

# ImmunoComb®

## BOVINE IBR - PI3 - BRSV ANTIBODY TEST KIT INSTRUCTION MANUAL SUFFICIENT FOR 30 ASSAYS

### I. INTENDED USE

This kit is designed to determine cow serum IgG antibody titers for Infectious Bovine Rhinotracheitis (IBR), Parainfluenza-3 (PI-3) and Bovine Respiratory Syncytial Virus (BRSV).

### 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 BHV-1 (IBR), PI-3 and BRSV antigens are attached.
- Either immerse paper disks in cow blood or take a serum specimen. Deposit into sample wells of the multi-compartment developing plate (wells in row A).
- Insert Comb into the sample wells (A) 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-cow IgG antibody labeled with an enzyme.
- Immerse the Comb in this "conjugate." The bound antibodies will be labeled.
- After 2 stages of wash, 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 lower spot's color intensity to the anti-BHV-1 immunoglobulin level, the second spot to anti-PI-3 IgG and the top spot to anti-BRSV IgG level.
- The ImmunoComb® may be divided into three separate sections. Each segment processes between 1-4 samples.

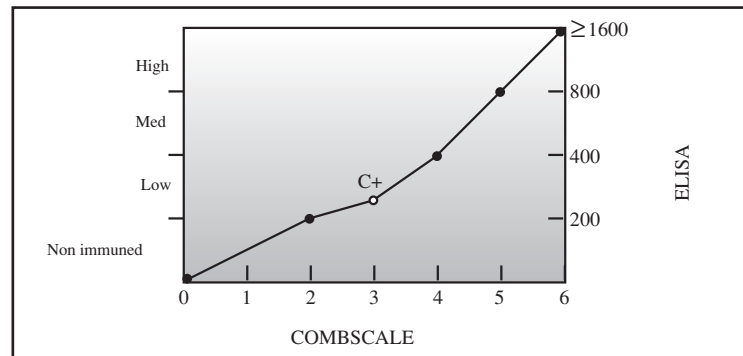
### IV. HANDLING & STORAGE

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. It is recommended to incinerate kit after use. Use large amounts of water to flush kit solutions down sewage/drainage system.

### V. READING AND INTERPRETING THE RESULTS

- To determine the IgG titer of I.B.R, PI-3 and BRSV in specimens, compare the color intensity of the ImmunoComb'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 I.B.R, the second spot tests for PI-3 and the top spot test for BRSV IgG. 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+) is calibrated to 200 ELISA units (0.2 in O.D; S=3 in CombScale).
- Specimens with an identical or higher color intensity than the positive control are considered positive.
- The negative control consists of non-immuned sera and should be read as zero (S=0). Specimens with a color intensity lower than the positive control are considered negative, non-immuned or suspected if read as S2.
- To evaluate the titer, first use the CombScale provided in the kit and determine the S value (refer to section VI). Then use Fig. A as reference to convert to titer value.

Fig. A Relationship between the CombScale's "S" value and the ELISA



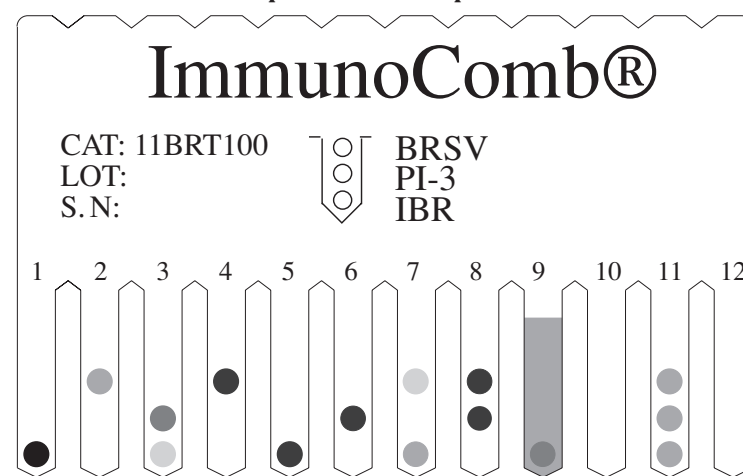
The scale on the right side illustrates ELISA units.

