



# **Interpretation and Application of Results Manual**

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## Application of results obtained with BioChek ELISA kits

For most of the poultry virus diseases (i.e. IB, IBD, ND, AE, REO) immunity is mainly antibody mediated and BioChek titers will give a good indication on how successful vaccination has been. In addition to monitoring the success of vaccination programs, other applications include:

- \* Evaluation of maternal antibody levels in day-old chicks, to select optimal timing of vaccination (IBD) and perform quality control on day-old chicks.
- \* Profiling of antibody levels in hyper-immunized breeder flocks.
- \* Identification of the nature and time of field infections
- \* Monitoring of SPF flocks
- \* Screening to confirm negative status
- \* Monitoring at processing

Although BioChek monitoring can be very useful in the design and maintenance of effective vaccination programs, one has to be a bit more careful when it comes to making predictions on protective titers. First, this is because the degree of protection depends on many variables such as, the vaccine strains used, the virulence of involved field challenge strains, type of bird, vaccination application method and schedule, and local variables (eg. temperature, feed quality, etc.). A protective BioChek titer of 4000 for NDV for one farm, may not protect birds on the farm next door. One needs to obtain protective titer values by testing flocks under their own local conditions.

Another reason why one has to be careful in correlating titers with degree of protection is that for many bacteria (i.e. *Pasturella multocida* and *Mycoplasmas* such as MG and MS), immunity is primarily cell-mediated which is not measured with ELISA. Therefore, titers will not directly indicate immune status. Also antibody titers of some viruses, i.e. Fowl Pox and ILT, do not reflect immune status. **However, ELISA titers for these disease agents are very useful to monitor success of vaccination and can also be used for diagnosis of field infection.**

## Good Sampling and Sample Size

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The value of serological monitoring depends largely on good sampling to give valid results. To be more precise, interpretation of flock titers is only meaningful, when flock titers are, in fact, reliable estimates of the flocks that you are looking at. In order to obtain reliable and representative flock titer results, one has to:

- 1) Use statistically valid sampling methods to ensure that samples are truly representative.
- 2) Use proper sampling handling to ensure a uniform quality of serum samples, and
- 3) Use proper ELISA assay techniques to ensure reproducibility and reliability of the test results.

This paper will mainly focus on the importance of proper sampling methods, sample handling, and sample size.

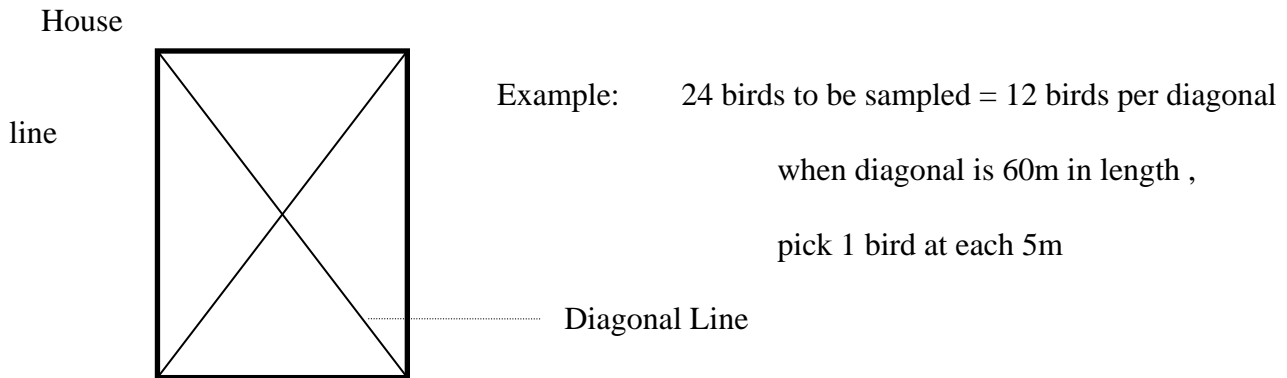
### Sample Methods

The application of statistically valid sampling methods is of vital importance to obtain reproducible and reliable estimates of mean flock titers. For sampling to be statistically valid there are 2 basic conditions that will have to be met:

- 1) Random selection of birds for sampling.
- 2) Proper sample size (for further discussion see “Sample size” page 6)

Random sampling of birds basically means that every bird in a flock has an equal chance of being selected from a flock. This is of course easier said than done, but there are in practice various different ways to approach random selection of birds in a house and an example is given in Figure 1 (below). Randomization of sampling is very important, as it tends to average out effects of what in statistics is called “uncontrollable variation” (i.e. uneven vaccine uptake through drinking water, uneven feed uptake, effects of uneven temperature, moisture etc.). To illustrate this point, suppose that in a house the drinking water system is defect. In the front half of the house the drinking water system is not working and in the second half of the house the system works properly. The birds are vaccinated through the drinking water system. If samples are taken from the first half of the house, one would conclude that the entire flock has not been vaccinated at all. If samples are taken only from the second half of the house, one would conclude that the entire flock has been correctly vaccinated. If random samples are collected throughout the whole house, one would notice that the vaccination has only been partially successful, which is the correct indication of what really happened to the flock.

