ImmunoComb

CANINE BRUCELLA ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 12/120 assays

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Instruction Cat. No: 63CBR311

I. INTENDED USE OF THE KIT

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

II. GENERAL INFORMATION

Brucella canis is sexually transmitted by the mating of infected males and females. *Brucella canis* in the female dog will live in the vaginal and uterine tissue and secretions for years, usually for life. In males, the Brucella bacteria live in the testicles and seminal fluids. An infected male is just as contagious as the female since he can spread the Brucella bacteria via urine or semen.

Transmission between dogs occurs via mucous membranes, so the bacteria may enter the body through the nose, mouth, conjunctiva of the eye and vagina. The majority of bacteria in infected dogs are secreted in semen and vaginal secretions. Bacteria may also be present in milk, urine and saliva, thus any body fluids can infect another dog.

When an infected bitch aborts, spread throughout a kennel can be very rapid. Infected bitches may deliver both living and dead puppies. These surviving puppies are infected and will shed bacteria in their secretions.

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 *Brucella canis* 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 Brucella antigen 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. DESCRIPTION OF DISEASE

Usually clinical signs are absent in a non-gravid bitch, and are hard to be noticed during a physical examination. *Brucella canis* affects the reproductive system both in female and male dogs, and is characterized by reproductive disturbances.

Litters are commonly aborted, usually in the last two weeks of gestation, or the puppies may die shortly after birth. A bitch that aborts after 45 days of gestation should be highly suspected of Brucellosis. Usually the fetuses are partially decayed and accompanied by a gray to green vaginal discharge. This discharge may contain very high numbers of the bacteria. If embryos die early, they may be reabsorbed and the female may never appear to be pregnant at all. In males, there are often no signs, except for advanced cases when the testicles may be uneven in size.

Since many infected dogs have no clinical signs, and since *Brucella canis* bacteria are very persistent and rapidly transmitted, it is highly recommended to screen all dogs as a routine procedure. This is mostly important in breeding kennels and wherever dogs are kept together.

VI. DIAGNOSIS:

The diagnosis of canine Brucellosis is largely based on serology. As most serological methods have been proven to have false positive results, it is recommended to confirm infection by either *B. canis* culture or PCR. Until a confirmation is done, and decision is accepted with the aid of the vet, it is recommended to keep the dog isolated and take precautions while handling it.

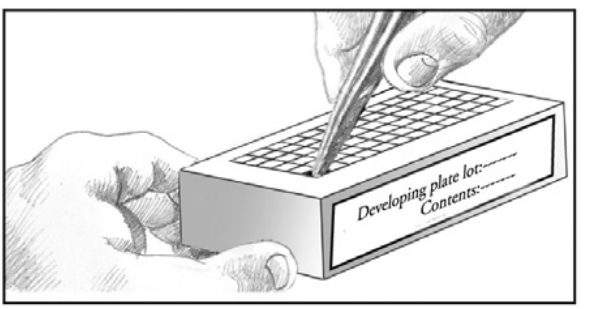
VII. STEP BY STEP WITH IMMUNOCOMB

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.

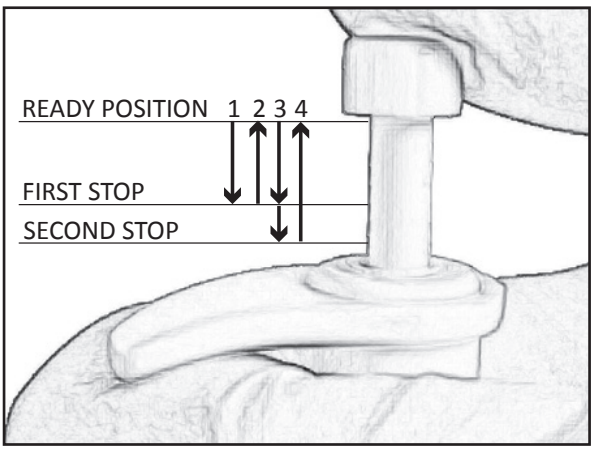
For testing serum or plasma use 5µl.
For testing whole blood use 10µl*.

Raise and lower pipette plunger several times to achieve mixing. (See Pipetting Technique 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 50CBR201 , 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.