Assign a lower value when the color falls between two CombScale color windows.

#### Important

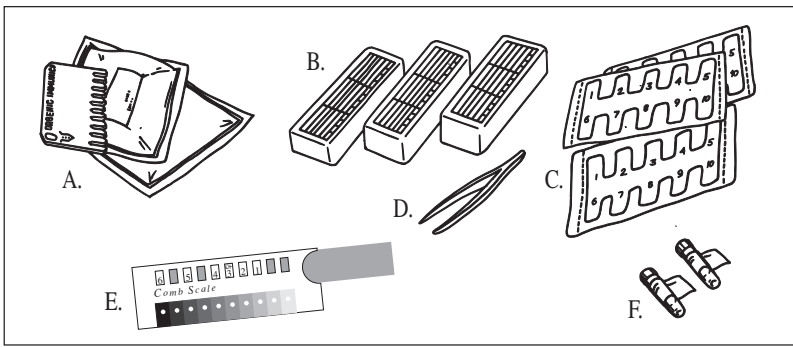
The margin of error is similar to that of other enzyme Immunoassay kit procedures. Therefore, an error in one color scale window will not result in a wrong diagnosis.

#### Example of a developed Comb



TOOTH No.	RESULT & REMARKS
1,5	High positive for IBR, and neg. for PI-3 & BRSV.
2	Positive for BRSV, and neg. for PI-3 & IBR.
3	Medium positive for PI-3, low positive for IBR, neg. for BRSV.
4	High positive for BRSV and neg. for IBR & PI-3.
6	High positive for PI-3, and neg. for IBR & BRSV.
7	Low reaction to BRSV, neg. for PI-3 and positive for IBR.
8	High positive for BRSV and PI-3, and neg. for IBR.
9	High back ground color - test not valid.
10	Negative for IBR, PI-3 and BRSV.
11	Positive control.
12	Negative control.

# STEP-BY-STEP WITH ImmunoComb®

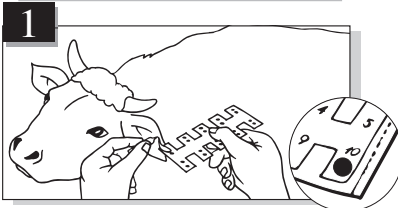


The ImmunoComb® kit includes: A. 3 ImmunoComb® cards, each separately wrapped in an aluminum envelope; B. 3 developing plates; C. 3 specimen papers with pre-punched disks; D. One disposable tweezers; E. One calibrated CombScale color card; F. One tube of positive control serum and one tube of negative control serum, a CombScore sheet and a user manual.

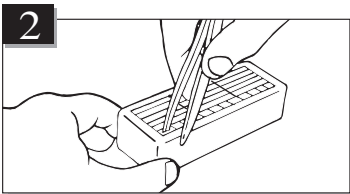
A pipette or capillary tubes are also needed. The capillary tubes are available at Biogal, or through your supplier:  
40 capillary tubes + 1 piston, cat. no. 10000140.

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

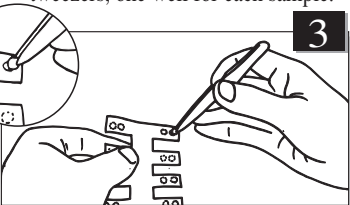
## When using a paper disk



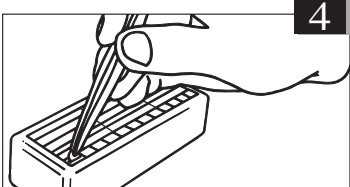
Pierce one of the cow's veins. Take a specimen paper and saturate a pre-punched disk with the blood.



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



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



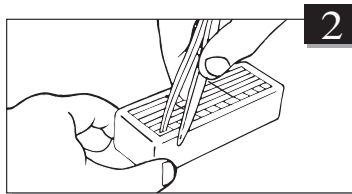
Insert the disk into well #1 of compartment A. Dip it well into the liquid. Proceed with the other samples.

