**Introduction**

Infectious Bursal Disease (IBD) or Gumboro, is a viral disease of young chickens. Those up to 5 weeks of age are severely affected, in birds from 9-15 weeks of age the effect is less severe. The bursa becomes swollen and the immune system is suppressed often leading to secondary infections. Infection is of a sudden and short duration with deaths occurring two days after first signs of disease. (Mortality about 5% but may be 30% or more).

**When to Test**

The FLOCKSCREEN™ Infectious Bursal Disease Antibody ELISA test can be used:

(i) To estimate the most appropriate time for vaccination
(ii) To monitor vaccination response
(iii) To confirm exposure to disease

**Sampling Recommendations**

As a guide, a 1% sample is usually sufficient for vaccination or disease monitoring. In practice, for IBD, between 20-50 birds per house would usually be tested.

**Assay Description**

The FLOCKSCREEN™ IBD Antibody ELISA Kit provides a rapid, simple and sensitive method of detecting antibodies to IBD Virus in chicken serum or egg yolk.

Microtitre plates are supplied pre-coated with purified viral antigens. Diluted samples are incubated in the wells where any antibody specific to IBD binds and forms a complex. Unbound material is washed from the wells and an alkaline phosphatase labelled donkey anti-chicken IgG conjugate reagent is added which binds to the chicken antibodies attached to IBD antigens. Unbound conjugate is washed away and PMP substrate is added to the wells. The degree of colour developed (optical density) is directly related to the amount of antibody to IBD present in the sample.
FLOCKSCREEN™ Infectious Bursal Disease/ Gumboro (IBD) 
Antibody Detection ELISA: V090/V094

Interpretation of Results
For calculation of results, an S/P ratio is required (Sample value related to Positive Control value). The following formula is applied (using mean absorbance values for controls and paired samples):

\[
\frac{\text{SAMPLE ABSORBANCE} - \text{NEGATIVE CONTROL ABSORBANCE}}{\text{POSITIVE CONTROL ABSORBANCE} - \text{NEGATIVE CONTROL ABSORBANCE}} = S/P
\]

The different IBD vaccines come with different data sheet recommendations for flock vaccination and these should always be adhered to.

The following titre calculation, together with the formula* for estimating when the maternally derived antibody level is likely to have fallen below the target titre, may be used where relevant:

\[
\text{Log} \_{10} \text{ Titre} = 0.557 \times (\text{Log} \_{10} \ S/P) + 3.6845
\]

\[
\text{Titre} = \text{Antilog of } \text{Log} \_{10} \text{ Titre}
\]

The IBD S/P ratio and/or ELISA titre value of the samples may be interpreted using the following guide (N.B. ‘Hot’ vaccines can be administered at much higher titres. The target titre of 500 is intended only as a guide):

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<th>S/P Ratio</th>
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The following formula may be used for estimating the number of days until the antibody level has fallen below the target titre*:

\[
\text{Number of days until antibody is below target titre} = \left( \sqrt{\text{mean of titres}} - \sqrt{\text{target titre}} \right) + 1 / 2.82
\]

*Developed by the Poultry Health Institute in Doorn, The Netherlands.

Guildhay also offers a data acquisition and analysis software programme called SOFTScreen™.

This Microsoft Windows™ based programme can be run with the majority of the most popular microplate readers and collects the data from the FLOCKSCREEN tests, calculates the results and generates reports automatically.

FLOCKSCREEN™ Product Range:

| V010: | S. enteritidis | V130: | IBV |
| V020: | S. typhimurium | V140: | ART |
| V050: | Mg             | V150: | REO |
| V080: | CAV            | V160: | ILT |
| V090: | IBD            | V170: | AI  |
| V110: | EDS            | V710: | Duck Se |
| V120: | NDV            | V720: | Duck St |

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The FLOCKSCREEN™ IBD Antibody ELISA Kit provides a rapid, simple and sensitive method of detecting antibodies to IBD Virus in chicken serum or egg yolk.