**Figure 1. Random sampling of birds in a free-range house.**



When sampling from cages or boxes, one can number each box, let's say number 1 to 102. Then with a random number table, draw a number and take 1 bird from the box with the corresponding number.

### **Sample Handling and Storage**

During and after sampling, the next important step is to make sure that the blood samples are correctly handled and stored to ensure uniformity and good quality samples. The following are some guidelines for the proper handling and storage of samples:

- Collect 2-3 ml blood, this will yield 0.5 - 0.75 ml serum, which is more than sufficient for ELISAs
- Use disposable syringes
- Separate serum from blood by centrifugation or by natural coagulation (1-2 hrs at room temp)
- ALWAYS LABEL SAMPLES PROPERLY  
(Company ID, Flock ID, Age, Type Bird etc.)
- Store samples 4- 8° C for short term storage ( < 48 hrs)
- Store samples -20° C in plastic storage vials with airtight cap (microcentrifuge tubes 1.5 ml) and in labelled zip-lock bag for long term storage
- Avoid many freeze/thaw cycles of serum samples
- Diluted serum samples (in sample diluent) are more stable than non-diluted samples.
- Do not use spoiled serum samples (bad smell). Spoiled samples contain proteolytic enzymes which may affect ELISA results.

## Sample size

Next to randomization of sample taking, the number of blood samples taken from a flock is of vital importance to get reliable estimates of the “true mean flock titer”. The sample size varies with the level of uniformity/non-uniformity of titers found within a flock. In general, the larger the titer variability (non-uniformity) in a flock, the larger your sample size has to be to correctly estimate the “true mean titer”. Therefore, the best indication of proper sample size is to examine historical titer data within flocks.

In practice, the number of samples taken from flocks is often a compromise between the statistically correct number and the costs of sample taking and analysis. However, compromising too much on sample size to save money may have a negative effect on the reliability of the calculated means. Table 1 shows what happens to the mean titer of a group of 100 samples taken from day-old broilers, when one splits them up (at random) into smaller group of samples.

The 100 individual samples were run on a BioChek IBD ELISA. The mean maternal antibody titer for these day-old chicks was 4057 with a %CV of 48 %. The predicted “Days To Vaccination” (DTV) with the LOG2 method and the Deventer Log2 method was 19 days and 21 days, respectively. The individual samples were then randomly divided into groups of various sample sizes and the mean titers together with the DTV were calculated. This was repeated 16 times.

**Table 1. Relationship of Sample Size on estimation of Mean Flock Titer and Predicted Days to Vaccination (DTV) (Log2 method).**

No.of Samples/group	Mean Titer		%CV		DTV	
	Min	Max	Min	Max	Min	Max
46	3712	4449	42	58	20	22
30	3333	4779	37	57	20	22
23	3388	4753	40	64	20	22
15	3390	4973	33	62	19	22
10	2374	5260	25	71	17	23
5	2221	5371	23	71	16	23

The above table clearly shows that with decreasing sample size, the deviation of the mean titer, % CV, and “Predicted DTV” increases. This means for instance that for this flock, a sample size of 23 samples, will give a mean titer in between 3388 and 4753, with a CV in between 40% and 64%, and with a vaccination date prediction varying in between 20 and 22 days. For 10 samples, the estimated mean titer will fluctuate in between 2374 and 5260, with a CV in between 25 and 71 %!

The data also shows that sample sizes from 46 to 23 seem to give similar results in terms of deviations from the mean titer, but sample sizes below 23 samples significantly increase the deviations for estimating the mean titer. The increasing variability with decreasing sample size also affects the reliability of the calculated mean titers and vaccination date prediction.

Table 2 shows how many % of predicted mean titers per sample size fall within a 20% range of the real mean titer of the 100 samples. The % predicted DTV’s per sample size, which fall within 1 day variation of the real mean DTV is also shown below.

**Table 2. Relationship of sample size and % reliable prediction of the real population mean.**

No.of Samples/group	Mean Titer % within range of 4057 +/- 20%	DTV	
		% within range of Mean +/- 1 day LOG2	DEV LOG2
46	100%	100%	100%
30	100%	100%	100%
23	100%	88%	100%
15	88%	88%	94%
10	50%	43%	57%

- True Population Mean Titer (TMT) was 4057. Estimated mean titers within TMT +/- 20% variation range were defined as acceptable estimations.

