

The Synbiotics ProFLOK® **Hemorrhagic Enteritis Virus (HEV) ELISA kit** is a rapid and specific USDA-licensed test for the detection of HEV antibodies in turkey serum samples.

Hemorrhagic enteritis is an acute disease of turkeys four weeks of age or older. HE is characterized by depression, bloody droppings, and variable but often high mortality¹. In addition, HE may play a role in triggering or exacerbating secondary infections (i.e. colibacillosis) within affected turkey flocks².

The Synbiotics HEV ELISA kit offers the following benefits:

- Reliable, consistent results
- USDA-licensed
- High-volume, easy-to-use format
- Long shelf life. All Synbiotics kits have an 18-month shelf life.
- Powerful database management software system
- Excellent field-oriented technical support

Plate Antigen

The HEV ELISA utilizes an affinity purified HE virus hexon protein³ which ensures specific and reproducible test results.

Suggested Uses

1. HEV Vaccination Evaluation and optimization for commercial turkey flocks: Collect and assay 30 serum samples per each turkey flock receiving HEV vaccine as follows:

- 1 to 3 days of age and 10 to 14 days of age (measures maternal HEV antibody levels)
- The day of vaccination (pre-vaccination)
- 3 to 5 weeks post-vaccination
- 7 to 9 weeks post-vaccination

2. Presumptive sero-diagnosis of HEV infection in unvaccinated turkey flocks:

- Collect and assay 30 or more serum samples from clinically affected (acute phase) flocks and assay with the Synbiotics HEV ELISA kit.
- Rebleed clinically affected flocks 14 to 21 days after first serum collection (convalescent phase).
- Compare the acute and convalescent serum sample HEV ELISA results. An increase of three ELISA titer groups or greater between the acute and convalescent phases, is suggestive of HEV infection.

NOTE: Additional information such as flock history, clinical signs, necropsy findings, histopathologic evaluation, etc. are needed for a confirmed diagnosis of HEV infection.

Kit Performance

The HEV ELISA has been extensively tested for the following parameters:

1. Specificity

The results shown in table 1 below indicate that the HEV ELISA kit demonstrates an extremely high degree of specificity and only detected HEV antibody when used to assay reference turkey sera to numerous infectious agents of turkeys.

Table 1. Specificity.
Average sample-to-positive (SP) ratio, SP ranges, and titer values for a reference serum panel assayed with the SBIO HEV ELISA test.

Reference Turkey Serum	Average SP ^{A,B} Ratio Values	SBIO HEV ELISA SP Range ^C	SBIO HEV ELISA Titer Values ^D
Normal turkey control (NTS)	0.000	-	0
<i>Bordetella avium</i> (BA)	0.040	-	0
Rotavirus (RTV)	0.060	-	0
Coronavirus (CRV)	0.000	-	0
Avian reovirus (REO)	0.000	-	0
Herpesvirus of turkeys (HVT)	0.010	-	0
Newcastle disease virus (NDV)	0.000	-	0
Inactivated vaccine components	0.000	-	0
<i>Mycoplasma gallisepticum</i> (Mg)	0.000	-	0
<i>Mycoplasma synoviae</i> (Ms)	0.000	-	0
<i>Mycoplasma meleagridis</i> (Mm)	0.010	-	0
Hemorrhagic enteritis (HEV)	1.130	+	1882

^AValues are the arithmetic mean of 15 replicate samples (3 replicates / 5 ELISA plates)

^BLog₁₀ titer = (1.464 x Log₁₀ SP) + 3.197

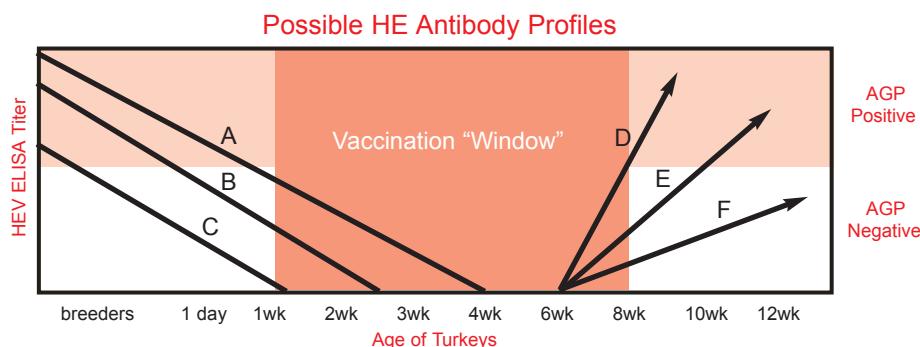
^CSample SP Ratio = $\frac{\text{Optical Density (OD) sample} - \text{Avg. OD Normal Control}}{\text{Avg. OD Positive Control} - \text{Avg. OD Normal Control}}$