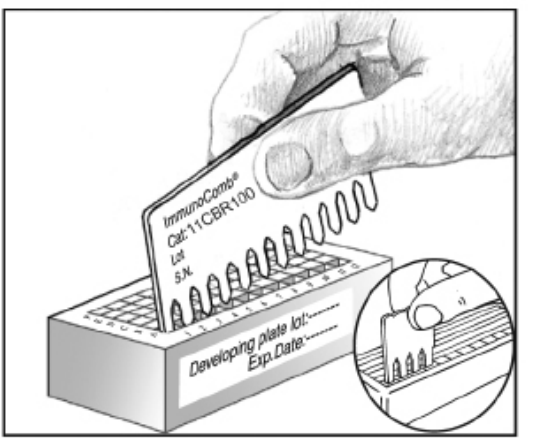
2- Dip the tip attached to the pipette into the sample to a depth of about 1 cm and slowly release the operating button.
Wait for a while, then withdraw it from the liquid touching it against the edge of the reservoir to remove excess liquid adhering to the outer surface of the tip.

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.**

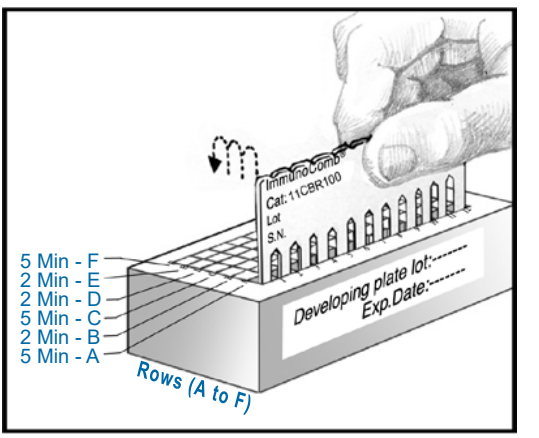


Avoid scratching the front active side of the Comb by leaning it to the back while mixing.
Gently shake off excess liquid from Comb teeth onto a tissue before moving it to the next row.

- Insert the Comb into the open well(s) in row A (printed side facing you) and incubate for 5 minutes. Mix as described above.**

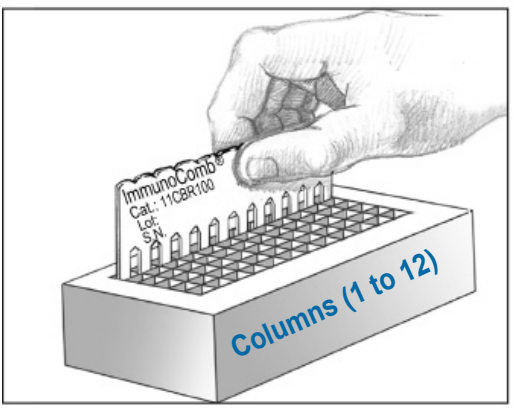
- Use tweezers to pierce the foil of the next well(s) in row B. Shake off excess liquid and insert Comb for 2 minutes. Mix as described above.**

- 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

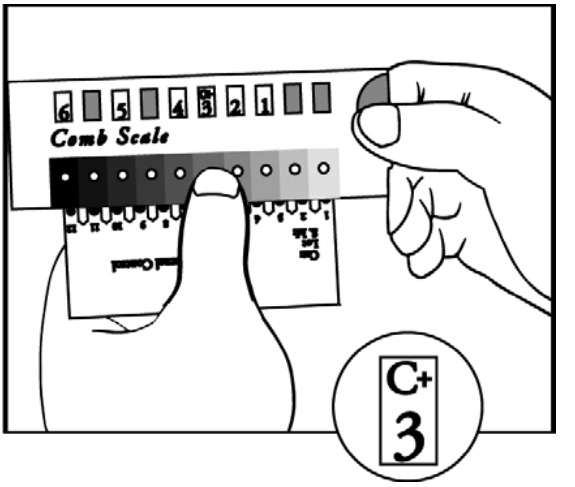
- The upper spot is the Positive Reference spot and it should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive IgG response. When using the CombScale, this spot should be read as S3 (see section IX). S3 is considered the "cut-off" level of IgG antibody, which is roughly equivalent to a positive immune response at a titer 1:200 by the Immunofluorescent assay (IFA).
- The bottom spot on the Comb gives the result of *Brucella canis* IgG antibodies in the specimen. Compare the color tone of *B. 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

