SPECIFIC REQUIREMENTS FOR THE PRODUCTION AND CONTROL OF LIVE AND INACTIVATED VACCINES INTENDED FOR FISH

Guideline Title	General Requirements for the Production and Control of Live and Inactivated Vaccines intended for Fish.
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Additional Notes	This note for guidance is intended to provide general guidance on the type of data which should be included in applications for marketing authorisations for bacterial and viral vaccines intended for administration to fish. It is intended to supplement Directive 81/852/EEC as amended, and should be read in conjunction with that Directive. Cross-references to the notes for guidance on *General Requirements for the Production and Control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use* and *General Requirements for the Production and Control of Inactivated Mammalian Bacterial and Viral Vaccines for Veterinary Use* are made.

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SPECIFIC REQUIREMENTS FOR THE PRODUCTION AND CONTROL OF LIVE AND INACTIVATED VACCINES INTENDED FOR FISH

This document is intended to provide guidance on the type of data which should be included in applications for marketing authorisations for viral and bacterial vaccines for fish. It is intended to supplement Directive 81/852/EEC as amended by Directive 92/18/EEC and must be read in conjunction with that Directive. A special attention should be given to the investigation into the possible spread of live vaccine strains to wild fish species.

The principle for the production and control of mammalian vaccines apply equally to fish vaccines. Where requirements have been included in the *General Requirements for the Production and Control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use* (GRLMV) or the *General Requirements for the Production and Control of Inactivated Mammalian Bacterial and Viral Vaccines for Veterinary Use* (GRIMV), these requirements have not been repeated here, but a cross-reference has been given.

I. GENERAL REQUIREMENTS FOR FISH VACCINES

All vaccines intended for use in fish shall normally comply with the GRIMV and GRLMV, as modified by this note for guidance.

Any material of piscine origin in the production and control of virus vaccines must originate from hatchery and fish farms of specified health status as defined in Annex A to this note.

II. LIVE VACCINES

PRODUCTION

1. STARTING MATERIAL

1.2 Cell substrates

1.2.1 General requirements

The general requirements of GRLMV apply.

1.2.2 Requirements for cell lines

If a virus can be grown efficaciously on cell cultures based on a seed lot system of established cell lines, no primary cells should be used.

Cell lines of piscine origin used for the production of vaccines for fish should be free from extraneous agents listed in the Annex to this note.

Cell lines of mammalian origin should comply with GRLMV section 1.2.

Cell substrates of avian origin should be derived from SPF flocks meeting the requirements specified in the General Monograph for Veterinary Vaccines in the European Pharmacopoeia.

1.2.3 Requirements for primary cells

The requirements for GRLMV 1.2.3 to 1.2.3.2 apply.

If a vaccine has to be produced on primary fish cells, the seed material should be obtained from a specific pathogen free farm with a complete protection from introduction of disease. The farm shall comply with the requirements in the annex.

1.3 Virus seed

The requirements of GRLMV 1.3 to 1.3.5 apply.

1.3.1 Extraneous agents

The test for extraneous agents specified in GRLMV 1.3.5 should be carried out as indicated in item 1.2.2 of this document.

1.4 Bacterial seed

The requirements of GRLMV 1.4 to 1.4.3 apply.

2. FINISHED PRODUCT – ASSAY RESULTS REQUIRED IN THE APPLICATION FOR MARKETING AUTHORISATION

All assays conducted with groups of fish should be duplicated or triplicate in order to take the tank effect into account.

2.1 Safety

The samples for the safety testing shall be taken from batches produced according to the manufacturing process described in the application for marketing authorisation.

The test shall employ the target species of fish. If a vaccine is intended for use in several species the test must be employed in each species.

The fish shall be observed and any morbidity/mortality recorded. After at least 14 days the remaining fish should be slaughtered and examined for signs of systemic and/or local reactions.

Any possible injection site reactions shall be addressed and suitable studies to reveal the occurrence or magnitude of such reactions should be documented.

The withdrawal period before slaughtering shall be documented.

2.2 Efficacy

Efficacy experiments shall be performed on a sufficient number of target species and fish. The following should be well documented: The efficacy of all the vaccine strains, the efficacy for all the species and age/size for which the vaccine is intended, e.g. oral, injection, dipping and the vaccination scheme recommended by the manufacturer, the temperature in the environments recommended for vaccinations.

If established, the nature and level of immune response following vaccination shall be documented. The duration of immunity, assessed by challenge, and the tolerance/side effects shall be documented in each case.

Field trials

Safety and efficacy must be studied in field trials performed on a sufficient number of target species distributed in more than one premises. The parameters mentioned for the experimental safety and efficacy studies should be looked for in the field trials and temperature in the environments should be recorded.