^DSBIO HEV ELISA SP threshold ranges: Negative (-) = ≤ 0.200
Positive (+) = > 0.200

2. Sensitivity

Figure 1. Relative sensitivity of the ELISA versus AGP. Possible HEV ELISA profiles of HE antibodies over time (from the breeders to the poults) are illustrated. The AGP detection threshold is marked to demonstrate the increased sensitivity offered by the ELISA. Three possible flock maternal antibody levels are illustrated as A, B, and C. Three possible flock post vaccination or field HE virus infection responses are illustrated as D, E, and F.

The enhanced sensitivity of the ELISA allows maternal antibody levels below the level of AGP detection to be measured within the critical vaccination period or “window”. The development of HEV ELISA serologic profiles as suggested (see Interpreting Results Section), allows for more accurate timing of HE vaccination based on each flock’s maternal antibody pattern. For example, in Figure 1 below, high maternal antibody levels (A) may interfere with HE vaccination if given too early, while low maternal antibody levels (C) may leave flocks susceptible to HE virus field infection if HE vaccine is administered too late. More accurate monitoring of post-vaccination responses (D, E, and F), combined with flock performance parameters, are good indicators of flock health. For example, within a given operation, good performing flocks may exhibit an “A-F” ELISA profile, whereas poor performing flocks may exhibit a “C-D” HE ELISA profile.



The data presented in Table 2 below indicate that the Synbiotics HEV ELISA kit demonstrates excellent intra- and inter- plate reproducibility. All Synbiotics ELISA kits are optimized to provide valid, reproducible results test after test, day after day.

Table 2. Intra-plate and inter-plate reproducibility: Average optical density (OD) values and percent coefficient of variation (%CV) for high, medium, and low-titered HEV sera assayed 30 times on each of five SBIO HEV ELISA test plates.

SBIO HEV ELISA Plate	High-Titered HEV Serum		Medium-Titered HEV Serum		Low-Titered HEV Serum	
	OD ^A	CV(%)	OD ^A	CV(%)	OD ^A	CV(%)
1	1.074	2.88	0.515	5.64	0.267	6.16
2	1.002	5.74	0.493	5.15	0.247	5.87
3	1.067	3.22	0.522	1.90	0.282	3.46
4	0.907	6.79	0.444	8.21	0.231	7.93
5	0.875	8.48	0.435	7.81	0.249	11.08
Avg. Mean Values ^B	0.985	5.42	0.482	5.74	0.255	6.90

^AValues are the arithmetic mean of 30 replicates per each SBIO HEV ELISA plate.
^BValues are the arithmetic mean of 150 replicates per 5 HEV ELISA kit plates..

Interpreting Results

References

- Whitman, C.E. and A.A. Bickford, Hemorrhagic enteritis of turkeys. Avian Disease Manual. H.J. Barnes et al. eds. Colorado State University, CO. pp. 20-22. 1979.
- Domeruth, C.H., W.B. Gross and F.W. Pierson. Hemorrhagic enteritis of turkeys. A Laboratory Manual for the Isolation and Identification of Avian Pathogens. 3rd ed. H.G. Purchase et al. eds. Kendall/Hunt Publishing Co. Dubuque, IA. pp. 82-84. 1989.
- Used under license from Biostar and the Veterinary Infectious Disease Organization of the University of Saskatchewan.

Evaluate the Synbiotics HEV ELISA results for the following:

- 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 HEV titer value uniformity (i.e. CV% of less than 45%).
 - Titer Patterns
 - Evaluate the HEV ELISA titer pattern, over time, for each flock and compare to flock performance records at market age (i.e. % livability, body weight gain rate, body weight uniformity, etc.)
 - Compare the HEV ELISA flock profiles of good vs. poor performance turkey flocks, over time, and note differences, if any.
- NOTE: The goal of HEV vaccination is to adequately and uniformly vaccinate each flock to minimize its susceptibility to infection by an HEV field strain. Therefore, the age of the flock at HEV vaccination, source of HEV vaccine and vaccination administration procedures may each need to be evaluated and adjusted to achieve maximum flock performance.