Wait 60 minutes for extraction of antibodies.

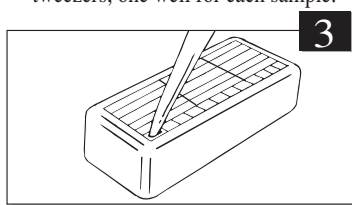
## When using a serum



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



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



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.

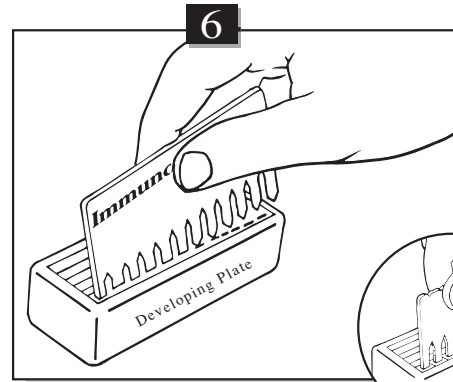
**5**

For control serum open the next 2 consecutive wells.

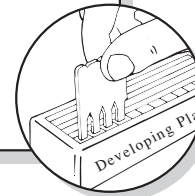
Take 5 µl positive control serum (C+) and insert into well A next to the last sample.

Mix the serum in the well.

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

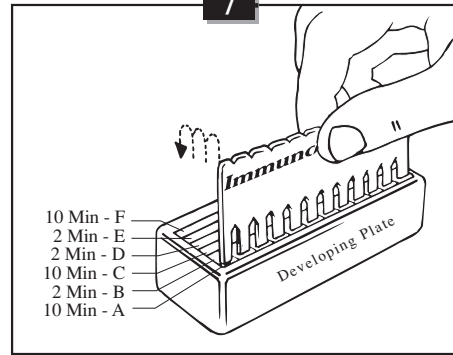


Remove one Comb from its protective wrapping and insert (print side facing you) in compartments of **Row A**. Gently move Comb up and down several times, then let incubate in **Row A**'s compartments for **10 minutes**.



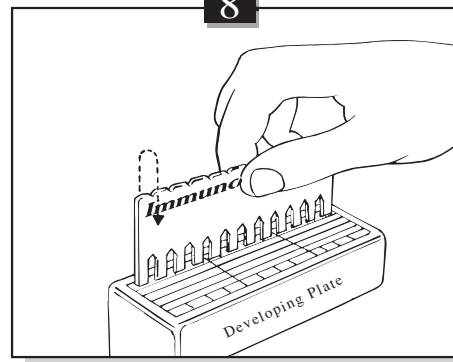
When using 1/3 or 2/3 of a Comb break the Comb by folding back on notch 4 or 9 respectively. Keep the rest in its original sleeve for further use.

**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**

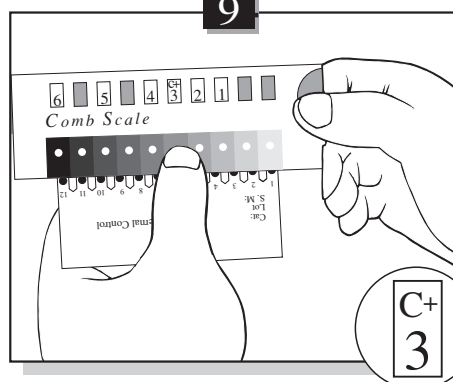


After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** to fix color.

**AIR DRY AND READ RESULTS**

## VI. READING RESULTS WITH THE CombScale

**9**

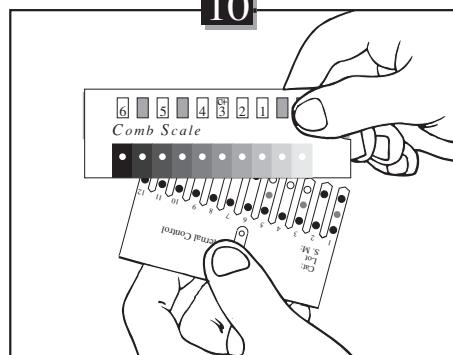


### A. Adjust scale with positive control:

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 !!!**