- True Mean DTV LOG2 method was 19 days and 21 days for Deventer Log2 method. Estimated mean DTV's within 1 day variation range of true mean DTV were defined as acceptable estimations.

### Conclusions Sample size:

The sample size "cut-off" in terms of reliability and reproducibility seems to be around 23 samples. Below 23 samples, there is a marked decline of reliability of mean titer and vaccination date estimations.

Based on the above data, practical experience, and statistical information, a sample size of 23 samples seems to be a reasonable number for establishing baselines or profiles within a flock, or for vaccination date prediction. Once baselines are established, 15 samples can show trends, by detecting the most common titer groups, but this number is insufficient for quantitative analysis.

Compromising on sample size to save money may have an adverse effect on reliability of calculated mean titers and may lead to misleading results and conclusions.

### Sample size for Disease Monitoring

For disease monitoring, sample size varies according to % infection rate and sampling frequency. One may have to increase sample size beyond 23 samples, when titer detection at low infection or incidence rates (< 2-5 %) is desired (i.e. Salmonella monitoring). Further guidelines are given in Table 3.

**Table 3. Disease Monitoring: Relation of sample size, flock size, and infection rate.**

**NUMBER OF SAMPLES NEEDED TO BE 95% CONFIDENT THAT THE DISEASE WILL BE DETECTED IF PRESENT AT VARIOUS LEVELS OF PREVALENCE OR CONTAMINATION**

FLOCK SIZE = N	CONTAMINATION RATE						
	20%	10%	5%	2%	1%	0.5%	0.1%
	Sample size = n						
N	n	n	n	n	n	n	n
20	10	15	19	48	20	20	20
50	12	22	34	77	50	50	50
100	13	25	44	94	96	100	100
200	13	26	48	105	155	190	200
500	14	28	55	128	225	349	500
1000	14	28	56	138	258	450	950
5000	14	28	58	146	290	564	2253
10000	14	28	58	147	294	581	2588
100000	14	28	58	148	299	596	2995

**To pick up 10% infection rate in a flock of 5000 - 10000 birds, one needs to take 28 random samples**

**To pick up a 2% infection rate in a flock of 5000 - 10000 birds, one needs to take 147 random samples**



## Interpretation of BioChek Results

It is only possible to interpret BioChek results when the following conditions are met:

1. **Valid Assay.** To be sure that the results in the laboratory are valid.

Check to see if positive and negative controls, and reference control values, are within specified range (see Certificate of Analysis).

2. **Proper numbers of samples are analyzed.** For assessment of mean quantitative titer of a flock, the optimal sample size, in terms of reliability and reproducibility, is 23 samples per flock. Minimum sample size is 18 samples/flock. The sample size varies with the level of uniformity/non-uniformity of titers (% CV) found within a flock. In general, the larger the titer variability (non-uniformity) in a flock, the larger your sample size has to be to correctly estimate the “true mean titer”. Therefore, the best indication of proper sample size is to examine historical titer data within flocks. (For more information on Sampling and correct Sample Size see pages 6-8.
3. **Vaccination Baseline values have been established.** Important in determining if the resulting BioChek ELISA titers are within the expected normal range.

Interpretation of results requires experience, obtained by building and storing data and by comparing new data with previously obtained data. Regular serological flock monitoring allows BioChek users to set up their own standards for expected titer values, the so-called “baseline values”. One should always realize that these baseline values are “farm specific” and that they may vary according to differences in the breed of birds, age, vaccine type and vaccination program, and other differences in the operating environments of the flock.

Once baselines for titers and CV's have been established, one can start monitoring the effectiveness of the vaccine program or start monitoring for potential health problems in flocks. For instance, if elevated titers far above normally expected vaccine titers would be observed, it would suggest that a field infection could be running through the flock. If titers are below expected vaccine response values, it could indicate improper vaccine administration or poor quality of vaccines.

We often get questions concerning interpretation of results from new users. Therefore, some guidelines are provided in the Appendix to help with interpretation in the beginning. These are based off experience and information from clients and are very rough guidelines. As previously mentioned, titer values may vary according to type of bird, age, vaccine type, vaccination program, management program, and other factors and it is possible to see different results under different circumstances. Thus, it is strongly recommended to make one's own baselines.

***Please refer to Appendix I for BioChek Baselines***

## Notes on Baselines

- \* The serological response of a vaccination depends on maternal antibody levels at the moment of vaccination.
- \* Response will differ depending on vaccination method and vaccine brand used.
- \* Processing age will influence final titer levels for broilers.