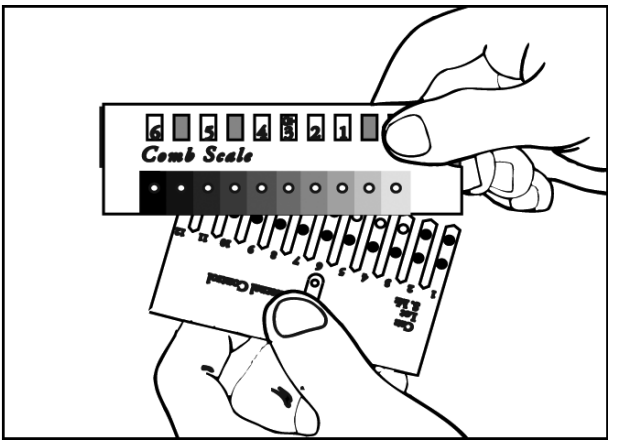
The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3.

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

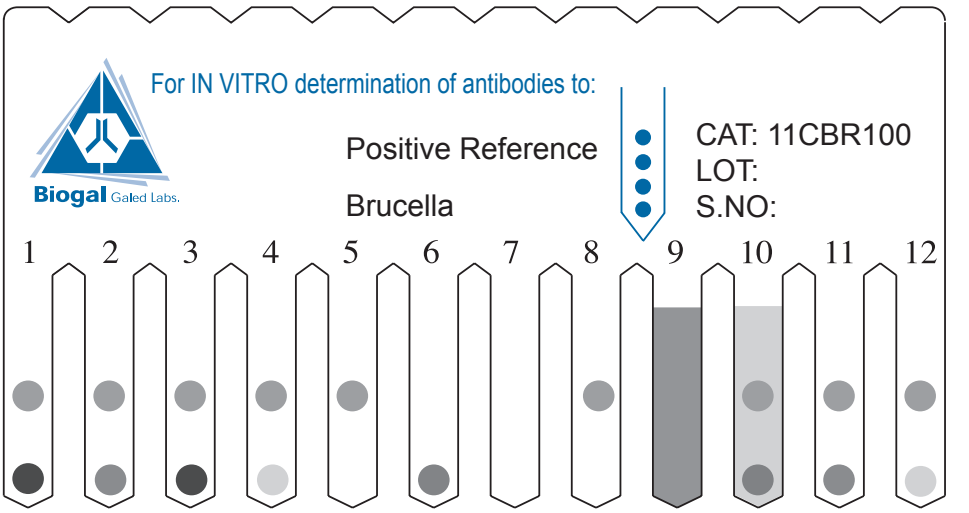
Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper spot). Slide the yellow ruler until the C+ mark appears in the window above that color you just found. **Hold the ruler in this position during the entire reading.** This step actually calibrates 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	
1	≥S5	High positive reaction to *B. canis*
2	S3-4	Medium positive reaction to *B. canis*
3	≥S5	High positive reaction to *B. canis*
4	S1-2	Low positive reaction to *B. canis*.
5	S0	Negative reaction to *B. canis*.
6		*No Positive Reference
7		*No Positive Reference
8	S0	Negative reaction to *B. canis*.
9		*High background color - interferes with reading
10	≥S3	Positive reaction with high background.
11	S3-4	Medium positive reaction to *B. canis*
12	S1-2	Low positive reaction to *B. canis*.

* Repeat Test

XI. STORAGE & HANDLING

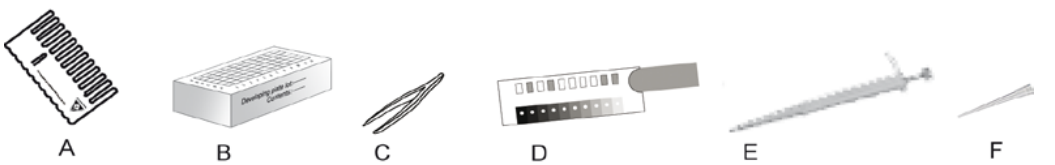
1. Store the kit under normal refrigeration (2° – 8° C / 36° – 46° F). **Do not freeze the kit.**
2. **Do not mix reagents from different kits or from different compartments of the same kit.**
3. The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance 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 (50CBR201)	12 Test Kit (50CBR301)	120 Test Kit (50CBR110)
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

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- Wanke, MM. (2004). Canine brucellosis. Anim Report Sci., **82-83**: 195-207.

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