

ImmunoComb®

CANINE BRUCELLA ANTIBODY TEST KIT

INSTRUCTION MANUAL SUFFICIENT FOR 120 TESTS

I. INTENDED USE

This kit is intended to determine dog serum IgG antibody titer for *Brucella canis*.

II. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® is a self-contained portable kit. A sensitive test which detects antibody levels in blood or serum. The ImmunoComb® provides results within 21 minutes.

III. HOW DOES THE IMMUNOCOMB® WORK?

- Based on a solid phase immunoassay principle, the ImmunoComb® is a plastic card shaped like a comb, on which purified Brucella antigen is attached.
- Take a serum, plasma or whole blood specimen, deposit into each sample cell of the multi-compartment developing plate.
- Insert Comb into sample cells (well A) so that antibodies from samples, if present, bind themselves to the antigens on the Comb.
- Non-bound antibodies are washed out in the second compartment.
- The next compartment contains an anti-dog IgG antibody labeled with an enzyme. Immerse the Comb in this "conjugate". The bound antibodies will be labeled.
- After 2 stages of washes, insert the Comb into the compartment where the enzyme reaction takes place. This generates a color change which indicates the amount of antibodies present.
- Using the CombScale, convert the lower spot color intensity to the anti-Brucella immunoglobulin level by comparing it to the positive reference which is located on the upper spot. The color of this spot indicates a positive immune response equal to an IFA titer of 1:200.
- The Comb may be divided to individual teeth. Each segment can be processed independently. Simply follow the Step-by-Step instructions.

IV. HANDLING & STORAGE

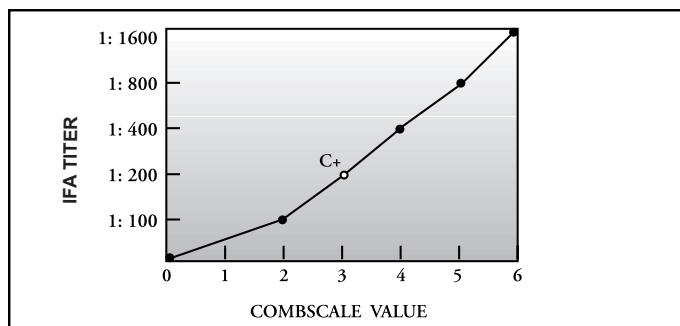
1. Store the kit under normal refrigeration: 2° - 8° C (36° - 46° F). **Do not freeze the kit.**
2. Before conducting the test, all kit elements and specimens must be at room temperature – preferably for 60 – 120 minutes (or 22 minutes at 37° C or 98.6° F). **Perform assay at room temperature of 20° - 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 the same kit.
6. Do not touch teeth of ImmunoComb® card.
7. When using developing plate, pierce cover of each compartment by strictly following test procedure instructions. **Do not rip off or remove cover of entire developing plate all at once.**

