BioChek
SMART VETERINARY DIAGNOSTICS

AE

Data Pack

Avian encephalomyelits Antibody detection ELISA
(Detects antibodies to avian encephalomyelitis virus)

CONTENTS

SUMMARY

Kit

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

Key Performance Features

Sensitivity:
Positive reactions 10 - 14 days after infection

Specificity:
Highly specific test, both on monospecific antisera as on sera of 60 week SPF old leghorns, less than 1% non-specific reactions.

Reproducibility:
Plate CV's lower than 10%, lot to lot reproducibility less than 15% of standard kit.

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" can be found by testing 2 - 5 weeks after vaccination. Successful vaccination is when 100 % of the birds have seroconverted 4-6 weeks after vaccination. Vaccination failure is when less than 60% of the birds seroconverted 4 to 6 weeks after vaccination.

Field infection:
About 10 - 20 days after infection seroconversion will show.

BioChek Poultry Immunoassays

Avian Encephalomyelitis Antibody Test Kit

Catalogue Code CK 123

Description of Test

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

Reagents provided:

1. **AE 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 sachets**. 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 AE 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 AE coated plate from sealed bag and record location of samples on template.
2. Add 100 ⌠l of negative control into wells A1 and B1.
3. Add 100 ⌠l of positive control into wells C1 and D1.
4. Add 100 ⌠l 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 (350⌠l per well). Invert plate and tap firmly on absorbent paper until no moisture is visible.
6. Add 100 ⌠l 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 ⌠l 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 ⌠l 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 0.30 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 AE positive control has been carefully standardised to represent significant amounts of antibody to AE 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 0.5 or greater contain anti-AE 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
0.499 or less	1070 or less	Negative
0.500 or greater	1071 or greater	Positive

Each Laboratory should establish its own criteria for immunity with respect to antibody titre based on correlation of BioChek AE to current laboratory test methodologies and on historical antibody responses to specific vaccines and vaccination protocols.

BioChek has available a software programme which can be used with the AE 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 AE ELISA

Procedure

16 samples from 60 week old SPF white Leghorns were obtained and assayed using the standard protocol for the BioChek AE ELISA.

Results/Conclusion

The results are shown in Table 1 and Graph1. The S/P value of each sample is plotted on the Y-axis, the sample number on the X-axis.

The data show all negative samples for AE demonstrating that the BioChek AE ELISA has 100% specificity on this panel.

Table 1 Sera of SPF flocks 60 weeks old

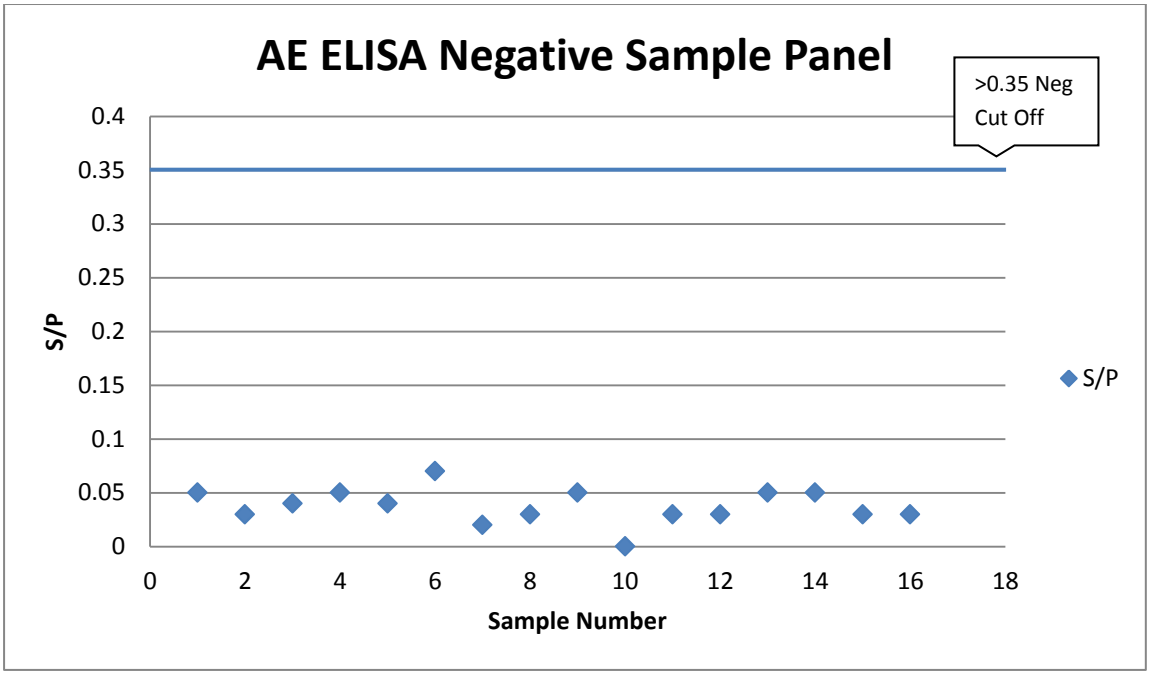
Assay :	AE	**Bleeding Date :**	26/04/2001
Mean Titer:	63	**Dilution :**	500
G.M.T.:	48	**%CV :**	50
		Lot No:	FS3649
	0	**%CV (≤10=OK)**	0

