



CAV

Data Pack

Chicken Anaemia Virus Antibody ELISA
(Detects antibodies to CAV)

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SUMMARY

Kit

- 5 plates
- Indirect ELISA
- Run at room temperature
- Incubation times: 60-60-30
- Read at 405nm
- 1:500 dilution

Key Performance Features

Sensitivity:

Will give positive results 10 – 21 days after infection (or vaccination)

Specificity

>98%

100% on serum from SPF leghorn flocks older than 50 weeks

Applications

Field infection:

About 10 - 20 days after infection seroconversion will show. Positive result means that the flock has been in contact with Chicken Anemia virus.

Positive alternative methods such as VN should be used for confirmation.

Pre-vaccination check:

Test flock prior to CAV vaccination in order to establish if the antibody titre level in the flock meets criteria for vaccination.

Vaccination check:

Test flock after CAV vaccination in order to establish efficiency of vaccination. Answers to key questions like “did the vaccine actually stimulate the immune system”, will be answered.

Test 2- 5 weeks after live vaccination and 5 - 10 weeks after vaccination with inactivated vaccine.

BioChek Poultry Immunoassays

Chicken Anemia Antibody Test Kit

Catalogue Code CK 126

Description of Test

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

Reagents provided

1. **CAV 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 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 CAV 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 °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. Wash buffer will remain stable for use for 1 month if stored at +4 °C.

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 CAV 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 **60 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 **60 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 **30 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 CAV positive control has been carefully standardised to represent significant amounts of antibody to CAV in chicken or turkey 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 .35 or greater contain anti-CAV antibodies and are considered POSITIVE.

1. Calculation of S/P ratio

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

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

$$\text{Log}_{10} \text{Titre} = 1.1 * \text{Log}(\text{SP}) + 3.361$$

$$\text{Antilog} = \text{Titre}$$

S/P value	Titre Range	Antibody status
.349 or less	723 or less	Negative
.350 or greater	724 or greater	Positive

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

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DATA SHEETS

MONOSPECIFIC PANEL

Monospecific samples containing antibodies to various viruses.

Purpose

To determine if the BioChek CAV 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 CAV ELISA.

Results / Conclusion

The results are shown in Table 2

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

Table 2 Monospecific Panel

BioChek sample panel of sera positive for antigens mentioned

The BioChek CAV test tests negative for all samples except the CAV monospecific sample.

Name : MONOSPECIFICS sample panel
 Test date : 08-02-2007
 Assay : BioChek CAV Lot No: CH2234
 Dilution : 1:500

Interpretation results		
S/P value	Titre Range	Antibody status
.349 or less	723 or less	Negative
.350 or greater	724 or greater	Positive

Sample ID	S/P Ratio
Ms	0.08
PMV1	0
CR88	0
IBV D1466	0.08
IBV D274	0
IBV M41	0
IBDQX	0
S. typh	0.01
S.ent	0.32
ILT	0
AE	0
E.coli	0.04

Sample ID	S/P Ratio
CAV	0.98
EDS	0.04
REV	0
ORT G	0
ART A	0.01
ADENO	0.04
REO	0
AI H5	0
IBD	0.01
PMV3	0.01
IBV D8880	0.22
ART B	0.06
ILT	0.21

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 AI ELISA.

Procedure

36 samples from SPF chickens older than 50 weeks were obtained (Deveter, Holland) and assayed using the standard protocol for the BioChek CAV ELISA. These are samples notorious for giving a high background signal

