2-3-2. **Bacterial endotoxins.** A test for bacterial endotoxins (2.6.14) is carried out on the final lot or, where the nature of the adjuvant prevents performance of a satisfactory test, on the bulk antigen or the mixture of bulk antigens immediately before addition of the adjuvant. The maximum acceptable amount of bacterial endotoxins is that found for a batch of vaccine that has been shown satisfactory in safety test (section 2-2-1). The method chosen for determining the maximum acceptable amount of bacterial endotoxins is used subsequently for testing each batch.

3. **BATCH TESTS**

3-1. **Identification.** When injected into SPF chickens (5.2.2), the vaccine stimulates the production of antibodies against each of the serovars of *P. multocida* in the vaccine.

3-2. **Bacteria and fungi.** The vaccine and, where applicable, the liquid supplied with it comply with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062).

3-3. **Safety.** For vaccines recommended for use in chickens, use not fewer than 10 chickens from an SPF flock (5.2.2) and of the minimum age recommended for vaccination. For vaccines recommended for use only in turkeys, ducks or geese, use not fewer than 10 birds of the species likely to be sensitive to the vaccine and, where applicable, immunogenicity. 2-2. The vaccine virus is grown in embryonated hens’ eggs or in cell cultures.

3-4. **Potency.** The vaccine complies with the requirements of the test mentioned under Immunogenicity (section 2-2-3) when administered by a recommended route and method.

**LABELLING**

The label states:
- the serovar(s) used to prepare the vaccine,
- the serovar(s) against which protection is claimed.

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**FOWL-POX VACCINE (LIVE)**

*Vaccinum variolae gallinacea vivum*

**1. DEFINITION**

Fowl-pox vaccine (live) is a preparation of a suitable strain of avian pox virus. This monograph applies to vaccines intended for administration to chickens for active immunisation.

**2. PRODUCTION**

2-1. **PREPARATION OF THE VACCINE**

The vaccine virus is grown in embryonated hens’ eggs or in cell cultures.

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 flocks free from specified pathogens (SPF) (5.2.2).

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. **SEED LOTS**

2-3-1. **Extraneous agents.** The master seed lot complies with the tests for extraneous agents in seed lots (2.6.2). In these tests on the master seed lot, the organisms used are not more than 5 passages from the master seed lot at the start of the tests.

2-4. **CHOICE OF VACCINE VIRUS**

The vaccine virus shall be shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) for the chickens for which it is intended.

The following tests for safety (section 2-4-1), increase in virulence (section 2-4-2) and immunogenicity (section 2-4-3) may be used during demonstration of safety and immunogenicity.

2-4-1. **Safety.** Carry out the test for each route and method of administration to be recommended for vaccination using in each case chickens not older than the youngest age to be recommended for vaccination. Use vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. For each test use not fewer than 20 chickens, from an SPF flock (5.2.2).

Administer to each chicken a quantity of the vaccine virus equivalent to not less than 10 times the maximum virus titre likely to be contained in a dose of the vaccine. Observe the chickens at least daily for 21 days. The test is not valid if more than 10 per cent of the chickens die from causes not attributable to the vaccine virus. The vaccine virus complies with the test if no chicken shows notable clinical signs of fowl pox or dies from causes attributable to the vaccine virus.

2-4-2. **Increase in virulence.** Administer by a suitable route a quantity of the vaccine virus that will allow recovery of virus for the passages described below to each of 5 chickens not older than the minimum age to be recommended for vaccination and from an SPF flock (5.2.2). Use the vaccine virus at the least attenuated passage level that will be present between the master seed lot and a batch of the vaccine. Prepare 4 to 7 days after administration a suspension from the induced skin lesions of each chicken and pool these samples. Administer 0.2 ml of the pooled samples by cutaneous scarification of the comb or other unfeathered part of the body, or by another suitable method to each of 5 other chickens not older than the minimum age to be recommended for vaccination and from an SPF flock (5.2.2).

Carry out this passage operation not fewer than 5 times; verify the presence of the virus at each passage. Care must be taken to avoid contamination by virus from previous passages. If the virus is not found at a passage level, carry out a second series of passages. Carry out the test for safety (section 2-4-1) using the unpassaged vaccine virus and the maximally passaged virus that has been recovered. Administer the virus by the route recommended for vaccination likely to be the least safe. The vaccine virus complies with the test if no indication of increase in virulence of the maximally passaged virus compared with the unpassaged virus is observed. If virus is not recovered at any passage level in the first and second series of passages, the vaccine virus also complies with the test.

