

The Synbiotics ProFLOK® Mg ELISA kit is a USDA-licensed, NPIP approved rapid presumptive ***Mycoplasma gallisepticum*** (Mg) screening test for the detection of Mg antibodies in chicken serum.

Mg infection of chickens is characterized by respiratory rales, coughing, and nasal discharge with clinical signs usually slow to develop¹. Airsacculitis can be a significant cause of condemnations at slaughter. The Synbiotics Mg ELISA kit is a reliable Mg screening test with the following benefits:

- Reliable, consistent results
- NPIP-approved screening test
- Long shelf-life. All Synbiotics kits have an 18-month shelf-life.
- High-volume (900 tests/kit), easy to use format
- Powerful database management software system
- Excellent technical support

Suggested Uses

The Synbiotics Mg ELISA kit was developed for the routine monitoring of chicken flocks for the presence of Mg antibody. Collect and assay 30 or more high quality serum samples per chicken flock on a routine basis (i.e. every four weeks).

Plate Antigen

The Mg ELISA plates have been coated with the R strain of *Mycoplasma gallisepticum*.

Test Features

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

1. Specificity

The results shown in Table 1 below indicate that the Synbiotics Mg ELISA kit demonstrated excellent specificity (i.e. no false positive reactions) to reference sera for numerous infectious agents of poultry including *M. synoviae*, *S. aureus*, and killed vaccine components. These antigens have been previously reported to cause false positive reactions with commercial serum plate agglutination antigens and other commercially available Mg ELISA kits². The Synbiotics Mg ELISA kit, like all Synbiotics ELISA kits, is highly specific to provide valid, reproducible test results.

Table 1. Specificity.

Average sample-to-positive (SP) ratio values, SP ranges, and titer values for a specificity serum panel assayed with the SBIO MG ELISA test.

Reference Serum	Average SP ^{A,B} Ratio Values	SBIO MS ELISA SP Range ^C	SBIO MS ELISA Titer Values ^D
Infectious Bursal Disease (IBD)	0.011	-	0
Newcastle Disease Virus (NDV)	0.012	-	0
Avian Reovirus (REO)	0.012	-	0
Infectious Bronchitis Virus (IBV)	0.006	-	0
Infectious Laryngotracheitis (ILT)	0.101	-	0
Avian encephalomyelitis (AE)	0.070	-	0
Pasteurella multocida (Pm)	0.012	-	0
Staphylococcus aureus	0.055	-	0
Anti-tissue culture medium	0.017	-	0
Commercial inactivated vaccine	0.033	-	0
Mg Negative Control Serum (NCS)	0.000	-	0
Mycoplasma meleagridis (Mm)	0.016	-	0
Mycoplasma synoviae; 40 HI (Ms)	0.000	-	0
Mycoplasma synoviae; 160 HI (Ms)	0.059	-	0
Mycoplasma gallisepticum; 40 HI (Mg)	1.088	+	1781
Mycoplasma gallisepticum; 160 HI (Mg)	1.565	+	3032

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

^DLog₁₀ titer = (1.464 x Log₁₀ SP) + 3.197

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

^CSBIO MG ELISA SP threshold ranges: Negative (-) = ≤ .199
Suspect (?) = 0.200 - 0.599
Positive (+) = ≥ .600

2. Sensitivity

The data shown in Table 2 below indicate that the SBIO Mg ELISA demonstrated excellent sensitivity when compared to the Mg HI test conducted in a commercial laboratory. The enhanced sensitivity of the Synbiotics Mg ELISA test enables improved detection of Mg antibodies within chicken serum samples.

Table 2. Sensitivity. Comparison of SBIO Mg ELISA sample-to-positive (SP) ratio and titer values, and Mg hemagglutination-inhibition (HI) titers for a Mg sensitivity serum panel assayed with the SBIO Mg ELISA kit and the Mg HI test in two commercial laboratories.