Results/Conclusion

The results are shown in Table 1

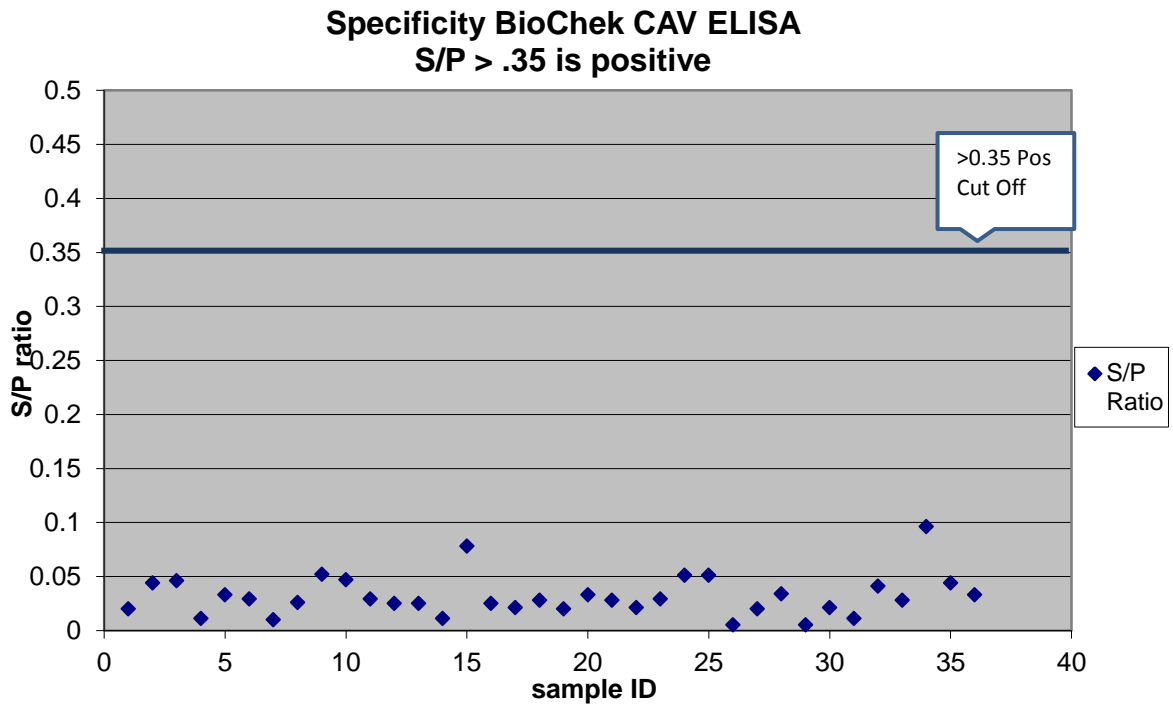
The results have been plotted on Graph 1 showing S/P value against sample number.

The data demonstrates that the BioChek CAV ELISA has 100% specificity on this sample panel. All samples test negative.

Table 1 Specificity Negative Panel

Sample ID	S/P Ratio	Result
1	0.02	Neg
2	0.044	Neg
3	0.046	Neg
4	0.011	Neg
5	0.033	Neg
6	0.029	Neg
7	0.01	Neg
8	0.026	Neg
9	0.052	Neg
10	0.047	Neg
11	0.029	Neg
12	0.025	Neg
13	0.025	Neg
14	0.011	Neg
15	0.078	Neg
16	0.025	Neg
17	0.021	Neg
18	0.028	Neg
19	0.02	Neg
20	0.033	Neg
21	0.028	Neg
22	0.021	Neg
23	0.029	Neg
24	0.051	Neg
25	0.051	Neg
26	0.005	Neg
27	0.02	Neg
28	0.034	Neg
29	0.005	Neg
30	0.021	Neg
31	0.011	Neg
32	0.041	Neg
33	0.028	Neg
34	0.096	Neg
35	0.044	Neg
36	0.033	Neg

