ImmunoComb®

OVINE BRUCELLA ANTIBODY TEST KIT

INSTRUCTION MANUAL

SUFFICIENT FOR 300 ASSAYS

I. INTENDED USE

This kit is designed to determine sheep serum IgG antibody titers for *Brucella melitensis.*

II. WHAT IS THE ImmunoComb® ASSAY?

The ImmunoComb® is a self-contained portable kit. A sensitive test which detects antibody levels in the blood or serum, the ImmunoComb® provides results within 40 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 antigens are attached.
- Either immerse paper disks in sheep blood or take a serum specimen. Deposit into sample wells of the multi-compartment developing plate.
- Insert Comb into the sample wells so that antibodies from samples bind themselves to the antigens on the Comb.
- Non bound antibodies are washed out in the second compartment.
- The next compartment contains an anti-sheep IgG antibody labeled with an enzyme. Immerse the Comb in this "conjugate". The bound antibodies will be labeled. Insert the Comb into a compartment where the enzyme reaction takes place. This generates a color change which indicates the amount of antibodies present.
- Using the CombScale, convert the bottom spot's color intensity to the anti-Brucella immunoglobulin level.
- An Internal control indicates that the development is completed.
- The ImmunoComb® may be divided into three separate sections. Each segment processes between 1-4 samples.

IV. HANDLING & STORAGE

1. Store the kit under normal refrigeration: 2°- 8° C (36° - 46° F).

Do not freeze the kit.

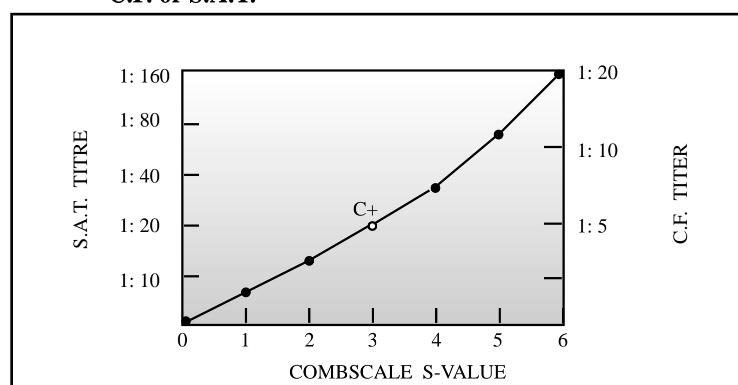
2. Before conducting the test, maintain all kit elements and specimens at room temperature -- preferably for 60 minutes. Perform assay at room temperature of 20° - 25° C (68° - 77° F).
3. Avoid spillage and cross-contamination of solutions.
4. Mix reagents by inverting developing plate several times 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 cover of each compartment while 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. Use large amounts of water to flush kit solutions down sewage/drainage system.

63OBR130 22/7/04

V. READING THE RESULTS

- To determine the IgG titer of Brucella specimens, compare the color intensity of the Comb's appropriate teeth with the color spot series on the enclosed CombScale table (see illustrations 9 &10 for details).
- The bottom spot on the ImmunoComb® tests for Brucella. Evaluate the results of each spot separately.
- Compare the specimen's color intensity with that of the positive control (C+) included in the kit, in order to determine its titer.
- The positive control (C+) for Brucella is calibrated to a 1:32 titer (C.F.).
- Specimens with an identical or higher color intensity than the positive control are considered positive.
- The negative control consists of non-immune sera and should be read as zero (S=0).
- Specimens with a color intensity lower than the positive control are considered negative or non-immune.
- When a test color is darker than S6, it may indicate either an acute or a recent infection. Proceed with the dilution process by transferring 5µl from the sample in compartment A to a new sample well. Repeat the entire process. Read results using “2nd dilution titer” (see Fig.A).

Fig. A Relationship between the CombScale's value and the C.F. or S.A.T.



The scale on the right side illustrates C.F. titer and the left side - S.A.T. titer, as outlined above. Assign a lower value when the color falls between CombScale color windows.

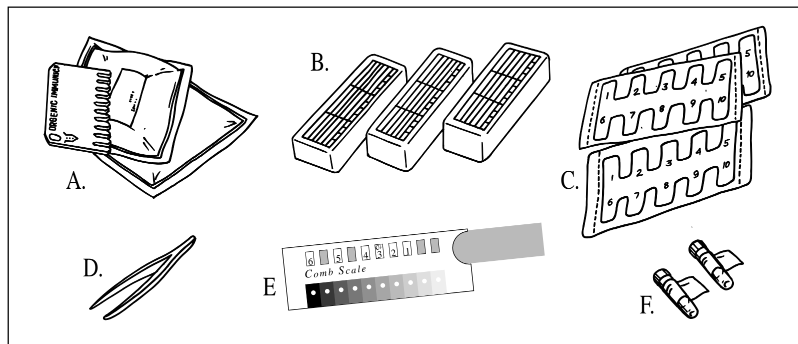
Important
The margin of error is similar to that of other enzyme immunoassay kit procedures. Therefore, an error in one color scale window will not result in a wrong diagnosis.

