BioChek
SMART VETERINARY DIAGNOSTICS

IBD

Data Pack

Infectious Bursal Disease Antibody ELISA
(Detects antibodies to Infectious Bursal Disease Virus)

CONTENTS

Summary **Page 3**
The Kit
Key Performance Features
Applications

Package Insert **Pages 4-6**
Description of test
Reagents provided
Materials and equipment required
Warnings and precautions
Reagent preparation
Sample preparation
Test procedure
Results
Interpretation of results

Data Sheets **Pages 7-18**
Specificity: Negative population
Monospecific sample panel
Sensitivity
Commercial ELISA's Vs VN
Comparison to VN

SUMMARY

Kit

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

Key Performance Features

Sensitivity

Detects antibodies to IBD 7 - 14 days after challenge or vaccination. The sensitivity is similar to VN.
Concerning passive immunity, BioChek IBD starts getting positive at a VN of 6 – 7.

The following IBD strains have been tested positive on the BioChek IBD ELISA:

D78
Lukert
Variant E
GLS
Winterfield

Correlation with VN

Active immunity:	95% (VN range 5 – 18)
Passive (maternal) immunity:	98% (VN range 6 – 16)

Specificity

Serum samples from 30 – 60 week old SPF leghorns: 99.5%

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 IBD pathogen
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 can be used to determine the serotype.

BioChek Poultry Immunoassays
Infectious Bursal Disease Antibody Test Kit

Catalogue Code CK113

Description of Test:

The IBD ELISA kit will measure the amount of antibody to IBD in the serum of chickens. Microtitre plates have been pre-coated with inactivated IBD antigen. Chicken serum samples are diluted and added to the microtitre wells where any anti-IBD 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-IBD antibodies 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-IBD antibody is present and the intensity is directly related to the amount of anti-IBD present in the sample.

Reagents provided:

1. **IBD Coated plates**. Inactivated viral antigen on microtitre plates.
2. **Conjugate reagent** 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 reagent**. Diethanolamine buffer with enzyme co-factors.
5. **Stop solution**. Sodium Hydroxide in Diethanolamine buffer.
6. **Sample diluent reagent**. Phosphate buffer with protein stabilisers 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 IBD in Phosphate buffer with protein stabilisers and sodium azide preservative (0.1% w/v).

Materials and Equipment required (not provided with kit):

Precision Pipettes 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 - 6 ml of substrate buffer and allow to mix until fully dissolved (+/- 10 minutes). The prepared reagent should be made on day of use but will be stable for one week if kept in dark at +4 °C. Drop tablets into clean container and add appropriate volume of Substrate Buffer.
   DO NOT HANDLE TABLETS 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.

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

Sample preparation:

1. Dilute each test sample 1:500

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING!!

Test procedure:

1. Remove IBD 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 (350µl 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 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 IBD positive control has been carefully standardised to represent significant amounts of antibody to IBD 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 0.2 or greater contain anti-IBD 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 sample at a 1:500 dilution to an end point titre.

Log10 Titre = 1.1 * Log (SP) + 3.361

Antilog = Titre

S/P value	Titre Range	Antibody status
0.199 or less	390 or less	Negative
0.200 or greater	391 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 IBD kit to calculate S/P values, titres and provide general flock profiling.

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KI/CK113REV01

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 IBD 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 IBD ELISA.