Please keep the following in mind when using the BioChek data:

- 1. The numbers given/expected as titers after vaccination are a very rough guideline.**
- 2. These titer values may vary according to type of bird, age, vaccine type, vaccination program, and other factors. One may find different results under different circumstances.**
- 3. We strongly recommend making one's own baselines.**

## How to make your own baselines

It is critical is to supply adequate information with the samples to the laboratory. In the laboratory one can input this information in the various code fields. Once data has been stored in the computer with proper flock information, baselines can be calculated using the fields.

### **Critical information for baseline calculation needed by the laboratory is the following:**

- Age of bird at moment of sampling
- Type of bird (Broiler Breeder, Layer, Commercial Broilers etc.)
- Reason for testing (routine screen or diagnosis)

Optional is:

- Vaccination schedule
- Brand (breed) of chicken

Only when above information has been consistently used, one can generate the report as in the following example:

Baseline Titer Calculations

Name:

Company:

Code:

Birthdate:

Age:

House no.:

Reason test: **SCREEN**

Type chicken: **BB**

Vacc. code: **IA**

Veterinarian:

Internal control:

Bleeding date:

Closest match searched flock codes:

Date	Name:	Company:	CC

Parameters

Period: **01-01-1998** <-> **29-09-1998**

Age: **24W** <-> **28W**

Assay's:

- Assay
- Gumboro Disease
- EDS
- NDV
- St

The following baseline report will be made:

The mean titer of all **BB** (=Broiler Breeder) flocks, with vaccination code **IA**, and with a reason for testing **SCREEN**, and with an age between **24W** and **28W** (see parameters) will be calculated for **Gumboro Disease**, and **NDV**.

WITH THE BIOCHEK II SOFTWARE

Select criteria for reporting

Flock/Herd

Labcode:

Sampletype:

Samplingdate:

Customer-Name:

Company: **XYZ**

Grower No.:

Flock:

House No.:

City:

Type:

Manager:

Parameters

Period: From: **09/06/2010** To: **06/19/2012**

Age: From: **22** Week(s) To: **25** Week(s)

Test:

- FAV 1 - FAV group 1
- Hps A - Hps OppA
- IBV - Infectious Bronchitis
- IBD - Infectious Bursal Disease
- ILT - Infectious Laryngotracheitis
- LOTH - Leukosis (Meconium, Cloacal Swabs)

Vacc. Program:

User: **<all users>**

Print reference controls

Search

Results

Select	Testdate	Assay	Customer-Name	Company
<input checked="" type="checkbox"/>	12/06/2010	IBD	Blue Moon	XYZ
<input checked="" type="checkbox"/>	12/06/2010	IBV	Blue Moon	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBD	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBD	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBD	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBD	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBV	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBV	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBV	Max Farm	XYZ
<input checked="" type="checkbox"/>	01/24/2011	IBV	Max Farm	XYZ

Unselect All Select All Skip

Reportstyle **Baseline Titer Calculati** Preview Report

The mean titer of all flocks for company XYZ between the ages of 22-25W will be calculated for IBD and IBV.

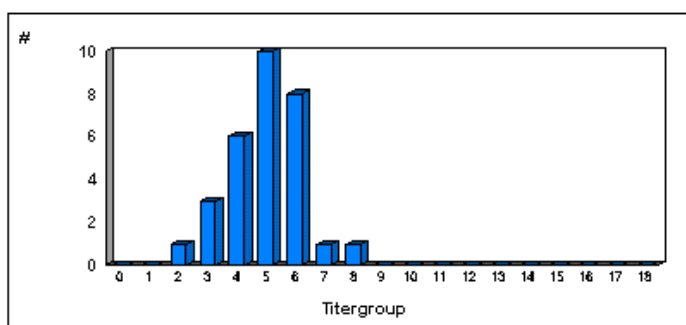
For further details on how to generate this report, please consult our software manual.

### Interpretation of qualitative results

For qualitative test the results are presented as “negative, and positive” (neg, and pos). Strictly speaking one positive sample in a flock makes the flock serologically positive. We strongly recommend the following when finding positive or suspect samples:

1. Retest suspect and positive sera in duplicate. When negative, consider the flock serologically negative; when positive go to point 2.
2. Retest flock 1 week later. When negative, consider flock serologically negative, when positive, go to point 3.
3. Confirm with other test method.

