BioChek
SMART VETERINARY DIAGNOSTICS

CastV-B

Data Pack

Chicken Astrovirus group B Antibody Test Kit
(Detects antibodies to Chicken Astrovirus group B)

CONTENTS

SUMMARY

The Kit

- 5 plates
- Indirect ELISA
- Run at room temperature
- Incubation times: 30-30-15
- Read at: 405nm
- 1:100 dilution

BioChek Software is available for data recording and analysis

Key Performance Features

General

Detects antibodies against Chicken Astrovirus group B

Specificity

>98% or better
The kit does not cross-react with Chicken Astrovirus group A (CastV-A) antibodies

Sensitivity

No data available

Repeatability

Inter-plate Coefficient of Variation: 6-11%

Applications

- Monitoring in screening and control programs
- Confirmation of success of vaccination
- Detection of infected animals

Chicken Astrovirus Group B Antibody Test Kit (CAstV GpB)

BioChek
SMART VETERINARY DIAGNOSTICS

BioChek Poultry Immunoassays

Product Number CK 133

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

Reagents provided:
1. **CAstV GpB Coated plates**. Inactivated recombinant 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 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 (0.2% 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 (0.2% w/v).
9. **Positive control**. Antibodies specific to CAstV GpB in Phosphate buffer with protein stabilisers and sodium azide preservative (0.2% 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 ml of substrate buffer and allow to mix until fully dissolved (approx. 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:

Dilute each test sample 1:100 in Sample diluent reagent.

POSITIVE AND NEGATIVE KIT CONTROLS DO NOT REQUIRE DILUTING.

Test procedure:

1. Remove CAstV GpB 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 until no moisture is visible.
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 assay to be valid the mean negative control absorbance should read below 0.30. The difference between the mean negative control and the mean positive control should be greater than 0.20.

The CAstV GpB positive control has been carefully standardised to represent significant amounts of antibody to CAstV GpB 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 0.7 or greater contain anti- CAstV GpB 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: 100 dilution to a titre

Log10 Titre = 1.1 * Log (SP) + 3.156

Antilog = Titre

S/P value	Titre Range	Antibody status
0.499 or less	667 or less	No antibody detected
0.500 - 0.699	668 - 966	Suspect
0.700 or greater	967 or greater	Positive

This test is highly specific for antibodies against Chicken Astrovirus Group B. However, be aware that false positive reactors can occur in rare circumstances. Therefore confirmation with an established reference method is required for a final diagnosis.

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

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KI/CK133REV02

DATA SHEETS

Background and intended use of the BioChek CastV-B antibody ELISA

Background of Disease

Chicken Astroviruses (CastV) belong to the Genus Avastrovirus in the Family Astroviridae. Two distinct groups have been identified group A, (i.e. ANV) and group B. Within group B, two types have been identified (Bi, Bii). A third type (Biii) is currently subject of discussion.

Infections with Chicken Astrovirus group B (CastV-B) have been associated with a wide variety of pathologies that include: Visceral gout, nephritis, enteritis, runting-stunting syndrome and "White Chick Syndrome" (WCS). WCS is a condition where baby chicks hatch weak, with pale coloration of feathers and show green livers on post-portem examination; in addition breeding flocks originating this kind of progeny had transient decreased hatchability.

The virus has been reported worldwide, including several countries in the Middle East, India, Northern Europe, the Far East, USA, Canada and Brazil.

There is evidence for vertical as well as horizontal transmission. Vertical transmission is believed to happen when naive breeders get exposed to the virus during lay, and for the time (approx. 2-4 weeks) they take to produce sufficient antibodies to neutralize the shedding of the virus.

Control is achieved by natural exposure of the breeding stock to contaminated environment or with the use of inactivated vaccines.

Diagnosis

History, clinical signs and necropsy. PCR, Indirect Fluorescent Antibody (IFA), and ELISA

ELISA can be used for detection of infected animals, flock profiling and monitoring.

Background of the test

An inactivated CastV-B recombinant capsid protein is used as antigen. This antigen will detect antibodies directed against CastV group B only. Antibodies due to natural infection and antibodies due to vaccination will be detected. The test results will be presented in a quantitative manner allowing for differentiation between negative, low, medium and high serological responses.