Results/Conclusion

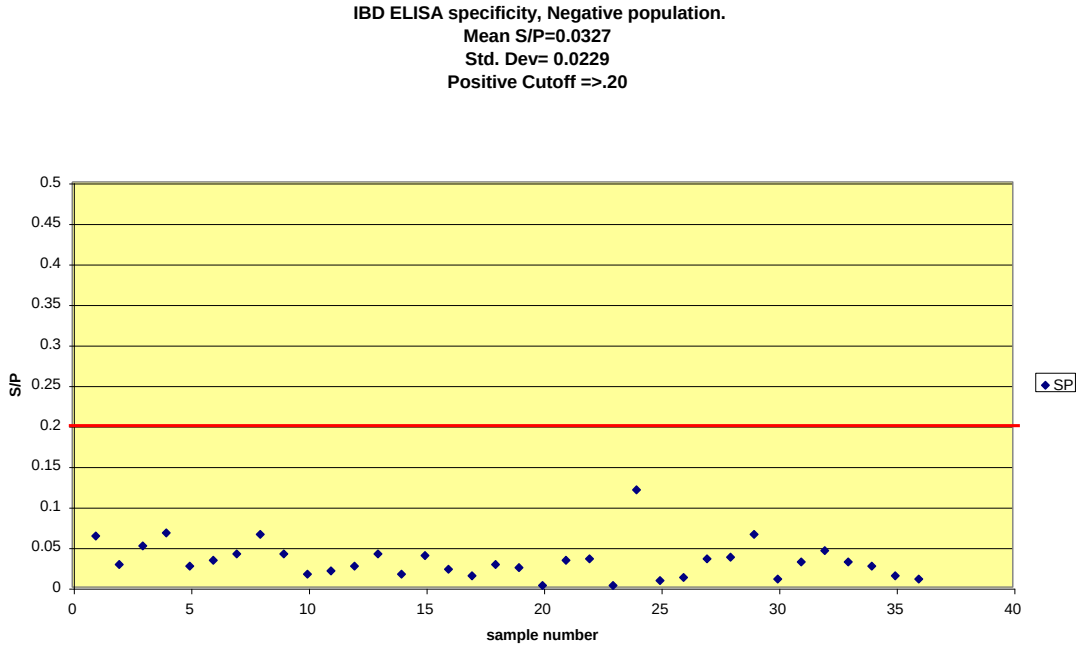
The results are shown in Table 1 and Graph 1.

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 .0327 and the Standard deviation was .00229.
The data demonstrate that the BioChek IBD ELISA has a 100% specificity on this sample panel.

Table 1 Specificity, Negative population

ELISA IBD SPF Negative Panel			Mean S/P	0.0327		Kit Lot No.	FS4210
			St dev	0.0229			
Sample							
Number	SP	Result					
1	0.063	NEG					
2	0.028	NEG					
3	0.051	NEG					
4	0.067	NEG					
5	0.026	NEG					
6	0.033	NEG					
7	0.041	NEG					
8	0.065	NEG					
9	0.041	NEG					
10	0.016	NEG					
11	0.02	NEG					
12	0.026	NEG					
13	0.041	NEG					
14	0.016	NEG					
15	0.039	NEG					
16	0.022	NEG					
17	0.014	NEG					
18	0.028	NEG					
19	0.024	NEG					
20	0.002	NEG					
21	0.033	NEG					
22	0.035	NEG					
23	0.002	NEG					
24	0.12	NEG					
25	0.008	NEG					
26	0.012	NEG					
27	0.035	NEG					
28	0.037	NEG					
29	0.065	NEG					
30	0.01	NEG					
31	0.031	NEG					
32	0.045	NEG					
33	0.031	NEG					
34	0.026	NEG					
35	0.014	NEG					
36	0.01	NEG					

Graph 1 Specificity



DATA SHEETS

MONOSPECIFIC SAMPLE PANEL

Serum samples negative for Infectious Bursal Disease antibody positive for other avian pathogens.

Purpose

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

Procedure

A sample panel, mono-specific for antibodies of pathogens, common in Poultry, was tested on the BioChek IBD test . The following monospecific sera were tested on the BioChek IBD test: Avian Paramyxovirus serotype 1 (Newcastle Disease Virus), Avian Paramyxovirus serotype 3 , Avian Pneumovirus Type A and C, Infectious Laringotracheitis, Infectious Bronchitis serotypes 4/91, cr88, cr98,M41, D274, D1466, D3128, D8880 , Egg Drop Syndrome (Adenovirus), Fowl Pox, Reo virus S1133 & S2534S, Mycoplasma Gallisepticum, Mycoplasma Synoviae, Infectious Bursal Disease, and Avian Encephalomyelitis.

Results/Conclusion

The results are shown in Table 2

Only the monospecific serum sample for IBD (Infectious Busal Disease) tested positive on the BioChek IBD ELISA.