### Interpretation of BioChek Histogram



**Assay :** IBD  
**Bleeding Date :** 08/01/199  
**Samples :** 30  
**Mean Titer :** 3278  
**Std.Dev. :** 2207  
**%CV :** 67  
**G.M.T.** 2433  
**Dilution :** 500

### Interpretation Titer groups :

**Titer group 0 = NEGATIVE**  
**Titer group ≥ 1 = POSITIVE**

### Vaccination C.V. Guidelines

% CV	Uniformity
< 40%	Excellent
40-60%	Good
> 60%	Need to Improve

**With live priming, complete seroconversion more important than %CV. Check if all birds (100%) test positive.**

### Background:

When evaluating BioChek ELISA titers, one always has to look at mean titer response of the tested birds, and the % CV.

The mean titer of the tested birds within a flock tells how strong the antibody response is of a flock after vaccination. It basically provides a measure of the immune response of your flock.

The second parameter, the coefficient of variation or CV%, provides an indication on how variable a mean titer response is of a flock. The lower the %CV, the more uniform the distribution of titers and the better the vaccination. **For most diseases, the % CV after a correctly applied inactivated vaccination should be less than 40%. For live vaccine applications, the % CV should be less than 60%. With live priming, complete seroconversion is more important than % CV and one should check if all birds test positive.**

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## **Introduction**

The use of ELISA serology in Poultry Health Management has been shown to be a useful tool to monitor the immune response following vaccination. Often the veterinarian judges the success of a vaccination by looking at two main components of a vaccination response: the Mean Titer Response (mean antibody level of a flock) and %CV (value that measures uniformity). As the relationship between Mean Titer and % CV is important for evaluation of vaccine responses, the idea was born to look at the ratio between Mean Titer and % CV. The ratio between mean titer and % CV was expressed in a new parameter, Vaccination Index (VI) :

$$VI = \frac{(\text{Mean Titer})^2}{(\text{St Dev} \times 100)} = \frac{\text{Mean Titer}}{\% \text{ CV}}$$

The VI seems to be a logical parameter, as VI is expected to give a high score with a good vaccination (high mean titer, low % CV) and a low score with a poor vaccination (Low Mean Titer, high % CV).

In order to test the concept of VI, vaccination results from flocks were examined after live IBV and NDV vaccination. The VI for all the flocks were calculated for healthy non-infected flocks and for confirmed clinically infected flocks after live and/or inactivated vaccination.

## **Materials and Methods**

In total more than 500 individual broiler flock results for BioChek ELISA were examined following different live IBV, NDV, and IBD vaccination schedules. Also before - and post-Infection Titer results were compared from 3 Broiler Breeder flocks for IBV after live priming and inactivation. Selection of flocks were based on the following criteria:

1. Mean titers in expected range for normal flocks and significantly elevated titers for clinically infected flocks.
2. Presence and absence of clinical symptoms to differentiate between normal healthy flocks and clinically infected flocks.
3. Reference controls were used and were in range, indicating proper reproduction of ELISA titers.

## **Results**

Results are summarized in table 1 for IBV, table 2 for NDV, table 3 for IBD in broiler flocks, and table 4 for broiler breeder flocks.

**Table 1. Vaccination Index (VI) results for live IBV vaccinated Broilers at 35-45D.**

Vaccination Program	VI Normal	VI Clinical Infection
H120, IB primer, Ma5	10-90	>100
H120 + CR88 or 4/91	50-200	>200

**Table 2. Vaccination Index (VI) results for live NDV vaccinated Broilers at 35-45D.**

Vaccination Program	VI Normal	VI Clinical Infection
up to 3x live Clone 30, VH, Hitchner B1, LaSota, Avinew, NDW	20-250	> 300
Inact 01D + 3x live	50-280	> 300

**Table 3. Vaccination Index (VI) results for live IBD vaccinated Broilers at 35-45D.**

Vaccination Program	VI Normal	VI Suspect Infection
Bursine2, Bursine Plus 1-2x	50-300	> 300
Gallivac IBD, D78 1-2X, Avipro Precise	100-400	> 500
Cevac IBD L , Bursa F ,Hipra GM97 1-2 X	100-500	> 500
Avipro IBD extreme 1-2x	200-500	> 500
228E 1-2x	200-550	> 600
Tabic MB 1-2x	200-600	> 700
Vaxxitek @ 01D S.C./ or In-Ovo @18D incubation	10-90	> 200
Cevac Transmune IBD @ 01D S.C./ or In-Ovo @18D incubation	100-500	
Bursaplex @ 01D S.C./ or In-Ovo @18D incubation	100-500	

**Table 4. Vaccination Indexes (VI) of live primed and inactivated IBV vaccinated Broiler Breeder flocks before and after IBV infection. Age of BB flocks before infection was 34-52W. Post-Infection titers taken at 3-6 W after first clinical symptoms.**

Mean Titer		% CV		VI	
Before Infection	After Infection	Before Infection	After Infection	Before Infection	After Infection
4850	16036	75	38	65	425
5288	15145	46	15	115	1010
5937	23456	58	28	102	838

## Discussion & conclusions

The above data shows that the Vaccination Index (VI) can be helpful to differentiate between:

1. Good vaccination responses (high VI score) and poor vaccination responses (low VI score).
2. Normal vaccination responses and possible field challenges of vaccinated flocks.