3. TEST ON THE FINISHED PRODUCT – BATCH TESTING

The requirements of GRLMV apply. Testing for safety and efficacy shall be carried out as follows:

3.1 Safety

The test shall employ the target species of fish. If a vaccine is intended for use in several species, the species most sensitive to infectious agents in question should be used.

For vaccines intended for individual administration, at least 30 susceptible fish should be vaccinated and for vaccines intended for oral-, spray-, bath- or dip-vaccination, at least 50 fish should be employed. Fish of minimum age and/or size recommended for vaccination should be used. Vaccination should be carried out according to label instructions. A control group of a composition similar to the vaccinated group should undergo mock-vaccination. During the following 21 days, vaccinates and controls should be held at an environmental temperature relevant to the species and observed every day for mortality and adverse effects. Mortality among vaccinates should not exceed that of controls, and no clinical symptoms of ill health should appear in any surviving vaccinates. The cause of mortality should be assessed. If in the case of 30 vaccinates, more than 2 fish die or if more than 3 out of 50 vaccinates die during the observation period, the safety test must be repeated once. If in the second test the specified limits for mortality are again exceeded, the batch is considered unsafe and must be discarded.

3.2 Potency

Where the viral or bacterial titre in the vaccine does not correlate with the protection and a potency test is required to comply with the requirements of GRLMV 3.7, the following test should be carried out.

The test shall employ the target species of fish. If a vaccine is intended for use in several species, the species most sensitive to the infectious agent in question should be used. Fish of the minimum age and /or size recommended for vaccination should be employed.

For each strain of organisms contained in the vaccine, at least 50 fish should be vaccinated according to label instructions.

Challenge should take place at least 28 days following vaccination. For each strain contained in the vaccine, one group of vaccinates and a simple group of mock-vaccinated, susceptible fish should be exposed to the corresponding virulent organism. The challenge should cause at least 70 per cent morbidity or mortality in control fish. Addition of virulent organisms to the water is the preferred method of exposure; however with some species of microorganisms, individual challenge, e.g. intra-muscular or intra-peritoneal

administration, may be necessary in order to obtain acceptable morbidity/mortality among controls.

For some diseases it may be acceptable to introduce the virulent organisms into the water by means of infected fish where vaccinates and controls are held in the same tank.

Following challenge, all groups shall be kept at an environmental temperature relevant to the species and must be observed each day until at least 21 days after challenge and until it is established that no further mortality is occurring.

For all strains of organisms contained in the vaccine, morbidity and mortality should be significantly lower in vaccinated fish than in mock-vaccinates.

III. INACTIVATED VACCINES

PRODUCTION

1. STARTING MATERIAL

1.2 Cell substrates

See requirements for live vaccines for fish.

1.3 Virus seed

See requirements for live vaccines for fish.

1.4 Bacterial seed

See requirements for live vaccines for fish.

2. FINISHED PRODUCT – ASSAY RESULTS REQUIRED IN THE APPLICATION FOR MARKETING AUTHORISATION

See requirements for live vaccines for fish.

3. TEST OF THE FINISHED PRODUCT – BATCH TESTING

See requirements for live vaccines for fish.

ANNEX

Health requirements for pisciculture establishments (fish farms) acting as suppliers of material of piscary origin used in the production and control of virus vaccines intended for fish.

The requirements are not exhaustive; they will be in accordance with the provisions of Directive 91/67/EEC and its amendments and adapted to progress in knowledge.

1. Fish farms acting as suppliers of material of piscary origin used for the production and control of virus vaccines intended for fish must maintain SPF status with respect to the following pathogens:

   Viral agents

   Viral Haemorrhagic Septicaemia Virus (VHSV) type I, II, III

   Infectious Haematopoletic Necrosis Virus (IHNV)

   Spring Viraemia of Carp Virus (SCVC)

   Infectious Pancreatic Necrosis Virus (IPNV)

   Protozoal agents

   Myxosma cerebralis

   Bacterial agents

   Yersinia ruckeri

   Vibrio anguillarum

   Aeromonas salmonicinarum

2. In order to document continuous freedom from the pathogens mentioned in section 1, the establishment must be subjected to a disease control on a regular basis, including laboratory examination of suitable specimens collected on the premises.

3. In order to become eligible as supplier, the establishment must have been subjected to official disease control carried out on a regular basis for at least 2 years, during which period no evidence of pathogens on the premises must be revealed. In addition, it must be documented that natural conditions and preventative measures make it highly unlikely for contamination of the premises with the pathogens to occur, e.g. through the water supply or through wild or restocking fish.