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Assay Procedure
1. Add Sample/Controls
2. Incubate 30 mins & Wash
3. Add Enzyme Conjugate
4. Incubate 30 mins & Wash
5. Add Substrate Reagent
6. Incubate 15 mins
7. Add Stop Sol. Read at 550nm
Kit Contents

<table>
<thead>
<tr>
<th>2 Plate Kit</th>
<th>4 Plate Kit</th>
</tr>
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<tr>
<td>1. 2 x 96 well plates pre-coated with IBD antigen (supplied as 2 well holders each containing 12 x 8-well strips). In a re-sealable foil pouch with silica gel.</td>
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<td>2. Positive Control with antibodies to IBD preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (500µl ready to use).</td>
<td>Positive Control with antibodies to IBD preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (2 x 500µl ready to use).</td>
</tr>
<tr>
<td>3. Negative Control with SPF chicken serum preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (500µl ready to use).</td>
<td>Negative Control with SPF chicken serum preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (2 x 500µl ready to use).</td>
</tr>
<tr>
<td>4. Enzyme Conjugate Reagent, containing alkaline phosphatase labelled donkey anti-chicken IgG in tris buffer with an inert blue dye and Sodium Azide 0.1% w/v. (11ml)</td>
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</tr>
<tr>
<td>5. ELISA Substrate Reagent, containing phenolphthalein monophosphate and enzyme co-factors in a diethanolamine buffer. (11ml)</td>
<td>ELISA Substrate Reagent, containing phenolphthalein monophosphate and enzyme co-factors in a diethanolamine buffer. (2 x 11ml)</td>
</tr>
<tr>
<td>6. ELISA Stop Solution, containing sodium hydroxide and a chelating agent in a diethanolamine buffer. (11ml). WARNING CAUSTIC!</td>
<td>ELISA Stop Solution, containing sodium hydroxide and a chelating agent in a diethanolamine buffer. (22ml). WARNING CAUSTIC!</td>
</tr>
<tr>
<td>7. Wash Buffer Concentrate, containing phosphate buffer with ProClin 0.63% v/v. (50ml) – sufficient to make up 1000ml of wash buffer.</td>
<td>Wash Buffer Concentrate, containing phosphate buffer with ProClin 0.63% v/v. (100ml) - sufficient to make up 2000ml of wash buffer.</td>
</tr>
<tr>
<td>8. Sample Diluent Concentrate, containing phosphate buffer with protein stabiliser and ProClin 0.63% v/v. (50ml) – sufficient to make up 500ml of sample diluent.</td>
<td>Sample Diluent Concentrate, containing phosphate buffer with protein stabiliser and ProClin 0.63% v/v. (100ml) - sufficient to make up 1000ml of sample diluent.</td>
</tr>
<tr>
<td>9. 6 adhesive ELISA microtitre plate covers for use during incubation.</td>
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Materials and Equipment Required (Not Supplied):
In order to run the FLOCKSCREEN™ assays, the following equipment is recommended:
1. Precision pipettes: 5µl (or variable 1-20µl) 50µl (or variable 10-200µl) 50µl repeater or an 8 or 12 channel 2.5ml (or variable 1-5ml)
2. Disposable tips for pipettes
3. Microtitre Plate Reader with 550nm filter
4. Microtitre Plate Washer
5. +37°C incubator
6. Distilled or deionised water
7. Disposable 5ml plastic tubes
It is possible to run the assays without the 50µl repeater, or an 8-channel pipette and to use a wash bottle for plate washing instead of an automated plate washer. The results will however, be less consistent.

Warnings and Precautions:
1. This kit is for IN VITRO use only.
2. Optimum results will be obtained by strict adherence to this protocol. Careful pipetting and washing are necessary to achieve good assay performance.
3. The assay has been developed with incubations at +37°C for more consistent results. This eliminates problems associated with varying room temperature conditions.
4. The plates are coated with purified inactivated viral antigens and the control sera have been filtered with a 0.2µm filter. However, because your sample sera may be infected with bacteria or viruses, all reagents should be treated as potentially biohazardous and handled appropriately.
5. Do not intermix reagents from different Lot numbers with the exceptions of wash buffer & sample diluent.
6. The Substrate Reagent is very sensitive and under no circumstances should the same pipette tips or containers used for other reagents be used with the Substrate Reagent. The Substrate Reagent should be yellow in colour before addition to the wells. An orange, brown or pink colour indicates deterioration or contamination and the reagent should not be used.
7. Caution should be exercised in the handling of alkaline or other hazardous chemicals in accordance with Good Laboratory Practice.
8. Never pipette by mouth.
9. Wash solution and waste should be properly decontaminated with bleach or other strong oxidising agents before disposal.
Reagent Preparation

1. Allow all reagents to come to room temperature before use.

2. The Wash Buffer Concentrate and Sample Diluent Concentrate may partly recrystallize. This is due to the high concentration of salts. Should crystallisation occur, simply shake the bottle prior to reconstituting as described in the next two steps. The crystals will dissolve readily upon mixing.