VI. INTERPRETATION OF RESULTS

It is generally accepted that specimens containing titer 1:20 S.A.T. or 1:5 C.F. or greater, for *B. melitensis*, are considered “positive” or immune. In areas where eradicetion has been carried out without vaccination, each result that is darker in color than the negative control should be taken as carrying an immune response and should be retested. In areas where the vaccination program is based on rev-1 serotype *B. melitensis*, the kit will show both responses to vaccination as well as to carriers. In this case, different samples from the flock may give the mean result of the vaccination, while typically high responses of aborting ewes should be assigned as positive . Fig. A shows a correlation between the CombScale’s “S” values (1-6) to C.F. (right hand side) and S.A.T. (left hand side) titers. With double dilution, titers should be multiplied by factor of 1:40.

Note: The “S” values are not indentical to C.F. or S.A.T. The correlation was calculated from results of clinical filed studies.

STEP-BY-STEP WITH ImmunoComb

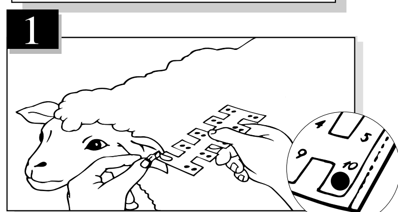


The ImmunoComb® kit includes: A. 30 ImmunoComb® cards,each separately wrapped in an aluminum envelope; B. 30 developing plates; C. 30 specimen papers with pre-punched disks; D. One disposable tweezers; E. One calibrated CombScale color card; F. One tube of positive control serum and one tube of negative control serum, and a user manual.

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

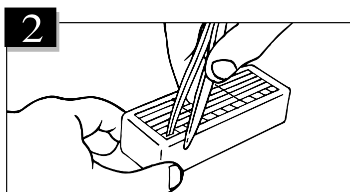
When using a paper disk

1



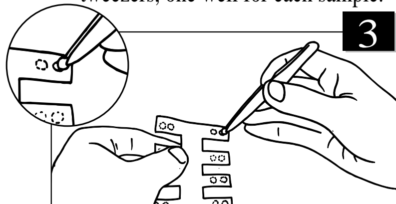
Pierce one of the sheep's veins. Take a specimen paper and saturate a pre-punched disk with the blood.

2



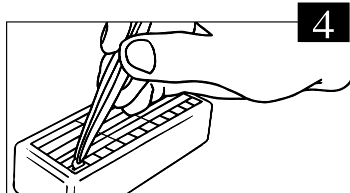
Slit open the protective aluminum covering of compartment A with the tweezers, one well for each sample.

3



When using a paper disk punch out a disk saturated with blood.

4

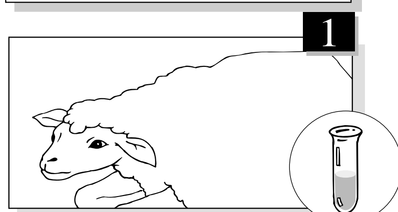


Insert the disk into well #1 of compartment A. Immerse totally in the liquid. Proceed with the other samples.

Wait 60 minutes for extraction of antibodies.

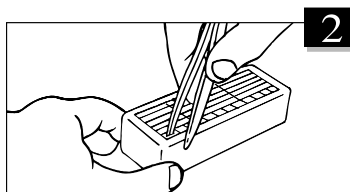
When using a serum

1



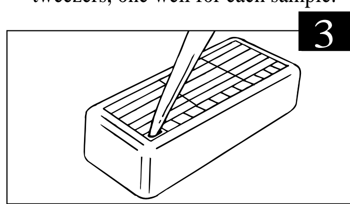
Use a pipette or a capillary tube. For testing a serum sample use 5 µl.

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. When using the capillary tubes raise and lower the piston several times to achieve mixing. When using a pipette, mix by depressing the plunger a number of times.

Proceed to step 5.

5

For control serum open the next 2 consecutive wells.

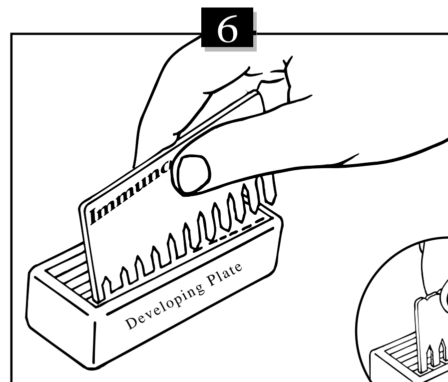
Take 5 µl positive control serum (C+) and insert into well A next to the last sample.