Sample ID / Well		Raw O.D.	S/P Ratio	Titer	Titergroup
-	A01	0.118	0.00		
-	B01	0.115	0.00		
+	C01	0.515	0.00		
+	D01	0.508	0.00		
01	C02	0.137	0.05	89	
02	D02	0.128	0.03	47	
03	E02	0.132	0.04	65	
04	F02	0.138	0.05	93	
05	G02	0.133	0.04	70	
06	H02	0.145	0.07	127	
07	A03	0.124	0.02	29	
08	B03	0.112	0.03	40	
09	C03	0.138	0.05	93	
10	D03	0.117	0.00	1	
11	E03	0.127	0.03	43	
12	F03	0.129	0.03	52	
13	G03	0.137	0.05	89	
14	H03	0.137	0.05	89	
15	A04	0.128	0.03	47	
16	B04	0.113	0.03	40	

Interpretation of results

S/P value	Titre Range	Antibody status
.349 or less	722 or less	Negative
.350 - 0.499	723 - 1070	Suspect
.500 or greater	1070 or greater	Positive

Graph 1Specificity, negative panel



DATA SHEETS

MONOSPECIFIC SAMPLE PANEL
Monospecific samples containing antibodies to various viruses.

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

Procedure
A sample panel monospecific for antibodies of pathogens common in poultry was tested on the BioChek AE ELISA.

Results / Conclusion
The results are shown in Table 2

The data demonstrates that only the monospecific serum sample for AE tested positive on the BioChek AE ELISA. This concludes that the test kit does not cross-react with antibodies directed at other avian pathogens.

Table 2 AE Specificity Panel

Antiserum	Source	S/P	Cutoff	Result
Adenovirus AGP	Deventer	0.056	0.350	**neg**
Fowlpox AGP	Deventer	0.044	0.350	**neg**
Gumboro AGP	Deventer	0.022	0.350	**neg**
IBV D1466	Deventer	0.060	0.350	**neg**
IBV D274	Deventer	0.078	0.350	**neg**
IBV D3128	Deventer	0.053	0.350	**neg**
IBV D8880	Deventer	0.122	0.350	**neg**
IBV M41	Deventer	0.044	0.350	**neg**
ILT AGP	Deventer	0.075	0.350	**neg**
Mg HI	Deventer	0.038	0.350	**neg**
Ms HI	Deventer	0.103	0.350	**neg**
NDV PMV1	Deventer	0.056	0.350	**neg**
REO 1133	Deventer	0.016	0.350	**neg**

REO 2534	Deventer	0.016	0.350	**neg**
NDV PMV3	Deventer	0.047	0.350	**neg**
ILT AGP	Deventer	0.028	0.350	**neg**
AE	Deventer	**1.028**	0.350	**pos**

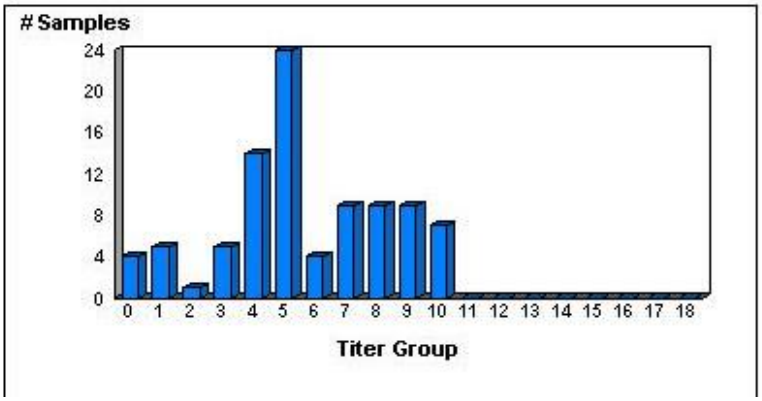
DATA SHEETS

SENSITIVITY

Breeders flocks vaccinated through drinking water at 13/14 weeks were tested at 15 and at 18 weeks of age.

