Mycoplasma Synoviae and *Mycoplasma Gallisepticum* Antibody Test Kit
Poultry Check MP® Ms-Mg
Insert

2016-09-07

Poultry Check MP® Ms-Mg Assay is a mutiplex immunoassay intended for determining the presence of antibodies to ***Mycoplasma synoviae*** **(Ms) and** ***Mycoplasma gallisepticum*** **(Mg)** in chicken serum. This test kit is not indicated for diagnosis of disease in individual birds.

PRINCIPLE OF THE ASSAY

Poultry Check MP® Ms-Mg Assay is a microsphere-based multiplex fluorescent immunoassay (MFIA)-type antibody detection test. A total of five distinct microsphere sets (i.e. three coated with Ms, Mg antigens or a non-specific reaction control and two coated with Chicken IgY or Rabbit anti-chicken IgY as internal controls) are incubated in diluted chicken serum and then washed. Successive incubations with biotinylated anti-chicken IgY (Detection Antibody) and streptavidin R-phycoerythrin (SA-PE) reporter follow, each succeeded by a wash step to remove unbound reagent. Users place plates in the plate analyzer, which captures the fluorescence intensity of the microspheres and SA-PE reporter. An S/P ratio of the SA-PE on the antigen-coated microspheres above the provided cut-offs indicates antibody is present in the sample. For the internal control microspheres, MFI results above the provided cut-off confirm the addition of both sample and Detection Antibody (MFI, median fluorescence intensity, is an arbitrary raw unit that denotes how much reporter fluorescence a given particle population carries; it is expressed and compared in linear numbers).

MATERIAL

Components Supplied in the Kit

Component	Volume	Storage
Sample Diluent	250 mL	2 to 7°C
Positive Control	100 µL	-10 to - 25°C
Negative Control	100 µL	-10 to - 25°C
Microsphere Mix	28 mL	2 to 7°C
Wash Buffer (10X)	500 mL	2 to 7°C
Detection Antibody	52 mL	2 to 7°C
SA-PE Conjugate	52 mL	2 to 7°C
Microtiter Plate	5	20 to 25°C

The materials provided are sufficient for testing up to 460 samples.

Microsphere Mix Constituent	Microsphere Code (Region)
Ms antigen	52
Mg antigen	54
Chicken IgY	35
Rabbit anti-chicken IgY	45
Non-specific control	46

Equipment and Consumables Required But Not Provided

- Purified water
- Table-top bath sonicator
- Vortex
- Plate shaker
- Magnetic microsphere separator
- Plate analyzer (e.g. LX200, Magpix)
- Data analysis software (e.g. xPONENT)
- Aluminum foil
- Bottles, tubes or plates for dilution
- Micropipettes and tips
- Microcentrifuge
- Plate washer (optional)
- Additional 96-well round bottom white non treated polystyrene microplates (if required)

PRECAUTIONS AND WARNINGS

- Handle all assay materials as if they are capable of infection. Although the viral antigens have been chemically inactivated prior to coating on the microspheres, follow proper biological safety procedures while handling the materials and wear appropriate personal protective equipment.
- All wastes should be properly decontaminated prior to disposal.
- Kit components contain ProClin® to prevent microbial growth. This can cause allergic reactions in some people. The ProClin content is <0.05%.
- The magnetic field generated by the magnetic separator interferes with electrical medical devices.
- **Microsphere Mix, Detection Antibody, and SA-PE Conjugate are light sensitive**. **Store them protected from light.**
- Store all materials as indicated in the Components Supplied in Kit table, above, and on the labels of the kit components.
- Reagents should be stored back at appropriate temperature as soon as possible after use.
- Limit freeze-thaw cycles for the Negative Control and Positive Control sera. Store these components at -20°C in a **manual-defrost freezer.**
- Centrifuge Positive Control and Negative Control prior to use to remove any material from the cap.
- Do not use reagents beyond their expiration dates and do not use them with components from different serials.
- To achieve reliable results, follow proper laboratory technique and pipette accurately. Take care to mix dilutions properly and use correct pipetting technique.
- This assay is for *in vitro* veterinary use only.

TECHNICAL SUPPORT

For technical support, send inquiries by e-mail to support@biovet-inc.com

ASSAY EXECUTION

Prepare the Samples and Reagents

1. Allow reagents to equilibrate to room temperature (19-26°C) before proceeding.
2. Centrifuge all controls to remove any liquid from the cap and vortex thoroughly.
3. Dilute the samples and controls 1/500 in Sample Diluent and mix thoroughly.
4. After homogenizing the 10X Wash Buffer (no evidence of crystals), dilute 1/10 with purified water. The Wash Buffer 1X solution is stable for 1 week at 2-7°C.
5. Vortex the Microsphere Mix for approximately 5 seconds, then sonicate using a bath sonicator (20 to 35 W, 40 to 60 kHz) for approximately 5 seconds.
6. Pipet the required volume of each reagent.