The conclusion is that the BioChek IBD ELISA does not cross-react with antibodies directed at other avian pathogens in above set of monospecific antisera.

Table 2 Monospecific Panel

BioChek sample panel of sera positive for antigens mentioned
The BioChek IBD test test negative for all samples exept IBD positive samples

Name : MONOSPECIFICS sample panel
Bleeding Date : 25-06-2002
Assay : BioChek IBD Lot No: FS3662
Dilution : '1:500

Interpretation results	
S/P value	Antibody status
.15 or less	Negative
.150 - .200	suspect
.200 or greater	Positive

Sample ID	S/P Ratio
4/91INT	0,046
793BVLA	0,046
adeno	0,044
AE	0,03
CR88	0,016
CR98	0,016
D1466	0,076
D1466INT	0,044
D274	0,131
D274INT	0,046
D3128	0,131
D8880	0,016
4/91DEV	0,071

Sample ID	S/P Ratio
Fpox	0,046
IBD	3,239
ILT	0,085
ILTAGP	0,046
M41	0,046
M41INT	0,002
Mg	0,046
Ms	0,046
PMV1	0,067
PMV3	0,046
REO1133	0,044
REO2534	0,057
TRTA	0,048
TRTC	0,046

DATA SHEETS

SENSITIVITY

Purpose

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

Procedure

A sample set was obtained from the AHS, Deventer, Holland. All samples except sample #5, were produced in a vaccination experiment in which SPF White Leghorn birds kept in Horsefall-Bauer isolators were individually vaccinated with a single dose of live IBDV vaccine according to the manufacturer's instructions. At 7, 10 or 14 days post vaccination(d.p.v.), birds wer removed for bleeding. The sera were pooled before freeze drying.

Sample #5 is a pooled serum from vaccinated broilers that have been challenged with the very virulent IBDV strain D6948.

Sample 2	SPF serum (1 year old layers; pooled sample)
Sample 5	Vaccinated and challenged (vvIBDV) chickens
Sample 8	D78 vaccination, pooled sample taken at 7 d.p.v
Sample 1	D78 vaccination, pooled sample taken at 14 d.p.v
Sample 6	Bursine 2 vaccination, pooled sample taken at 7 d.p.v.
Sample 7	Bursine 2 vaccination, pooled sample taken at 14 d.p.v
Sample 3	Gallivac vaccination, pooled sample taken at 7 d.p.v
Sample 4	Gallivac vaccination, pooled sample taken at 14 d.p.v

Results/Conclusion

The results are shown in Table 1

The BioChek IBD antibody detection test has an good sensitivity as it detects antibodies 7 -14D days post vaccination.

Table 1: S/P results IBD ringtrial 2005

	rep 1	rep 2
# sample	S/P	S/P
1	5,75	5,27
2	0,11	0,12
3	4,89	4,64
4	5,37	5,56
5	3,85	3,99
6	0,24	0,28
7	3,27	3,29
8	2,36	2,85

Positive cutoff S/P > .200
Interpretation:
All samples test positive except sample 2.

