CastV-B

Data Pack

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

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

2. Calculation of Antibody Titre

The following equation relates the S/P of a sample at a 1: 100 dilution to a titre

\[
\log_{10} \text{Titre} = 1.1 \times \log (SP) + 3.156
\]

\[
\text{Antilog} = \text{Titre}
\]

<table>
<thead>
<tr>
<th>S/P value</th>
<th>Titre Range</th>
<th>Antibody status</th>
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</thead>
<tbody>
<tr>
<td>0.499 or less</td>
<td>667 or less</td>
<td>No antibody detected</td>
</tr>
<tr>
<td>0.500 - 0.699</td>
<td>668 - 966</td>
<td>Suspect</td>
</tr>
<tr>
<td>0.700 or greater</td>
<td>967 or greater</td>
<td>Positive</td>
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</table>

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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fax: +31 182 599 360
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Website: www.biochek.com

Manufacturer:
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Millfield Road, Hounslow
London TW4 5PY

KI/CK133REV02
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, running-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 but not to CastV A.
Panel 1: Monospecific samples containing antibodies to various viruses.

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<th>Analyte</th>
<th>Strain</th>
<th>SP</th>
<th>Titre</th>
<th>Interpretation</th>
<th>Sample No</th>
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Panel 2: Monospecific samples containing antibodies to various Chicken Astrovirus strains.

### Panel 2: AFBINI CAstV Monos

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<td>4</td>
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<td>2,14</td>
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### +ve Control Sera

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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.

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