trol Serum samples from the dilution plate to the corresponding wells of the MM coated test plate. Discard pipette tips after each row of sample is transferred. Transfer 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 as directed.

ADDITION OF ANTI-TURKEY IgG PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION

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

i) Incubate for 30 minutes at room temperature.

j) **WASH** as in steps f and g above.

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

l) Incubate 15 minutes at room temperature.

m) Using an 8 or 12 channel pipette (or transplating device), add 100 μl diluted Stop Solution (prepared 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 plate 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 H11. Calculate the average Normal Control Serum absorbance using values obtained from wells A2, H10 and H12. Record both averages.

c) Subtract the average normal control absorbance from the average positive control 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:

$$SP = \frac{\text{(SAMPLE ABSORBANCE) - (AVERAGE NORMAL CONTROL ABSORBANCE)}}{\text{CORRECTED POSITIVE CONTROL ABSORBANCE}}$$

e) An MM-T ELISA titer can be calculated by the following suggested equation:

$\text{LOG}_{10}\ \text{TITER} = (1.464 \times \text{LOG}_{10}\ \text{Sp}) + 3.197$

$\text{TITER} = \text{ANTILOG OF LOG}_{10}\ \text{TITER}$

Example:

1. Example Positive Control Absorbance:
   0.585, 0.610, 0.590
   Average = (0.585 + 0.610 + 0.590) / 3 = 0.595
2. Example Normal Controls:
   0.082, 0.085, 0.079
   Average = (0.082 + 0.085 + 0.079) / 3 = 0.082
3. Corrected Positive Control:
   (0.595) - (0.082) = 0.513
4. Example Sp value calculation:
   Absorbance of sample = 0.560
   (0.560) - (0.082) / 0.513 = 0.931
5. Example of calculation of titer using the Sp from above:
   $\text{Log}_{10}\ \text{Titer} = 1.464 \times (\text{Log}_{10}\ 0.931) + 3.197$
   Titer = ANTILOG 3.15
   Titer = 1413

RESULTS

Assay Control Values:

Valid MM-T 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 MM test results should be considered invalid and the samples should be retested. Samples testing with an Sp value of less than or equal to 0.299 will receive a 0 titer value and are considered negative for MM antibody.

Under optimal conditions* the **suggested** O.D. value ranges of **0.045 to 0.095 for MM-T Normal Control Serum** and **0.350 to 0.800 for MM-T Positive Control Serum** should be strived for to ensure the most consistent laboratory test results. Please note that a plate with O.D. values which do not fall within the suggested O.D. ranges above <u>does not</u> 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 MM-T Sp ratio values and/or ELISA titer values obtained for sera should be interpreted using the following value ranges:

Sample to Positive (Sp) Value	MM ELISA Titer Range	MM Presumed Antibody Status
Less than 0.300	0	Negative[a]
0.300 to 0.599	270 to 743	Probable[b,c]
Greater or equal to 0.6	744 or greater	Positive[c]

a. **Negative.** Serum samples with an MM-T Sp ratio value of less than 0.300 receive a "0" titer value and are presumed negative for MM antibody. However, a variety of factors, such as the biological and antigenic properties of various *Mycoplasma meleagridis* strains[1,2], prevalence of an MM strain within a flock and timing and randomness of serum sample collection procedures could result in an MM-infected turkey flock yielding MM-negative ELISA results. It is therefore recommended that each turkey flock only be considered to be MM negative after (a) each flock has been adequately sampled and repeatedly tested several times and has yielded negative MM ELISA results each time **and** (b) each flock has been adequately sampled and repeatedly tested by standard conventional serologic tests (SPA and HI) and MM culture techniques[2] and has yielded MM negative serologic and culture results each time.

b. **Probable.** Presumed MM antibody probable denotes the ELISA Sp value range within which MM-T ELISA and conventional (SPA and HI) test data may suggest but **may not conclusively** detect MM antibody within a sample. The probable range represents a "suspect" or "gray" area in which MM-T 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** MM culture techniques[2] be conducted on serum and culture samples collected from MM-T ELISA probable turkey flocks, as recommended in parts a and c, to confirm whether each flock is an MM negative or MM positive-infected flock.

c. **Positive.** Additional conventional serologic testing (SPA and HI) and culturing of samples collected from presumed MM-T ELISA antibody **probable** and **positive** turkey flocks, using standard techniques[2], are needed to obtain a confirmed positive diagnosis of MM infection within a turkey flock.

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Please contact Synbiotics Technical Service at
800-247-1725 or (816) 454-7246 with questions and comments.

03-3036-1200

MYCOPLASMA MELEAGRIDIS ANTIBODY TEST KIT

(FOR USE IN TURKEY)

ITEM NO. 96-8018

ProFLOK®

SYNBIOTICS CORPORATION
11011 VIA FRONTERA SAN DIEGO, CA 92127
800 228 4305 (OR outside U.S./Canada 858 451 3771)

U.S. VET LIC NO. 312

MYCOPLASMA MELEAGRIDIS ANTIBODY TEST KIT (TURKEY)

GENERAL INFORMATION AND INTENDED USES

Mycoplasma meleagridis (MM) infection of turkeys is the etiological agent of an egg-transmitted disease of turkeys characterized by decreased hatchability, skeletal abnormalities, reduced growth performance and airsacculitis in the progeny.

