

ImmunoComb

CANINE LEPTOSPIRA ANTIBODY TEST KIT

INSTRUCTION MANUAL
Sufficient for 12/120 assays
14 FEB 2018



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I. INTENDED USE OF THE KIT

The Canine *Leptospira* Antibody Test kit is designed to determine dog serum antibody titers to different pathogenic serovars of *Leptospira interrogans*. A mixture of antigens: *L. ichterohaemorrhagiae* (copenhageni and RGA), *L. canicola*, *L. pomona* and *L. grippityphosa*, bind antibodies to the most widespread variants found in dogs. A positive result indicates current infection. The kit is NOT intended to distinguish the specific serotype and is not intended to monitor vaccination.

II. GENERAL INFORMATION

Leptospirosis, a spirochetal disease that occurs worldwide in numerous animal hosts, is reemerging as an important zoonotic disease. Reported cases are on the rise throughout the world. Different serovars of *Leptospira interrogans* are maintained in nature in numerous sub-clinically infected wild and domestic animal reservoir hosts. Reservoir hosts are a source of infection for humans and other incidental hosts.

Canine infection is seen mostly in hunting and sporting dogs that eat wildlife, or dogs that have access to wooded areas with riverbanks or marshes. Standing water is a natural environment for *Leptospira*. Infection occurs via direct contact with contaminated urine and by transplacental and venereal routes. Indirect infection is also possible via exposure to contaminated water, vegetation, soil or food.

Leptospira does not multiply outside a host and its survival depends on the environmental conditions in which they are found. *Leptospira* are susceptible to drying, and pH changes can be detrimental. There is a higher incidence of Leptospirosis during the rainy

season when there is an abundance of standing water and swampy conditions. In an attempt to control the disease, vaccination programs have been instituted. However, the vaccine does not always contain relevant serovars, and the duration of immunity achieved is very short (6-12 months).

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 Canine *Leptospira* 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.
- A test spot of *Leptospira* serovar mix is attached to the lowest spot of each tooth on the Comb. The upper most spot is a Positive Reference.
- The first step of the test is to deposit a serum, plasma or whole blood specimen into 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. CLINICAL SIGNS

The canine disease presents as an acute infection of the kidney and liver and even as a septicemia. Chronic kidney disease is a common manifestation of infection. Abortions may occur in pregnant bitches. The prevalence of Leptospirosis in dogs may be underestimated because of asymptomatic infection.

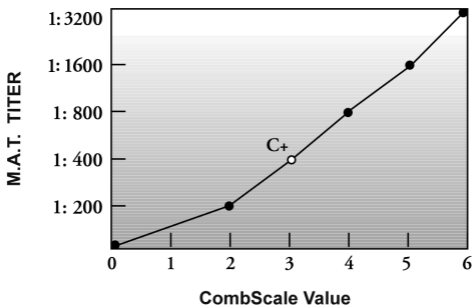
VI. DIAGNOSIS:

The diagnosis of canine *Leptospira* is largely based on clinical signs. Serologic testing is useful in establishing the diagnosis. The Microscopic Agglutination Test (MAT) is the standard laboratory method to serologically diagnose Leptospirosis. A four-fold rise in antibody titer to a *Leptospira* serovar is considered significant. It is not unusual to see elevated titers to multiple serovars due to cross-reactions. If there is a positive cross-reaction the serovars with the highest titers are considered to be most significant.

The ImmunoComb test which is based on the "dot"-ELISA technology, is more sensitive than the MAT, yet it does not presume to identify the specific

serotype. The ImmunoComb Canine Leptospira test kit is suitable for the detection of rising antibody levels due to infection with any of the following serovars: *L. canicola*, *L. icterohaemorrhagiae*, *L. grippityphosa* and *L. pomona* serovars.

FIG.1: RELATIONSHIP BETWEEN THE COMBSCALE'S "S" VALUE AND THE M.A.T. TITER



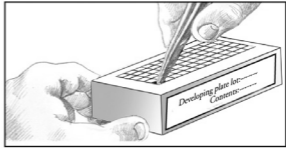
VII. STEP BY STEP WITH IMMUNOCOMB

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.