Age :	15W
Bleed Date :	14/06/2001

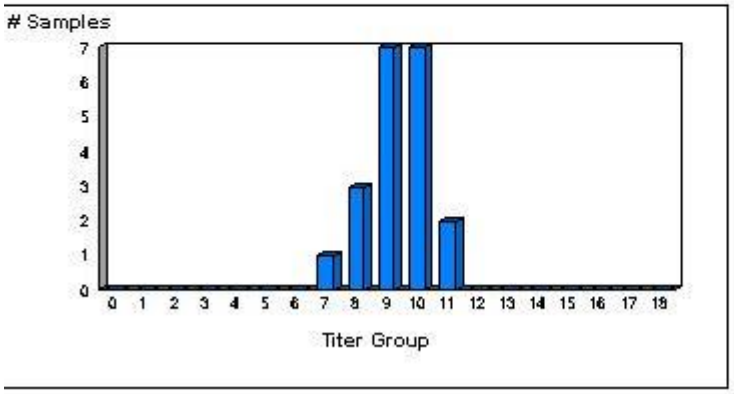
Assay :	**AE**	Dilution :	**500**
Bleeding Date :	**14/06/2001**	Samples :	**91**



Mean Titer :	**5607**
G.M.T. :	**4388**
%CV :	**58**
Std.Dev. :	**3255**

Age :	18W
House No. :	01
Type of Bird:	BB
Bleed Date :	08/06/2001

Assay :	**AE**	Dilution :	**500**
Bleeding Date :	**08/06/2001**	Samples :	**20**



Mean Titer :	**10390**
G.M.T. :	**10269**
%CV :	**16**
Std.Dev. :	**1623**

DATA SHEETS

COMPARITIVE STUDY

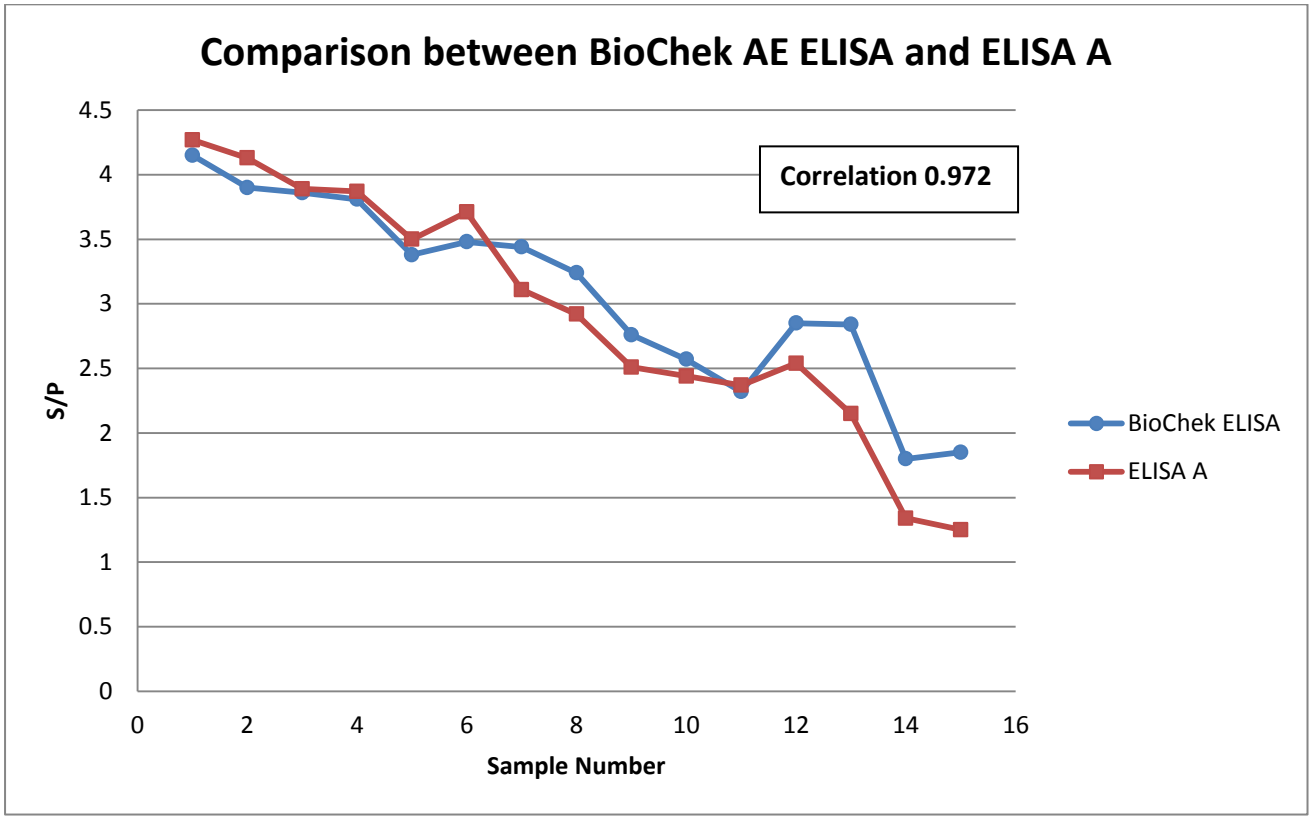
A study was carried out to compare the BioChek AE ELISA with another ELISA. Samples were taken from Broiler Breeders and Broiler Flocks at various ages and the correlation calculated.