DATA SHEETS

COMMERCIAL ELISA'S VS. VN

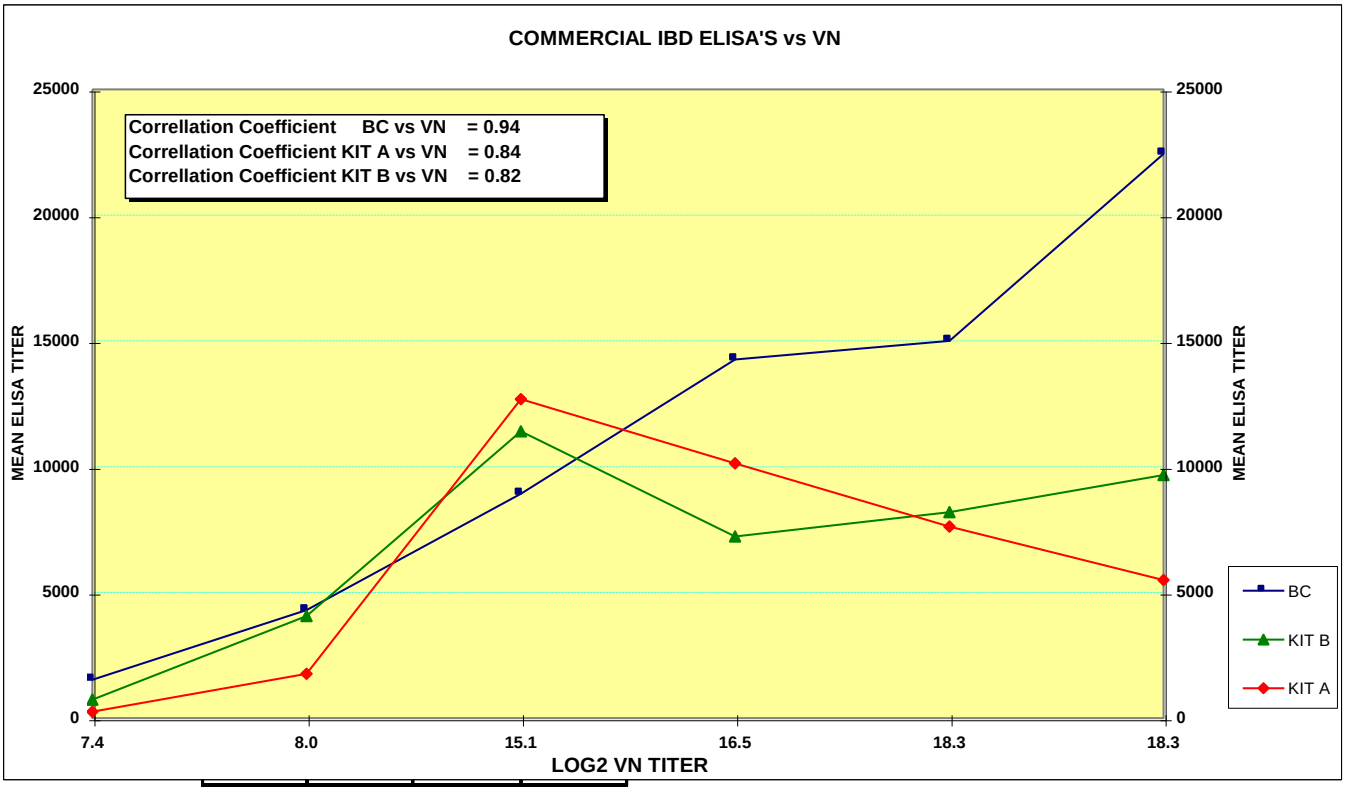
Serum Samples and VN results provided by Intervet International B.V., Boxmeer, Holland

Vaccination Titers vs. VN

Correlation study of Mean Log2 ELISA Titers with Log2 VN Titers. BC shows a good correlation (95%) in the range of VN 5 to 18.

