ImmunoComb

CANINE EHRLICHIA ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 12/120 assays

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Instruction Cat. No: 63CEH911

I. INTENDED USE OF THE KIT

The ImmunoComb Canine Ehrlichia Antibody Test Kit is designed to determine dog serum antibody titers to *Ehrlichia canis*.

II. GENERAL INFORMATION

Canine Ehrlichiosis is a worldwide recognized infectious blood disease caused by the rickettsia *Ehrlichia canis*. This infection is usually spread via the tick vector *Rhipicephalus sanguineum*, which is better known as "brown tick" and that is why the disease is often called "Tick fever". Another way of transmission of the rickettsia is by blood transfusion from an infected blood donor.
Canine Ehrlichiosis is a multisystemic disease and its clinical signs manifestation may vary in their nature, severity and duration (see chapter V), yet it infects primarily monocytes in dogs, giving the disease its name Canine Monocytotrophic Ehrlichiosis (CME).

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 *Ehrlichia canis* tests are obtained within 20 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.

- A test spot of *E.canis* antigen is attached to the lowest spot of each tooth on the Comb. The upper spot is a Positive Reference. (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 IgG antibodies from the specimen, if present, bind to the antigen at the test spots and will be labeled in row C, which contains an enzyme labeled anti-dog IgG antibody.
- At the end of the developing process, a purple-grey color results are developed in all Positive Reference spots and in any positive sample tested spot.
- 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 CombScale (see section IX).

V. DESCRIPTION OF DISEASE

In general, the course of the disease is delineated into three stages:

1st Stage: The early or acute stage of the infection includes nonspecific clinical signs, which may be mild and sometimes pass unnoticed by the dog owner. Some dogs have a decreased appetite with fever and lethargy. Upon physical examination, the veterinarian may detect lymphadenopathy and splenomegaly.
A decreased platelet count is the most consistent hematologic finding. If left untreated, most dogs may well "recover" from the acute stage. However, they do not

necessarily eliminate the organism from their system, and thus enter the second stage of infection.

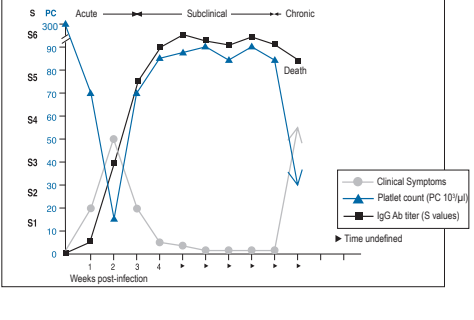
2nd Stage: This is the subclinical stage (i.e. there are no signs of illness), which may last for an indefinite period. Correct treatment at this stage, specially in high risk animals, is important in order to prevent further progress of the disease.

3rd Stage: It is unclear what causes some dogs to pass from the subclinical stage into the chronic (3rd) stage of Ehrlichiosis. It is recognized, however, that after dogs have reached this stage, treatment attempts are often unrewarding. Clinical signs of chronic Ehrlichiosis include lethargy, fever, inappetence, weight loss, bleeding tendencies and ultimately death.

VI. DIAGNOSIS:

The diagnosis of CME is largely based on clinical signs during the acute and chronic stages. Whereas a decreased platelet count is the most consistent hematologic finding, serology is the preferred method for confirming infection by E. canis, especially during the 2nd stage of the disease, when clinical manifestations are absent. (See fig. 1)

Fig. 1 Canine Ehrlichiosis: Three Stages of Infection



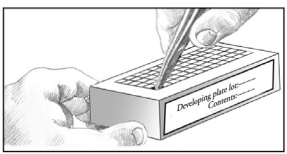
VII. STEP BY STEP WITH IMMUNOCOMB

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.

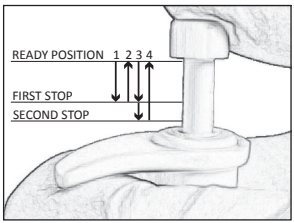
For testing serum or plasma use 5µl.
For testing whole blood use 10µl*.

