

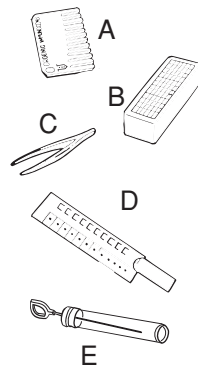
XI. STORAGE & HANDLING

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

XII. KIT CONTENTS

Component	12 Test Kit Cat.50CLC201	120 Test Kit Cat.50CLC210
A. ImmunoComb® card (wrapped in an aluminum envelope)	1	10
B. Developing plate*	1	10
C. Disposable tweezers	1	1
D. Calibrated CombScale color card	1	1
E. Unit of 12 capillary tubes & one piston	1	—
Instruction manual	1	1

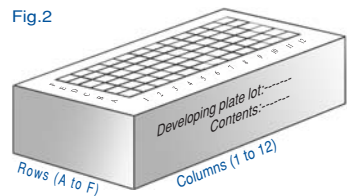
* The developing plate is illustrated below.



XIII. REFERENCES

- 1). Noel R. (2002). An overview of canine leptospirosis. HYPERLINK "<http://www.vet.uga.edu/vpp/clerk/noel/>" <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. HYPERLINK "<http://www.ivis.org/>" www.ivis.org
- 3). Levett, P. Leptospirosis. (2001). *Clinical Microbial Reviews*, 14(2), 296-326.

Fig.2



ImmunoComb®

CANINE LEPTOSPIRA ANTIBODY TEST KIT

INSTRUCTION MANUAL

Product Cat. No: 50CLC201 & 50CLC210

6.6.2007



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I. INTENDED USE OF THE KIT

This kit is designed to determine dog serum antibody titers to different pathogenic serovars of *Leptospira interrogans*. A mixture of antigens: *L. icterohaemorrhagiae* (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.

II. GENERAL INFORMATION

Leptospirosis, a spirochetal disease that occurs worldwide in numerous animal hosts, is re-emerging 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 do not multiply outside of a host and their 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 (6-7 months).

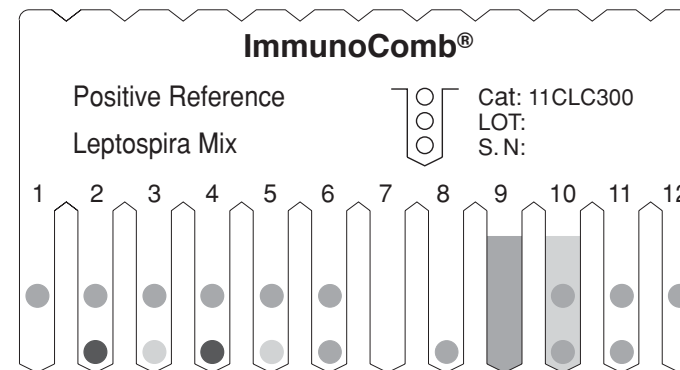
III. WHAT IS THE IMMUNOCOMB ASSAY?

The ImmunoComb® test is a modified ELISA, which can be described as an enzyme labeled "dot assay", that detects antibody levels in serum or whole blood. The kit contains all the necessary reagents for developing the test. Results for the Canine *Leptospira* 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 is 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 in a well in row A of the multi-compartment developing plate.
- Next, the Comb is inserted into the well with the sample(s) and transferred to the remaining wells (B-F) at timed intervals, according to the instructions that follow. Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots.

X. EXAMPLE OF A DEVELOPED COMB



TOOTH No.	RESULT	REMARKS
1, 12	Negative reaction for <i>Leptospira</i> .	Negative
2, 4	High positive reaction for <i>Leptospira</i> .	S 5-6
3, 5	Low positive reaction for <i>Leptospira</i> .	S 1-2
6, 11	Positive reaction for <i>Leptospira</i> .	S 3-4
7, 8	No positive reference - Development failed.	Invalid test
9	High background color - interferes with reading.	Invalid test
10	Positive reaction with high background	Positive

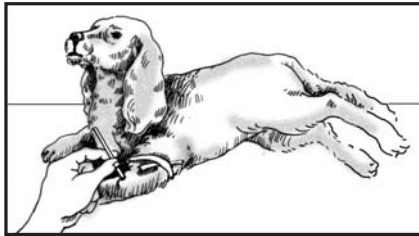
Another way to read the results is by using the CombScan 2000. This is a software program that utilizes a computer and a twain compatible scanner. When a comb is placed on the scanner, the program translates the color results into numerical values. The CombScan 2000 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

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.

