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

Porcine Enzootic Pneumonia Vaccine (Inactivated)

General Notices

(Ph. Eur. monograph 2448)

Ph Eur

1 DEFINITION

Porcine enzootic pneumonia vaccine (inactivated) is a preparation of a suitable strain of *Mycoplasma hyopneumoniae* that has been inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of pigs against enzootic pneumonia caused by *M. hyopneumoniae*.

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. The identity of the strain 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 of the harvest is verified using a suitable method.

After cultivation, the mycoplasma suspension is collected and inactivated by a suitable method. 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*) for the pigs for which it is intended.

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.

2-2-1 Safety

2-2-1-1 Laboratory tests. Carry out the test for each route and method of administration to be recommended for vaccination and where applicable, in pigs of each category for which the vaccine is intended, using in each case pigs not older than the minimum age to be recommended for vaccination. Use a batch of vaccine containing not less than the maximum potency that may be expected in a batch of vaccine.

For each test, use not fewer than 8 pigs that do not have antibodies against *M. hyopneumoniae*. Administer to each pig 1 dose of the vaccine. If the schedule to be recommended requires a 2nd dose, administer another dose after an interval of at least 14 days. Observe the pigs at least daily until at least 14 days after the last administration. Record body

temperature the day before vaccination, at vaccination, 4 h later and then daily for 4 days; note the maximum temperature increase for each pig.

The vaccine complies with the test if no pig shows notable signs of disease or dies from causes attributable to the vaccine, and, in particular, if the average body temperature increase for all pigs does not exceed 1.5 °C and no pig shows a rise greater than 2.0 °C.

2-2-1-2 Field studies. The animals used for field trials are also used to evaluate safety. Carry out a test in each category of animals for which the vaccine is intended. Use not fewer than 3 groups each of not fewer than 20 animals with corresponding groups of not fewer than 10 controls. Examine the injection site for local reactions after vaccination. Record body temperature the day before vaccination, at vaccination, at the time interval after which a rise in temperature, if any, was seen in test 2-2-1-1, and daily during the 2 days following vaccination; note the maximum temperature increase for each animal.

The vaccine complies with the test if no animal shows notable signs of disease or dies from causes attributable to the vaccine, the average body temperature increase for all animals does not exceed 1.5 °C, and no animal shows a rise in body temperature greater than 2.0 °C.

2-2-2 Immunogenicity

A test is carried out for each route and method of administration to be recommended using in each case pigs not older than the minimum age to be recommended for vaccination. The vaccine to be administered to each pig is of minimum potency.

Use not fewer than 20 pigs that do not have antibodies against *M. hyopneumoniae* and that are from a herd or herds where there are no signs of enzootic pneumonia and that have not been vaccinated against *M. hyopneumoniae*. Vaccinate not fewer than 12 pigs according to the schedule to be recommended. Maintain not fewer than 8 non-vaccinated pigs as controls. Challenge each pig at least 14 days after the last vaccination by the intranasal or intratracheal route or by aerosol with a sufficient quantity of a virulent strain of *M. hyopneumoniae*. The challenge strain used is different from the vaccine strain. 21-30 days after challenge, euthanise the pigs. Conduct a post-mortem examination on each pig in order to evaluate the extent of lung lesions using a validated lung lesion scoring system that is adapted to the age of the animals. The following scoring system may be used.

A weighted score is allocated to each of the 7 lobes of the lungs according to the relative weight of the lung lobes.

Lobes	**Left**	**Right**
Apical	5	11
Cardiac	6	10
Diaphragmatic	29	34
Intermediate	5	

The vaccine complies with the test if the vaccinated pigs, when compared with controls, show a significant reduction in the lung lesion score.

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. hyopneumoniae*). 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, 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 test described under Potency. A quantification of the antigen (i.e. an *in vitro* test using a reference vaccine that has given satisfactory results in the test described under Potency) together with a test for adjuvant quantification may be used as an alternative method provided the antigen that is measured has been proven to be protective and/or immunorelevant.

Alternatively, a test measuring induction of antibody response in laboratory animals may be used. The following method is given as an example.

Use at least 5 mice weighing 18-20 g and that do not have antibodies against *M. hyopneumoniae*. Vaccinate each mouse by the subcutaneous route with a suitable dose. Maintain not fewer than 5 mice as controls. Where the recommended schedule requires a booster injection to be given, a booster vaccination may also be given in this test provided it has been demonstrated that this will still provide a suitably sensitive test system. Before the vaccination and at a given interval within the range of 14-21 days after the last injection, collect blood from each mouse and prepare serum samples. Determine individually for each serum the titre of specific antibodies against each antigenic component stated on the label, using a suitable validated test such as enzyme-linked immunosorbent assay (*2.7.1*).

The vaccine complies with the test if the mean antibody levels are not significantly lower than those obtained for a batch that has given satisfactory results in the test described under Potency.

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. hyopneumoniae* using a culture method (for example *2.6.7*, using media shown to be suitable for *M. hyopneumoniae*). The vaccine complies with the test if no live mycoplasmas are detected.

3-4 Potency

The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-2) when administered by a recommended route and method.