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

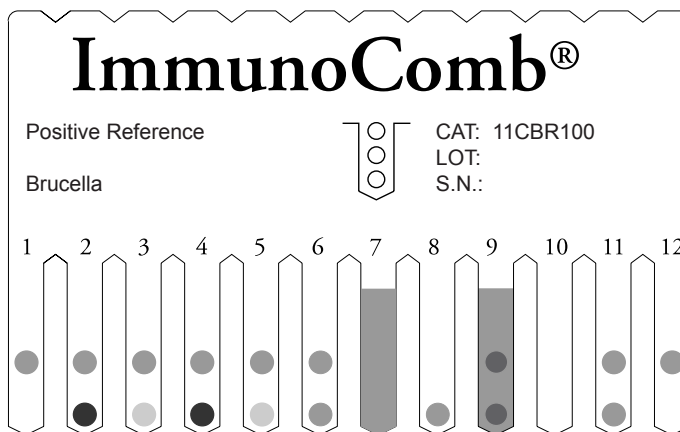
V. READING & INTERPRETING THE RESULTS

- The upper spot on the ImmunoComb® is the positive reference spot. It should give a bright grey color. This color is the same color that is generated by a positive immune specimen that contains anti-Brucella antibodies at 1:200 titer by an IFA test. When using the CombScale it should be read as S=3.
- The lower spot on the ImmunoComb® tests for *Brucella canis*.
- Specimens with a color intensity lower than the positive reference are considered suspicious. A result darker than the positive reference spot means high titer.
- The intensity of the color spot is proportional to the level of antibodies toward *Brucella canis*. To evaluate the titer, use the CombScale provided in the kit, and determine the titer using Fig. A as reference. The procedure is detailed in section VI.

Fig. A A correlation between the CombScale "S" Value & the IFA Titer

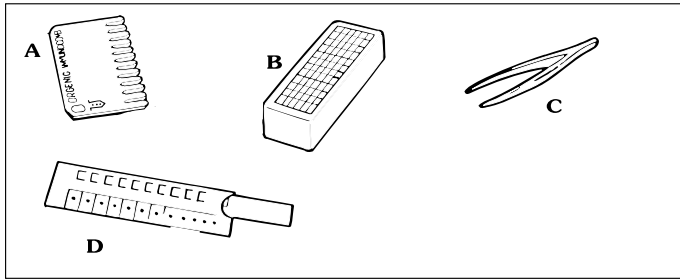


Example of a Developed Comb



.TOOTH No	RESULTS & REMARKS
1, 12	Negative reaction for <i>Brucella canis</i> .
2, 4	High positive reaction for <i>Brucella canis</i> .
3, 5	Low positive reaction - suspected <i>Brucella canis</i> .
6, 11	Positive reaction for <i>Brucella canis</i> .
7	High background color - Invalid test.
8, 10	No positive reference - Invalid test.
9	High background color - A positive reaction for <i>Brucella canis</i> .

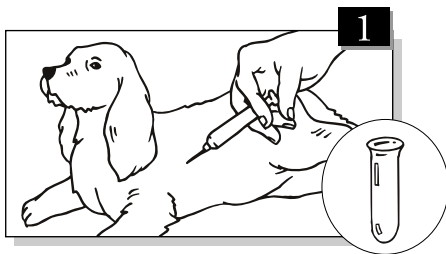
The ImmunoComb® kit includes:



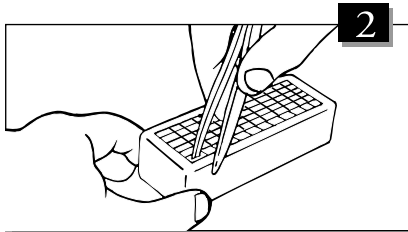
A. Ten Comb cards wrapped in an aluminum envelope; B. Ten developing plates; C. One disposable tweezers; D. One CombScale color card; and one user manual.

Step-by-Step with the ImmunoComb®

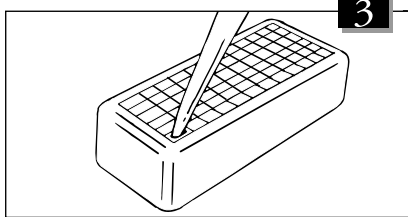
Perform assay at room temperature of 20° - 25° C (68° - 77° F).



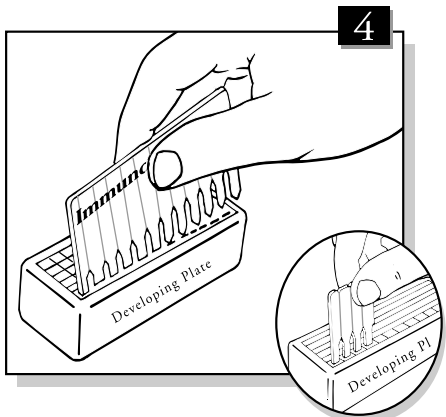
1 Obtain blood sample from dog.



