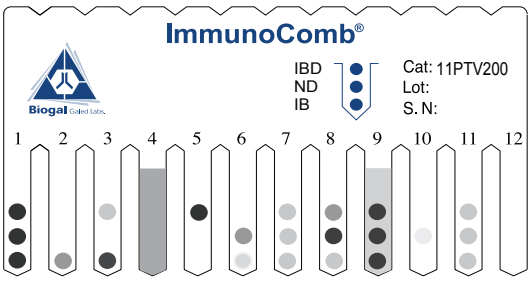
## VII. READING & INTERPRETING THE RESULTS

- The upper spot on the ImmunoComb tests for IBD, the middle spot tests for ND and the lower spot tests for IB. Evaluate the results of each disease separately.
- IBD, ND, IB IgG levels are determined by comparing each specimen's color intensity to the Positive Control (C+). Reading instructions are described in section VI. See illustrations 8 & 9 for details.
- Specimens with 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).
- Non-specific reactions around 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.
- Refer to CombScore instructions for score profiling (S) of each specimen antibody level. To determine the immunity profile of your flock use the enclosed CombScore tables (Illustration 10).
- To assist you interpreting the results, please refer to the example of the developed Comb and the remarks below.

**Important:** The margin of errors is similar to that of other enzyme Immunoassay kit procedures. Therefore, an error in one color tone will not result in a wrong diagnosis.

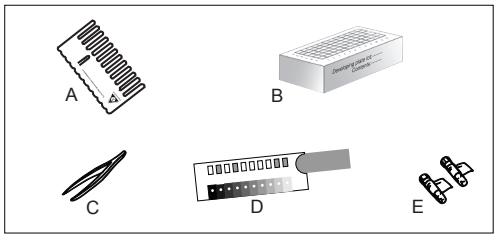
### Example of a developed Comb




| Tooth No. | Results | | |
|---|---|---|---|
| | IB | ND | IBD |
| 1 | ≥S5 - High Positive | ≥S5 - High Positive | ≥S5 - High Positive |
| 2 | S4 - Positive | Negative | Negative |
| 3 | ≥S5 - High Positive | Negative | S3 - Positive |
| 4 | *Invalid | *Invalid | *Invalid |
| 5 | Negative | Negative | ≥S5 - High Positive |
| 6 | S2 - Inadequate Immunity | S4 Positive | Negative |
| 7 | S3 - Positive | S3 - Positive | S3 - Positive |
| 8 | S3 - Positive | ≥S5 - High Positive | S4 - Positive |
| 9 | ≥S5 - **Positive | ≥S5 - **Positive | ≥S5 - **Positive |
| 10 | Negative | Negative | Negative |
| 11 | S3 - Positive Control | S3 - Positive Control | S3 - Positive Control |
| 12 | S0 - Negative Control | S0 - Negative Control | S0 - Negative Control |

*High background ** High background with positive reaction

## VIII. THE IMMUNOCOMB KIT CONTAINS




**A. Thirty Comb cards**, each separately wrapped in an aluminum envelope; **B. Thirty Developing Plates** divided into compartments A-F that are subdivided into 12 wells. The plate compartments are pre-filled with reagent solutions, **C. One pair of plastic tweezers**, **D. One CombScale** color card; **E. One** tube of **Positive Control** serum and **one** tube of **Negative Control** serum. **A CombScore sheet** and an **instruction manual** are included.



# ImmunoComb

**Poultry IBD - ND - IB**
**Antibody Test Kit**

**50PTV230**
**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 **chicken and turkey** flocks.

## II. WHAT IS THE IMMUNOCOMB ASSAY?

The ImmunoComb is a self-contained portable kit based on a solid phase immunoassay principle. A sensitive test, which detects antibody levels in plasma, serum or egg yolk. The ImmunoComb provides results within 38 minutes.

## III. HOW DOES THE IMMUNOCOMB WORK?

- The ImmunoComb is a plastic card shaped like a comb, on which purified IBD, ND and IB antigens are attached.
- Samples from chicken or turkey (serum, plasma or egg yolk ) are deposited into separate wells in row **A** of the developing plate.
- Positive Control and Negative Control samples, supplied in the kit, should be applied in separate wells in Row A.
- Comb is inserted into sample wells so that antibodies from samples bind to the antigens on the Comb's teeth.
- Each plate may be used to test individual or any number of chicken/turkey up to 10, by breaking off the desired number of teeth from the Comb and using the corresponding column of wells in the developing plate. Each run should include Positive Control and Negative Control wells/teeth.
- Non-bound antibodies are washed off in the second row.
- The next row contains an anti-chicken/turkey IgG antibody labeled with an enzyme. While Comb is immersed in this "conjugate", the bound antibodies will be labeled.
- After two washing steps the Comb is inserted into a row where the enzyme reaction takes place. This generates a color change, its intensity indicates the amount of antibodies present in each sample.
- Purple-grey color intensity obtained at each spot should be converted to the antibody levels by using the CombScale.

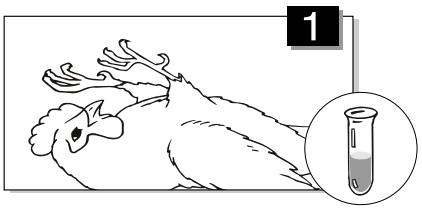
## IV. HANDLING & STORAGE

- Store the kit under normal refrigeration: 2º - 8º C (36º - 46º 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 incubate only the developing plate for 22 minutes at 37ºC/98.6º F). **Perform assay at room temperature of 20º - 25º C (68º - 77º 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 rows of the same kit.
- Do not touch teeth of ImmunoComb card.
- When using developing plate, pierce cover of each row 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.