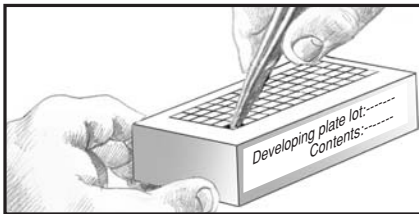
VII. STEP BY STEP WITH THE IMMUNOCOMB®

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

(1) Obtain blood sample from dog.



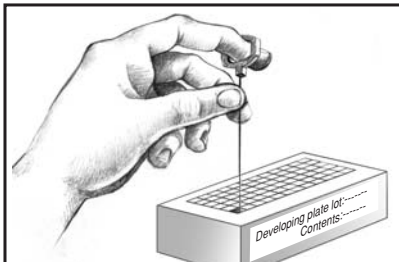
(2) Use the tweezers to pierce the protective aluminum cover of row A*. One well for each specimen.



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



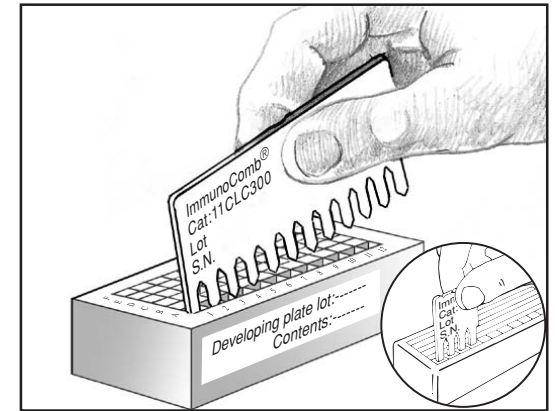
(4) Deposit a sample into well A*. Raise and lower the piston /pipette plunger several times to achieve mixing.



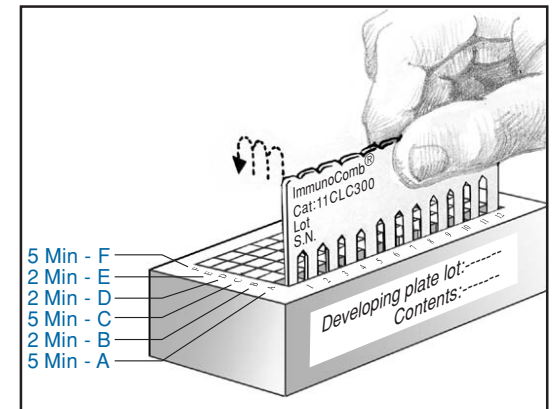
* See Fig 2, page 7.

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

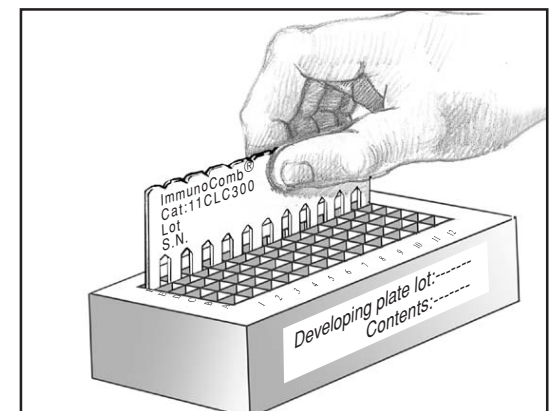
(5) Remove the Comb from its protective wrapping. For testing less than 12 samples, cut or fold Comb in allocated notches for the number of tests required. Insert the Comb into the open well(s) in **row A*** (printed side facing you) and incubate for **5 minutes**. To improve mixing, gently jiggle the Comb up and down. 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 the Comb from one well to the next, pierce the foil of the next well, and gently shake off excess liquid from the Comb tooth onto a tissue. Insert Comb into the next well (**row C***) for **5 minutes**. Place Comb in the remaining wells (**rows D* & E***) for **2 minutes** 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** to fix color. Take the Comb out and let it dry.



* See Fig 2, page 8.