IBD: CORRELLATION OF COMMERCIAL IBD ELISA's WITH VN

Mean ELISA Titers of vaccinated flocks, corresponding to VN titer range of 7-18

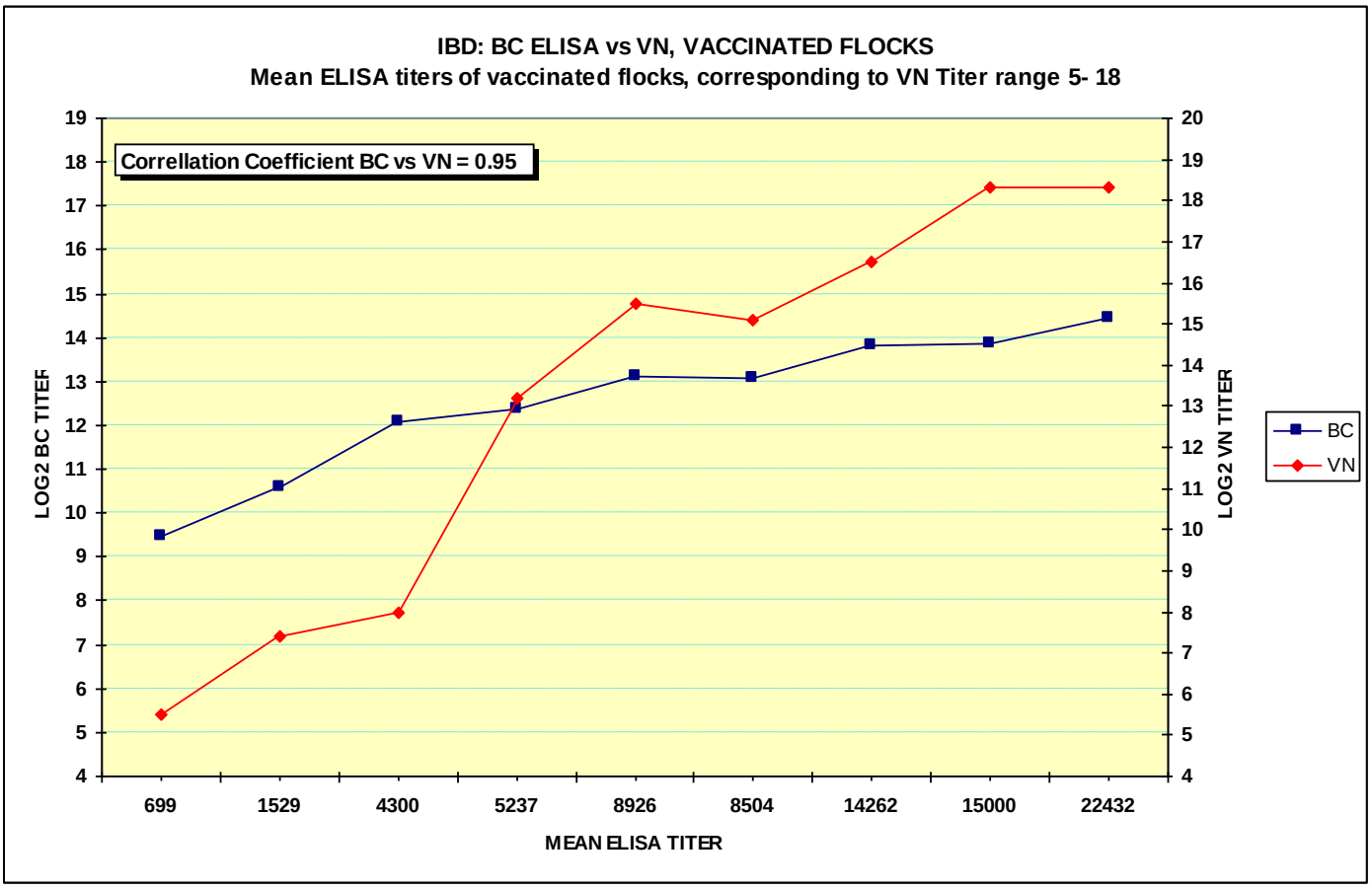


DATA SHEETS

COMPARISON BIOCHEK IBD RESULTS WITH VIRUS NEUTRALIZATION (VN)

Correlation study of Log2 ELISA Titers with Log2 VN Titers. Sera of SPF birds, challenged with increasing vaccine. BC shows high correlation with VN (94%) for VN's ranging from 7 to 18. Other kits show decreasing (rather then increasing) titers, with increasing VN Titers. This is called "Hook effect " (negative interference causes titers to decrease after point of saturation).