Traditional ELISA evaluation of vaccination responses include the comparison of obtained mean titer results with the expected mean titers after vaccination, the so called “vaccination baselines”.

As the VI score takes Mean Titer response and % CV in to account, it can make serological ELISA evaluation of vaccination more simple and complete than looking at “baseline titers” alone.

Also, the VI score has a logical dimension, in the fact that increasing scores indicate better and stronger immunological responses after vaccination and/or infection.

Although the data show that excessive VI scores seem to correlate well with the presence of a field challenge after vaccination, it should be stressed, however, that VI scores by itself, cannot be used to establish diagnosis. **Diagnosis can only be established when one combines VI scores with clinical symptoms and isolation of the pathogen and/or confirmation testing with PCR.**

**Table 5. Vaccination Indexes (VI) of vaccinated BB flocks from 5 different operations.**

Test	VI Index BB at Age in Weeks			
	14W-18W	22W-24W	30W-40W	45W-60W
IBD	100-500	300-1500	300-1000	200-1000
NDV	100-400	300-2000	300-1000	200-1000
IBV	50-300	50-300	50-300	50-200
REO		200-800		
CAV	100-300	100-250	100-200	
AE	50-500			
ART		100-500	50-300	50-250



# Appendix

- I. Baselines Broilers, Breeders, Layers
- II. Half-life values Maternal Antibodies IBD
- III. Optimal Titers to Vaccinate IBD (Target Titers)

# BIOCHEK VACCINATION BASELINES BROILERS

Titer values may vary according to age and type of bird , vaccine type, vaccination program, and other factors such as placement programs. You may find different results under different circumstances.

TEST	VACCINE TYPE	MEAN TITER RANGE AT PROCESSING (35D- 45D)	VI Index	SUSPECT TITER INFECTION
IBV	live, 1x@ 01D (H120/mild Mass, Arkansas, DE072, MA5, IB primer, 4/91, IB Bird)	300 - 1 500	< 50	> 3 000 VI > 70
	live, 2x@ 01D (H120 , MA5, IB Primer + 4/91, CR88, IB Bird)	500 - 2 500	< 60	> 3 000 VI > 70
	live, 2x (H120, MA5, IB Primer, MMark )	1 000 - 4 000	< 90	> 5 000 VI > 90
	live, 2x (H120/MA5 + 4/91 or H120 + CR88) < 13D	1 000 - 3 000	< 90	> 4 000 VI > 90
	live, 2x (H120/MA5 + 4/91 or H120 + CR88) > 13D	1 000 - 6 000	< 150	> 8 000 VI > 200
NDV	live , C2 strain, VG/GA, Avinew, Ulster NDW, VH	1 000 - 4 000	< 280	> 10 000 VI > 300
	live, 2x Dr. Water (Clone 30, LaSota )	2 000 - 5 000	< 280	> 10 000 VI > 300
	live, 2x Spray (Clone 30, NDW, LaSota )	4 000 - 8 000	< 280	> 10 000 VI > 300
	Inact . 01D + 2x Live LaSota	4 000 - 12 000 ( 100% Pos)	< 280	> 14 000 VI > 300
	r-HVT/NDV (in Ovo or S.C. 01D)	500 - 1 500 ( 10 – 50% Pos)	< 40	> 3 000 (100% Pos) VI > 100
	r-HVT/NDV (in Ovo or S.C. 01D + 2x live )	4 000 - 10 000 ( > 90 % Pos)	< 280	> 12 000 (100% Pos) VI > 300
REO	none	0 - 2 500	< 40	> 3 000* VI > 50
ART	live, A strain	1 000 - 2 000		
	live, B strain	1 000 - 4 000		
ILT	TCO	300 - 600 ( < 30 % Pos)		> 1000 ( ≥ 40% Pos)
	CEO	1 000 - 3 000 ( > 50 % Pos)		no differentiation
	r-HVT/ILT (in Ovo or S.C. 01D)	500 - 1 500 ( 10 – 60 % Pos)	< 40	> 2000 ( > 80% Pos) VI > 50
ORT	none	0 - 1 500	< 20	> 3 000* VI > 20
CAV	none	0 - 2 500	< 30	> 3 000* VI > 50
FAV-1	none	0 - 3 000	< 40	> 6 000* VI > 50

\* Mean Titers of non-vaccinated birds above indicated suspect levels, may indicate enhanced invasion and often correlate with clinical symptoms.