Prepare the Plate with Diluted Samples and Controls

7. Dispense 50 µL of Microsphere Mix to each well of the sample plate.
8. Dispense 50 µL of the diluted Negative Control to wells A1 and B1.
9. Dispense 50 µL of diluted Positive Control to wells C1 and D1.
10. Dispense 50 µL of diluted sample to the appropriate wells of the plate.
11. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
12. Place the plate on a plate shaker and incubate at room temperature (19 to 26°C) for 60 minutes at 800 rpm.
13. Move the plate to a 96-well magnetic separator for a minimum of 60 seconds.
14. While leaving the plate on the magnetic separator, discard the supernatant, ensuring that there is no disruption to the microspheres.
15. Do not remove the plate from the magnetic separator and dispense 100 µL of 1X Wash Buffer into each well, incubate for one minute, then discard the Wash Buffer as in step 14.
16. Repeat step 15 to perform another wash cycle (for a total of 2 wash cycles), remove the plate from the magnetic separator.

Add the Detection Antibody

17. Dispense 100 µL of the Detection Antibody to each well of the plate.
18. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
19. Place the plate on the plate shaker and incubate at room temperature (19 to 26°C) for 15 minutes at 800 rpm.
20. Discard the supernatant and perform 2 wash cycles as described in steps 13 through 16.

Add the SA-PE Conjugate

21. Dispense 100 µL of SA-PE Conjugate to each well of the plate.
22. Cover the entire plate with aluminum foil or an equivalent opaque material to protect it from light.
23. Place the plate on the plate shaker and incubate at room temperature (19 to 26°C) for 15 minutes at 800 rpm.
24. Discard the supernatant and perform 2 wash cycles as described in steps 13 through 16.

Analyse the Plate

25. Dispense 100 µL of 1X Wash Buffer into each well and resuspend the microspheres manually or using a plate shaker.
26. Load the plate into the plate analyzer and run the batch.

RESULT VALIDITY AND INTERPRETATION

Result Validity

Assay results must meet the following criteria to be valid:

1. Each sample requires the analysis of at least 50 microspheres per antigen or internal control for valid data. Rerun any sample with a lower microsphere count for any antigen or control.
2. Poultry Check MP® Ms-Mg Assay includes two internal control microspheres to confirm that both the sample and Detection Antibody were added to the assay.
    a. The chicken antibody-coated microsphere (IgY) binds to the Detection Antibody and indicates that the conjugate has been added to the corresponding well.
    b. The rabbit anti-chicken antibody-coated microsphere (Rb) binds to chicken IgY in samples and indicates that the sample has been added to the corresponding well.
    c. The non-specific beads indicate the non specific signal of the sample.
    d. If one or both of the internal controls provides a signal <5000 MFI, there was incorrect pipetting of sample or Detection Antibody into that particular well. In this case, the affected sample must be rerun.
3. The mean "corrected MFI" signal for the Negative Control must be <500 for Ms and Mg. Higher Negative Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.
4. The mean "corrected MFI" signal for the Positive Control must be ≥1000 for Ms and Mg. Lower Positive Control signals can indicate a systematic error for the assay plate and require repeating the assay plate.

Data Analysis

The S/P ratio is intended to differentiate positive from negative specimens. Calculate the S/P ratio for each sample using the formulas in the Calculations section.

- For Ms, an S/P ratio ≥ 0.2 indicates antibody presence to Mycoplasma synoviae (Ms).
- For Mg, an S/P ratio ≥ 0.2 indicates antibody presence to Mycoplasma gallisepticum (Mg).

Each laboratory is responsible for correlating Poultry Check MP® Ms–Mg results to the method currently used.

Calculations

1. For each well, calculate the "corrected MFI" for each antigen bead set: "antigen bead" MFI - "non-specific control bead" MFI.
2. Calculate Positive Control and Negative Control mean "corrected MFI" for each antigen bead set:
   Positive Control mean "corrected MFI" : (Well C1 "corrected MFI" + Well D1 "corrected MFI") / 2
   Negative Control mean "corrected MFI" : (Well A1 "corrected MFI" + Well B1 "corrected MFI") / 2
3. For each sample calculate S/P Ratio for each antigen = Sample "corrected MFI" / Positive Control mean "corrected MFI"

Examples

1. Positive control Well C1: MFI for Ms: 10,050, MFI for non-specific beads: 25.
   - Well C1 "corrected MFI" for Ms: 10,050 MFI – 25 MFI = 10,025 MFI
2. Positive control Well D1: MFI for Ms: 10,000, MFI for non-specific beads: 25.
   - Well D1 "corrected MFI" for Ms: 10,000 MFI – 25 MFI = 9,975 MFI
3. Positive Control mean "corrected MFI" for MS: 10,025 + 9,975 / 2 = 10,000 MFI
4. Sample well: MFI for Ms: 5,025, MFI for non-specific beads: 25.
   - Sample "corrected MFI" for Ms: 5,025 MFI – 25 MFI = 5,000 MFI
5. Sample S/P ratio for Ms: 5,000 MFI / 10,000 MFI = 0.50