Log 2	Dilution Ratio	SBIO MG SP Values ^A	SBIO MG SP Range ^{AB}	SBIO MG ELISA Titer Values ^{AD}	Laboratory A Average HI Titer ^C	Laboratory B Average HI Titer ^A
-1	1:2	3.371	+	9325	186.7	146.7
-2	1:4	3.005	+	7881	66.7	80.0
-3	1:8	2.218	+	5052	66.7	60.0
-4	1:16	1.600	+	3132	53.3	40.0
-5	1:32	1.000	+	1574	26.7	33.3
-6	1:64	0.560	?	674	20.0	20.0
-7	1:128	0.320	?	297	13.3	13.3

^AValues are the arithmetic mean of 6 replicate samples

^BSBIO MG ELISA SP threshold ranges: Negative (-) = ≤ 0.199
Suspect (?) = 0.200 - 0.599
Positive (+) = ≥ 0.600

^CValues are the arithmetic mean of 3 replicate samples

^DLog₁₀ titer = (1.464 x Log₁₀ SP) + 3.197

3. Reproducibility

The data presented in Table 3 below demonstrate the excellent intra- and inter-plate reproducibility of the Synbiotics Mg ELISA kit. All Synbiotics ELISA kits are optimized to provide valid, reproducible results test after test, day after day.

Table 3. Intra-plate and inter-plate reproducibility: Average optical density (OD) values and percent coefficient of variation (CV (%)) for a Mg positive chicken serum assayed 84 times on each of ten SBIO Mg ELISA plates.

SBIO MG ELISA Plate	Average Optical Density ^A	CV(%)	Average Plate CV (%) ^B
1	.642 ± .007	7.49	5.88
2	.687 ± .033	4.81	
3	.575 ± .040	6.95	
4	.605 ± .005	8.73	
5	.590 ± .005	6.05	
6	.686 ± .005	4.44	
7	.703 ± .006	5.28	
8	.617 ± .005	4.75	
9	.634 ± .005	4.58	
10	.621 ± .006	5.68	

^AValues are the arithmetic mean ± SEM of one Mg positive chicken serum assayed 84 times per Mg ELISA plate.
^BValue is the mean % Coefficient of Variation (CV%) of one Mg positive chicken serum assayed 84 times per ELISA plate on each of ten Mg ELISA plates.

Interpreting Results

When evaluating Synbiotics Mg ELISA results, keep in mind that the ELISA is a flock test. Flock decisions should not be made on individual samples or very small flock samples (less than 10 serum samples). Evaluate Synbiotics Mg ELISA results as follows:

References

1. Yoder, Jr., H.W. Mycoplasma gallisepticum Infection. In: *Diseases of Poultry*. 8th ed. M.S. Hofstad et al eds. Iowa State University., Ames, IA. pp. 190-202. 1988.
2. Avakian, A.P., S.H. Kleven, and J.R. Glisson. Evaluation of the specificity and sensitivity of two commercial enzyme-linked immunosorbent assay kits, the serum plate agglutination test, and the hemagglutination-inhibition test for antibodies formed in response to Mycoplasma gallisepticum. *Avian Diseases* 32: 262-272. 1988.

Presumed MS Antibody Status

Negative (-)^a
Probable (?)^{b,c}
Positive (+)^c

SP Range

0.199 or less
0.200 - 0.599
0.600 and greater

Titer Range

0
149 - 743
744 and greater

a. Negative.

Serum samples with an Mg SP value of less than 0.200 receive a "0" titer value and are presumed negative for Mg antibody. However, a variety of factors, such as possible Mg strains that may exhibit atypical biological and/or antigenic properties^{1,2}, prevalence of an Mg strain within a flock, and timing and randomness of serum sample collection procedures could result in an Mg-infected chicken flock yielding Mg-negative ELISA results. It is therefore recommended that each chicken flock only be considered to be Mg negative after (a) each flock has been adequately sampled and repeatedly tested several times and has yielded negative Mg ELISA results each time and (b) each flock has been adequately sampled and repeatedly tested by standard conventional serologic tests (SPA and HI) and Mg culture techniques² and has yielded Mg negative serologic and culture results each time.

b. Probable.

Presumed Mg antibody probable denotes the ELISA SP value range within which Mg ELISA and conventional (SPA and HI) test data may suggest but do not conclusively detect Mg antibody within a sample. The probable range represents a "suspect" or "gray" area in which Mg ELISA results may or may not be supported by conventional serologic (SPA and HI) test results. It is highly recommended that additional conventional serologic tests and Mg culture techniques² be conducted on serum and culture samples collected from Mg ELISA probable chicken flocks, as recommended in parts a and c, to confirm whether each flock is an Mg negative or Mg positive-infected flock.

c. Positive.

Additional conventional serologic testing (SPA and HI) and culturing of samples collected from presumed Mg ELISA antibody probable and positive chicken flocks, using standard techniques², are needed to obtain a confirmed positive diagnosis of Mg infection within a chicken flock.