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Equine Influenza Vaccine, Inactivated

General Notices

(Equine Influenza Vaccine (Inactivated), Ph. Eur. monograph 0249)

Ph Eur

1 DEFINITION

Equine influenza vaccine (inactivated) is a preparation of one or more suitable strains of equine influenza virus, inactivated while maintaining adequate immunogenic properties. Suitable strains contain both haemagglutinin and neuraminidase. This monograph applies to vaccines intended for the active immunisation of horses against equine influenza.

2 PRODUCTION

2-1 PREPARATION OF THE VACCINE

Each strain of virus is grown separately in embryonated hens' eggs or in cell cultures. The viral suspensions may be purified and concentrated. The antigen content of the vaccine is based on the haemagglutinin content of the viral suspensions determined as described under Manufacturer's tests; the amount of haemagglutinin for each strain is not less than that in the vaccine shown to be satisfactory in the test for potency. The vaccine may be adjuvanted.

2-2 SUBSTRATE FOR VIRUS PROPAGATION

2-2-1 Embryonated hens' eggs

If the vaccine virus is grown in embryonated hens' eggs, they are obtained from a healthy flock (*5.2.13*).

2-2-2 Cell cultures

If the vaccine virus is grown in cell cultures, they comply with the requirements for cell cultures for production of veterinary vaccines (*5.2.4*).

2-3 CHOICE OF VACCINE COMPOSITION

The choice of strains used in the vaccine is based on epidemiological data. The World Organisation for Animal Health (OIE, formerly the *Office international des épizooties*) reviews the epidemiological data periodically and if necessary recommends new strains corresponding to prevailing epidemiological evidence. Such strains are used in accordance with the regulations in force in the signatory states of the Convention on the Elaboration of a European Pharmacopoeia.

The vaccine is shown to be satisfactory with respect to safety (*5.2.6*) and efficacy (*5.2.7*) for the horses for which it is intended. Where a particular breed of horse is known to be especially sensitive to the vaccine, horses from that breed are

included in the tests for safety.

The following tests for safety (section 2-3-1) and immunogenicity (section 2-3-2) may be used during the demonstration of safety and efficacy.

2-3-1 Safety

Carry out the test for each route and method of administration to be recommended for vaccination and in horses of each category 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.

Use for the test not fewer than 8 horses that preferably do not have antibodies against equine influenza virus or, where justified, use horses with a low level of such antibodies as long as they have not been vaccinated against equine influenza and administration of the vaccine does not cause an anamnestic response. Administer to each horse 1 dose of the vaccine, then another dose after at least 14 days. Observe the horses at least daily until at least 14 days after the last administration.

The vaccine complies with the test if no horse shows abnormal local or systemic reactions or dies from causes attributable to the vaccine during the 28 days of the test.

2-3-2 Immunogenicity

The test described under 2-3-2-1 is suitable to demonstrate the immunogenicity of the strains present in the vaccine.

A test with virulent challenge is carried out for at least one vaccine strain (see test under 2-3-2-1). For other strains in the vaccine, demonstration of immunogenicity may, where justified, be based on the serological response induced in horses by the vaccine (see tests under 2-3-2-2); justification for protection against these strains may be based on published data on the correlation of the antibody titre with protection against antigenically related strains.

Where serology is used, the test is carried out as described under 2-3-2-1 but instead of virulent challenge, a blood sample is drawn 2 weeks after the last vaccination and the antibody titre of each serum is determined by a suitable immunochemical method (*2.7.1*), such as the single radial haemolysis test or the haemagglutination-inhibition test shown below; a reference serum is used to validate the test. The acceptance criteria depend on the strain and are based on available data; for A/equine-2 virus, vaccines have usually been found satisfactory if the antibody titre of each serum is not less than 85 mm^2 where the single radial haemolysis test is used, or not less than 1:64 (before mixture with the suspension of antigen and erythrocytes) where the haemagglutination-inhibition test is used.

Equine influenza subtype 1 strain A/eq/Newmarket/77 horse antiserum BRP, *equine influenza subtype 2 European-like strain A/eq/Newmarket/2/93 horse antiserum BRP*, *equine influenza subtype 2 American-like strain A/eq/Newmarket/1/93 horse antiserum BRP*, *equine influenza subtype 2 American-like strain A/eq/South Africa/4/03 horse antiserum BRP* and *equine influenza subtype 2 American-like strain A/eq/Richmond/1/2007 horse antiserum BRP* are suitable for use as reference sera for the single radial haemolysis test.

The claims for the product reflect the type of immunogenicity demonstrated (protection against challenge or antibody production).

2-3-2-1 Protection from signs of disease and reduction of virus excretion. Carry out the immunogenicity test using a challenge strain against which the vaccine is stated to provide protection. Use where possible a recent isolate.

A test is carried out for each route and method of administration to be recommended, using in each case horses not less than 6 months old. The vaccine administered to each horse is of minimum potency.

Use for the test not fewer than 10 horses that do not have antibodies against equine influenza virus. Draw a blood sample from each horse and test individually for antibodies against equine influenza virus to determine seronegativity. Vaccinate not fewer than 6 horses according to the schedule to be recommended. Maintain not fewer than 4 horses as controls. Draw a second blood sample from each vaccinated horse 7 days after the first vaccination and test individually for antibodies against equine influenza virus, to detect an anamnestic sero-response. Horses showing sero-conversion at this stage are excluded from the test. At least 2 weeks after the last vaccination, challenge each horse by aerosol with a quantity of equine influenza virus sufficient to produce characteristic signs of disease such as fever, nasal discharge and coughing in a susceptible horse. Observe the horses at least daily for 14 days. Collect nasal swabs daily from each individual horse to isolate the virus.