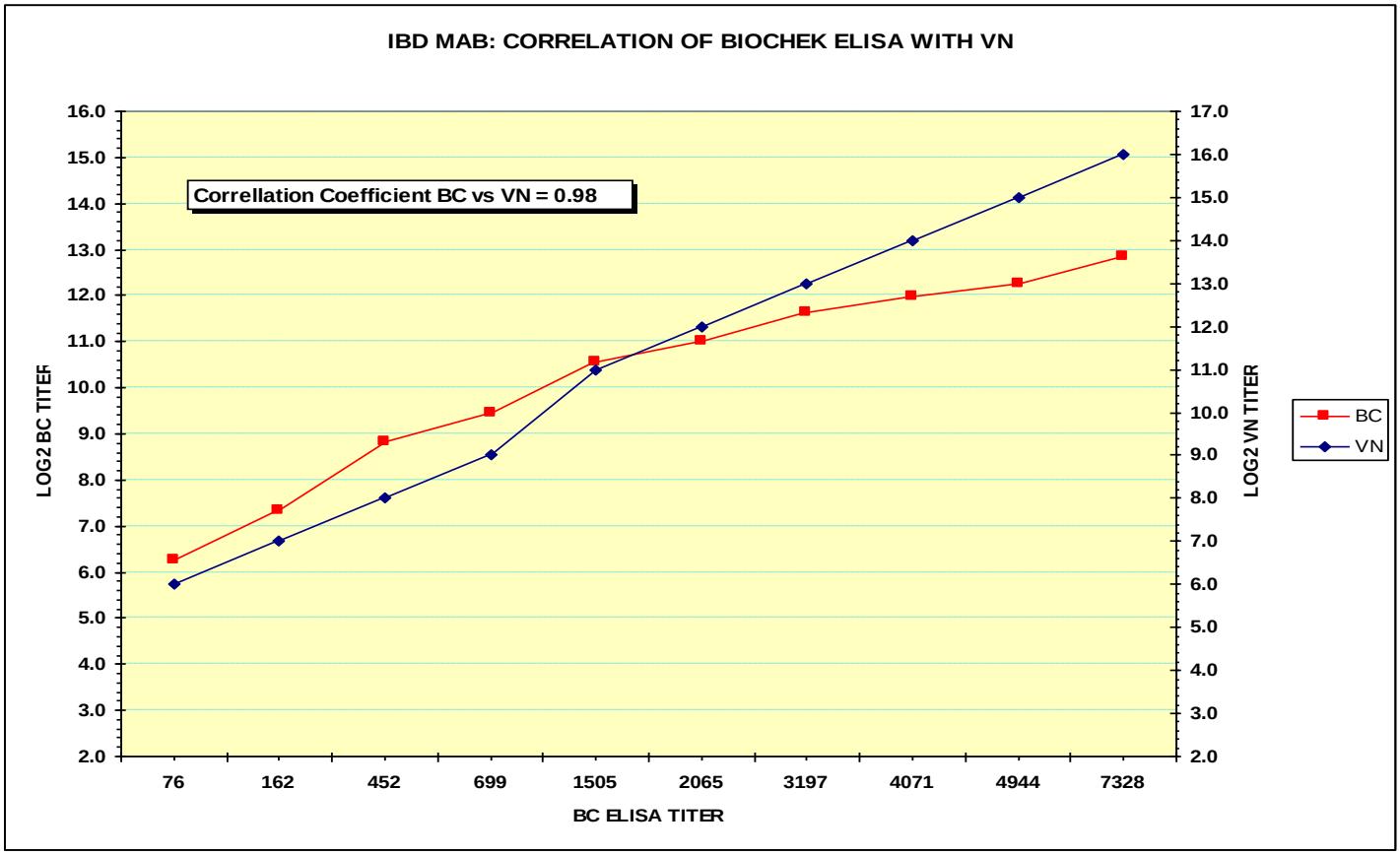
The BioChek IBD ELISA kit has an extended range of detection, without loss of sensitivity. The BioChek kit covers a VN titer range of 8 to 20, whereas most other commercial kits have a range from VN 8 - 14 (see data sheet). The extended range of the BioChek kit will provide more accurate serological information after live and inactivated vaccination and will also allow more accurate vaccination date prediction of birds with high maternal antibody content.