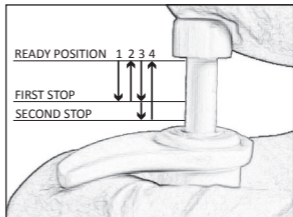
***For whole blood only: If dispensing the sample with a fix pipette provided with kits catalogue number 50CLC301, use the same tip to deposit twice 5 μ l into the same well in row A.**

Pipetting Technique

Forward Pipetting

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.

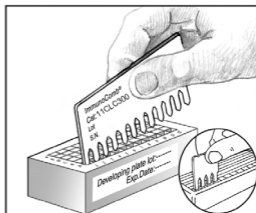
4- Release the operating button to the ready position.

(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.**

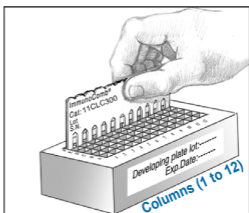
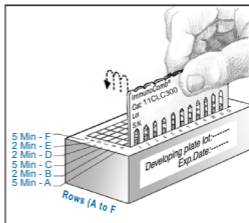
■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 5 minutes. Mix as described above.**

■ 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 should give a distinct purple-grey color. This is the same color tone generated by a significant positive IgG response. This spot should be read as S3 on a scale of S0 to S6. (S3 is considered the "cut-off" level of IgG antibodies, which is roughly equivalent to a positive immune response at a titer

of 1:400 by the Microscopic Agglutination Assay - MAT). See Fig. 1, section VI.

■ The bottom spot on the Comb gives the result of the anti *Leptospira* serovars mix IgG antibodies reaction in the specimen tested. Compare the color tone of the anti *Leptospira* serovars mix spot (bottom one) with the Positive Reference spot (upper one). A clear, visible purple-grey dot indicates a positive response. A result darker than the positive reference means a higher titer. Color fainter than the positive reference indicates a low response, which is approximately equivalent to MAT titer of 1:100 to 1:200.

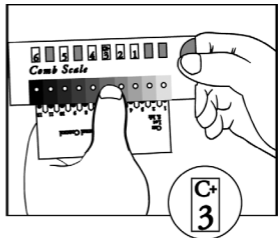
■ To evaluate the antibodies score, use the CombScale provided in the kit.

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.

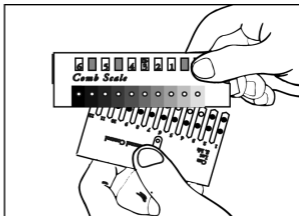
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.

Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper spot). Slide the



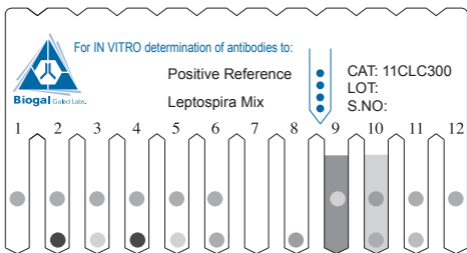
yellow ruler until the C+ mark appears in the window above that color you just found. **Hold the ruler in this position during the entire reading.** This step actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.

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.



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.

X. EXAMPLE OF A DEVELOPED COMB



Tooth No.	Results	
1	S0	Negative reaction to <i>Leptospira</i>
2	≥S5	High positive reaction to <i>Leptospira</i>
3	S1-2	Low positive reaction to <i>Leptospira</i>
4	≥S5	High positive reaction to <i>Leptospira</i>
5	S1-2	Low positive reaction to <i>Leptospira</i>
6	≥S3	Positive reaction to <i>Leptospira</i>
7*		Invalid
8*		Invalid
9**		Invalid
10***	≥S3	Positive reaction to <i>Leptospira</i>
11	≥S3	Positive reaction to <i>Leptospira</i>
12	S0	Negative reaction to <i>Leptospira</i>

Remarks:

- * No Positive Reference. Repeat test.
- ** High background. Repeat test.
- *** High background with positive result.

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 (50CLC301)	12 Test Kit (50CLC501)	120 Test Kit (50CLC210)
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



A



B



C



D



E



F

XIV. REFERENCES

- 1). Noel R. (2002). An overview of canine leptospirosis.
<http://www.vet.uga.edu/vpp/clerk/noel/>
- 2). McDonough, P.L. (2001). Leptospirosis in dogs current status, Recent Advances in Canine Infectious Diseases. *International Veterinary Information Services, July*.
<http://www.ivis.org>
- 3). Levett, P. Leptospirosis. (2001). *Clinical Microbial Reviews*, 14(2), 296-326.

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.