**ELISA TEST PROCEDURE**

**PREPARING THE TEST PLATE**

a) Remove an MG antigen coated test plate from the protective bag and label according to dilution plate identification.

b) Add 50 µl Dilution Buffer to all wells on the test plate.

c) Add 5 µl diluted MG Positive Control Serum to wells A1, A3, and H1. Discard pipette tip.

d) Using an 8 or 12 channel pipette transfer 50 µl/well of each of the diluted serum samples and Normal Control Serum samples from the dilution plate to the corresponding wells of the MG coated test plate. Discard pipette tips after each row of sample is transferred. Transfers of samples to the ELISA plate should be done as quickly as possible.

e) Incubate plate for 30 minutes at room temperature.

**WASH PROCEDURE**

f) Tap out liquid from each well into an appropriate vessel containing bleach or other decontamination agent.

g) Using an 8 or 12 channel pipette (or comparable automatic washing device), fill each well with approximately 300 µl Wash Solution. Allow to soak in wells for 3 minutes; then discard contents into an appropriate waste container (waste container should contain bleach solution). Tap inverted plate to ensure that all residual liquid is removed. Repeat wash procedure 2 more times.

NOTE: The wash procedure is a very critical step in any ELISA procedure. Please follow the above steps directed.

**ADDITION OF ANTI-CHICKEN IgG**

**PERoxidase CONJUGATE, SUBSTRATE AND STOP**

h) Using an 8 or 12 channel pipette (or transplicating device) dispense 100 µl diluted conjugate (prepared as described above) into each assay well. Discard pipette tip.

i) Incubate for 30 minutes at room temperature.

j) WASH as steps f and g above.

k) Using an 8 or 12 channel pipette (or transplicating device) dispense 100 µl Substrate Solution into each test well. Discard pipette tip.

l) Incubate 5 minutes at room temperature.

m) Using an 8 or 12 channel pipette (or transplicating device) add 100 µl diluted Stop Solution (pre pared as described above) to each test well.

n) Allow bubbles to dissipate before reading plate.

**MANUAL PROCESSING OF DATA**

a) Read the plate using an ELISA reader set at 405-410 nm. Be sure to blank the reader as directed.

b) Calculate the average Positive Control Serum absorbance (Optical Density (O.D.)) using the absorbance values of wells A1, A3 and H1. Calculate the average Normal Control Serum absorbance using values obtained from wells A2, A10 and H12. Record both averages.

c) Subtract the average normal control absorbance from the average positive absorbance. The difference is the Corrected Positive Control.

d) Calculate a sample to positive (Sp) ratio by subtracting the average normal control absorbance from each sample absorbance. The difference is divided by the corrected positive control. Use the following equation format:

\[ \text{Sp = } \frac{\text{Sample O.D.} - \text{Normal Control O.D.}}{\text{Corrected Positive Control O.D.}} \]

\[ \text{MF ELISA} \text{ TITER = ANTILOG OF Sp TITER} \]

\[ \text{Example:} \text{ Positive Control Absorbance:} \frac{0.585 + 0.610 + 0.590}{3} = 0.595 \]

\[ \text{Corrected Positive Control:} \frac{0.090 + 0.087 + 0.098}{3} = 0.092 \]

\[ \text{Corrected Positive Control:} \frac{0.585}{0.092} = 5.305 \]

\[ \text{Example Calculation of titer using the Sp from above:} \text{LODg TITER = } (1.464 \times \text{LODg Sp}) + 3.197 \]

\[ \text{TITER = ANTILOG OF LODg TITER} \]

**RESULTS**

**Assay Control Values**

Valid MG ELISA results are obtained when the average optical density (O.D.) value of the Normal Control Serum is less than 0.200 and the Corrected Positive Control value range is between 0.250 and 0.900. If either of these values are out of range, the MG test results should be considered invalid and the samples should be retested. Samples testing with an Sp value lower than or equal to 0.199 will receive a titer value of 0 and are considered negative for MG antibody.

Under optimal conditions* the suggested O.D. value ranges of 0.060 to 0.120 for MG Normal Control Serum and 0.550 to 0.750 for MG Positive Control Serum should be strived for to ensure the most consistent laboratory test results. Please note that tests with O.D. values which do not fall within the suggested O.D. ranges above do not constitute an invalid test.

*Optimal conditions are at room temperature (70 to 75°F (21 to 24°C)). Higher room temperatures may result in slightly higher O.D. values.

**Interpretation of Results**

The MG Sp ratio values and/or ELISA TITER values obtained for sera should be interpreted using the following value ranges:

<table>
<thead>
<tr>
<th>Sample Positive (Sp) Value</th>
<th>MF ELISA TITER</th>
<th>MG Presumed Positive Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 0.200</td>
<td>0</td>
<td>Negative*</td>
</tr>
<tr>
<td>0.200 to 0.599</td>
<td>1 to 743</td>
<td>Probable Positive</td>
</tr>
<tr>
<td>Greater than or equal to 0.6</td>
<td>744 or greater</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**a. Negative.** Serum samples with an MG Sp ratio 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 strain variations that may exhibit atypical biological and/or antigenic properties, a variance 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 techniques 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 results may suggest but may 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 techniques 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 techniques, are needed to obtain a confirmed positive diagnosis of MG infection within a chicken flock.

**BIBLIOGRAPHY**


Please contact Zoetis Veterinary Investigations Product Support (VIPS) team at 1-800-866-5288 with questions and comments.