Serum Samples and VN results provided by Intervet International B.V., Boxmeer, Holland

Correlation of Maternal Antibody Titers vs. VN

BC shows good correlation (98%) in maternal VN range of 6 to 16. In these studies, a VN maternal titer of 8 (Target Titer for "Intermediate PLus Vaccines") , corresponds with a BC titer of about 450-500. Recommended use of the Target Titer for intermediate vaccines (VN titer 6-7) is a BC titer of 125-250



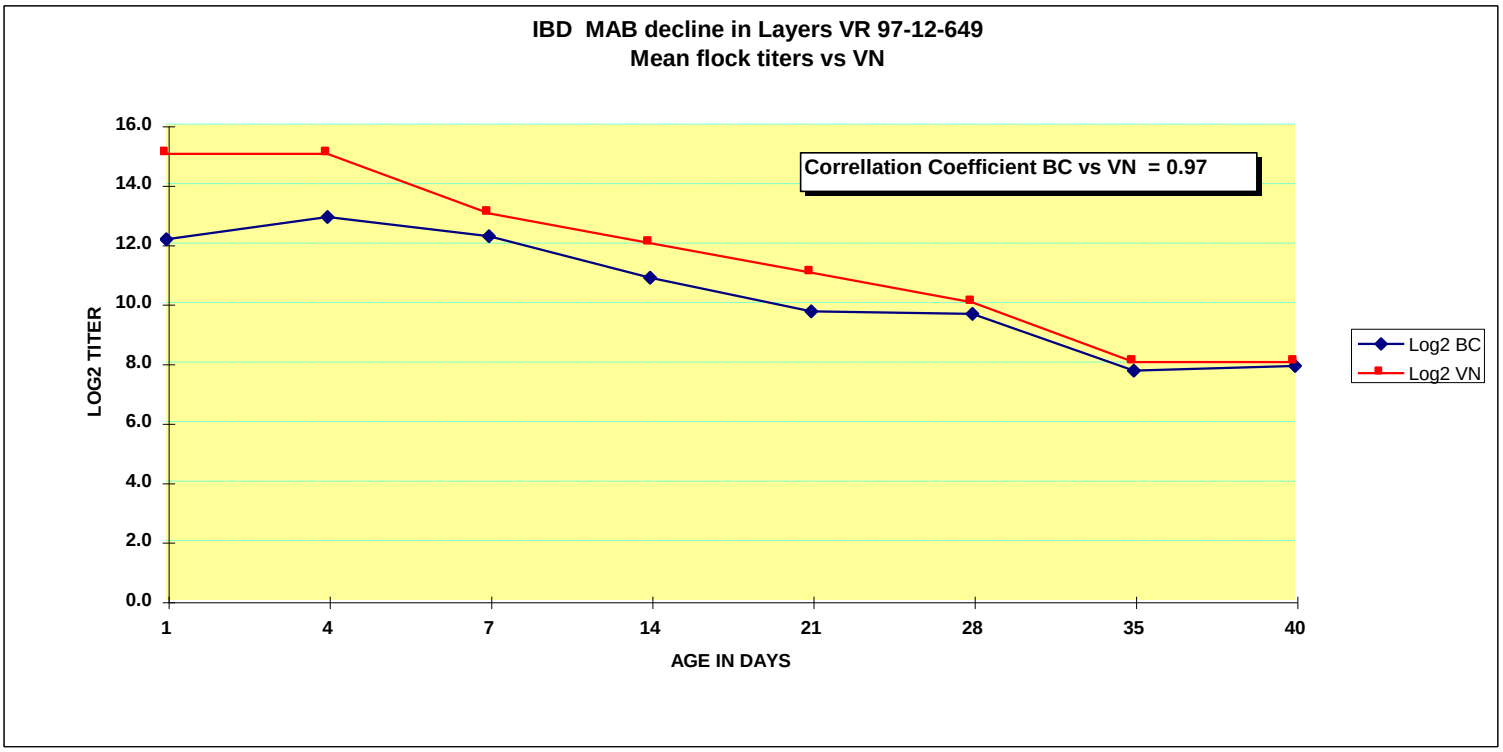
Serum Samples and VN results provided by Intervet International B.V., Boxmeer, Holland

MAB decline curve in Layers: BC vs VN.

MAB decline study of Mean Maternal Log2 ELISA Titers vs Mean Maternal Log2 VN Titers in Layers.
Mean maternal BC titers correlate very well with VN (97% correlation) in the range of VN 6 to 15.

Decline of IBD MAB Titers in Layers VR 97-12-649

Mean flock titers vs VN



Serum Samples and VN results provided by Intervet International B.V., Boxmeer, Holland