The results are presented in Table 1 and Graph 1

Table 3 S/P values of samples for the two ELISAs

Samples taken from Broiler Breeders and Broiler flocks at various ages.							
Sample No	S/P Biochek	S/P ELISA A	Correlation	Sample No	S/P Biochek	S/P ELISA A	Correlation
1	3.76	3.2	0.949	1	1.05	0.54	0.977
2	3.6	3.74		2	1.05	0.52	
3	3.71	3.17		3	1.3	0.75	
4	3.25	3.02		4	1.43	1.13	
5	1.4	0.95		5	1.34	1.18	
6	2.68	2.8		6	1.52	0.88	
7	3.87	4.04		7	1.59	1.48	
8	3.27	3.28		8	1.61	1.68	
9	2.24	1.69		9	1.77	1.7	
				10	1.99	1.63	
1	1.95	0.72	0.902	11	2.14	1.72	
2	3	3.14		12	3.03	2.73	
3	3.32	2.24		13	3.4	2.9	
4	0.52	0.3		14	3.64	3.37	
5	1.06	1.09		15	3.55	3.14	
6	2.74	2.24					
7	3.23	3.55		1	4.15	4.27	0.972
8	2.41	2		2	3.9	4.13	
9	1.25	0.24		3	3.86	3.89	
10	0.93	0.97		4	3.81	3.87	
11	2.72	2.72		5	3.38	3.5	
12	0.59	0.71		6	3.48	3.71	
13	1.9	0.84		7	3.44	3.11	
14	3.87	3.14		8	3.24	2.92	
15	2.8	2.66		9	2.76	2.51	
16	4	4.85		10	2.57	2.44	
17	2.85	2.51		11	2.32	2.37	
18	3.17	3.01		12	2.85	2.54	
19	2.44	2.38		13	2.84	2.15	
				14	1.8	1.34	
1	1.01	0.98	0.829	15	1.85	1.25	
2	0.77	0.44					
3	1.46	0.59		**Average**	0.926		
4	2.44	2.52		**Correlation**			
5	2.09	1.45					
6	4.57	4.97					
7	1.69	1.4					
8	3.14	2.72					
9	2.35	2.29					
10	3.18	2.48					
11	3.26	2.22					
12	3.44	2.18					
13	2.05	2.07					
14	2.63	3.63					
15	2.88	1.39					

Graph 2 One set of data comparing the two ELISAs



DATA SHEETS

AVIAN ENCEPHALOMYELITIS & SEROLOGY:

Important characteristics
Diagnosis
Immunity
Prevention
Field case

Important characteristics:
Avian Encephalomyelitis is an infectious viral disease affecting young chickens, turkeys, quail and pheasant. Typical symptoms are ataxia and tremor, because of the latter it was often called epidemic tremor. AE occurs world wide, incidence of clinical disease is very low due to vaccination of breeders. Infection of breeders results in reduced egg production, decreased hatchability and clinical AE in chicks hatched from eggs laid during the outbreak of the disease. The disease spreads both horizontally as vertically. The source of infection of susceptible flocks is most likely people or fomites.

