Mycoplasma Synoviae and *Mycoplasma Gallisepticum* Antibody Test Kit

Poultry Check MP® MS-MG

Now fully approved as screening test by the National Poultry Improvement Plan (NPIP)

Simultaneously detecting antibodies to *Mycoplasma synoviae* & *Mycoplasma gallisepticum* in chicken serum in a single assay

Major advantages of Poultry Check MP® MS-MG

- **Enables testing for two important diseases in a single well assay** (multiplex assay)
- **Multiplexing allows saving time, labor and consumables**
- **Minimal sample volume required** (1 µL of serum)
- **Two internal controls include in each assay (well) for addressing possible pipetting error**
- **The entire assay is performed at room temperature**
- **92 samples may be tested at once** (one plate)p
- **Most of the reagents are ready to use**
- **The assay may be partially automated**
- **The test is highly sensitive, specific, and reproducible**

Kit Components	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

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

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

IMPORTANT:

- Requires a permit to release veterinary biologics from CFIA in Canada. This product is not licensed by CFIA and any claims made have not been substantiated by CFIA.
- Requires a Research and Evaluation import permit from the USDA in the US. This product is not licensed by the USDA and any claims made have not been substantiated by the USDA.

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Poultry Check MP® MS-MG

Poultry flocks are susceptible to a variety of respiratory pathogens, including several Mycoplasma species, the most important being Mycoplasma synoviae (MS) and Mycoplasma gallisepticum (MG). MS and MG causes chronic respiratory infections, especially in the presence of environmental stresses and/or other respiratory pathogens (viruses, bacteria). MG and MS strains vary in virulence and infections may sometimes remain unapparent. MS and MG infections usually result in clinical signs characterized by coryza, conjunctivitis, sneezing and coughing. MS may also cause synovitis. Lesions of the respiratory tact take the form initially of excess mucous exudate in the upper airways and sometimes fibrino-purulent exudates in the air sacs (aerosacculitis). Mycoplasmosis can result in loss of production, growth retardation in broilers, and loss of egg production in layers.

MS and MG infections can be diagnosed either by identifying the mycoplasmas in clinical samples or detecting mycoplasma specific antibodies in the serum samples. MS and MG can be cultivated from clinical samples such as swabs of tissues, tissue homogenates, exudates, and aspirates from joint cavities using special mycoplasma media. They may also be detected in clinical samples or in cultures using molecular (DNA) methods based on the polymerase chain reaction (PCR). PCR testing is usually more sensitive that cultivation as cultures are frequently overwhelmed by bacterial contaminants.

Several serological tests may be used to detect MG or MS antibodies in serum samples. They are used for flock screening rather than for testing individuals. The most commonly used tests are the serum plate agglutination test (PAT) test, the haemagglutination inhibition (HI) test, and the enzyme-linked immunosorbent assay (ELISA). PTA has a low specificity and suspect or positive samples have to be tested by either ELISA or HI for confirmation. In contrast to PTA and HI test ELISAs easily automated and are especially convenient for large scale testing.

Recently a new technology called Multiplexed Fluorometric Immuno Assay (MFIA) has been developed. MFIA allows a single small volume of sample to be used to screen for antibodies to many antigens at one time in a single well (multiplex assays). The MFIA utilizes suspensions of 6.5 µm magnetic microspheres (beads) with unique internal fluorescent dyes. Several bead sets with specific « fluorescent tag » are available. These beads are coupled to their surface with unique antigens (eg MS or MG antigens).

Bead sets and sera are added to 96 well microtitre plates. Antigen-antibody complexes formed during incubation are then detected through successive incubations with biotinylated species-specific anti-immunoglobulins (Ig) followed by streptavidin coupled to R-phycoerythrin (SA-PE). Incubations are followed by wash steps to remove unbound serum constituents and reagents.

In addition two internal controls are incorporated into the assay to evaluate sample suitability and assay function and to ensure the accuracy of results. Controls consist in a species-specific Ig bead set which confirms that the serum has been properly added and an anti-species immunoglobulin bead set which demonstrates that the labelled reagents have been properly added.

MFIA plates are read and analyzed using a microtiter plate suspension microarray fluorescence analyser controlled by a specific software. In the analyser, beads are exposed to two lasers: a red laser excites the internal dyes that identify the bead's color set corresponding to a particular antigen and a green laser excites the phycoerythrin reporter dye captured during the assay.

A minimum number of beads are read per assay and the intensity of R-phycoerythrin fluorescence is reported as a Median Fluorescence Index (MFI). MFI is an arbitrary raw unit that denotes how much reporter fluorescence of a given microsphere set carries. An S/P ratio of the SA-PE on the antigen-coated microspheres above provided threshold (cut off) indicates that antibodies to the corresponding antigen are present in the sample.

Biovet has recently developed a MFIA-based kit allowing to detect and differentiate antibodies to both MS and MG in a single assay (well). The required volume of serum sample is only 1 µL. The results are available in less than 3 hours. The test has demonstrated excellent sensitivity, specificity and reproducibility compared to reference tests such as HI and ELISA.

	MG BIOVET MFIA	MG ELISA
False neg	0 (0.00%)	0 (0.00%)
False pos	4 (1.42%)	3 (1.07%)
True neg	165 (58.72%)	166 (59.07%)
True pos	112 (39.86%)	112 (39.86%)
Total	281 (100%)	281 (100%)
Sensitivity	100.00%	100.00%
Specificity	98.58%	98.93%

	MS BIOVET MFIA	MS ELISA
False neg	1 (0.33%)	3 (1.00%)
False pos	5 (1.66%)	1 (0.33%)
True neg	105 (34.88%)	108 (35.88%)
True pos	190 (63.12%)	189 (62.79%)
Total	301 (100%)	301 (100%)
Sensitivity	99.67%	99.00%
Specificity	98.34%	99.67%

MS and MG status based on testing with USDA approved MS and MG ELISA kits and HI test.

For further information visit www.biovet-inc.com or contact customer service:

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