ELISA TEST PROCEDURE

PREPARING THE TEST PLATE

a) Remove an IBD antigen coated test plate from the protective bag and label according to dilution plate identification.
b) Add 50 μl Dilution Buffer to all wells on the test plate.
c) Add 50 μl diluted IBD Positive Control Serum to wells A1, A3 and H11. Discard pipette tip.
d) Using an 8 or 12 channel pipette transfer 50 μl/well of each of the diluted serum samples and Normal Control Serum samples from the dilution plate to the corresponding wells of the IBD coated test plate. Discard pipette tips after each row of sample is transferred. Transfer of samples to the ELISA plate should be done as quickly as possible.
e) Incubate plate for 30 minutes at room temperature.

WASH PROCEDURE

f) Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.
g) Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 μl Wash Solution. **Allow to soak in wells for 3 minutes;** then discard contents into an appropriate waste container (waste container should contain bleach solution). Tap inverted plate to ensure that all residual liquid is removed. **Repeat wash procedure 2 more times.**

NOTE: The wash procedure is a very critical step in any ELISA procedure. Please follow the above steps as directed.

ADDITION OF ANTI-CHICKEN IgG PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION

h) Using an 8 or 12 channel pipette (or transplating device) dispense 100 μl diluted conjugate (prepared as described above) into each assay well. Discard pipette tips.
i) Incubate for 30 minutes at room temperature.
j) **WASH** as in steps f and g above.
k) Using an 8 or 12 channel pipette (or transplating device) dispense 100 μl Substrate Solution into each test well. Discard pipette tips.
l) Incubate 15 minutes at room temperature.
m) Using an 8 or 12 channel pipette (or transplating device) add 100 μl diluted Stop Solution (prepared as described above) to each test well.
n) Allow bubbles to dissipate before reading plate.

MANUAL PROCESSING OF DATA

a) Read the plate using an ELISA plate reader set at 405-410 nm. Be sure to blank the reader as directed.
b) Calculate the average Positive Control Serum absorbance (Optical Density [O.D.]) using the absorbance values of wells A1, A3 and H11. Calculate the average Normal Control Serum absorbance using values obtained from wells A2, H10 and H12. Record both averages.
c) Subtract the average normal control absorbance from the average positive absorbance. The difference is the Corrected Positive Control.
d) Calculate a sample to positive (Sp) ratio by subtracting the average normal control absorbance from each sample absorbance. The difference is divided by the corrected positive control. Use the following equation format:

$$SP = \frac{\text{(SAMPLE ABSORBANCE)} - \text{(AVG NORMAL CONTROL ABSORBANCE)}}{\text{CORRECTED POSITIVE CONTROL ABSORBANCE}}$$

e) An IBD ELISA titer can be calculated by the following suggested equation:
LOG_{10} TITER = (1.172 X LOG_{10} Sp) + 3.614
TITER = ANTILOG OF LOG_{10} TITER

Example:

1. Example Positive Control Absorbance:
0.585, 0.610, 0.590
Average = (0.585 + 0.610 + 0.590) / 3 = 0.595

2. Example Normal Controls:
0.110, 0.102, 0.112
Average = (0.110 + 0.102 + 0.112) / 3 = 0.108

3. Corrected Positive Control:
(0.595) - (0.108) = 0.487

4. Example Sp value calculation:
Absorbance of sample = 0.560
(0.560) - (0.108) / 0.487 = 0.928

5. Example of Calculation of titer using the Sp from above:
Log_{10} Titer = 1.172 X (Log_{10} 0.928) + 3.614
Titer = ANTILOG 3.57
Titer = 3767

RESULTS

Assay Control Values:

Valid IBD ELISA results are obtained when the average optical density (O.D.) value of the Normal Control Serum is less than 0.250 and the Corrected Positive Control value range is between 0.250 and 0.900. If either of these values are out of range, the IBD test results should be considered invalid and the samples should be retested. Samples testing with an Sp value of less than or equal to 0.180 will receive a 0 titer value and are considered negative for IBD antibody.

Under optimal conditions*, the **suggested** O.D. value ranges of **0.100 to 0.160 for IBD Normal Control Serum** and **0.400 to 0.850 for IBD Positive Control Serum** should be strived for to ensure the most consistent laboratory test results. Please note that tests with O.D. values which do not fall within the suggested O.D. ranges above <u>does not</u> constitute an invalid test.