Intended use of the test

- Detection of infected animals
- Confirmation of success of vaccination
- Monitoring in screening and control programs

MONOSPECIFIC SAMPLE PANEL

Panel 1: Monospecific samples containing antibodies to various viruses.

Purpose
To determine if the BioChek CastV-B ELISA 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 CastV-B ELISA test kit.

Results / Conclusion
The results are shown in Table 1

The data demonstrates that there was no positive result for any of the other avian pathogens sera. This concludes that the test kit does not cross-react with antibodies directed at other avian pathogens.

Panel 2: Monospecific samples containing antibodies to various Chicken Astro strains.

Purpose
To determine if the BioChek CastV-B ELISA test kit cross-reacts with antibodies generated by CastV type A, Bi, Bii and Biii.

Procedure
A sample panel monospecific for antibodies to above antigens was tested on the BioChek CastV-B ELISA test kit.

Results / Conclusion
The results are shown in Table 2

The data demonstrates that the BioChek CastV-B ELISA detects antibodies to CastV Bi, Bii and Biii buit not to CastV A.

Panel 1: Monospecific samples containing antibodies to various viruses.

Sample No	Analyte	Strain	SP	Titre	Interpretation
1	Adenovirus	AGP	0	1	NEG
2	Adenovirus	Type 1	0,02	19	NEG
3	Adenovirus	Type 3	0,08	90	NEG
4	Adenovirus	Type 5	0,00	1	NEG
5	Fowlpox	AGP	0,00	1	NEG
6	IBD	D78	0,00	1	NEG
7	IBV	D1466	0,00	1	NEG
8	IBV	D274	0,01	8	NEG
9	IBV	D3128	0,01	8	NEG
10	IBV	D8880	0,00	1	NEG
11	IBV	M41	0,00	1	NEG
12	IBV	793/B	0,00	1	NEG
13	IBV	4 91	0,01	8	NEG
14	IBV	4 91	0,00	1	NEG
15	IBV	CR88	0,00	1	NEG
16	IBV	D1466	0,00	1	NEG
17	IBV	D274	0,00	1	NEG
18	IBV	M41	0,00	1	NEG
19	IBV	QX	0,00	1	NEG
20	IBV	Italian 02	0,00	1	NEG
21	IBV	Arkansas	0,38	488	NEG
22	IBV	Connneti	0,03	28	NEG
23	IBV	DE072	0,05	57	NEG
24	Fowl plague	PMV3	0,00	1	NEG
25	Newcastle	PMV1	0,00	1	NEG

Sample No	Analyte	Strain	SP	Titre	Interpretation
26	REO	AGP	0	1	NEG
27	REO	S92008	0,00	1	NEG
28	REO	320	0,00	1	NEG
29	Influenza	AGP	0,00	1	NEG
30	Influenza	H5	0,00	1	NEG
31	Influenza	H7	0,00	1	NEG
32	ART	Gp B	0,00	1	NEG
33	ART	Colorado	0,00	1	NEG
34	ART	Gp A	0,00	1	NEG
35	Ort	Gp A	0,00	1	NEG
36	Ort	Gp B	0,00	1	NEG
37	Ort	Gp G	0,00	1	NEG
38	Mg		0,00	1	NEG
39	Ms		0,00	1	NEG
40	S Typh		0,00	1	NEG
41	S Ent		0,00	1	NEG
42	ILT	AGP	0,00	1	NEG
43	AE	AGP	0,00	1	NEG
44	Ecoli		0,01	11	NEG
45	GR98		0,00	1	NEG
46	CAV		0,00	1	NEG
47	EDS		0,00	1	NEG
48	REV	THV	0,00	1	NEG
49	Mareks		0,00	1	NEG
50	SPF	Turkey	0,00	1	NEG

Panel 2: Monospecific samples containing antibodies to various Chicken Astro strains.

