Edition: BP 2025 (Ph. Eur. 11.6 update)

Mycoplasma Gallisepticum Vaccine (Inactivated)

General Notices

(Ph. Eur. monograph 1942)

Ph Eur

1 DEFINITION

Mycoplasma gallisepticum vaccine (inactivated) is a preparation of one or more suitable strains of *Mycoplasma gallisepticum* that have been inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of chickens and/or turkeys.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

Production of the vaccine is based on a seed-lot system. The seed material is cultured in a suitable solid and/or liquid medium to ensure optimal growth under the chosen incubation conditions. Each strain is cultivated separately and identity is verified using a suitable method. During production, various parameters such as growth rate are monitored by suitable methods; the values are within the limits approved for the particular vaccine. Purity and identity of the harvest are verified using suitable methods. After cultivation, the mycoplasma suspensions are collected separately and inactivated by a suitable method. The mycoplasma suspensions may be treated to fragment the mycoplasmas and the fragments may be purified and concentrated. The vaccine may contain an adjuvant.

2-2 CHOICE OF VACCINE COMPOSITION

The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) in the target animals. The following tests for safety (section 2-2-1) and immunogenicity (section 2-2-2) may be used during the demonstration of safety and efficacy. If the indications for the vaccine include protection against a drop in laying performance or protection against infectious sinusitis in turkeys, further suitable immunogenicity testing is necessary.

2-2-1 Safety

The test is carried out for each route of administration to be recommended for vaccination and for each avian species for which the vaccine is intended. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test performed in birds younger than 3 weeks of age, use not fewer than 10 birds not older than the minimum age to be recommended for vaccination. For each test performed in birds older than 3 weeks of age, use not fewer than 8 birds not older than the minimum age to be recommended for vaccination. In the case of chickens, use chickens from a flock free from specified pathogens (SPF) (*5.2.2*) and in the case of turkeys, use birds that have not been vaccinated and that do not have antibodies against *M. gallisepticum*. Administer by a route and method to be recommended to each bird 1 dose of vaccine. If the schedule to be recommended requires a 2nd dose, administer 1 dose to each bird after an interval of at least 14 days. Observe the birds at least daily for at least 14 days after the last administration of the vaccine.

The test is not valid if more than 10 per cent of the birds younger than 3 weeks of age show abnormal signs of disease or die from causes not attributable to the vaccine. For birds older than 3 weeks of age, the test is not valid if non-specific mortality occurs.

The vaccine complies with the test if no bird shows abnormal signs of disease or dies from causes attributable to the vaccine.

2-2-2 Immunogenicity

The test is carried out for each recommended route of administration and for each avian species for which the vaccine is intended. Use for each test not fewer than 40 birds not older than the minimum age to be recommended for vaccination. Use chickens from an SPF flock (5.2.2) or turkeys that have not been vaccinated and are free from antibodies against *M. gallisepticum*. For each test, administer to each of not fewer than 20 birds a quantity of the vaccine not greater than a single dose. If re-vaccination is recommended, repeat this operation after the recommended interval. Maintain not fewer than 20 birds as controls. Challenge each bird from both groups not more than 28 days after the last administration by a suitable route with a sufficient quantity of virulent *M. gallisepticum* (R-strain). Observe the birds at least daily for 14 days after challenge. Evaluation is carried out 14 days after challenge, at which point the birds are euthanised. Record the deaths and the number of surviving birds that show clinical signs of disease (e.g. respiratory distress, nasal discharge), and record air sac lesions.

The test is not valid if:

— during the observation period after challenge, fewer than 70 per cent of the controls die or show lesions or clinical signs of disease; and/or

— during the period between vaccination and challenge, more than 10 per cent of the birds from the control group or from the vaccinated group show abnormal clinical signs of disease or die from causes not attributable to the vaccine.

Thoracic and abdominal air sacs are evaluated individually on each side of the animal. The scoring system presented below may be used. The vaccine complies with the test if the score for the vaccinated birds is significantly lower than that for the controls and if the reduction is not less than 30 per cent.

0	no air sac lesions
1	in a limited area of 1 or 2 air sacs: cloudiness with slight thickening of the air sac membrane or flecks of yellowish exudate
2	in 1 air sac or portions of 2 air sacs: greyish or yellow, sometimes foamy exudate, with thickening of the air sac membrane
3	in 3 air sacs: extensive exudate, with clear thickening of most air sacs
4	severe air-sacculitis with considerable exudate and thickening of most air sacs.

2-3 MANUFACTURER'S TESTS

2-3-1 Residual live mycoplasmas

A validated test for residual live mycoplasmas is carried out using a culture method (for example 2.6.7, using media shown to be suitable for *M. gallisepticum*). The inactivated mycoplasma harvest complies with the test if no live mycoplasmas are detected.

2-3-2 Batch potency test

It is not necessary to carry out the potency test (section 3-4) for each batch of the vaccine if it has been carried out using a batch of vaccine with a minimum potency. Where the test is not carried out on a batch, an alternative validated method is used, the criteria for acceptance being set with reference to a batch of vaccine that has given satisfactory results in the potency test (section 3-4). The following test may be used.

Use not fewer than 13 chickens, 3-4 weeks old, from an SPF flock (5.2.2) or not fewer than 15 turkeys, 3-4 weeks old, that have not been vaccinated against *M. gallisepticum*, do not have antibodies against *M. gallisepticum*, and are obtained from a healthy flock. Collect serum samples from each vaccinate and control bird just before vaccination and check for the absence of antibodies against *M. gallisepticum*. Administer to each of not fewer than 10 birds 1 dose of the vaccine by a recommended route. Maintain not fewer than 3 birds as controls. Collect serum samples 5 weeks after vaccination from each vaccinated and control bird. Measure the titres of serum antibodies against *M. gallisepticum* using a suitable method.

Calculate the mean titres for the group of vaccinates. The test is not valid if specific *M. gallisepticum* antibodies are found in any serum samples from the control birds 5 weeks after the time of administration of the vaccine. The vaccine complies with the test if the mean antibody titres of the group of vaccinates are equal to or greater than the titres obtained with a batch that has given satisfactory results in the potency test (section 3-4).

3 BATCH TESTS

3-1 Identification

The vaccine contains the antigen or antigens stated under Definition.

3-2 Bacteria and fungi

The vaccine, including where applicable the diluent supplied for reconstitution, complies with the test for sterility prescribed in the monograph *Vaccines for veterinary use (0062)*.

3-3 Residual live mycoplasmas

This test may be omitted for batch release, as stated in the monograph Vaccines for veterinary use (0062).

Carry out a validated test for residual live mycoplasmas to confirm inactivation of *M. gallisepticum* using a culture method (for example *2.6.7*, using media shown to be suitable for *M. gallisepticum*). The vaccine complies with the test if no live mycoplasmas are detected.

3-4 Potency

The vaccine complies with the test for immunogenicity (section 2-2-2).