*Optimal conditions are at room temperature [70 to 75°F (21 to 24°C)]. Higher room temperatures may result in slightly higher OD values.

Interpretation of Results

The IBD ELISA titer values obtained represent a comparison of the IBD antibody level within each field chicken serum tested and the IBD ELISA kit positive and normal control sera. Therefore, it is important to first determine that the IBD ELISA positive and normal control sera values obtained are valid as detailed above in the "Assay Control Values" section of this pamphlet before IBD ELISA results are interpreted.

A "0" IBD ELISA titer represents a chicken serum sample that contains an extremely low to insignificant IBD antibody level compared to the IBD ELISA kit positive and normal control sera.

An IBD ELISA titer value above "0" indicates only that a chicken serum sample contains a significant and ELISA-detectable IBD antibody level compared to the IBD ELISA kit positive and normal control sera. However, these titers do **not** imply or ensure "protection" nor provide serologic differentiation between an IBD vaccine response or IBD field infection.

Optimal IBD vaccine administration practices and "protective" flock IBD titer target values must be determined by each IBD ELISA kit user by comparing **flock** pre- and post-vaccination IBD ELISA results [i.e. coefficient of variation (%CV) and geometric titer (GMT) values] with flock performance parameters (i.e. morbidity, mortality, flock body weight gain or uniformity) over time.

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1. Cosgrove, A.S., Apparently New Disease of Chicken - Avian Nephrosis. Avian Diseases: 6: 385-389. 1962.
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Please contact Synbiotics Technical Service at 800-247-1725 or (816) 454-7246 with questions and comments.

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INFECTIOUS BURSAL DISEASE VIRUS ANTIBODY TEST KIT

ITEM NO. 96-6503

ProFLOK®

SYNBIOTICS CORPORATION
11011 VIA FRONTERA SAN DIEGO, CA 92127
800 228 4305 (OR outside U.S./Canada 858 451 3771)

U.S. VET LIC NO. 312

INFECTIOUS BURSAL DISEASE VIRUS ANTIBODY TEST KIT

GENERAL INFORMATION AND INTENDED USES

Infectious Bursal Disease (IBD) was first described by Cosgrove in 1962[1]. IBD is one of the most economically important diseases that affects commercial chickens. High levels of maternal antibody against IBD, passed from the hen to the chick, provide young chickens with passive protection against field varieties of IBD. Antibody against IBD in hen or chick sera has previously been measured by the enzyme linked immunoassay (ELISA).[2]

The ProFLOK® IBD ELISA Kit is a rapid serologic test for the detection of IBD antibody in chicken serum samples. It was developed primarily to aid in the detection of pre and post-vaccination IBD antibody levels in chickens.

The assay is designed to measure IBD antibody bound to IBD antigen coated plates. The principle of the test is as follows: Serum obtained from chickens exposed to IBD antigens contains specific anti-IBD antibodies. Serum, diluted in Dilution Buffer, is added to an IBD antigen coated plate. Specific IBD antibody in the serum forms an antibody-antigen complex with the IBD antigen bound to the plate. After washing the plate, an affinity purified goat anti-chicken IgG (H+L) peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step binds with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contains a chromagen (ABTS), is added to each well. Chromagen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. The relative intensity of color developed in 15 minutes (compared to controls) is directly proportional to the level of IBD antibody in the serum. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm.

REAGENTS REQUIRED TO PERFORM 90 TESTS

a) 1 IBD antigen coated plate
b) 10 µl IBD Positive Control Serum
c) 10 µl Normal Control Serum
d) 100 µl Goat anti-Chicken IgG (H+L) Peroxidase Conjugate Solution
e) 40 ml Dilution Buffer
f) 10 ml ABTS-Hydrogen Peroxide Substrate Solution
g) 2.5 ml 5X Stop Solution (dilute [1:5] with laboratory grade water)
h) 20 ml 20X Wash Solution (dilute [1:20] with laboratory grade water)

NOTE: Store all reagents provided in the kit at 2-7°C.