Panel 2: AFBINI CAstV Monos

477	Sample Information				SP	Titre
3		CAstV Bi: 35 days after inoculation per os and after intramuscular boost	11672	d35	2,70	4267
4			11672	d35	2,14	3310
5			11672	d35	3,01	4816
6	CAstV Bii	CAstV Bii: 08-29 (Bii) 35 days after inoculation per os and after intramuscular boost.	Dun	d35	0,75	1040
7			Dun	d35	1,05	1514
8			Dun	d35	2,37	3703
9			Dun	d35	1,93	2958

475	Sample Information					SP	Titre
1	CAstV A	EF 91/121-80	612 A/S	041	-	0,27	333
2	CAstV Bi	EF03-268 A	11672 A/S	-	12-02-04	0,88	1244

+ve Control Sera	Sample Information			SP	Titre
B0090	Pooled Field Sera		-	0,81	1131
B0251	CAstV Bi	EF 03-268 A		1,52	2274
B0282	CAstV Biii	EF13/27		2,00	3074

DATA SHEETS

SPECIFICITY

Purpose

To determine the specificity chicken serum originating from SPF (Specific Pathogen Free) chickens free of CastV-B, were tested on the BioChek CastV-B ELISA.

Procedure

459 samples from SPF chickens (Ref. B0229) at various ages were obtained and assayed using the standard protocol for the BioChek CastV-B ELISA

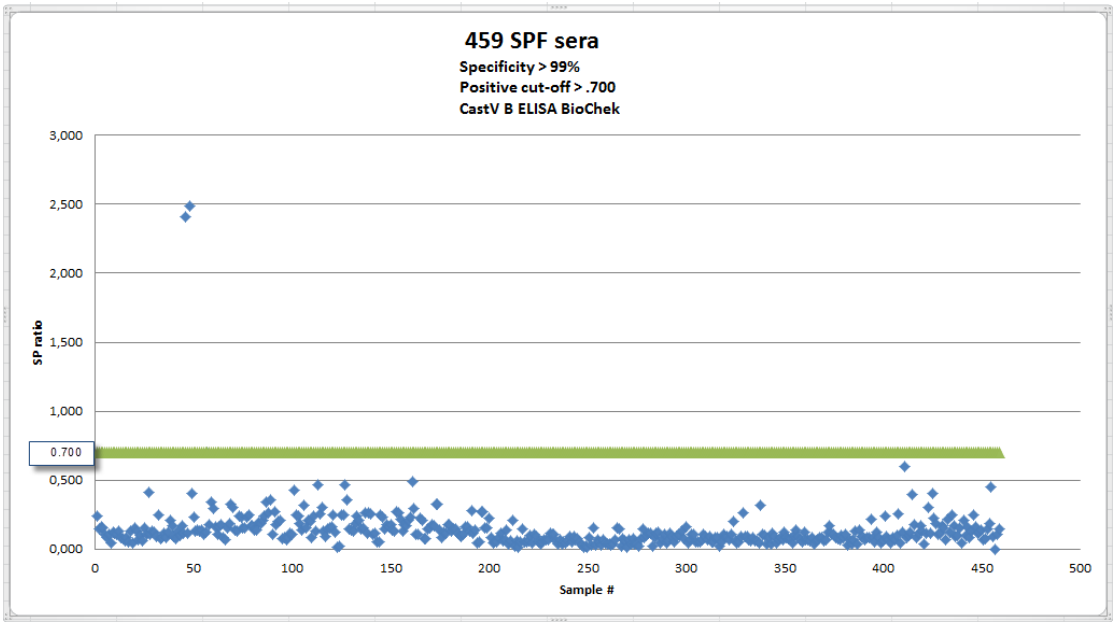
Results/Conclusion

The results are shown in Table 3

The data demonstrates that the BioChek CastV-B ELISA test kit has specificity of > 99%.

Table 3 SPF Panel (VALO)

459 sera from SPF chickens were tested on the BioChek CastV-B ELISA. Only 2 of those sera or 0.44% had a S/P ratio higher than the positive cutoff of 0.7. This results in a specificity > 99%.
4 sera or 0.87% had a S/P ratio higher than 0.5.



Field data

Sera from the parent birds from broiler flocks suffering from White Chick Disease were collected. The parent flocks were bled about when the clinical signs in the broilers became manifest. No sera prior to clinical manifestation were available.
The diagnosis White Chick disease was based on clinical signs.
As a negative control serum samples from parents chickens from broilers with no signs of White Chick disease were taken.

Conlusion: there is a clear difference between the 2 groups. However in flock D one can some seroconversion.

