BioChek
SMART VETERINARY DIAGNOSTICS

IBV

Data Pack

Infectious Bronchitis Antibody ELISA
(Detects antibodies to avian Corona virus)

CONTENTS

SUMMARY

Kit

- 5 plates
- Indirect ELISA
- Run at room temperature
- Incubation times: 30-30-15
- Read at: 405nm
- 1:500 dilution

Key Performance Features

General

Detects antibodies to avian Corona virus in Chicken sera
Picks up antibodies to IBV 7 - 14 days after challenge or vaccination. Good affinity for a wide range of variant IBV strains.

IBV strains reacting positive on the BioChek IBV ELISA are:

Arkansas	Mass	Connecticut	JMK
IOWA 609	IOWA 97	Chile	Allen
Australian nephritic	Gray	Holte	793 B
491(dev)	CR88 FR	D8880	D274
D1466	D3128	D3540	M41

Correlation mean Flocktiters with HI

Breeders and layers (HI range 4 - 11) ≥85%

Applications

Vaccination check

Test flock after vaccination in order to establish efficiency of vaccination. Answers to key questions like “did the vaccine actually stimulate the immune system” and how well the vaccine spread in the flock can be found by testing 2 - 5 weeks after vaccination. Test 2- 5 weeks after live vaccination and 5 - 10 weeks after vaccination with inactivated vaccine. See our interpretation manual for details on expected response to vaccination.

Field infection

about 10 - 20 days after infection seroconversion will show. Positive results means that the flock has been in contact with Avian Corona virus.
For flocks which haven't been vaccinated, positive samples mean field infection
For vaccinated flocks, compare obtained mean flock titer with expected (baseline) mean titers for similar flocks.

When positive alternative methods such as VN or HI can be used to determine the serotype.

BioChek Poultry Immunoassays

Infectious Bronchitis Antibody Test Kit

Catalogue Code CK119

Description of Test

The IBV ELISA kit will measure the amount of antibody to IBV in the serum of chickens. Microtitre plates have been pre-coated with inactivated IBV antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-IBV antibodies present will bind and form an antigen-antibody complex. Non specific antibodies and other serum proteins are then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase is then added to the wells and binds to any chicken anti-IBV antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate is added in the form of pNPP chromogen. A yellow colour is developed if anti-IBV antibody is present and the intensity is directly related to the amount of anti-IBV present in the sample.

Reagents provided

1. **IBV Coated plates**. Inactivated viral antigen on microtitre plates
2. **Conjugate reagent**. Sheep anti-Chicken: Alkaline Phosphatase in Tris buffer with protein stabilisers, inert red dye and sodium azide preservative (0.1% w/v)
3. **Substrate tablets**. PNPP (p-Nitrophenyl Phosphate) tablets to dissolve with Substrate buffer.
4. **Substrate buffer**. Diethanolamine buffer with enzyme co-factors
5. **Stop Solution**. Sodium Hydroxide in Diethanolamine buffer
6. **Sample Diluent**. Phosphate buffer with protein stabalisers and sodium azide preservative (0.1% w/v)
7. **Wash Buffer**. Powdered Phosphate Buffered Saline with Tween
8. **Negative control**. Specific Pathogen Free serum in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)
9. **Positive Control**. Antibodies specific to IBV in Phosphate Buffer with protein stabilisers and sodium azide preservative (0.1% w/v)

Materials and Equipment Required (not provided with kit)

Precision Pipettors and disposable tips
8 or 12 channel pipette / repeater pipette
Plastic tubes for sample dilution
Distilled or deionised water
Microtitre Plate Reader with 405 nm filter
Microtitre Plate Washer

Warnings and Precautions

1. Handle all reagents with care. STOP SOLUTION contains STRONG ALKALI which can be CAUSTIC. If in contact with skin or eyes, wash with copious amounts of water.
2. Treat all biological materials as potentially biohazardous, including all field samples. Decontaminate used plates and waste including washings with bleach or other strong oxidising agent before disposal.
3. NEVER pipette anything by mouth. There should be no eating, drinking or smoking in areas designated for using kit reagents and handling field samples.
4. This kit is for IN VITRO use only.
5. Strict adherence to the test protocol will lead to achieving best results.