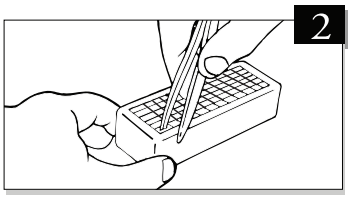
## V. STEP-BY-STEP DEVELOPMENT PROCESS

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

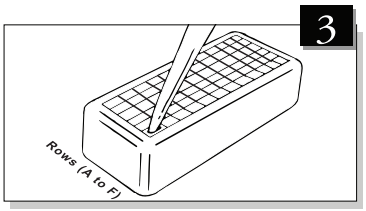
### When using serum specimens




Prepare serum/plasma samples.
Apply **5µl** sample by using either a pipette or a capillary tube.



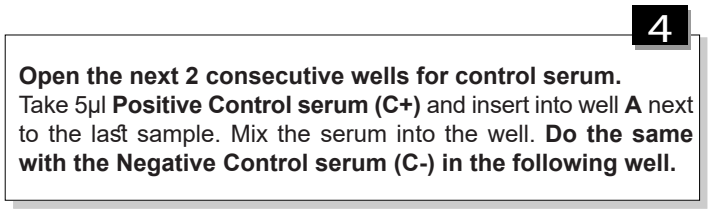

Using the tweezers, slit open the protective aluminum cover of wells in **row A.**



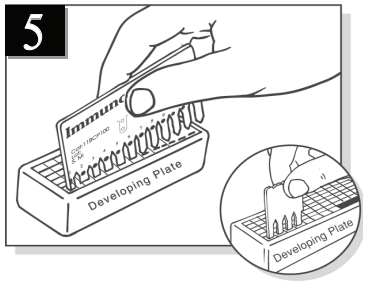

Dispense sample into each well. Using a pipette, mix by depressing carefully the plunger a number of times.

### When using egg yolk specimens

Separate the entire egg yolk and wash gently with tap water. Withdraw 1 ml yolk 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 into respective well. Mix by withdrawing and expelling with the pipette several times. Proceed to the next step immediately.



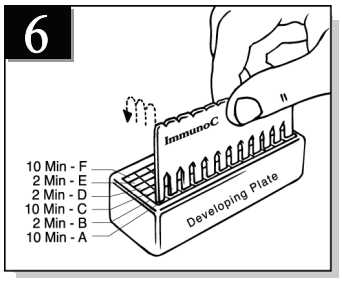

**Open the next 2 consecutive wells for control serum.**
Take 5µl **Positive Control serum (C+)** and insert into well **A** next to the last sample. Mix the serum into the well. **Do the same with the Negative Control serum (C-) in the following well.**



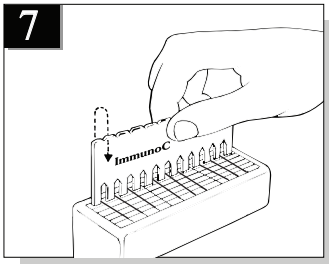

Remove one Comb from its protective wrapping and insert it (printed side facing you) into **Row A**. Incubate for **10 minutes**. To improve mixing, gently move Comb **up and down** at the start of each incubation (each row). Repeat this motion at least twice in all of the remaining rows.

**When using a partial Comb**, cut the number of teeth needed including Positive and Negative Controls. Keep the remaining unused teeth sealed in its original envelope for further use. In each further step, open and use only the corresponding wells in the developing plate.



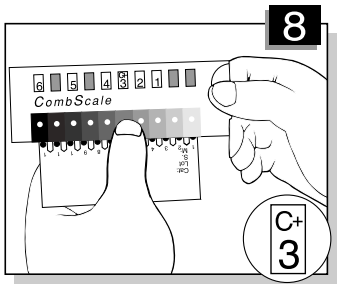



Pierce the cover of wells in **Row B** with the tweezers. Gently shake off excess liquid from the Comb onto a tissue (follow the same procedure for remaining rows at the end of each step). Insert Comb into wells of **Row B** and incubate for **2 minutes**, shake off and transfer the Comb to **Row C** and incubate for **10 minutes**. 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.

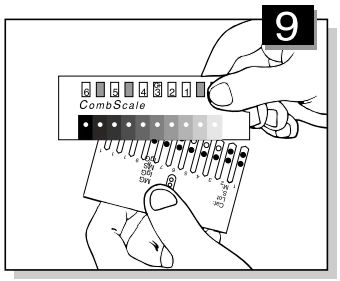



After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** color fixation.

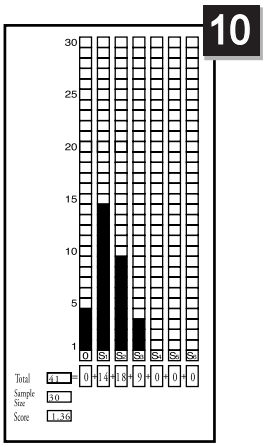
## VI. READING RESULTS USING THE COMBSCALE




**A.** When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit. Find the tone of the purple-grey on the CombScale that most closely matches the **Positive Control spot**. Slide the yellow ruler until the C+ mark appears in the window above the color you have found. **Hold the slide 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.




**B. Read each of the spots separately:** Choose the most suitable color and read the titer in the yellow windows.




**CombScore Table**

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