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

***For whole blood only: If dispensing the sample with a fix pipette provided with kits catalogue number 50CEH301 , use the same tip to deposit twice 5µl into the same well in row A.**

Pipetting Technique

Forward Pippeting



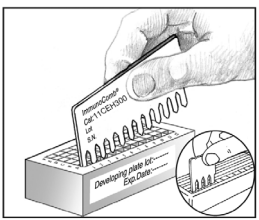
1- Press the operating button to the first stop.

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.

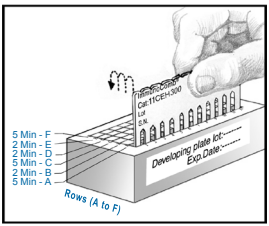
(4) Remove the Comb from its protective envelope. Do not touch the teeth of ImmunoComb card. For testing less than 12 samples, cut or break the Comb by folding in allocated notches for the number of tests required.



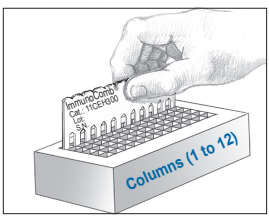
Note: Mixing during incubation according to instructions is critical for valid results.

****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 IgG ANTIBODY RESULTS

■ The upper 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 significant positive IgG response. When using the CombScale, this spot should be read as S3 (see section IX). S3 is considered the "cut-off" level of IgG antibody, which is roughly equivalent to a positive immune response at a titer 1:80 by the Immunofluorescent assay (IFA).

■ The bottom spot on the Comb gives the result of *E. canis* IgG antibodies in the specimen.Compare the color tone of *E. canis* spot with the Positive Reference spot (separately).

■ A color tone that is equal or darker than the reference spot is considered a positive response.

■ Color fainter than the Positive Reference indicates a low response.

■ To evaluate the antibodies score use the CombScale provided in the kit (see section IX).

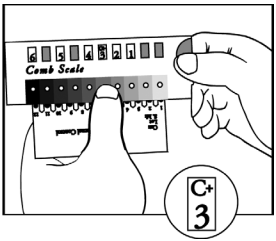
■ The dry Comb may be kept as record.

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.

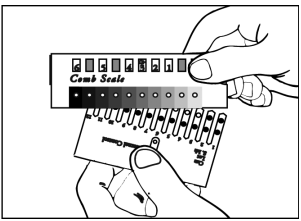
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.
Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper

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

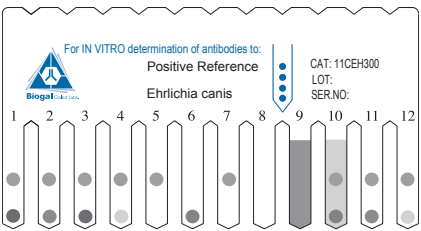


Hold the ruler in this position during the entire reading. This step actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.

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 Nº	Results		Remarks
1	≥S5	High positive reaction to *E. canis*	Titer 1:320-1280
2	S3-S4	Medium positive reaction *E. canis*.	Titer 1:80-1:160
3	≥S5	High positive reaction to *E. canis*	Titer 1:320-1280
4	S1-2	Low positive reaction to *E. canis*.	Titer 1:20-1:40
5	S0	Negative reaction to *E. canis*.	Negative
6		*No Positive Reference	Invalid test
7		*No Positive Reference	Invalid test
8	S0	Negative reaction to *E. canis*.	Negative
9	*	High background color - interferes with reading.	Invalid test
10	≥S3	Positive reaction with high background.	Positive
11	S3-S4	Medium positive reaction *E. canis*.	Titer 1:80-1:160
12	S1-2	Low positive reaction to *E. canis*.	Titer 1:20-1:40

*** Repeat Test**

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.

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 (50CEH301)	12 Test Kit (50CEH401)	120 Test Kit (50CEH210)
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 S et al. (2002). *Vet Microbiol.* **86 (4)**, 361-368.
Waner T et al. (2000). *Jl of Vet Diag Invest.* **12**, 240-244.
Waner T (1999). Diag of Canine Monocytic Eh Thesis, Utrecht U.
Waner T et al. (1997). *Vet Para.* **69**, 307-317.

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