These guidelines are based on our experience and information from our clients. BioChek does not accept any responsibility for the results using these guidelines.

# BIOCHEK IBD VACCINATION BASELINES BROILERS

Titer values may vary according to age and type of bird , vaccine type, vaccination program, and other factors such as placement programs. You may find different results under different circumstances.

VACCINE TYPE	MEAN TITER RANGE AT PROCESSING (35D- 45D) WHEN VACCINATED IN PRESENCE OF MATERNAL ANTIBODIES	VI Index	SUSPECT TITER OF INFECTION / OR WHEN VACCINATED WITHOUT PRESENCE OF MATERNAL ANTIBODIES
<b><u>Intermediate vaccines, up to 2x live vaccination:</u></b>			
Bursine-2, Bursine Plus	2 500 - 6 500	< 300	> 9 000 VI > 300
D78, Cevac Gumbo L, Avipro precise	2 500 - 8 000	< 500	> 10 000 VI > 500
Gallivac IBD	3 000 - 9 000	< 500	> 11 000 VI > 500
<b><u>Intermediate vaccines, up to 3x live vaccination:</u></b>			
Bursine-2	4 000 - 7 500	< 500	> 10 000 VI > 500
<b><u>Intermediate Plus vaccines, 1x application :</u></b>			
228E, Bursa plus	6 000 - 10 000	< 500	> 14 000 VI > 500
Cevac IBD L, Vladimir Inst. BG, Avipro IBD Extreme	6 000 - 12 000	< 500	> 14 000 VI > 500
Abic MB	6 000 - 14 000	< 600	> 16 000 VI > 600
<b><u>Intermediate Plus vaccines, 2x application :</u></b>			
228E	6 000 - 12 000	< 550	> 14 000 VI > 600
<b><u>Immune Complex Vaccines</u></b>			
Transmune IBD (In Ovo vac., or 01D s.c.)	4 000 - 12 000 (100% Pos)	< 500	> 14 000 VI > 500
<b><u>Recombinant Vector Vaccines</u></b>			
r-HVT +IBD (In Ovo vac. , or 01D s.c.)	800 - 2 500 (≥80 % Pos.)	< 50	> 4 000 ( 100% Pos) VI > 100

These guidelines are based on our experience and information from our clients. BioChek does not accept any responsibility for the results using these guidelines.

# BIOCHEK VACCINATION BASELINES LAYERS/BREEDERS

Titer values may vary according to age & type of bird , vaccine type, vaccination program, and other factors such as placement programs. You may find different results under different circumstances.

TEST	VACCINE TYPE	MEAN TITER RANGE	WKS AFTER VAC. TO TEST	% POS	VI Index	SUSPECT TITER INFECTION
IBV	live (H120)	1 000 - 2 000	3 - 5 wks	100%		> 4 000
	live (MA5)	1 000 - 4 000	3 - 5 wks	100%		> 6 000
	live (1 <sup>st</sup> Priming H120, 2 <sup>nd</sup> 4/91)	6 000 - 10 000	3 - 5 wks	100%	50- 300	> 12 000 VI > 300
	inact.	6 000 - 12 000	5 - 8 wks	100%	50 - 400	VI > 400
IBD	live intermed. (D78, Bursine-2)	4 000 - 12 000	3 - 5 wks	100%	100 - 500	> 14 000 VI > 600
	inact.	7 000 - 20 000	5 - 8 wks	100%	300 - 1 500	
	rHVT/IBD	1 000 - 4 000	7 - 10 wks	100%	50 - 250	> 5 000 VI > 300
NDV	live (Clone30, NDW, Lasota )	2 000 - 8 000	3 - 5 wks	100%	100 - 400	
	inact.	10 000 - 25 000	5 - 8 wks	100%	100 - 2 000	
REO	live	2 000 - 5 000	3 - 5 wks			> 6 000* and > 90% Pos
	inact.	7 000 - 15 000	5 - 8 wks	100%	200 - 800	VI > 800
ART	live	2 000 - 6 000	3 - 5 wks			
	inact.	7 000 - 25 000	5 - 8 wks	100%	50 - 500	
AE	live 1x	5 000 - 12 000	4 - 6 wks	> 80%	50 - 500	
MG	live 6/85 (spray)	negative	6 -12 wks	0%		positive
	live Ts-11, (eye drop)	1 000 - 3 000	6 -12 wks	30-70%		> 5000 and > 90% Pos
	F-strain (dr. water, spray)	2 000 - 8 000	6 -12 wks	100%		
	inact. 2x (Gallimune MG)	3 000 - 6 000	4 - 6 wks after 2 <sup>nd</sup>	> 90%		
		500 - 1 000	10 -12 wks after 2 <sup>nd</sup>	40-90%		

\* REO: Suspect titer for infection with potentially more virulent strains

- Above titers are based on two times live priming and one time inactivated boosting at 16-18 weeks.