3. To prepare sample diluent buffer, add the Sample Diluent Concentrate (50ml) to distilled or deionised water and make up to a total volume of 500ml. This sample diluent can be stored at +4°C for up to 3 months and can be used for preparing samples for any of the FLOCKSCREEN™ Kits.

4. To prepare the wash buffer, add the Wash Buffer Concentrate (50ml) to distilled or deionised water and make up to a total volume of 1000ml. This is stable at room temperature for 3 months and can be used with any of the FLOCKSCREEN™ Kits.

5. **DO NOT DILUTE THE POSITIVE AND NEGATIVE CONTROLS.**

Sample Preparation

**Serum Samples:** These should be as fresh and clean as practicable and stored at +4°C (up to 2 days) or at -20°C for longer term storage. Make a 1:500 dilution of each test sample in sample diluent buffer by adding 2.5ml of reconstituted sample diluent to 5µl of serum in a disposable 5ml plastic tube. Invert gently 2 or 3 times to mix. Alternatively a 2-step dilution protocol using dilution plates may be followed using a minimum of 5µl of sample.

**Yolk Samples:** Take 200µl of fresh yolk and add to 1.8ml wash buffer. Dilute a further 1:50 in sample diluent buffer (50µl in 2.5ml).

Diluted samples can be kept for several days at +4°C for re testing or at -20°C for longer term storage.

Assay Procedure

1. Remove the pre-coated plates from their sealed bags and record sample and control locations on a 12 x 8 template sheet. Each sample should be run in duplicate for optimum results. The positive and negative controls should always be run in duplicate.

2. Add 50µl of the undiluted controls and diluted samples to the appropriate wells. Diluted samples should be retained at +4°C until successful results are confirmed. Cover the plate with an adhesive cover and incubate at +37°C for 30 minutes. Mix on a plate shaker or by gently tapping the side of the plate.

3. Remove adhesive cover and wash the plate 4 times with wash buffer (300µl per well), invert and tap firmly on absorbent paper. **N.B. To reduce the possibility of sample carryover, it is recommended where possible, that the plate washer is programmed to wash each strip individually four times before washing the next strip.**

4. Add 50µl of Enzyme Conjugate Reagent to each well. Mix on a plate shaker or by gently tapping the side of the plate.

5. Cover the plate with the adhesive cover and incubate at +37°C for 30 minutes.

6. Remove adhesive cover and wash the plate 4 times with wash buffer (300µl per well), invert and tap firmly on absorbent paper.

7. Add 50µl ELISA Substrate Reagent to each well. The reagent must be at room temperature to achieve maximum colour development. Mix on a plate shaker or by gently tapping the side of the plate.

8. Cover the plate with the adhesive cover and incubate at +37°C for 15 minutes. Colour development is pale pink, which deepens on addition of ELISA Stop Solution.

9. Remove adhesive cover and add 50µl ELISA Stop Solution to each well. Mix on a plate shaker to obtain full colour development.

10. Wipe the under surface of the plate free of dust etc. with a soft tissue. Read the plate using a Microtitre Plate Reader at 550nm having first blanked on air. In order to obtain optimum results the plate should be read within 15 minutes of adding the ELISA Stop Solution.

**Results**

For the test to be valid:
(a) Mean Negative control absorbance must be <0.2
(b) Mean Positive control absorbance must be >0.6

It is important that the results fall within these parameters in order to prove that the components of the kit are all in good condition and that there have been no operator errors.

**Interpretation of Results**

For calculation of results, an S/P ratio is required (Sample value related to Positive Control value). The following formula is applied (using mean absorbance values for controls and paired samples):

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\]

*Developed by the Poultry Health Institute in Doorn, The Netherlands.

**Storage and Stability**

All reagents should be stored at +4°C on delivery. Do not freeze.

Avoid exposure to sunlight.

Do not use after the stated expiry date.

Do not use if silica gel desiccant in the pouch containing the microtitre plates is pink.

Any unused strips should be resealed in the re-sealable foil pouch together with the silica gel.

**ONCE A KIT HAS BEEN OPENED IT HAS A MAXIMUM SHELF-LIFE OF 3 MONTHS**