

# ImmunoComb®

## Poultry IBD - ND - IB Antibody Test Kit

### INSTRUCTION MANUAL SUFFICIENT FOR 300 TESTS

#### I. INTENDED USE

This kit is designed to determine IgG antibody titers to Infectious Bursal Disease (IBD), Newcastle Disease (ND) and Infectious Bronchitis virus (IB; Massachusetts strain) in poultry.

#### II. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® is a self-contained portable kit. A sensitive test which detects antibody levels in serum or egg yolk. The ImmunoComb® provides results in 38 minutes.

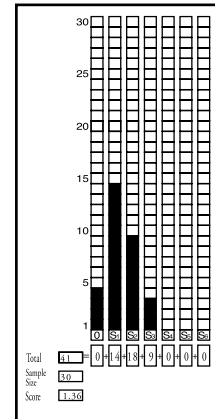
#### III. HOW DOES THE IMMUNOCOMB® WORK?

- Based on a solid phase immunoassay principle, the ImmunoComb® is a plastic card shaped like a comb, on which purified IBD, ND and IB antigens are attached.
- Use chicken or turkey serum, plasma or egg yolk specimen. Deposit sample into cells of the multi-compartment developing plate.
- Insert Comb into sample cells 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-chicken 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 upper spot color intensity to the anti-IBD-immunoglobulin level, convert the middle spot color intensity to the anti-ND-immunoglobulin level and convert the lower spot color intensity to the anti-IB-immunoglobulin level.

#### IV. READING & INTERPRETING THE RESULTS

- Read the results using the CombScale as described in section VIII.
- The upper spot on the ImmunoComb® tests for IBD, the middle spot test for ND and the lower spot tests for IB.
- In order to determine its titer, compare the specimen's color intensity with that of the positive control (C+) included in the kit.
- Specimens with identical or higher color intensity than the positive control (C+) are considered positive.
- The negative control consists of non-immune sera and should be read as zero (S=0).
- Non-specific reactions equal to or greater than S1 (i.e., false positives) occurs occasionally due to various reasons and may be associated with the use of certain commercial vaccines. To avoid misinterpretation of non-specific reactions and possible confusion with true low positive results, it is recommended to confirm results by retesting at a one week interval.
- A test color darker than S6, indicates either an acute disease or a highly immune flock.
- To determine the IgG titer of IBD, ND and IB specimens, compare the color intensity of the Comb's appropriate teeth with the color spot series on the enclosed CombScale.

- Refer to CombScore instructions for the profiling of each antibody level. To determine the immunity profile of your flock use the enclosed CombScore.

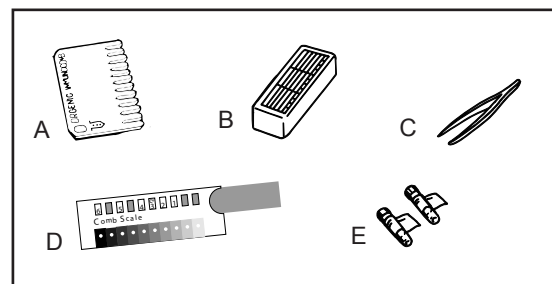


CombScore Table

#### V. HANDLING & STORAGE

- Store the kit under normal refrigeration: 2° - 8° C (36° - 46° F). **Do not freeze the kit.**
- 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).**
- Avoid spillage and cross-contamination of solutions.
- Mix reagents by inverting developing plate several times prior to use.
- Do not mix reagents from different kits or from different compartments of the same kit.
- Do not touch teeth of ImmunoComb® card.
- 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.**
- The ImmunoComb® kit contains inactivated biological material. Kit must be handled and disposed of in accordance with accepted sanitary requirements.

#### VI. THE IMMUNOCOMB® KIT INCLUDES:



**A.** Thirty Comb cards, each separately wrapped in an aluminum envelope; **B.** Thirty developing plates; **C.** One disposable tweezers; **D.** one CombScale color card; **E.** One tube of positive control serum and one tube of negative control serum; a CombScore sheet and a user manual.

Note: A pipette or capillary tubes are needed. The capillary tubes are available at Biogal or through your supplier: 40 capillary tubes & 1 piston, CAT. NO. 1000140.