Reagent preparation

1. **Substrate Reagent**. To make Substrate Reagent, add 1 tablet to 5.5 ml of Substrate Buffer and allow to mix for 3 minutes or until fully dissolved. The prepared reagent should be made on day of use *but will be stable for one week if kept in dark at +4 ℃.*

*D*rop tablets into clean container and add appropriate volume of Substrate Buffer
DO NOT HANDLE TABLET WITH BARE FINGERS

2. **Wash Buffer**. Empty the contents of one wash buffer sachet into one litre of distilled or deionised water and allow to dissolve fully by mixing. Wash buffer will remain stable for use for 1 month if stored at *+4 ℃.*

3. All other kit components are ready to use but allow to come to room temperature (22 – 27 °C) before use.

Sample preparation
Dilute each test sample 1 : 500 by adding 1 ul to .5 ml of sample diluent

1. Mix well by vortexing or shaking the tube
2. A fresh pipette tip must be used for each separate sample.
3. Identify dilution tube clearly with sample number

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING !!

Test procedure:
1. Remove IBV coated plate from sealed bag and record location of samples on template.
2. Add 100 µl of negative control into wells A1 and B1
3. Add 100 µl of positive control into wells C1 and D1
4. Add 100 µl of diluted samples into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
5. Aspirate contents of wells and wash 4 times with wash buffer (300µl per well). Invert plate and tap firmly on absorbent paper.
6. Add 100 µl of Conjugate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **30 minutes**.
7. Repeat wash procedure as in 5.
8. Add 100 µl of Substrate Reagent into the appropriate wells. Cover plate with lid and incubate at room temperature (22-27°C) for **15 minutes**.
9. Add 100 µl of Stop Solution to appropriate wells to stop reaction.
10. Blank the microtitre plate reader on air and record the absorbance of controls and the samples by reading at 405 nm.

Results:

For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15.

Variance in lab temperatures will lead to lower or higher absorbance values. Test sample values will be relative to the control values and the test will still be valid.

The IBV positive control has been carefully standardised to represent significant amounts of antibody to IBV in Chicken serum.
The relative amounts of antibodies in chicken samples can then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio)

Interpretation of results

Samples with an S/P of .2 or greater contain anti-IBV antibodies and are considered POSITIVE.

1. **Calculation of S/P ratio**

$$\frac{\text{Mean of Test Sample - Mean of negative control}}{\text{Mean of Positive control - Mean of negative control}} = \text{S/P}$$

2. **Calculation of Antibody Titre**

The following equation relates the S/P of a samples at a 1 : 500 dilution to an end point titre

Log10 Titre = 1.0 * (log10 S/P) + 3.62

Antilog = Titre

S/P value	Titre Range	Antibody status
0.149 or less	624 or less	Negative
0.150 - 0.199	925 - 833	Suspect
.200 or greater	834 or greater	Positive

Each Laboratory should establish its own criteria for non protected and protected

BioChek has available a software programme which can be used with the IBV kit to calculate S/P values, titres and provide general flock profiling.

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DATA SHEETS

SPECIFICITY

Purpose

To determine the distribution and characteristics of chicken serum originating from SPF (Specific Pathogen Free) chickens, when tested on the BioChek IBV ELISA.

Procedure

36 samples from 12 week old SPF white Leghorns were obtained (Lohmann Cuxhaven Germany) and assayed using the standard protocol for the BioChek IBV ELISA.

Results/Conclusion

The results are shown in Table 1 and Graph 1.

The S/P value of each sample is plotted on the Y-axis, the sample number on the X-axis.
The mean S/P value was .0236 and the Standard deviation was .0133.
This means that the mean of the negative population is generously more than 3 Standard deviations from the cut-off (.20 S/P)

