

Chicken Anemia Virus Antibody ELISA

The Synbiotics **Chicken Anemia Virus (CAV) ELISA kit** is a rapid, specific and sensitive immunoassay for the detection of CAV antibodies in chicken sera.

Chicken anemia virus is a member of the newly classified Circoviridae family that is characterized by a single and circular DNA strand. CAV virus in susceptible chickens causes anemia and immunosuppression. This is followed by an increased susceptibility to other pathogens which could lead to viral, bacterial, and fungal infections. As a result, CAV infections can be economically devastating with high morbidity and mortality with poor and uneven growth.

Suggested Uses

- 1. Monitoring of Pullet and Breeder Flocks:** It is important that pullet flocks are CAV antibody positive before going into production. Assay 30 or more randomly collected serum samples per flock. Test pullet flocks initially between 8-14 weeks of age using the CAV ELISA kit. Breeder flocks should also be tested routinely to check the uniformity and level of CAV antibody following vaccination or field exposure.
- 2. Monitoring Broiler Flocks:** Flocks can be monitored at day of age for level and uniformity of CAV maternal antibody. Collect and assay 30 serum samples per flock.
- 3. Monitoring CAV vaccinations:** The CAV ELISA can be used to monitor CAV vaccinations. Collect and assay 30 serum samples per flock immediately prior to vaccination and 4 to 6 weeks post vaccination.

Kit Performance

The Synbiotics CAV ELISA kit has been extensively tested for the following parameters:

1. Specificity

The CAV ELISA kit is highly specific for CAV antibody and has been tested against a variety of serum samples. The data shown in tables 1 and 2 below indicate that the Synbiotics CAV ELISA demonstrated very high correlations with both the Indirect Fluorescence Antibody (IFA) test and the Virus Neutralization (VN) test.

Table 1. Two hundred eight sera were tested by IFA for antibody to CAV by SPAFAS, Inc. (Storrs, CT) prior to shipment to SBIO. CAV ELISA results generated at SBIO were compared to the IFA results. The % agreement between the IFA results and the SBIO CAV ELISA Kit is 94.7%*.

Comparison of the SBIO CAV ELISA Kit and the Indirect Fluorescence Antibody (IFA) Test

Number of Samples Tested	ELISA Results	IFA Results
113	+	+
84	-	-
11	+	-
2	-	+
Total 208		

* % agreement is $(113 + 84 / 208) \times 100 = 94.7\%$

KEY

■	two serological methods agreed
■	two serological methods did not agree, ELISA detected positive samples.
■	two serological methods did not agree, ELISA did not detect positive samples.

Table 2. 53 CAV antibody positive and negative field sera were collected from pullet flocks, ages 9 to 18 weeks and tested by the SBIO CAV ELISA Kit. The sera were then sent to Intervet America, Inc. (Millsboro, DE) for VN analysis. The % agreement between the SBIO CAV ELISA Kit and the VN test is approximately 98%§.

Comparison of the SBIO CAV ELISA and the Virus Neutralization (VN) Test

Number of Samples Tested	ELISA Results	VN Results
23	+	+
29	-	-
1	+	-
Total 53		

§ % agreement is $(23 + 29 / 53) \times 100 = 98.1\%$

CAV ELISA Results

SP Ratio = $\frac{\text{Optical Density (O.D.) sample} - \text{Avg. O.D. NCS}}{\text{Avg. O.D. PCS} - \text{Avg. O.D. NCS}}$

SP ranges: Negative (-), $SP \leq .349$
Positive (+), $SP \geq .350$

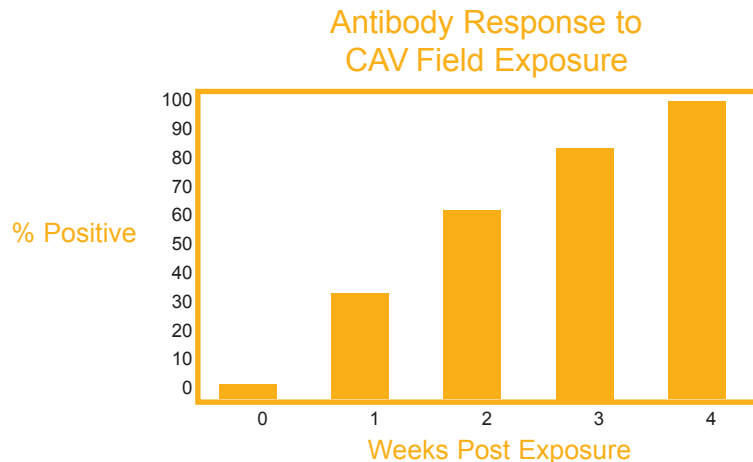
VN Results

Negative (-), $= < 4$
Positive (+), $= \geq 4$

2. Sensitivity

The Synbiotics CAV ELISA is also evaluated extensively for sensitivity. As illustrated in Figure 1, the CAV ELISA has demonstrated (in field studies) the ability to detect antibody to CAV within one week following field exposure.

Figure 1. Serum from a 15 week old pullet flock was found to be negative for CAV antibodies by the SBIO ELISA. The flock was then exposed to a CAV infected environment and tested for CAV antibodies (using the SBIO ELISA) every week for four weeks following exposure. Results are expressed as percent positive.



3. Stability

The CAV ELISA kit has a shelf-life of 18 months from the date of manufacture. The expiration date for each kit is clearly marked on the kit box label.

Interpreting Results

Evaluate Synbiotics CAV ELISA results using the following guidelines:

1. Uniformity

- Measured by the coefficient of variation value (CV%).
- The lower the CV% value for a flock tested, the better the titer value uniformity.
- Strive for the best 1-3 day old, pre-vaccination, and post-vaccination CAV titer value uniformity (i.e. CV% of less than 50%).

2. Titer Values

- The CAV ELISA titer values are directly proportional to the amount of CAV antibody in the test samples. Evaluate CAV titers using the following guidelines:

SP Threshold: Each Synbiotics ELISA kit has a sample-to-positive (SP) value threshold that clearly separates positive samples from negative samples. A negative sample is one that is not significantly different than the CAV kit normal control serum (NCS). The SP threshold for the CAV ELISA is as follows:

CAV Antibody Status	SP Value	Titer Range
Negative (-)	0.349 or less	0
Positive (+)	0.350 and greater	1472 and greater

*Note: Titer levels based on a single, live CAV vaccination administered, via wing web, to flocks that were 10-12 weeks of age when vaccinated. Flocks were bled approximately 6 weeks post-vaccination.

Vaccination Evaluation: Compare pre- and post-vaccination ELISA mean titers, geometric mean titers. A flock mean titer can be expected to increase 1500-2500 following vaccination. A titer level between 3000 and 5000 (mean titer) is a good target level to strive for in a CAV vaccinated flock*. Note that postvaccinal CAV ELISA values depend on a variety of factors such as CAV vaccine strain, route of administration, age of bird, etc. The goal of any vaccination program should be to achieve a uniform postvaccinal CAV ELISA titer response.

Routine Monitoring: When monitoring flocks for CAV antibody, samples with insignificant levels of CAV specific antibody receive a zero titer (see SP Threshold above). Therefore, samples from flocks receiving no CAV vaccination or exposure to CAV field virus, can be expected to have zero titers.