PASTEURELLA MULTOCIDA ANTIBODY TEST KIT

GENERAL INFORMATION AND INTENDED USES

Pasteurella multocida (PM) is the causative agent of fowl cholera, a contagious septicemic disease of domestic poultry and wild birds that is often associated with high mortality in flocks.

The ProFLEX® PM ELISA Kit is a rapid serologic test for the detection of PM antibody in chicken serum samples. The ProFLEX® PM ELISA test was developed primarily to aid in the serodiagnosis of Fowl Cholera and the detection of post-vaccination PM antibody levels in chickens.

The assay is designed to measure PM antibody bound to PM antigen coated plates. The principle of the test is as follows: Serum obtained from chickens exposed to Pasteurella multocida contains specific anti-PM antibodies. Serum, diluted in Dilution Buffer, is added to a PM antigen coated plate. Specific PM antibody in the serum forms an antibody-antigen complex with the PM antigen bound to the plate. After washing the plate, an affinity-purified goat anti-chicken IgG (F(ab')2) peroxidase conjugate is added to each well. The antibody-antigen complex remaining from the previous step reacts with the conjugate. After a brief incubation period, the unreacted conjugate is removed with a second wash step. Substrate, which contains a chromogen (AETS), 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 PM 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 ML PM antigen coated plate
- 10 µL PM Positive Control Serum
- 10 µL Normal Control Serum (NCS)
- 100 µL Gour anti-Chicken IgG (H+L) Peroxidase Conjugate Solution
- 40 µL Dilution Buffer
- 10 µL AETS-Hydrogen Peroxide Substrate Solution
- 2.5 µL 5X Stop Solution, 50 SV SDS (dilute 1:5) with laboratory grade water
- 20 µL 20X Wash Solution (dilute 1:20 with laboratory grade water)

NOTE: Store all reagents provided in the kit at 2-7°C. Reagents should not be frozen.

EQUIPMENT AND MATERIALS

REQUIRED BUT NOT PROVIDED

- High precision pipette (i.e. 1.2 µl microliter pipette)
- 0.2 ml, 1 ml and 5.0 ml pipettes
- 8 or 12 channel pipette (or dispensing device) and pipette tips
- 5 graduated cylinders (50 ml)
- 1 ml or 5 ml borosilicate glass test tubes
- Uncornered binding 96 well plates (i.e. Nunc catalog #209920)
- Laboratory grade (Distilled or R.O.) water
- 96 well plate reader spectrophotometer with 405-410 nm filter
- Plate washing apparatus
- Water container with bleach or other oxidizing agent

WARNING TO THE USERS OF REAGENTS AND PM ANTIGEN COATED PLATES

- Handle all reagents and samples as biohazardous material.
- Keep all reagents and sera in the refrigerator and eyes.
- If exposure should occur, immediately flush affected areas with cold water.
- 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.
- Take special care not to contaminate any of the test reagents with serum or bacterial agents.
- 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.
- The best results are achieved by following the protocols as they are described below, using good, safe laboratory techniques.
- Do not use this kit after the expiration date.
- 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 harvesting 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 PROCEDE

Dilute serum samples using Dilution Buffer in a clean, uncorked 96-well microtiter plate. Freeze serum samples should be completely thawed and thoroughly mixed before diluting. Set up samples and controls as shown in Figure 1.

PREPARATION OF THE SERUM DILUTION PLATE

- Add 300 µL Dilution Buffer to each well of an uncorked 96-well microtiter plate. This plate is referred to as the serum dilution plate.
- Add a µL unknown serum per well as per Figure 1 (providing a 1:50 dilution). Start with well A4 and well H19 (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.
- Add 4 µL of Normal Control Serum (producing a 1:50 dilution) to wells A2, H10, and H12.
- Aspirate and remove any liquid in dilution plate wells A1, A3 and H11.
- Allow all diluted sera to equilibrate in Dilution Buffer for 5 minutes before transferring to a PM antigen coated ELISA plate.
- Diluted serum should be tested within 24 hours.

This dilution format provides adequate quantities of diluted serum samples to conduct four additional ProFLEX® ELISA tests (i.e. IRB, REO, BVB or NDV) using the same serum dilution plate.

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Figure 1.

Preparation of PM Positive Control

A PM Positive Control Serum has been provided with this kit. Dilute the appropriate volume of PM Positive Control Serum with Dilution Buffer (1:50) in a clean, glass test tube. For example, dilute 10 µL of positive control serum in 300 µL Dilution Buffer. Mix well. 150 µL of diluted PM Positive Control is needed per ELISA plate.

Preparation of Conjugate Solution

The horseradish peroxidase conjugated anti-chicken 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 300 ml laboratory grade (distilled or R.O.) water (1:15). Mix well. Approximately 400 ml 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 example, 10 plates requires 100 ml substrate. 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 (1:1.25). Mix well. Approximately 12.5 ml 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 a PM antigen test plate from the protective bag and label according to serum dilution plate identification.

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

c) Add 50 µl diluted 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 Control Serum samples from the serum dilution plate to the corresponding wells of the PM control test plate (yields a 1:100 dilution). 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.

h) 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-CHICKEN IgG PEROXIDASE CONJUGATE, SUBSTRATE AND STOP SOLUTION
b) 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.

j) Incubate for 30 minutes at room temperature.

k) WASH as in step f and g above.

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

m) Incubate 15 minutes at room temperature.

n) Using an 8 or 12 channel pipette (or transplating device) add 100 µl Stop Solution (prepared as described above) to each test well.

o) 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 blanks the reader as directed.