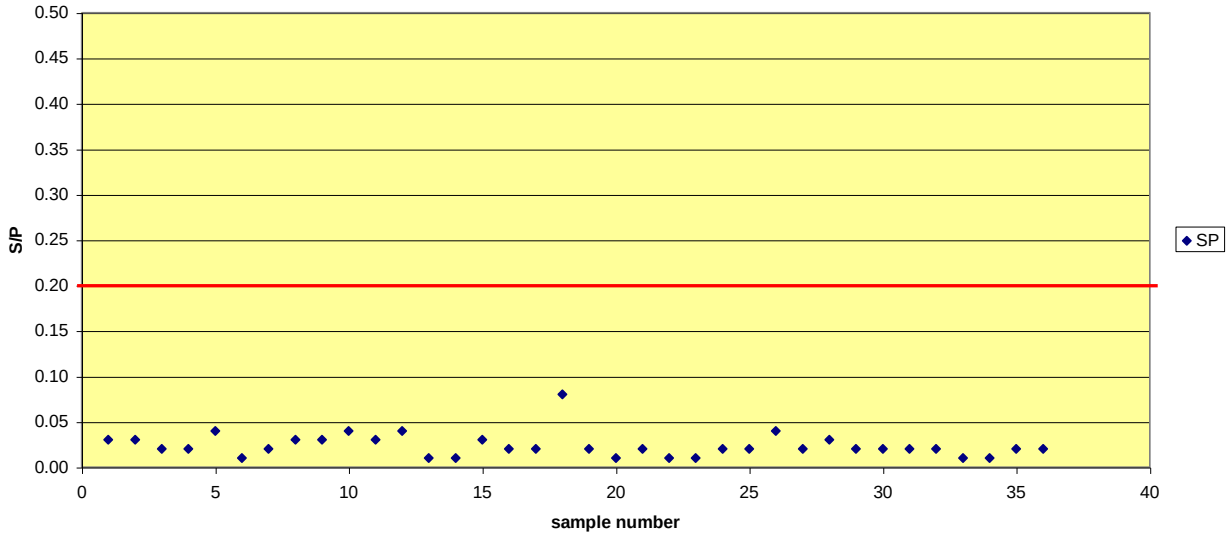
The data demonstrate that the BioChek IBV ELISA has good specificity.

Table 1 Specificity, Negative population

ELISA IBV SPF Negative Panel			Mean S/P	0.0236		Kit Lot No.	FS4081
			St dev	0.0133			
Sample							
Number	SP	Result					
1	0.03	NEG					
2	0.03	NEG					
3	0.02	NEG					
4	0.02	NEG					
5	0.04	NEG					
6	0.01	NEG					
7	0.02	NEG					
8	0.03	NEG					
9	0.03	NEG					
10	0.04	NEG					
11	0.03	NEG					
12	0.04	NEG					
13	0.01	NEG					
14	0.01	NEG					
15	0.03	NEG					
16	0.02	NEG					
17	0.02	NEG					
18	0.08	NEG					
19	0.02	NEG					
20	0.01	NEG					
21	0.02	NEG					
22	0.01	NEG					
23	0.01	NEG					
24	0.02	NEG					
25	0.02	NEG					
26	0.04	NEG					
27	0.02	NEG					
28	0.03	NEG					
29	0.02	NEG					
30	0.02	NEG					
31	0.02	NEG					
32	0.02	NEG					
33	0.01	NEG					
34	0.01	NEG					
35	0.02	NEG					
36	0.02	NEG					

Graph 1 Specificity

IBV ELISA specificity, Negative population.
Mean S/P=.0236
Std. Dev=.0133
Positive Cutoff =>.20



DATA SHEETS

MONOSPECIFIC SAMPLE PANEL

Purpose

To determine if the BioChek IBD test kit cross-reacts with antibodies generated by other pathogens common in chickens.

Procedure

A sample panel monospecific for antibodies of pathogens common in poultry was tested on the BioChek IBV kit

Results / Conclusion

The results are shown in Table 2

Only the monospecific serum samples for IBV tested positive on the BioChek IBV ELISA. This concludes that the test does not cross-react with antibodies directed at other avian pathogens in the set of monospecific antisera.

