

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

ImmunoComb
Canine VacciCheck[®]

INSTRUCTION MANUAL
November 25, 2018

In Vitro Use Only



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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.

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 12-tests kit

- **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.
- **Junior fix pipette 5 µl** - calibrated for dispensing 5 microliters serum or whole blood into compartments A of the developing plate.
- **10 µl universal grad tip** - A bag with 15 tips intended for use with the Junior fix pipette.
- **Documentation** - Instructions manual with detailed instructions.

The 120-tests kit

- **The ImmunoComb Card** - Ten Combs sufficient for 120 tests.
- **Developing Plate** - Ten plates, each 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.
- **Documentation** - Instruction manual with detailed instructions.

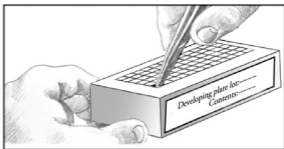
VII. STEP BY STEP WITH IMMUNOCOMB

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.



(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 the kit, use the same tip to deposit twice 5 μ l into the same well in row A.**

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

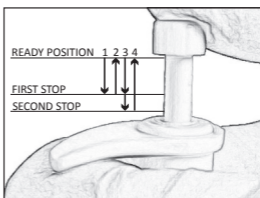
Do not remove aluminum cover of developing plate all at once.

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

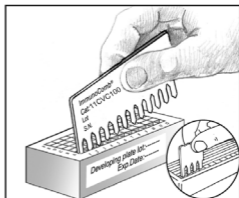


from the liquid touching it against the edge of the reservoir to remove excess liquid adhering to the outer surface of the tip.

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.

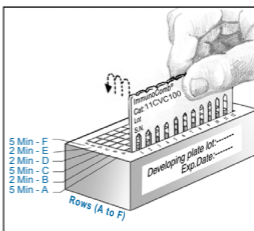
****To improve mixing, move the Comb up and down 3-4 times. During incubation, repeat the same mixing process 2-3 times.**

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.**

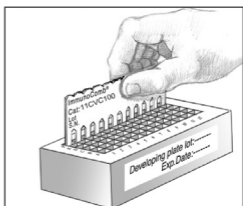
■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 5 minutes. Mix as described above.**

■ 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 IgG ANTIBODY RESULTS

■ The upper most spot should give a distinct purple-gray 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.

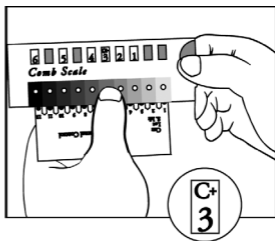
- The lower middle spot on the Comb gives the result of CPV IgG antibodies in the specimen.
- The bottom spot on the Comb gives the result of CDV IgG antibodies in the specimen.
- Compare the color tone of ICH, CPV and CDV test spots with the Positive Reference spot (separately).
- A color tone equal 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 a weak positive result.
- A test spot with a washed blue appearance is invalid. Refer to Biogal for further advice.

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.

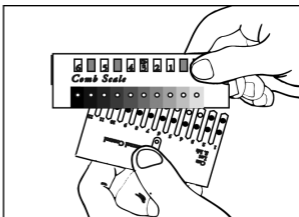
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.

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.



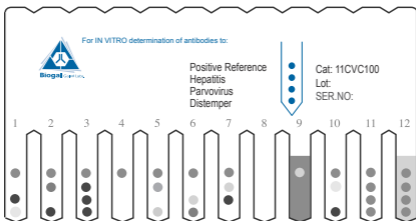
Hold the ruler in this position during the entire reading. This step actually calibrates the C+ to S3, which is the “cut-off” point to which test spots will be compared.

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, version 2.2.0. 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. See CombScan insert for complete instructions on validating and operating the program.

X. EXAMPLE OF A DEVELOPED COMB



Tooth N°	Results of Inf. Hepatitis V.		Results of Parvovirus		Results of Distemper V.	
	Result	Interpretation	Result	Interpretation	Result	Interpretation
1	S0	Negative	≥S5	High pos.	<S1	Negative
2	S4	Positive	S0	Negative	S6	High pos.
3	≥S5	High pos.	≥S5	High pos.	≥S5	High pos.
4	S0	Negative	S0	Negative	S0	Negative
5	≥S3	Positive	S0	Negative	S2	Weak pos.
6	S0	Negative	S2	Weak pos.	S4	Positive
7	S2	Weak pos.	≥S5	High pos.	S0	Negative
8*		Invalid		Invalid		Invalid
9**		Invalid		Invalid		Invalid
10	<S1	Negative	S0	Negative	≥S5	High pos.
11	≥S3	Positive	≥S3	Positive	≥S3	Positive
12***	≥S3	Positive	≥S3	Positive	≥S3	Positive

Remarks:

*No Positive Reference. Repeat test.

**High background. Repeat test.

***High background with positive results.

XI. LIMITATION 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 below 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.

XII. STORAGE AND HANDLING

■ Store the kit under normal refrigeration: 2°– 8° C (35° – 46° F). **Do not freeze the kit.**

■ **Do not mix reagents from different kits or from different compartments of the same kit.**

■ The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

XIII. REFERENCES

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Pollock & Carmichael. (1982). *JAVMA*, 180(1), 37-42.

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