VII. READING & INTERPRETING THE RESULTS

- The upper spot on the ImmunoComb® tests for MM (for turkeys only), the middle spot tests for MG and the lower spot tests for MS. Evaluate the results of each disease separately.
- MM, MG and MS 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 9 & 10 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 the profiling of each specimen antibody level. To determine the immunity profile of your flock use the enclosed CombScore tables (Illustration 11).

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

![Comb Image](Image)

VIII. THE IMMUNOCOMB® KIT CONTAINS

- A. Three Comb cards, each separately wrapped in an aluminum envelope;
- B. Three Developing Plates divided into compartments A-F that are subdivided into 12 wells. The plate compartments are pre-filled with reagent solutions;
- C. Three specimen papers with pre-punched disks;
- D. Four Blood Lancets;
- E. One pair of plastic tweezers;
- F. One CombScore color card;
- G. One tube of Positive Control serum and one tube of Negative Control serum.

A CombScore sheet and an instruction manual are included.

Note: A pipette or capillary tubes are needed. The capillary tubes are available at Biogal or through your supplier: 40 capillary tubes & 1 pistion, CAT. NO. 10000140.

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°C - 25°C (68°F - 77°F).

When using egg yolk specimens

1. When using a paper disk, pierce one of the chick's veins. Take a specimen paper and saturate a pre-punched disk with the blood.

2. Punch out a disk saturated with blood.

3. Insert disks into wells of row A, dipping into the diluent. Wait 60 minutes for extraction of antibodies.

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

5. 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.

6. 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.

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

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