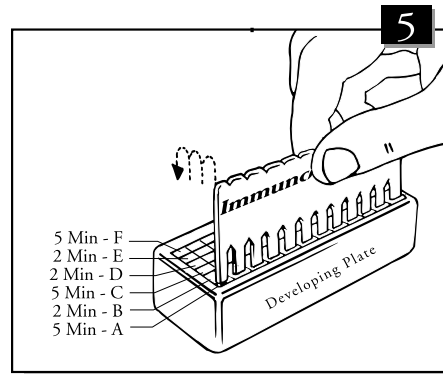
2 Slit open the protective aluminum cover of compartment A with the tweezers, one well for each sample.



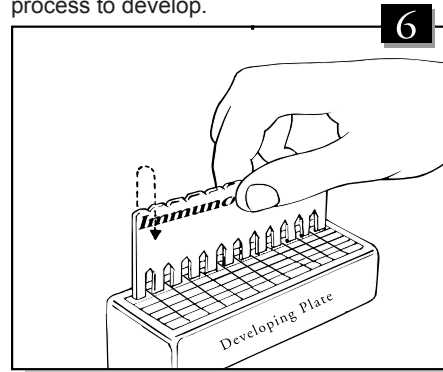
3 Dispense a sample into each well. Use a pipette or a capillary tube. For testing whole blood use 10µl. For testing a serum sample use 5µl. Mix by depressing the plunger a number of times.



4 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 remaining rows.

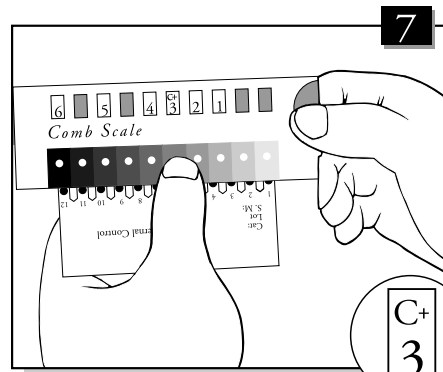


5 Pierce the cover of well(s) of Row B with the tweezers. Follow same procedure for remaining rows at end of each incubation period. Gently shake off excess liquid onto a tissue. Insert Comb in well(s) of Row B and incubate for 2 minutes. Shake off and transfer the Comb to well(s) of Row C and incubate for 5 minutes. Similarly, place the Comb in well(s) of Row D for 2 minutes, Row E for 2 minutes, and Row F for 5 minutes, allowing the color reaction process to develop.

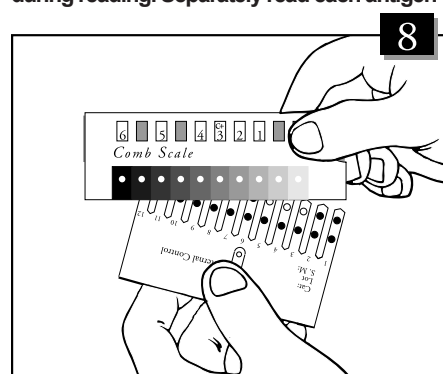


6 Upon completion of color development in row F, move the Comb back to row E for 2 minutes for color fixation. Take the Comb out and let dry.

VII. READING RESULTS WITH THE COMBSCALE



7 A. Match "C+" with the positive reference: When the Comb is completely dry, align it with the calibrated color CombScale. Compare the color resulting from the positive reference (upper spot) to the color scale by sliding the yellow ruler until the "C+" mark appears in the window corresponding to the color. Hold the slide in this position during reading. Separately read each antigen-spot.



8 B. Match the color scale to the test result: Find the shade of grey on the CombScale that most closely matches the test result (lower spot). The number that appears in the window above is the Comb score.

Important! The margin of error is similar to that of other enzyme immunoassay kit procedures. Therefore, an error in one color scale window will not result in a wrong diagnosis.

Another way to read the results is by using the CombScan 2007. 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 2007 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.