Diagnosis:
Clinical signs: Chicks nervous signs, mortality 25 - 50%
Mature birds: drop in egg production, decreased hatchability
PM: whitish areas in the muscularis of the proventriculis
Serology: seroconversion

Immunity
Humoral but not cellular immunity is the most important factor for protection. Flocks with positive serology rarely have recurrent outbreaks of AE.

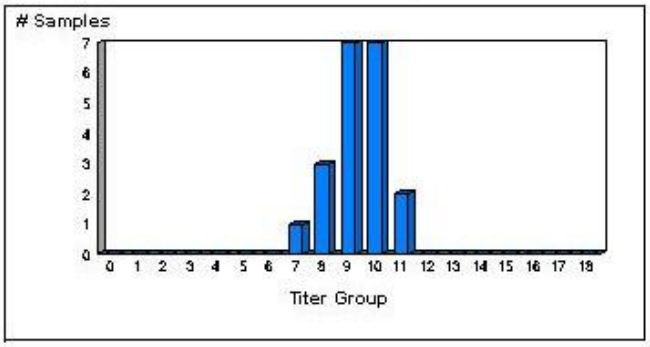
Prevention of the disease
Vaccination of the breeder flocks and commercial egg laying flocks in order to get humoral protection is commonly applied. When giving a live vaccination it's important not to vaccinate to early (>8 weeks of age) and not to late (not within 4 weeks of the onset of lay).
In order to ensure that the vaccination was successful, serological confirmation of the vaccination is recommended. Criteria for a successful vaccination are: > 60 percent must have seroconverted at 18 weeks of age. When less revaccinate.

Examples of different vaccination methods vaccination.

These breeders received a live vaccination through drinking water at 14 weeks of age. Bloods taken at 18 weeks.

Age :	18W
House No. :	01
Type of Bird:	BB
Bleed Date :	08/06/2001

Assay :	**AE**	Dilution :	**500**
Bleeding Date :	**08/06/2001**	Samples :	**20**



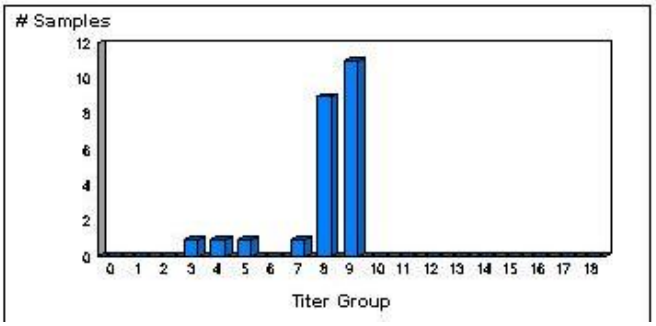
Mean Titer :	**10390**
G.M.T. :	**10269**
%CV :	**16**
Std.Dev. :	**1623**

Beak dipping 5% of the flock at 14 weeks, blood samples taken at 18 weeks:

Flock:

Name :	VACCINATION AE
Company :	INTERV
Code :	AE 5% BEAK DIP
Age :	19W
House No. :	ABCD
Reason for Testing:	VAC@14W
Type of Bird:	LP
Bleed Date :	08/04/2001

Assay :	**AE**	Dilution :	**500**
Bleeding Date :	**08/04/2001**	Samples :	**24**



Mean Titer :	**8355**
G.M.T. :	**7849**
%CV :	**27**
Std.Dev. :	**2214**

AE field case:

A broiler breeder farm routinely tested their breeders at 23weeks of age in order to confirm successful AE vaccination. At a certain point in time a flock tested negative at 23 weeks. As vaccinating at that age would cause the same problem as an AE infection and no inactivated AE vaccine was available, they could do nothing else but wait and hope that AE wouldn't infect the flock. However when the flock was 50 weeks of age, AE infection occurred: There was a 30% drop in egg production and a 10% drop in egg production. The economical damage was enormous.

Economic Damage:

Drop in egg production/drop in hatchabiliy	23 000 hatching eggs
Destruction of hatching eggs	35 000 hatching eggs
Mortality /culling day old chicks	22 000 chicks
Total:	80 000 hatching eggs/chicks (+/ EURO 16 000).

Not calculated is the economical damage due to damage control to customers etc.

Summary:

The lesson to be learnt is that it's important to confirm the success of the vaccination +/4 weeks after vaccination at a moment that corrective action can be taken

The economic damage could have simply been prevented by checking the vaccination serologically at about 4 weeks after vaccination and taking corrective action by re-vaccination.