The vaccine complies with the test if the vaccinated horses show no more than slight signs; the controls show characteristic signs. The average number of days on which virus is excreted and the respective virus titres are significantly lower in vaccinated horses than in control horses.

2-3-2-2 Presence of antibodies after vaccination

2-3-2-2-1 Single radial haemolysis. Heat each serum at 56 °C for 30 min. Perform tests on each serum using respectively the antigen or antigens prepared from the strain(s) used in the production of the vaccine. Mix 1 mL of sheep erythrocyte suspension in barbital buffer solution (1 volume of erythrocytes in 10 volumes of final suspension) with 1 mL of a suitable dilution of the influenza virus strain in barbital buffer solution and incubate the mixture at 4 °C for 30 min. To 2 mL of the virus/erythrocyte mixture, add 1 mL of a 3 g/L solution of *chromium(III) trichloride hexahydrate R*, mix and allow to stand for 10 min. Heat the sensitised erythrocytes to 47 °C in a water-bath. Mix 15 mL of a 10 g/L solution of *agarose for electrophoresis R* in barbital buffer solution, 0.7 mL of sensitised erythrocyte suspension and the appropriate amount of diluted guinea-pig complement in barbital buffer solution at 47 °C. Pour the mixture into Petri dishes and allow the agar to set. Punch holes in the agar layer and place in each hole 5 µL of the undiluted serum to be tested or the control serum. Incubate the Petri dishes at 37 °C for 18 h. Measure the diameter of the haemolysis zone and calculate its area, which expresses the antibody titre, in square millimetres.

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2-3-2-2-2 Haemagglutination-inhibition test. Inactivate each serum by heating at 56 °C for 30 min. To 1 volume of each serum add 3 volumes of *phosphate buffered saline pH 7.4 R* and 4 volumes of a 250 g/L suspension of *light kaolin R* in the same buffer solution. Shake each mixture for 10 min. Centrifuge, collect the supernatant and mix with a concentrated suspension of chicken erythrocytes. Allow to stand at 37 °C for 60 min and centrifuge. The dilution of the serum obtained is 1:8. Perform tests on each serum using each antigen prepared from the strains used in the production of the vaccine. Using each diluted serum, prepare a series of 2-fold dilutions. To 0.025 mL of each of the latter dilutions add 0.025 mL of a suspension of antigen treated with *ether R* and containing 4 haemagglutinating units. Allow the mixture to stand for 30 min and add 0.05 mL of a suspension of chicken erythrocytes containing 2×10^7 erythrocytes/mL. Allow to stand for 1 h and note the last dilution of serum that still completely inhibits haemagglutination.

2-4 MANUFACTURER'S TESTS

2-4-1 Residual live virus

The test for residual live virus is carried out using method 2-4-1-1 or method 2-4-1-2, whichever is more sensitive. The quantity of inactivated virus harvest used is equivalent to not less than 10 doses of vaccine.

2-4-1-1 *Test in cell cultures*. Inoculate the inactivated virus harvest into suitable cells; after incubation for 8 days, make a subculture. Incubate for a further 6-8 days. Harvest about 0.1 mL of the supernatant and examine for live virus by a haemagglutination test. If haemagglutination is found, carry out a further passage in cell culture and test for haemagglutination; the inactivated virus harvest complies with the test if no haemagglutination occurs.

2-4-1-2 *Test in embryonated eggs*. Inoculate 0.2 mL into the allantoic cavity of each of 10 embryonated eggs and incubate at 33-37 °C for 3-4 days. The test is invalid unless not fewer than 8 of the 10 embryos survive. Harvest 0.5 mL of the allantoic fluid from each surviving embryo and pool the fluids. Inoculate 0.2 mL of the pooled fluid into a further 10 embryonated eggs and incubate at 33-37 °C for 3-4 days. The test is invalid unless at least 8 of the 10 embryonated embryos survive. Harvest about 0.1 mL of the allantoic fluid from each surviving embryo and examine each individual harvest for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out a further passage of that fluid in eggs and test for haemagglutination; the inactivated virus harvest complies with the test if no haemagglutination occurs.

2-4-2 Batch potency test

It is not necessary to carry out the potency test (section 3-3) for each batch of 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(s) described under Potency. The following test may be used.

Use 5 guinea-pigs that do not have specific antibodies. Vaccinate each guinea-pig by the subcutaneous route with one dose of vaccine. 21 days later, collect blood samples and separate the serum. Carry out tests on the serum for specific antibodies by a suitable immunochemical method (*2.7.1*) such as single radial haemolysis or haemagglutination-inhibition, using reference sera to validate the test. The vaccine complies with the test if the antibody titres are not significantly lower than those obtained in guinea-pigs with a reference batch of vaccine shown to have satisfactory potency in horses.

2-4-3 Bacterial endotoxins

For vaccines produced in eggs, the content of bacterial endotoxins is determined on the virus harvest to monitor production.

2-4-4 Haemagglutinin content

The content of haemagglutinin in the inactivated virus suspension, after purification and concentration where applicable, is determined by a suitable immunochemical method (*2.7.1*), such as single radial immunodiffusion, using a suitable haemagglutinin reference preparation; the inactivated virus suspension complies with the test if the content is shown to be within the limits shown to allow preparation of a satisfactory vaccine.

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 Potency

The vaccine complies with the requirements of the test(s) mentioned under Immunogenicity (section 2-3-2) when administered by a recommended route and method.