**MYCOPLASMA GALLISEPTICUM ANTIBODY TEST KIT**

**ITEM NO. 96-6533**

**Manufactured by:** Zoetis Inc.

**Distributed by:** Zoetis Inc.

**Synbiotics Corporation**

A Wholly-Owned Subsidiary of Zoetis Inc.

16420 Via Espliego

San Diego, CA 92127

888-963-0471

U.S. VET LIC. NO. 312
MYCOPLASMA GALLISEPTICUM ANTIBODY TEST KIT

GENERAL INFORMATION AND INTENDED USES
Mycoplasma gallisepticum (MG) infection of chickens is associated with aracnoidalitis, reduced feed conversion and egg production efficiency, and increased condemn rate at slaughter. MG infection is one of the costliest disease problems confronting the poultry industry.

The ProFLOK® MG ELISA Kit is a rapid and specific presumptive seroenzynotic test for the detection of antibody to most conventional MG strains in chicken serum samples. It was designed for screening large numbers of chicken sera from numerous flocks; however, additional conventional MG serologic testing (i.e. serum plate agglutination (SPA) and hemagglutination-inhibition (HI) test) and culture techniques are needed to confirm MG negative and MG-infected chicken flocks.

The assay is designed to measure MG antibody bound to MG antigen coated plates. The principle of the test is as follows: Serum obtained from chickens exposed to MG antigens contains specific anti-MG antibodies. Serum, diluted in Dilution Buffer, is added to an MG antigen coated plate. Specific MG antibody in the serum forms an antibody-antigen complex with the MG antigen bound to the plate. After washing the plate, an affinity purified goat anti-chicken IgG (H+L) peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step binds with the conjugate. After a brief incubation period, the unbound conjugate is removed by a second wash step. Substrate, which contains a chromagen (ABTS), is added to unbound conjugate is removed by a second wash step. After the substrate has incubated, Stop Solution (controls) is directly proportional to the level of MG antibody in the serum. After the substrate has incubated, Stop Solution is added to each well to terminate the reaction and the plate is read using an ELISA plate reader at 405-410 nm.

REAGENTS REQUIRED TO PERFORM 90 TESTS
1. MG antigen coated plate
2. 10 µl MG Positive Control Serum
3. 10 µl Normal Control Serum
4. 100 µl Goat anti-Chicken IgG (H+L) Peroxidase Conjugate Solution
5. 40 µl Dilution Buffer
6. 10 µl A1BT-Hydrogen Peroxide Substrate Solution
7. 2.5 ml 5X Stop Solution (dilute 1:2) with laboratory grade water
8. 20 ml 2X Wash Solution (dilute 1:20) with laboratory grade water

NOTE: Store all reagents provided in the kit at 2-7°C.

EQUIPMENT AND MATERIALS REQUIRED
1. High precision pipette (i.e. 1-20 microliter pipette)
2. 0.2 ml, 1.0 ml and 5.0 ml pipettes
3. 8 or 12 channel pipette (or transatlanting device)
4. 2 graduated cylinders (50 ml)
5. 1 ml and 5 ml borosilicate glass test tubes
6. Uncoated 96 well plates (i.e. Nunc cat# 360450)
7. Laboratory grade (Distilled or R.O.) water
8. 96 well plate reading spectrophotometer with 405-410 nm filter

WARNING TO THE USERS OF REAGENTS AND MG ANTIGEN COATED PLATES
1. Handle all reagents and samples as biohazardous material.
2. Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
3. Wash solution, control sera, test plates, field samples and all other test kit reagents should be properly decontaminated with bleach or other strong oxidizing agent before disposal.
4. Take special care not to contaminate any of the test reagents with serum or bacterial agents.
5. Humidity indicators are supplied with each plate. If any of the indicators exhibit a pink color, the plate may be compromised in some way. Do not use this kit after the expiration date.
6. NEVER PIPEETE BY MOUTH.

PREPARATION OF THE SERUM
1. Add 500 µl Dilution Buffer to each well of an uncoated 96 well microtiter plate. Set up samples and controls as shown in Figure 1.

PREPARATION OF THE SERUM DILUITION PLATE
a) Add 500 µl Dilution Buffer to each well of an uncoated 96 well microtiter plate. This plate is referred to as the serum dilution plate.

b) Add 6 µl unknown serum per well as per Figure 1 (producing a 1:50 dilution). Start with well A4 and end with well H9 (moving left to right, row by row of wells).

Preparation of the Substrate Solution
1. Add 6 µl of Normal Control Serum (producing a 1:50 dilution) to wells A2, H10 and H12.
2. Aspirate and remove any liquid in dilution plate wells A1, A3 and H11.
3. Allow all diluted sera to equilibrate in Dilution Buffer for 5 minutes before transferring to an MG antigen coated ELISA plate.

Preparation of the Substrate Solution
1. Dilute 20 µl concentrated Wash Solution in 380 ml laboratory grade (distilled or R.O.) water (1:20). Mix well. Approximately 400 µl Wash Solution is needed for each 96 well ELISA plate.

Preparation of IX Wash Solution
1. Dilute 2.5 ml concentrated Wash Solution in 10 ml laboratory grade (distilled or R.O.) water (1:5). Mix well. Approximately 12.5 ml Stop Solution is needed for each 96 well ELISA plate.

Storage of 5X Stop Solution at refrigerated temperatures may cause the formation of a white solid. This does not affect product performance. Warm at room temperature or 37°C to dissolve before use.

Figure 1.