Graph 1 SPF Panel

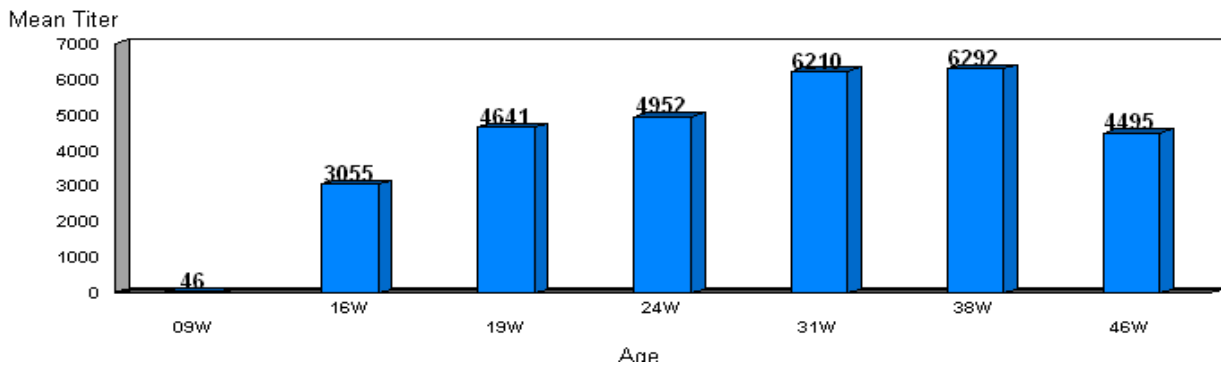


DATA SHEETS

FIELD RESULTS

Vaccinated flock

Broiler Breeder Flock vaccinated at 14 weeks of age with a live CAV vaccine. At 16 weeks of age 1 out of 40 samples tested negative.



DATA SHEETS

COMPARISON TO VN

The BioChek CAV ELISA demonstrates a good correlation with VN. Results are shown in table 3 and Graph 2.

Interpretation of BioChek CAV results

S/P range	Titre range	Virus Neutralization		
<.350	< 724	<7	negative	no protection
.350 - 1.00	724 - 2295	8 - 10	positive	moderate protection
≥1.00	≥2296	≥ 11	positive	protective titres

Please use above information as a guideline only. Users need to establish their own baseline for protective or non-protective titres.

Table 3 VN and CAV ELISA

Name : CAV SERA
 Company : INTERVE
 Code : SL99-12-347
 TITER GROUPS ARE LOG2 TITERS

Sample ID	S/P Ratio	VN	BC LOG2
		0 INTERVET	
		0	
		0	
		0	
1	0.02	3	4.95
10	0.02	3	4.95
13	1.739	10	12.04
14	2.022	9	12.28
15	1.923	8	12.2
16	1.203	9	11.46
17	2.049	8	12.3
18	0.654	8	10.49
19	1.741	9	12.05
2	0.02	3	4.95
20	3.138	9	12.98
21	1.609	12	11.92
22	1.774	12	12.07
23	1.955	12	12.23
25	1.81	12	12.11
26	2.465	12	12.6
28	1.596	9	11.91
29	3.195	12	13.01
3	0.022	3	5.09
32	3.068	12	12.94

33	2.616	12	12.69
34	3.352	12	13.08
35	3.788	12	13.28
36	1.594	12	11.91
37	2.726	12	12.76
38	3.737	12	13.26
39	1.757	7	12.06
4	0.02	3	4.95
40	2.844	8	12.82
41	3.753	10	13.26
42	1.164	7	11.41
43	2.459	9	12.59
44	1.994	9	12.26
45	0.889	7	10.98
46	1.77	8	12.07
47	1.388	9	11.69
48	3.307	9	13.06
49	2.071	8	12.32
5	0.02	3	4.95
50	0.75	6	10.71
51	1.295	9	11.58
52	3.737	10	13.26
53	3.808	10	13.29
54	1.036	7	11.22
55	2.709	12	12.75
56	1.988	12	12.26
58	2.903	12	12.86
59	2.428	12	12.57
6	0.02	3	4.95
60	1.729	8	12.03
61	3.011	12	12.91
62	1.486	12	11.79
63	2.31	12	12.49
64	3.99	12	13.36
65	4.061	12	13.39
66	2.891	10	12.85
67	2.917	12	12.86
68	2.989	12	12.9
69	2.173	12	12.4
7	0.02	3	4.95
70	3.036	12	12.93
71	3.58	12	13.19
72	1.633	9	11.94
74	2.815	12	12.81
75	0.092	3	7.38
76	0.02	3	4.95
77	0.02	3	4.95
78	0.02	3	4.95
79	0.02	3	4.95
8	0.02	3	4.95
80	0.02	3	4.95
81	0.02	3	4.95
82	0.02	3	4.95
83	0.02	3	4.95

84	0.02	3	4.95
85	0.02	3	4.95
86	0.02	3	4.95
87	0.02	3	4.95
88	0.02	3	4.95
89	0.02	3	4.95
90	0.02	3	4.95
91	0.02	3	4.95
92	0.02	3	4.95

Cor. BC Log2 vs VN = 0.93533

Graph 2 BioChek CAV ELISA and VN

