**Introduction**

Mycoplasma gallisepticum (Mg) remains a major pathogen throughout the commercial poultry industry. There is a high incidence of infection in broiler breeders, broilers and commercial layers worldwide.

Mg infection can be of a long duration, the organism invades the respiratory tissues and for many weeks high levels can be shed into the environment and eggs. The level of infection and shedding decreases after several weeks, however, infection can persist in the flock and birds may become intermittent shedders especially following stress.

Spread of the disease takes place from bird to bird via direct or indirect contact, but more importantly from parent to offspring through egg transmission. Monitoring of the flock status is critical for the health and welfare of the flock.

**When to Test**

The FLOCKSCREEN™ Mg ELISA Kit can be used as a screening test to confirm whether a flock has been exposed to Mg and therefore whether there are likely to be carrier birds in the flock. Significantly elevated IgG levels are detectable by ten days post infection. Where vaccines are used for Mg, the test may be used to monitor vaccination response.

**Sampling Recommendations**

As a guide, 60 birds per flock of 500 or greater need to be tested to give a 95% confidence of detection. A 1% sample is usually sufficient for vaccination monitoring.

**Assay Description**

The FLOCKSCREEN™ Mg Antibody ELISA Kit provides a rapid, simple and sensitive method of detecting antibodies to Mg in chicken serum.

Microtitre plates are supplied pre-coated with purified antigens. Diluted samples are incubated in the wells where any antibody specific to Mg binds and forms a complex. Unbound material is washed from the wells and an alkaline phosphatase labelled donkey anti-chicken IgG conjugate reagent is added, which binds to the chicken antibodies attached to Mg antigens. Unbound conjugate is washed away and PMP substrate is added to the wells. The degree of colour developed (optical density) is directly related to the amount of antibody to Mg present in the sample.

**Assay Procedure**

1. Add Sample/Controls
2. Incubate 30 mins & Wash
3. Add Enzyme Conjugate
4. Incubate 30 mins & Wash
5. Add Substrate Reagent
6. Incubate 15 mins
7. Add Stop Sol.
8. Read at 550nm
Interpretation of Results
Generally, differentiation between negative and positive samples will be very clear.
For calculation of results, an S/P ratio is required (Sample value related to Positive Control value). The following formula is applied (using mean absorbance values for controls and paired samples):

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\frac{\text{SAMPLE ABSORBANCE} - \text{NEGATIVE CONTROL ABSORBANCE}}{\text{POSITIVE CONTROL ABSORBANCE} - \text{NEGATIVE CONTROL ABSORBANCE}} = \text{S/P}
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Where samples fall within the suspect range the flock should be re-tested within 10-14 days.

Guildhay also offers a data acquisition and analysis software programme called SOFTScreen™.

This Microsoft Windows™ based programme can be run with the majority of the most popular microplate readers and collects the data from the FLOCKSCREEN tests, calculates the results and generates reports automatically.

FLOCKSCREEN™ Product Range:
- V010: S. enteritidis
- V020: S. typhimurium
- V050: Mg
- V080: CAV
- V090: IBD
- V110: EDS
- V120: NDV
- V130: IBV
- V140: ART
- V150: REO
- V160: ILT
- V170: AI
- V710: Duck Se
- V720: Duck St
**FLOCKSCREEN™**

*Mycoplasmagallisepticum (Mg)*

Antibody ELISA Kit

Cat.No. V050/V054

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![Assay Procedure Diagram](attachment:image.png)
### Kit Contents

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<tr>
<th>Kit Contents</th>
<th>2 Plate Kit</th>
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</tr>
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<tr>
<td>1</td>
<td><strong>Kit Contents</strong></td>
<td>2 x 96 well plates pre-coated with inactivated Mg antigen (supplied as 2 well holders each containing 12 x 8-well strips).</td>
</tr>
<tr>
<td>2</td>
<td>Positive Control with antibodies to Mg preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (500µl ready to use).</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>3</td>
<td>Negative Control with SPF chicken serum preserved in phosphate buffer with protein stabiliser and ProClin 0.063% v/v. (500µl ready to use).</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>4</td>
<td>Enzyme Conjugate Reagent, containing alkaline phosphatase labelled donkey anti-chicken IgG in tris buffer with an inert blue dye and sodium azide 0.1% w/v. (11ml)</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>5</td>
<td>ELISA Substrate Reagent, containing phenolphthalein monophosphate and enzyme co-factors in a diethanolamine buffer. (11ml)</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>6</td>
<td>Wash Buffer Concentrate, containing phosphate buffer with ProClin 0.63% v/v. (50ml) - sufficient to make up 1000ml of wash buffer.</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>7</td>
<td>Sample Diluent Concentrate, containing phosphate buffer with protein stabiliser and ProClin 0.63% v/v. (50ml) - sufficient to make up 500ml of sample diluent.</td>
<td><strong>Kit Contents</strong></td>
</tr>
<tr>
<td>8</td>
<td>6 adhesive ELISA microtitre plate covers for use during incubation.</td>
<td><strong>Kit Contents</strong></td>
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</table>

### Materials and Equipment Required (Not Supplied)

In order to run the FLOCKSCREEN™ assays, the following equipment is recommended:

1. Precision pipettes: 5µl (or variable 1-20µl)
   50µl (or variable 10-200µl)
   50µl repeater or an 8 or 12 channel
   2.5ml (or variable 1-5ml)

2. Disposable tips for pipettes
3. Microtitre Plate Reader with 550nm filter
4. Microtitre Plate Washer
5. +37°C incubator
6. Distilled or deionised water
7. Disposable 5ml plastic tubes

It is possible to run the assays without the 50µl repeater, or an 8 channel pipette. It is also possible to use a wash bottle for plate washing instead of a plate washer. The results will however be less consistent.