Mix the serum in the well.

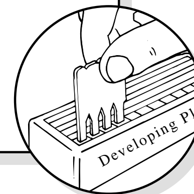
Do the same with the negative control serum in the next well.

63OBR130 22/7/04

6

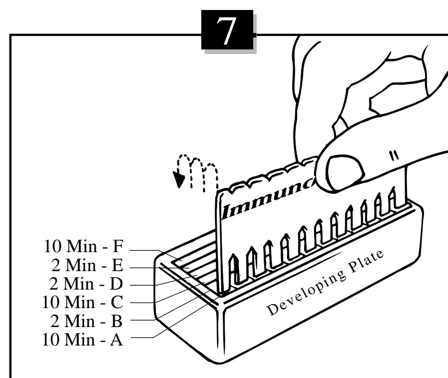


Remove one ImmunoComb® from its protective wrapping and insert (print side facing you) in compartments of **Row A**. Gently move Comb up and down several times, then let incubate in **Row A's** compartments for **10 minutes**.



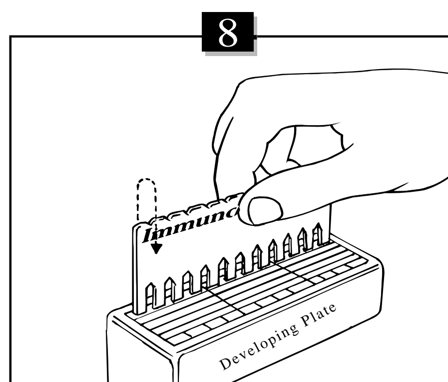
When using 1/3 or 2/3 of a Comb break the Comb by folding back on notch 4 or 9 respectively. Keep the rest in its original sleeve for further use.

7



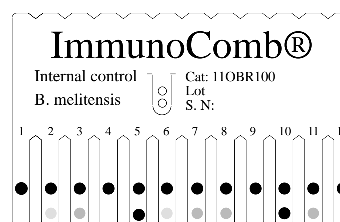
Pierce the cover of the appropriate section of compartment **B** with the tweezers. Follow same procedure for remaining rows at end of each incubation period. Gently shake off excess liquid onto a tissue. Insert Comb in **Row B**'s compartment and let incubate for **2 minutes**, shake-off and transfer Comb to **Row C** and incubate for **10 minutes**. Similarly, the Comb is placed in **Row D** for **2 minutes**, **Row E** for **2 minutes**, and **Row F** for **10 minutes**, allowing the color reaction process to develop.

8



After the Comb has completed the cycle for **Row F** ,transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** to fix color.

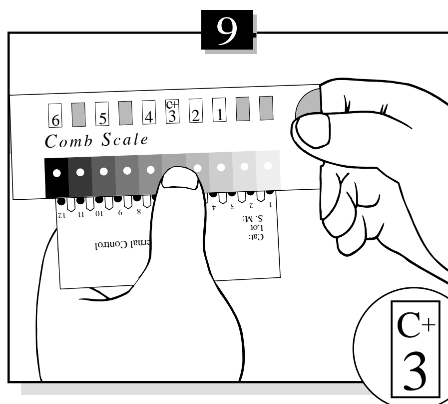
AIR DRY AND READ RESULTS



Developed *Brucella melitensis* ImmunoComb®

READING RESULTS WITH THE CombScale

9

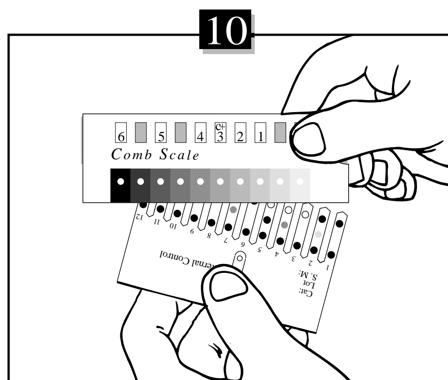


A. Adjust scale with positive control:

When the Comb is completely dry align it with the calibrated color CombScale. Compare the color resulting from the positive control (C+) sample to the color scale : slide the yellow ruler until the "C+" mark appears in the window corresponding to the color.

FINALLY, HOLD THE SLIDE IN THIS POSITION DURING READING.

10



B. Read each of the spots separately:

Choose the most suitable color and read the titer in the yellow windows.

REMEMBER: A DIFFERENCE OF ONE COLOR LEVEL WILL NOT AFFECT DIAGNOSIS !!!