EQUIPMENT AND MATERIALS REQUIRED

a) High precision pipette (i.e. 1-20 microliter pipette)
b) 0.2 ml, 1.0 ml and 5.0 ml pipettes
c) 8 or 12 channel pipette (or transplating device)
d) 2 graduated cylinders (50 ml)
e) 1 ml or 5 ml borosilicate glass test tubes
f) Uncoated low binding 96 well plates (i.e. Nunc catalog #269620)
g) Laboratory grade (Distilled or R.O.) water
h) 96 well plate reading spectrophotometer with 405-410 nm filter
i) Plate washing apparatus

WARNINGS TO THE USERS OF REAGENTS AND IBD ANTIGEN COATED PLATES

a) Handle all reagents and samples as biohazardous material.
b) Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
c) Wash solution, control sera, test plates, field samples and all other test kit reagents should be properly decontaminated with bleach or other strong oxidizing agent before disposal.
d) Take special care not to contaminate any of the test reagents with serum or bacterial agents.
e) Humidity indicators are supplied with each plate. If any of the indicators exhibit a pink color, the plate may be compromised in some way; decontaminate (i.e. wash the plate with bleach solution) and dispose of the plate.
f) The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
g) Do not use this kit after the expiration date.
h) **NEVER PIPETTE BY MOUTH.**

ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE BEFORE STARTING!

SAMPLE COLLECTION

For routine serologic flock monitoring, it is suggested that at least **30 or more sera per flock** be randomly collected at standard time intervals (i.e. every four weeks). Proper sample collection procedures, serum harvest and serum sample storage (4°C for up to four days or -20°C for longer periods) are needed to provide reliable test results.

SAMPLE DILUTION PROCEDURE

Dilute serum samples using Dilution Buffer in a clean, uncoated 96 well microtiter plate. Set up samples and controls as shown in Figure 1.

PREPARATION OF THE SERUM DILUTION PLATE

a) Add 300 µl Dilution Buffer to each well of an uncoated 96 well microtiter plate. This plate is referred to as the serum dilution plate.
b) Add 6 µl unknown serum per well as per Figure 1 (producing a 1:50 dilution). Start with well A4 and end with well H9 (moving left to right, row by row of wells). For example, wells 1 through 30 contain the diluted sera of flock 1, wells 31-60 contain the diluted sera of flock 2, etc.
c) Add 6 µl of Normal Control Serum (producing a 1:50 dilution) to wells A2, H10 and H12.
d) Aspirate and remove any liquid in dilution plate wells A1, A3 and H11.
e) Allow all diluted serums to equilibrate in Dilution Buffer for 5 minutes before transferring to an IBD antigen coated ELISA plate.
f) Diluted serum should be tested within 24 hours.

This dilution format provides adequate quantities of diluted serum samples to conduct four additional ProFLOK® ELISA tests (i.e. REO, NDV, IBV and ILT) using the same serum dilution plate.

Preparation of IBD Positive Control

An IBD Positive Control Serum has been provided with this kit. Dilute the appropriate volume of IBD Positive Control Serum with Dilution Buffer (1:50) in a clean, glass test tube. For example, dilute 6 µl of positive control serum in 300 µl Dilution Buffer. **Mix well.** 150 µl of IBD Positive Control is needed per ELISA plate.

Preparation of Conjugate Solution

The horseradish peroxidase conjugated anti-chicken IgG (H+L) is supplied in HRP Stabilizer. Dilute 100 µl stock conjugate in 10 ml Dilution Buffer (1:100 dilution). **Mix well.** This 10 ml preparation will supply sufficient conjugate for one 96 well ELISA plate.

Preparation of 1X Wash Solution

Dilute 20 ml concentrated Wash Solution in 380 ml laboratory grade (distilled or R.O.) water (1:20). **Mix well.** Approximately 400 ml Wash Solution is needed for each 96 well ELISA plate.

Preparation of the Substrate Solution

The Substrate Solution is ready to use. Each plate will require approximately 10 ml substrate solution. **For best results, the substrate solution must be equilibrated to room temperature before use.**

Preparation of 1X Stop Solution

Dilute 2.5 ml concentrated Stop Solution in 10 ml laboratory grade (distilled or R.O.) water (1:5). **Mix well.** Approximately 12.5 ml Stop Solution is needed for each 96 well ELISA plate.

NOTE: **Storage of 5X Stop Solution at refrigerated temperatures may cause the formation of a white solid. This does not affect product performance. Warm at room temperature or 37°C to dissolve before use.**

Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	-	+	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18	19	20	21
C	22	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44	45
E	46	47	48	49	50	51	52	53	54	55	56	57
F	58	59	60	61	62	63	64	65	66	67	68	69
G	70	71	72	73	74	75	76	77	78	79	80	81
H	82	83	84	85	86	87	88	89	90	-	+	-