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

The assay is designed to measure MM antibody bound to MM antigen coated plates. The principle of the test is as follows: Serum obtained from turkeys exposed to MM antigens contains specific anti-MM antibodies. Serum, diluted in Dilution Buffer, is added to an MM antigen coated plate. Specific MM antibody in the serum forms an antibody-antigen complex with the MM antigen bound to the plate. After washing the plate, an affinity purified goat anti-turkey 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 each well. Chromagen color change (from clear to green-blue) occurs in the presence of the peroxidase enzyme. The relative intensity of color developed in 15 minutes (compared to controls) is directly proportional to the level of MM 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

a) 1 MM-T antigen coated plate
b) 10 µl MM-T Positive Control Serum
c) 10 µl Normal Control Serum
d) 100 µl Goat anti-Turkey IgG (H+L) Peroxidase Conjugate Solution
e) 40 ml Dilution Buffer
f) 10 ml ABTS-Hydrogen Peroxide Substrate Solution
g) 2.5 ml 5X Stop Solution (dilute [1:5] with laboratory grade water)
h) 20 ml 20X 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

a) High precision pipette (i.e. 1-20 microliter pipette)
b) 0.2 ml, 1.0 ml and 5.0 ml pipettes
c) 8 or 12 channel pipette (or transplating device)
d) 2 graduated cylinders (50 ml)
e) 5 ml borosilicate glass test tubes
f) Uncoated low binding 96 well plates (i.e. Nunc catalog #269620)
g) Laboratory grade (Distilled or R.O.) water
h) 96 well plate reading spectrophotometer with 405-410 nm filter
i) Plate washing apparatus

WARNINGS TO THE USERS OF REAGENTS AND MM ANTIGEN COATED PLATES

a) Handle all reagents and samples as biohazardous material.
b) Keep all reagents away from skin and eyes. If exposure should occur, immediately flush affected areas with cold water.
c) 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.
d) Take special care not to contaminate any of the test reagents with serum or bacterial agents.
e) Humidity indicators are supplied with each plate. If any of the indicators exhibit a pink color, the plate may be compromised in some way; decontaminate (i.e. wash the plate with bleach solution) and dispose of the plate.
f) The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
g) Do not use this kit after the expiration date.
h) **NEVER PIPETTE BY MOUTH.**
ALLOW ALL REAGENTS TO COME TO ROOM TEMPERATURE BEFORE STARTING!

SAMPLE COLLECTION

For routine serologic flock monitoring, it is suggested that at least **30 or more sera per flock** be randomly collected at standard time intervals (i.e. every four weeks). Proper sample collection procedures, serum harvest and serum sample storage (4°C for up to four days or -20°C for longer periods) are needed to provide reliable test results.

SAMPLE DILUTION PROCEDURE

Dilute serum samples using Dilution Buffer in a clean, uncoated 96 well microtiter plate. Set up samples and controls as shown in Figure 1.

PREPARATION OF THE SERUM DILUTION PLATE

a) Add 300 µl Dilution Buffer to each well of an uncoated 96 well microtiter plate. This plate is referred to as the serum dilution plate. Label 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). For example, wells 1 through 30 contain the diluted sera of flock 1, wells 31-60 contain the diluted sera of flock 2, etc.
c) Add 6 µl of Normal Control Serum (producing a 1:50 dilution) to wells A2, H10 and H12.
d) Aspirate and remove any liquid in dilution plate wells A1, A3 and H11.
e) Allow all diluted serums to equilibrate in Dilution Buffer for 5 minutes before transferring to an MM antigen coated ELISA plate.
f) Diluted serum should be tested within 24 hours.

This dilution format provides adequate quantities of diluted serum samples to conduct four additional ProFLOK® ELISA tests (i.e. MS, MG, NDV and HE) using the same serum dilution plate.

Preparation of MM-T Positive Control

An MM-T Positive Control Serum has been provided with this kit. Dilute the appropriate volume of MM-T Positive Control Serum with Dilution Buffer (1:50) in a clean, glass test tube. For example, dilute 6 µl of positive control serum in 300 µl Dilution Buffer. **Mix well.** 150 µl of diluted MM-T Positive Control is needed per ELISA plate.

Figure 1.

	1	2	3	4	5	6	7	8	9	10	11	12
A	+	-	+	1	2	3	4	5	6	7	8	9
B	10	11	12	13	14	15	16	17	18	19	20	21
C	22	23	24	25	26	27	28	29	30	31	32	33
D	34	35	36	37	38	39	40	41	42	43	44	45
E	46	47	48	49	50	51	52	53	54	55	56	57
F	58	59	60	61	62	63	64	65	66	67	68	69
G	70	71	72	73	74	75	76	77	78	79	80	81
H	82	83	84	85	86	87	88	89	90	-	+	-

Preparation of Conjugate Solution

The horseradish peroxidase conjugated anti-turkey IgG (H+L) is supplied in HRP Stabilizer. Dilute 100 µl stock conjugate in 10 ml Dilution Buffer (1:100 dilution). **Mix well.** This 10 ml preparation will supply sufficient conjugate for one 96 well ELISA plate.

Preparation of 1X Wash Solution

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

Preparation of the Substrate Solution

The Substrate Solution is ready to use. Each plate will require approximately 10 ml substrate solution. **For best results, the substrate solution must be equilibrated to room temperature before use.**

Preparation of 1X Stop Solution

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

NOTE: 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.

ELISA TEST PROCEDURE

PREPARING THE TEST PLATE

a) Remove an MM-T 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 50 µl diluted MM-T Positive Control Serum to wells A1, A3 and H11. Discard pipette tip.
d) Using an 8 or 12 channel pipette, transfer 50 µl/well of each of the diluted serum samples and Normal Con-