## BIOCHEK VACCINATION BASELINES LAYERS/BREEDERS (Continued)

Titer values may vary according to age & type of bird , vaccine type, vaccination program, and other factors such as placement programs. You may find different results under different circumstances.

TEST	VACCINE TYPE	MEAN TITER RANGE	WKS AFTER VAC. TO TEST	% POS	VI Index	SUSPECT TITER INFECTION
MS	live MS-H (eye drop)	500 - 3 000	6 -12 wks	30- 70% Pos		> 5000 and > 90%
AI	Inact 2x H5N2	1 000 - 4 000	6 – 10 wks	100% Pos		
	Inact 2x H9N2	2 000 - 6 000	6 – 10 wks	100% Pos		
EDS	inact. 1x	1 000 - 4 000	4 – 6 wks			
SE (Salm D)	live 3x DW (Salmonella Vac E)	100 - 500	5 - 6 wks	< 15% Pos		
	inact. 2x Salenvac T, Gallimune Se +St	3 000 - 10 000	4 - 6 wks after 2 <sup>nd</sup>	90-100% Pos	50 - 500	
	Talovac 109 SE, Poulvac SE, Layermune SE, Avipro SE4	1 000 - 5 000	10 -12 wks after 2 <sup>nd</sup>	50-100% Pos	10 - 100	
SE/ST (Salm B&D)	live 3x DW (Salmonella Vac E+T)	500 - 1 500	5 - 6 wks	< 70% Pos		
	inact. 2x Salenvac T, Gallimune Se +St	3 000 - 12 000	4 - 6 wks after 2 <sup>nd</sup>	90-100% Pos	50 - 500	
	Talovac 109 SE, Poulvac SE, Layermune SE, Avipro SE4	1000 - 6 000	10 -12 wks after 2 <sup>nd</sup>	50-100% Pos	10 - 100	
CAV	live (Tymovac, PG4, CAV-Vac, Circomune)	3 000 - 8 000	4 - 6 wks	80-100% Pos	100 - 300	

\* **ORT: Titers > 10 000 often correlate with clinical disease**

These guidelines are based on our experience and information from our clients.

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## BIOCHEK VACCINATION BASELINES LAYERS/BREEDERS (Continued)

Titer values may vary according to age & type of bird , vaccine type, vaccination program, and other factors such as placement programs. You may find different results under different circumstances.

TEST	VACCINE TYPE	MEAN TITER RANGE	WKS AFTER VAC. TO TEST	% POS	VI Index	SUSPECT TITER INFECTION
ORT	none	negative				> 10 000*
ILT	Live TCO	1000 - 3 000	6 - 10 wks	0 - 30% Pos		> 50% positive
	Live CEO	1000 - 6 000	6 - 10 wks	80 - 100% Pos		no differentiation
	r-HVT/ILT (in Ovo or S.C. 01D)	500 - 3 000	6- 20wks	40 - 100% Pos	10 - 100	> 5000 and 100% Pos

These guidelines are based on our experience and information from our clients.  
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## **II. Half-lives of Maternal Antibodies IBD**

Following are the values we've found for the different type of chickens:

<b>TYPE BIRD</b>	<b>BLEEDING AGE</b>	<b>HALF-LIFE</b>
BROILERS	DAY 1	3.8 DAYS
	DAY 3 - 7	3.0 DAYS
BR. BREEDERS	DAY 1	4.5 DAYS
	DAY 4 - 10	4.0 DAYS
LAYERS	DAY 1	6.0 DAYS
	DAY 4- 10	5.0 DAYS

## **III. Optimal Titers to Vaccinate IBD (Target Titers)**

INTERMEDIATE PLUS VACCINES :      OPTIMAL TITER TO VACCINATE 400 - 500  
INTERMEDIATE VACCINES:              OPTIMAL TITER TO VACCINATE 100 - 250

There are several methods in the BioChek software with which one can predict the date to vaccinate, for now, we recommend using the DEVENTER method. This method corrects for variation in titers. (For more information on this method please consult our information leaflet on vaccination date prediction).