### Reagent Preparation

1. Allow all reagents to come to room temperature before use.
2. The Wash Buffer Concentrate and Sample Diluent Concentrate may partly recrystallize. This is due to the high concentration of salts. Should crystallisation occur, simply shake the bottle prior to reconstituting as described in the next two steps. The crystals will dissolve readily upon mixing.
3. To prepare sample diluent buffer, add the Sample Diluent Concentrate (50ml) to distilled or deionised water and make up to a total volume of 500ml. This sample diluent can be stored at +4°C for up to 3 months, and can be used for preparing samples for any of the FLOCKSCREEN™ kits.
4. To prepare the wash buffer, add the Wash Buffer Concentrate (50ml) to distilled or deionised water and make up to a total volume of 1 litre. This is stable at room temperature for 3 months and can be used with any of the FLOCKSCREEN™ kits.
5. **DO NOT DILUTE THE POSITIVE AND NEGATIVE CONTROLS.**
Sample Preparation

Serum Samples: These should be as fresh and clean as practicable and stored at +4°C (up to 2 days) or for longer term storage keep at -20°C. Make a 1:500 dilution of each test sample in sample diluent buffer by adding 2.5ml of reconstituted sample diluent to 5µl of serum in a disposable 5ml plastic tube. Invert gently 2 or 3 times to mix. Alternatively a 2-step dilution protocol using dilution plates may be followed using a minimum of 5µl of sample. Diluted samples can be kept for several days at +4°C for retesting or at -20°C for longer term storage.

Warnings and Precautions

1. This kit is for IN VITRO use only.
2. Optimum results will be obtained by strict adherence to this protocol. Careful pipetting and washing are necessary to achieve good assay performance.
3. The assay has been developed with incubations at +37°C for more consistent results. This eliminates problems associated with varying room temperature conditions.
4. Plates are coated with purified inactivated bacterial antigens and control sera have been filtered with a 0.2µm filter. However, because your sample sera may be infected with bacteria or viruses, all reagents should be treated as potential biohazards and handled appropriately.
5. Do not intermix reagents from different Lot numbers with the exceptions of wash buffer and sample diluent.
6. The Substrate Reagent is very sensitive and under no circumstances should the same pipette tips or containers used for other reagents be used with the Substrate Reagent. The Substrate Reagent should be yellow in colour before addition to the wells. An orange, brown or pink colour indicates deterioration or contamination and the reagent should not be used.
7. Caution should be exercised in the handling of alkaline or other hazardous chemicals in accordance with Good Laboratory Practice.
8. Never pipette by mouth.
9. Wash solution and waste should be properly decontaminated with bleach or other strong oxidising agents before disposal.

Assay Procedure

1. Remove the pre-coated plates from their sealed bags and record sample and control locations on a 12 x 8 template sheet. Each sample should be run in duplicate for optimum results. The positive and negative controls should always be run in duplicate.
2. Add 50µl of the undiluted controls and diluted samples to the appropriate wells. Diluted samples should be retained at +4°C until successful results are confirmed. Cover the plate with an adhesive cover and incubate at +37°C for 30 minutes. Mix on a plate shaker or by gently tapping the side of the plate.
3. Remove adhesive cover and wash the plate 4 times with wash buffer (300µl per well), invert and tap firmly on absorbent paper. N.B. To reduce the possibility of sample carryover, it is recommended where possible, that the plate washer is programmed to wash each strip individually four times before washing the next strip.
4. Add 50µl of Enzyme Conjugate Reagent to each well. Mix on a plate shaker or by gently tapping the side of the plate.
5. Cover the plate with the adhesive cover and incubate at +37°C for 30 minutes.
6. Remove adhesive cover and wash the plate 4 times with wash buffer (300µl per well), invert and tap firmly on absorbent paper.
7. Add 50µl ELISA Substrate Reagent to each well. The reagent must be at room temperature to achieve maximum colour development. Mix on a plate shaker or by gently tapping the side of the plate.
8. Cover the plate with the adhesive cover and incubate at +37°C for 15 minutes. Colour development is pale pink, which deepens on addition of ELISA Stop Solution.
9. Remove adhesive cover and add 50µl ELISA Stop Solution to each well. Mix on a plate shaker to obtain full colour development.
10. Wipe the under surface of the plate free of dust etc. with a soft tissue. Read the plate using a Microtitre Plate Reader at 550nm having first blanked on air. In order to obtain optimum results the plate should be read within 15 minutes of adding the ELISA Stop Solution.
**Results**
For the test to be valid:

a) Mean Negative control absorbance must be < 0.2

b) Mean Positive control absorbance must be at least 5 times the Mean Negative Absorbance.

It is important that the results fall within these parameters in order to prove that the components of the kit are all in good condition and that there have been no operator errors.

**Interpretation of Results**
Generally, differentiation between negative and positive samples will be very clear.

For calculation of results, an S/P ratio is required (Sample value related to Positive Control value). The following formula is applied (using mean absorbance values for controls and paired samples):

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Where samples fall within the suspect range the flock should be re-tested within 10-14 days.

**Storage and Stability**
All reagents should be stored at +4°C on delivery. Do not freeze.

Avoid exposure to sunlight.

Do not use after the stated expiry date.

Do not use if silica gel desiccant in the pouch containing the microtitre plate is pink.

Any unused strips should be resealed in the re-sealable foil pouch together with the silica gel.

**ONCE A KIT HAS BEEN OPENED IT HAS A MAXIMUM SHELF-LIFE OF 3 MONTHS**