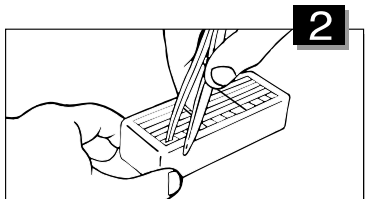
## VII. STEP-BY-STEP DEVELOPMENT PROCESS

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

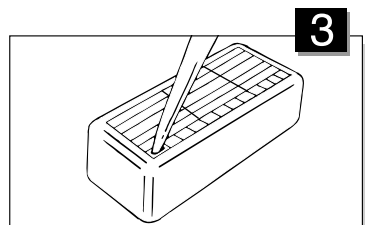
### When using serum specimen(s)



1 When using a serum sample use 5µl. Use either pipette or a capillary tube.



2 Slit open the protective aluminum covering of **compartment (well) A** with the tweezers.



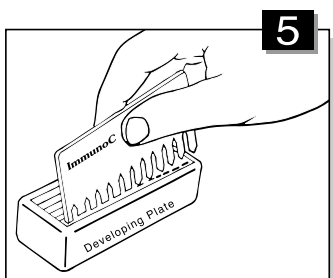
3 Dispense 5µl sample into each well. When using the capillary tubes raise and lower the piston several times to achieve mixing. When using a pipette, mix by depressing the plunger a number of times.

### When using egg yolk specimen(s)

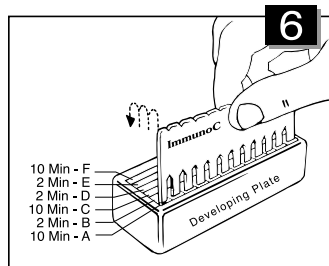
Separate the entire egg yolk and wash gently with tap water. Withdraw 1 ml yolk from the outer layer and transfer to a test tube; add 1 ml isotonic saline solution (0.85% NaCl) and mix thoroughly. Deposit 10µl of each diluted yolk specimen in the respective wells. Mix by withdrawing and expelling with the pipette several times. Proceed to the next step immediately.

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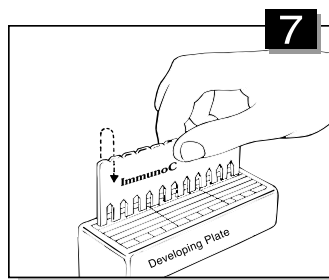
**Open the next 2 consecutive wells for control serum.** Take 5µl positive control serum (C+) and insert into well A next to the last sample. Mix the serum in the well. **Do the same with the negative control serum (C-) in the following well.**



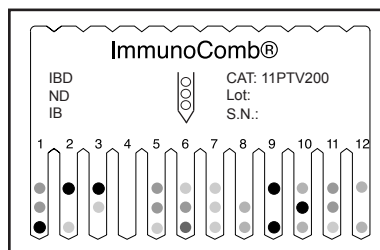
5 Remove one Comb from its protective wrapping and insert (printed side facing you) into well of **Row A**. Gently move Comb up and down several times, then incubate in wells of **Row A** for **10 minutes**.



6 Place the Comb in **Row D** for **2 minutes**, **Row E** for **2 minutes**, and **Row F** for **10 minutes**, allowing the color reaction process to develop.

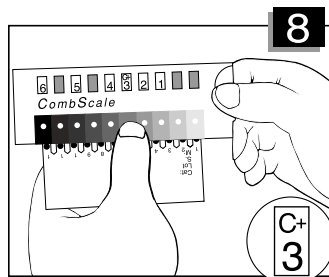


7 After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** to fix color.

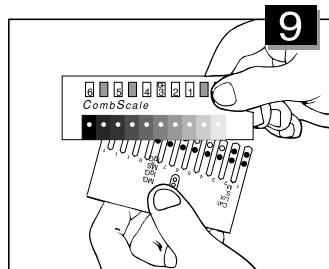


An Example of a Developed Comb for IBD, ND and IB Antibodies

## VIII. READING RESULTS WITH THE COMBSCALE



8 **A. Adjust scale with positive control:** When the Comb is completely dry, align it with the calibrated color CombScale. Compare color resulting from the positive control (C+) sample to the color scale by sliding the yellow ruler until the "C+" mark appears in the window corresponding to the color. Finally, hold the slide in this position during reading, **separately read each antigen-spot.**



9 **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 THE 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.