Table 2 BioChek IBV antibody detection ELISA, Biochek Monospecific sample panel

BC IBV test done on 6/26/2001 FS3628

Interpretation BC IBV results:		
S/P value	**Titre Range**	**Antibody status**
0.149 or less	**624 or less**	**Negative**
0.150 - 0.199	**925 - 833**	**Suspect**
.200 or greater	**834 or greater**	**Positive**

	S/P				**S/P**	
M41	**2.781**	**1.701**		ILT AGP	0.034	0.05
491deventer	**2.086**	**0.87**		ADENO	0.018	0.035
793B	**0.462**	**0.336**		AE	0.013	0.021
CR88 FR	**0.405**	**0.237**		Mg	0.055	0.113
D1466	**0.897**	**0.537**		Ms	0.059	0.185
D274	**1.457**	**0.826**		PMV1	0.133	0.218
D3128	**2.617**	**1.583**		PMV3	0.042	0.005
D8880	**1.568**	**0.794**		POX	0.016	0.045
IBD	0.013	0.021		reo1133	0.047	0.035
ILT	0.023	0.083		reo2534	0.016	0.024

DATA SHEETS

SENSITIVITY

Purpose

To establish the time it takes for the BioChek IBV antibody detection assay to detect antibodies after active immunization.

Procedure

A flock of 01D old commercial broilers were housed separately in House 4.
At day 16 they were vaccinated with live 4/91 vaccine (Intervet) through drinking water.
Serum samples were taken at 01D, 07D, 15D, 21D and 39D and frozen until analysis.
When the last samples were collected, all samples were analysed using the BioChek IBV ELISA.

Results / Conclusion

The Results are shown in Table 3 and Graph 2

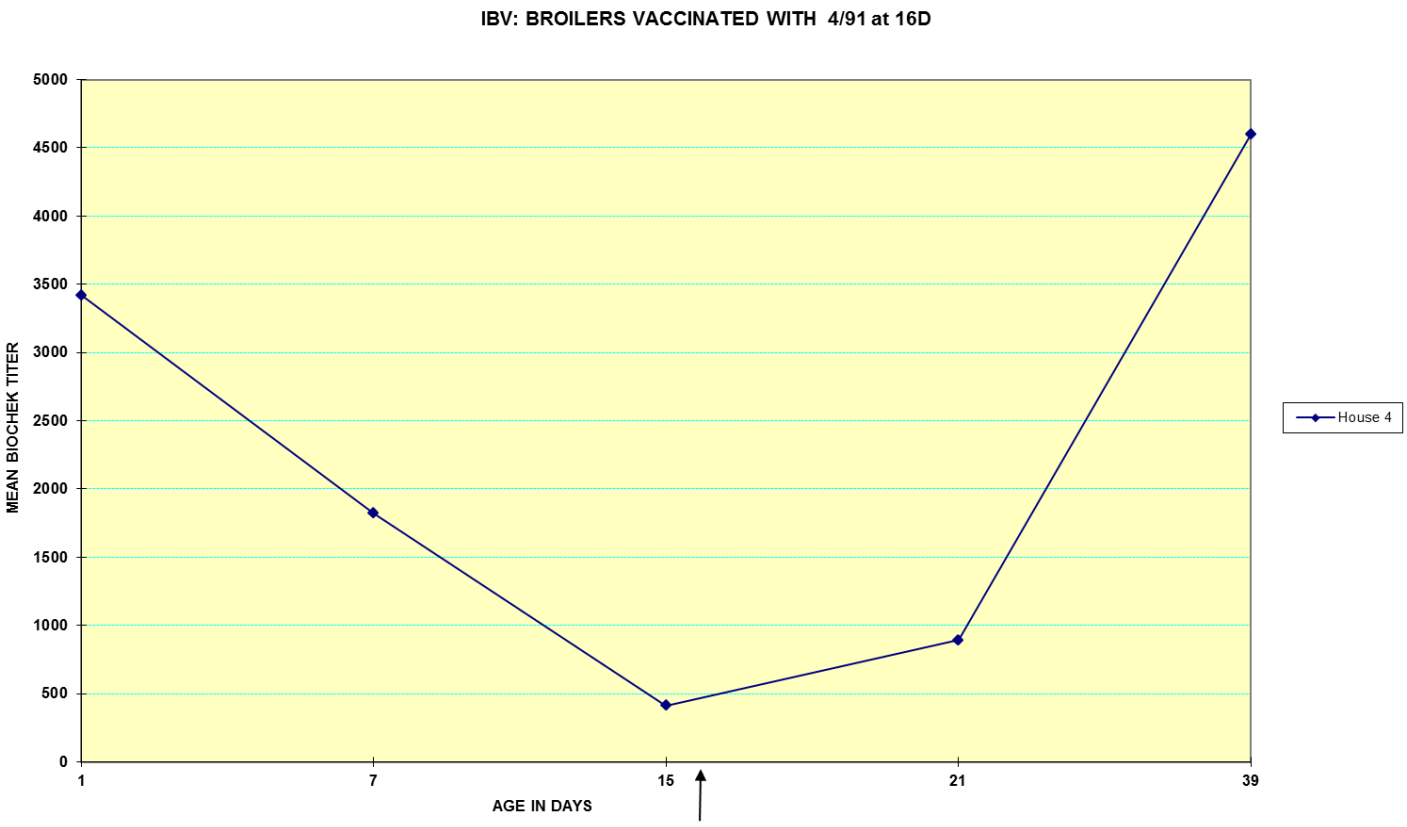
The Maternal antibody titers declined from 3421 to 415 at 01D to 15D respectively.
At 6 days post vaccination (d.p.v), 46% of the vaccinated broilers tested positive.
At 14 d.p.v, 100% of the vaccinated birds tested positive.

