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 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 score profiling (S) of each color card; Avoid spillage and cross-contamination of solutions.

The ImmunoComb® kit contains inactivated biological material. Kit must be handled and disposed of in accordance with accepted sanitary requirements.

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

#### Table: Tooth No. - Results

<table>
<thead>
<tr>
<th>Tooth No.</th>
<th>IB</th>
<th>ND</th>
<th>IBD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>≥S5 - High Positive</td>
<td>≥S5 - High Positive</td>
<td>≥S5 - High Positive</td>
</tr>
<tr>
<td>2</td>
<td>S4 - Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>≥S5 - High Positive</td>
<td>Negative</td>
<td>S3 - Positive</td>
</tr>
<tr>
<td>4</td>
<td>*Invalid</td>
<td>*Invalid</td>
<td>*Invalid</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>Negative</td>
<td>≥S5 - High Positive</td>
</tr>
<tr>
<td>6</td>
<td>S2 - Inadequate Immunity</td>
<td>S4 Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>S3 - Positive</td>
<td>S3 - Positive</td>
<td>S3 - Positive</td>
</tr>
<tr>
<td>8</td>
<td>S3 - Positive</td>
<td>≥S5 - High Positive</td>
<td>S4 - Positive</td>
</tr>
<tr>
<td>9</td>
<td>≥S5 - **Positive</td>
<td>≥S5 - **Positive</td>
<td>≥S5 - **Positive</td>
</tr>
<tr>
<td>10</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>S3 - Positive Control</td>
<td>S3 - Positive Control</td>
<td>S3 - Positive Control</td>
</tr>
<tr>
<td>12</td>
<td>S0 - Negative Control</td>
<td>S0 - Negative Control</td>
<td>S0 - Negative Control</td>
</tr>
</tbody>
</table>

*High background ** High background with positive reaction

### 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 (whole blood saturated paper disks, 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

When using egg yolk specimens

When using serum/plasma

When using blood paper disk

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

Another way to read the results is by using the CombScan, a 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 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.