2-4-3. **Immunogenicity.** A test is carried out for each route and method of administration to be recommended using in each case chickens not older than the youngest age to be recommended for vaccination. The quantity of the vaccine virus administered to each chicken is not greater than the minimum virus titre to be stated on the label and the virus is at the most attenuated passage level that will be present in a batch of the vaccine. Use for the test not fewer than 30 chickens of the same origin and from an SPF flock (5.2.2). Vaccinate by a recommended route not fewer
Furunculosis vaccine (inactivated) for salmonids

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than 20 chickens. Maintain not fewer than 10 chickens as controls. Challenge each chicken after 21 days by the feather-follicle route with a sufficient quantity of virulent fowl-pox virus. Observe the chickens at least daily for 21 days after challenge. Record the deaths and the number of surviving chickens that show clinical signs of disease. Examine each surviving chicken for macroscopic lesions: cutaneous lesions of the comb, wattle and other unfeathered areas of the skin and diphtheritic lesions of the mucous membranes of the oro-pharyngeal area.

The test is not valid if:

- during the observation period after challenge fewer than 90 per cent of the control chickens die or show severe clinical signs of fowl pox, including notable macroscopical lesions of the skin or mucous membranes of the oro-pharyngeal area,

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

The vaccine virus complies with the test if during the observation period after challenge not less than 90 per cent of the vaccinated chickens survive and show no notable clinical signs of disease, including macroscopical lesions of the skin and mucous membranes of the oro-pharyngeal area.

3. BATCH TESTS

3-1. Identification. Carry out an immunostaining test in cell cultures to demonstrate the presence of the vaccine virus. For egg adapted strains, inoculate the vaccine into eggs and notice the characteristic lesions.

3-2. Bacteria and fungi

Vaccines intended for administration by injection, scarification or piercing of the wing web comply with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062).

Vaccines not intended for administration by injection, scarification or piercing of the wing web either comply with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062) or with the following test: carry out a quantitative test for bacterial and fungal contamination; carry out identification tests for microorganisms detected in the vaccine; the vaccine does not contain pathogenic microorganisms and contains not more than 1 non-pathogenic microorganism per dose.

Any liquid supplied with the vaccine complies with the test for sterility prescribed in the monograph Vaccines for veterinary use (0062).

3-3. Mycoplasmas. The vaccine complies with the test for mycoplasmas (2.6.7).

3-4. Extraneous agents. The vaccine complies with the tests for extraneous agents in batches of finished product (2.6.25).

3-5. Safety. Use not fewer than 10 chickens from an SPF flock (5.2.2) of the youngest age recommended for vaccination. For vaccines recommended for use in chickens older than 6 weeks, chickens 6 weeks old may be used. Administer 10 doses of the vaccine to each chicken by a recommended route. Observe the chickens at least daily for 21 days. The test is not valid if more than 20 per cent of the chickens show abnormal clinical signs or die from causes not attributable to the vaccine.

The vaccine complies with the test if no chicken shows notable clinical signs of disease or dies from causes attributable to the vaccine.

3-6. Virus titre. Titrate the vaccine virus by inoculation into embryonated hens’ eggs from an SPF flock (5.2.25) or into suitable cell cultures (5.2.4). The vaccine complies with the test if 1 dose contains not less than the minimum virus titre stated on the label.

3-7. Potency. The vaccine complies with the requirements of the test prescribed under Immunogenicity (section 2-4.3) when administered according to the recommended schedule by a recommended route and method. It is not necessary to carry out the potency test for each batch of the vaccine if it has been carried out on a representative batch using a vaccinating dose containing not more than the minimum virus titre stated on the label.

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FURUNCULOSIS VACCINE
(INACTIVATED, OIL-ADJUVANTED, INJECTABLE) FOR SALMONIDS

Vaccinum furunculosidis ad salmonidas
inactivatum cum adiuvatione oleosa
ad injectionem

1. DEFINITION

Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids is prepared from cultures of one or more suitable strains of *Aeromonas salmonicida* subsp. *salmonicida*, inactivated while maintaining adequate immunogenic properties. This monograph applies to vaccines intended for the active immunisation of salmonids against furunculosis.

2. PRODUCTION

2-1. PREPARATION OF THE VACCINE

The strains of *A. salmonicida* are cultured and harvested separately. The harvests are inactivated by a suitable method. They may be purified and concentrated. Whole or disrupted cells may be used and the vaccine may contain extracellular products of the bacterium released into the growth medium.

The vaccine contains an oily adjuvant.

2-2. CHOICE OF VACCINE STRAIN

The strains included in the vaccine are shown to be suitable with respect to production of antigens of assumed immunological importance. The vaccine is shown to be satisfactory with respect to safety (5.2.6) and efficacy (5.2.7) in the species of fish 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 test. During development of the vaccine, safety is tested on 3 different batches.

Carry out the test in each species of fish for which the vaccine is intended, using in each case fish of the minimum body mass 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 50 fish from a population that does not have specific antibodies against *A. salmonicida* subsp. *salmonicida* and has not been vaccinated against nor exposed to furunculosis. The test is carried out in the conditions to be recommended for the use of the vaccine with a water temperature not less than 10 °C. Administer to