To conclude, the BioChek IBV antibody detection test has excellent sensitivity as it detects antibodies already 6-14 d.p.v. Also it is shown that the BioChek IBV antibody detection test detects both maternal antibodies as well as antibodies derived from active immunization.

Table 3 Mean IBV Titer and % Positives of Broilers at various ages before and after vaccination with Live vaccine at 16D (4/91, Drinking Water).

AGE	Mean Titer ELISA	% Positives ELISA
1	3421	100%
7	1822	58%
15	415	8%
21	894	46%
39	4597	100%

Graph 2 Mean titer with vaccination



DATA SHEETS

REPRODUCIBILITY

Trial 1: Batch to batch reproducibility
Trial 2: Intra assay reproducibility

Trial 1: Batch to Batch Reproducibility

Purpose

In this trial a pre-diluted chicken serum sample IBV MEDIUM containing antibodies to IBV was tested on several batches of the BioChek IBV ELISA.
The purpose of the trial is to assess batch to batch reproducibility.

Procedure

A known, pre-diluted IBV sample, (IBV MEDIUM) is assayed in duplicate on 7 different production batches of IBV kits. Mean S/P values, standard deviation, and C.V. are calculated to assess the amount of variability between the different batches of kits.

Results/Conclusion

As can be seen in the corresponding table (table IV reproducibility) the batch variability on the 7 batches is as following:

For the IBV MEDIUM sample results were:

Mean Titre	6852
SD	818
%CV	12

The data demonstrate that there is limited variation (< 15%) when comparing results from various production batches of the BioChek IBV ELISA

Table IV reproducibility

Assay:	IBV		
Mean Titer For All Flocks		6852	
Standard Deviation of Mean Flock Titers:		818	
Coefficient of Variation of Mean Flock Titers:		12	%

Name	Company	MEAN
MEDIUM	FS4601	7479
MEDIUM	FS4634	8473
MEDIUM	FS4649	6795
MEDIUM	FS4651	7418
MEDIUM	FS4672	5390
MEDIUM	FS4681	5546
MEDIUM	FS4699	6590
MEDIUM	FS4699	6921
MEDIUM	FS4699	6052
MEDIUM	FS4699	6700
MEDIUM	FS4601	6926
MEDIUM	FS4651	6889
MEDIUM	FS4699	7062
MEDIUM	FS4699	6182
MEDIUM	FS4699	7112
MEDIUM	FS4699	8100

Trial 2: Intra-Assay Reproducibility

Purpose

The purpose of the trial is to assess intra-plate reproducibility. The plate CV of the IBV test kit should be less than 10%.

Procedure

A standard pre diluted sample known positive for IBV is assayed on 90 wells of an IBV plate. IBV test is run according to package insert.

Results/Conclusion

The %CV of the sample (IBV HIGH) is 4.12 %.

DATA SHEETS

COMPARISON BIOCHEK IBV RESULTS WITH GOLDEN STANDARDS

1. **Layers, Mean Titer Comparison**
2. **Layers, Individual Titer Comparison**

1. LAYERS, Mean Titer Comparison

Purpose

To compare and validate results obtained with BioChek IBV antibody detection assay with HI (M41) and VN (4/91) tests.