Test results on Parents from broilers without any signs of White Chick Disease

	A - 32 weeks Br #1			B - 32 weeks Br #2			C - 32 weeks Br #3			D - 32 weeks Br #4		
Sample	SP	mean titer	Cut off	SP	mean titer	Cut off	SP	mean titer	Cut off	SP	mean titer	Cut off
		468	0,7		708	0,7		436	0,7		805	0,7
1	0,56	758	Neg	0,46	604	Neg	0,20	245	Neg	0,41	540	Neg
2	0,16	187	Neg	0,70	968	Pos	0,41	539	Neg	0,39	515	Neg
3	0,45	596	Neg	0,28	358	Neg	0,35	449	Neg	0,66	912	Neg
4	0,28	350	Neg	0,46	611	Neg	0,22	267	Neg	0,42	556	Neg
5	0,31	392	Neg	0,52	702	Neg	0,19	228	Neg	0,64	877	Neg
6	0,39	512	Neg	0,44	576	Neg	-		-	0,98	1405	Pos
7	0,38	494	Neg	0,59	803	Neg	0,43	569	Neg	0,73	1007	Pos
8	0,24	294	Neg	0,44	584	Neg	0,39	510	Neg	0,37	476	Neg
9	0,25	314	Neg	0,41	542	Neg	0,24	292	Neg	0,76	1058	Pos
10	0,34	443	Neg	0,59	803	Neg	0,12	136	Neg	0,33	424	Neg
11	0,60	814	Neg	0,44	588	Neg	0,70	963	Neg	0,46	611	Neg
12	0,41	542	Neg	0,51	683	Neg	0,54	727	Neg	0,92	1308	Pos
13	0,31	395	Neg	0,75	1044	Pos	0,36	464	Neg	0,34	441	Neg
14	-		-	0,34	444	Neg	0,22	276	Neg	0,78	1092	Pos
15	-		-	0,92	1303	Pos	-		-	0,62	852	Neg

Test results on Parents from broilers diagnosed White Chick disease

	A - LP			B - LP			C - LP			D - LP		
Sample	SP	mean titer	Cut off	SP	mean titer	Cut off	SP	mean titer	Cut off	SP	mean titer	Cut off
		1807	0,7		1243	0,7		1543	0,7		1384	0,7
1	0,96	1372	Pos	0,86	1211	Pos	0,94	1333	Pos	0,85	1192	Pos
2	1,00	1439	Pos	0,57	766	Neg	0,77	1081	Pos	0,93	1326	Pos
3	1,99	3058	Pos	1,26	1839	Pos	0,92	1307	Pos	0,83	1173	Pos
4	0,41	530	Neg	1,14	1654	Pos	0,80	1114	Pos	1,23	1804	Pos
5	2,13	3282	Pos	0,74	1026	Pos	0,90	1276	Pos	0,66	907	Neg
6	1,06	1520	Pos	1,42	2113	Pos	1,71	2584	Pos	0,72	998	Pos
7	1,77	2692	Pos	1,00	1432	Pos	1,00	1437	Pos	0,54	725	Neg
8	1,53	2285	Pos	0,78	1097	Pos	1,63	2449	Pos	0,68	937	Neg
9	0,64	877	Neg	0,80	1118	Pos	0,90	1278	Pos	1,42	2098	Pos
10	0,88	1240	Pos	0,85	1197	Pos	0,75	1048	Pos	0,98	1393	Pos
11	0,90	1276	Pos	1,12	1620	Pos	1,39	2058	Pos	1,05	1510	Pos
12	1,16	1679	Pos	1,30	1911	Pos	1,16	1684	Pos	0,58	785	Neg
13	1,01	1452	Pos	0,62	847	Neg	1,16	1691	Pos	0,85	1197	Pos
14	2,46	3853	Pos	1,33	1956	Pos	1,72	2600	Pos	0,95	1352	Pos
15	0,99	1410	Pos	1,11	1607	Pos	1,61	2426	Pos	1,96	3001	Pos
16	1,05	1510	Pos	0,66	900	Neg	0,92	1302	Pos	1,21	1760	Pos
17	1,52	2274	Pos	1,29	1901	Pos	1,00	1437	Pos	0,58	792	Neg
18	0,45	597	Neg	0,49	657	Neg	0,70	965	Neg	1,18	1718	Pos
19	0,47	617	Neg	-		-	1,22	1790	Pos	1,34	1978	Pos
20	2,07	3183	Pos	-		-	-		-	0,74	1036	Pos