Perform assay at room temperature of 20°-25° C (68° - 77°F).

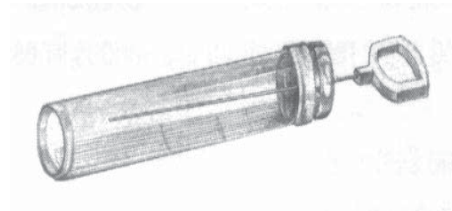
- (1) Obtain blood sample from dog.



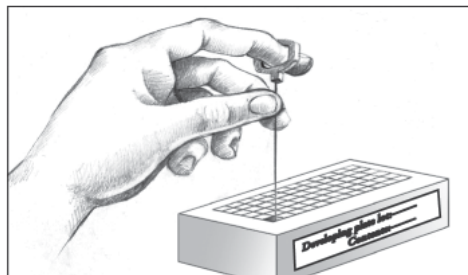
- (2) Use the tweezers to pierce the protective aluminum cover of row A. One well for each specimen.



- (3) Use a pipette or a capillary tube. For testing whole blood use 10µl. For testing serum use 5µl.

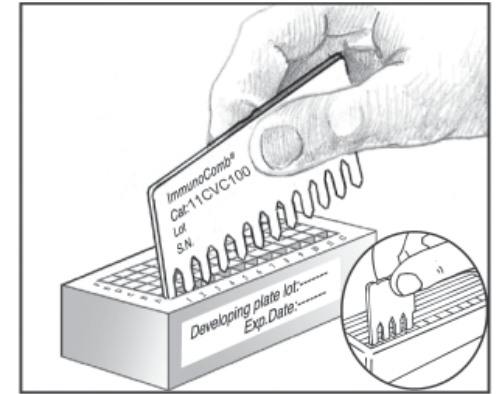


- (4) Deposit a sample into a well in row A. Raise and lower pipette/piston plunger several times to achieve mixing.

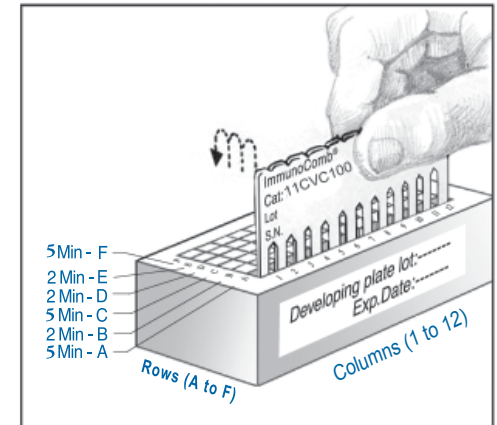


Do not open any well of row A or other rows which you do not intend to use.

- (5) Remove the Comb from its protective wrapping. For testing less than 12 samples, cut or break the Comb by folding in allocated notches for the number of tests required. Insert the Comb into the open well(s) in **row A** (printed side facing you) and incubate for **5 minutes**. To improve mixing, gently move Comb **up and down** at the start of each incubation (each row). Repeat this motion at least twice in all of the remaining rows.



- (6) Use tweezers to pierce the foil of the next well (**row B**), and insert Comb for **2 minutes**. Before transferring Comb from one well to the next, pierce the foil of the next well and gently shake off excess liquid from Comb teeth onto a tissue. Insert Comb into the next well (**row C**) for **5 minutes**. Place Comb into the remaining wells (**rows D & E**) for **2 minutes** each and the last well (**row F**) for **5 minutes**.



- (7) 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 2-5 minutes).