Procedure

A total of 143 samples were taken from 7 commercial layer flocks.
Samples for HI and VN were analyzed at Laboratory of Intervet International, Boxmeer, Holland.
Samples were then send to BioChek B.V. for analysis on BioChek IBV ELISA.
Mean HI (M41) log2 titers and Mean VN (4/91) Log2 Titers were then compared to Mean Titers and Mean Log2 BioChek ELISA titers. A total of 88 samples were taken from 7 commercial vaccinated layer flocks.

Results/Conclusion

See Table 4 and Figure 1.
Mean BC titers with corresponding HI titers (range of HI titers 5 to 11), and corresponding VN 4/91 titers (range VN titers 4 to 10) of 7 Layer flocks were correlated.

Results show Mean Log2 BC ELISA titers to correlate well (> 90%) with HI and VN.

The BioChek IBV antibody detection test has an good correlation with HI (M41) and VN (4/91) test in detecting antibodies in vaccinated Layer Flocks. Results show Mean Log2 BC ELISA titers to correlate well (> 90%) with Mean HI and VN Titers. Also Mean BC ELISA titers correlate well (>87%) with Mean HI and VN Log2 Titers.

Table 4 IBV: CORRELLATION OF BIOCHEK TITERS WITH HI(M41) & VN 4/91

Mean titers of 7 Layer Flocks , Age 18-45W (Total no.samples = 88)
Interpretation titers: VN <4 negative, VN =>4 positive
Interpretation titers: HI <3 negative, HI = 4-5 suspect, HI =>6 positive
Interpretation BC titers: titer < 624 negative, titer >624 and < 834 suspect, titer =>834 positive.

Flock	Mean Flock Titers			
	Titer BC	LOG2 BC	HI M41	VN 4/91
97-207	1418	10.5	5.6	4.6
97-305	2270	11.1	6.7	7.1
97-305	3765	11.9	8.7	6.1
97-308	5911	12.5	7.9	7.9
97-330	8819	13.1	10.5	9.7
97-330	10300	13.3	9.5	9.8
97-172	13289	13.7	10.3	10.3

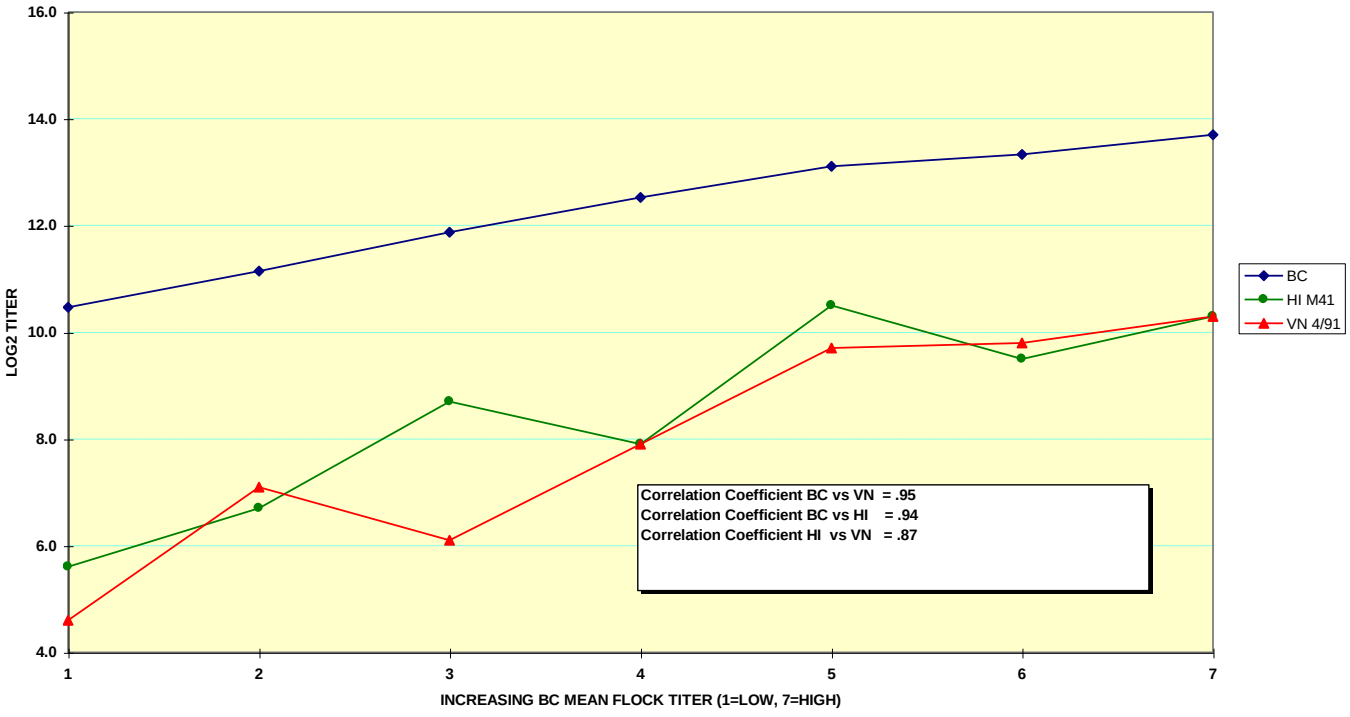
Log2 Titers:

CORRELLATION COEFFICIENT BC VS VN	0.945
CORRELLATION COEFFICIENT HI VS VN	0.869
CORRELLATION COEFFICIENT BC VS HI	0.935

BC IBV Titers:

CORRELLATION COEFFICIENT BC VS VN	0.930
CORRELLATION COEFFICIENT BC VS HI	0.878

FIGURE 1. IBV: Mean Log2 Titers of ELISA vs HI M41 and VN 4/91
Mean Titers of 7 flocks, with HI range of 5-10, VN range 4.5-10



2. Layers, Individual Titer Comparison

Purpose

To compare and validate results obtained with BioChek IBV antibody detection assay with HI (M41), on an individual sample-to-sample basis.

Procedure

A total of 44 samples were taken from 2 commercial layer flocks.
Samples for HI and VN were analyzed at Laboratory of Intervet International, Boxmeer, Holland.
Samples were then send to BioChek B.V. for analysis on BioChek IBV ELISA.
Individual HI (M41) log2 titers were then compared to Individual BioChek ELISA Titers.

Results/Conclusion

See Table 5 and Figure 2.
Individual BC titers with corresponding individual HI titers (range of HI titers 4 to 11) were correlated. Results show individual Log2 BC ELISA titers to correlate well (81%) with individual HI Log2 Titers.
When individual BC titers were compared with Log2 HI titers, the correlation was 85%.

Individual BioChek IBV titers correlate well with individual Log2 HI (M41) titers.
In vaccinated Layer Flocks , individual BioChek titers correlated at =>80% level with HI titers.

Table 5 IBV: Correlation Of Individual Biochek Titers With Hi M41

TITER	LOG2 TITERS	
BC	BC	HI M41
2518	11.3	4.0
517	9.0	4.0
500	9.0	4.0
638	9.3	5.0
3643	11.8	6.0
1025	10.0	5.0
734	9.5	6.0
2426	11.2	6.0
4060	12.0	6.0
1734	10.8	5.0
129	7.0	6.0
3844	11.9	5.0
154	7.3	4.0
179	7.5	5.0
154	7.3	7.0
2380	11.2	8.0
2768	11.4	4.0
500	9.0	7.0
225	7.8	6.0
825	9.7	5.0
1334	10.4	8.0
917	9.8	7.0
14232	13.8	11.0
18872	14.2	11.0
15312	13.9	11.0
8821	13.1	11.0
8021	13.0	10.0
7345	12.8	9.0
9734	13.2	8.0
13294	13.7	11.0
16095	14.0	10.0
10797	13.4	11.0
11072	13.4	10.0
21044	14.4	11.0
10117	13.3	10.0
16712	14.0	11.0
11827	13.5	10.0
11843	13.5	11.0
26113	14.7	11.0
8112	13.0	10.0
12506	13.6	9.0
10597	13.4	9.0
16483	14.0	10.0
11752	13.5	11.0
14941	13.9	11.0

Log2 Titers:

CORRELLATION COEFFICIENT BC VS HI	**0.807**

BC IBV Titers:

CORRELLATION COEFFICIENT BC VS HI	**0.851**

FIGURE 2. IBV Layers: Correlation of Individual Log2 BC Titers vs HI M41. (2 Layer flocks, with low and high mean titers)