UNIVERSITY OF STIRLING

General Overview of Fish Vaccination

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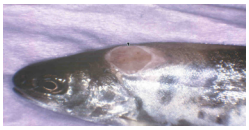
EDQM Symposium on Challenges of Quality Requirements for Fish Vaccines
10-11th May 2106, Norwegian University of Life Sciences, Oslo, Norway

Fish Diseases

-disease is still considered a major constraint to aquaculture production globally

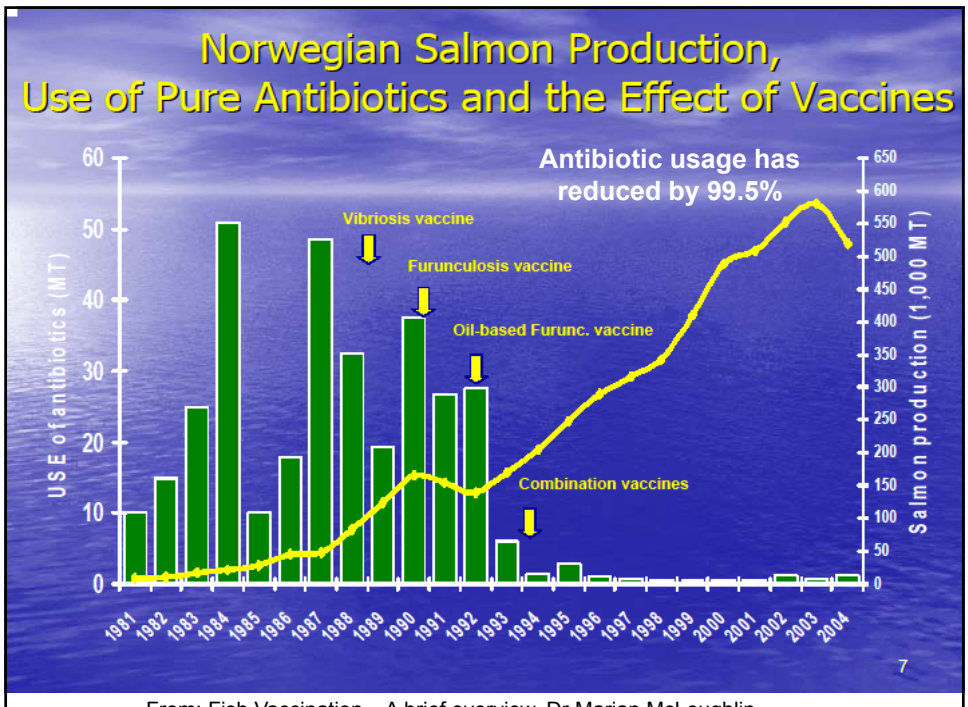
* Bacterial
* Fungal
* Viral
* Parasitic



Photograph courtesy of Peter Dixon, CEFAS

Vaccines

* Major area for growth in aquaculture
* Reduce concerns over residue levels and environmental impacts
* Reduce the need for antibiotics and chemicals
* Save costs
* Control significant diseases
* Reduce problems with antibiotic resistance



From: Fish Vaccination – A brief overview. Dr Marian McLoughlin

Size of Aquaculture Industry Worldwide

* ~ 70 million Tonnes

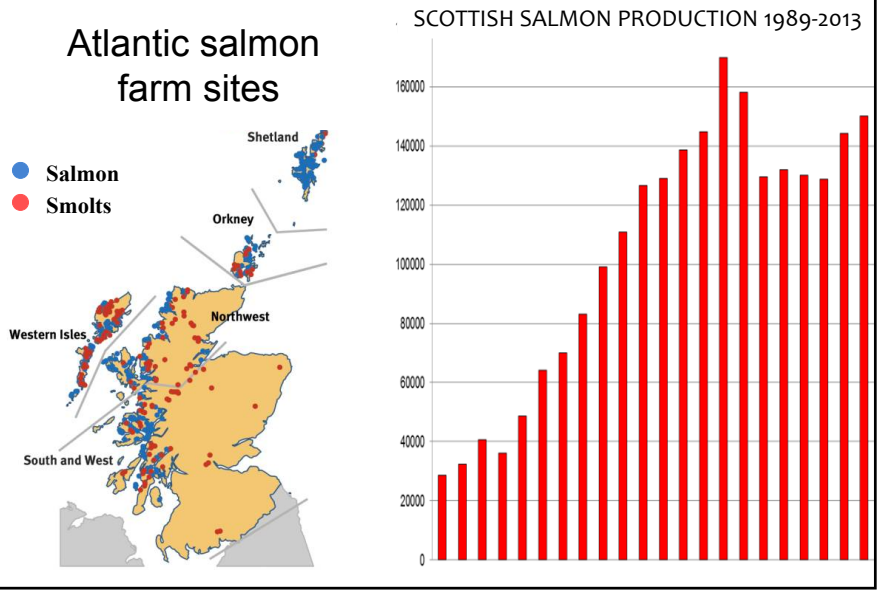
China 45 million tonnes , Norway 900,000 tonnes

* Value = US$ 130 billion
* Increased by 10-12% from 59 million tonnes in 2011 (fastest growing animal production sector) compared to farmed meat = 2.8% growth: capture fisheries = 0%)
* Key drivers = Food Security, Health, & Zero Increment
* Marine, freshwater, finfish, crustaceans, shellfish
* 40% of total world fish production

expected to reach 50% by 2030

* Over 30 species currently farmed

Mainly Atlantic Salmon farming in Scotland



Value

* Largest producer in EU
* Largest food export
* Worth £540M, retail value >£1billion
* I million fresh salmon meals eaten each day in UK
* Scottish Aquaculture currently contributes an aggregate economic impact of over £1.4 Billion per annum and 8,000 jobs to the Scottish economy

Each Year

In Scotland ~20 million trout and ~40 million salmon vaccinated
Globally ~90 million trout and 418 million salmon vaccinated

AIM of Vaccination

* Induce long term immunity by stimulating the memory component of the specific immune system
* PROTECT against disease (but carriers may still exist)

* Note: EARLIEST a fish can be vaccinated varies between species and between vaccines

Early Commercial Vaccines

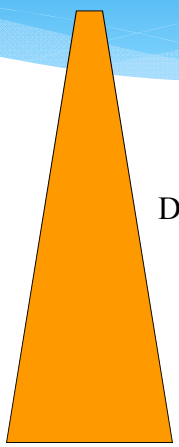
* Vaccines licensed in 1970's in USA
* Vaccines introduced to Scotland and Scandinavia in mid 1980's
* ERM and Vibriois vaccines administered by immersion
* Furunculosis vaccine-adjuvanted and administered by injection to increase immunogenicity

Primary considerations for developing a vaccine

- Safety
- Cost-effectiveness
- Long term protection
- Serotypic/genetic variation of the pathogen
- Time-/age when fish most susceptible to disease
- Species
- Route of administration
- Method of vaccine preparation

Types of Vaccine

- **Inactivated whole cell**
- Adjuvanted
- Sub-unit
- Recombinant
- Live attenuated
- Synthetic (peptide)
- DNA vaccines



First DNA vaccine in the EU recommended for use in salmon (SPD)

Commercially-Available Fish Vaccines

1982

1 Enteric Redmouth (ERM) vaccine
2 *Vibrio anguillarum* vaccine

TOTAL = 2

2016

1 Enteric Redmouth (ERM) vaccine
2 *Vibrio anguillarum* vaccine
3 Furunculosis vaccine
4 *Vibrio salmonicida* vaccine
5 Combined Vibriosis/Furunculosis vaccine
6 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa* vaccine
7 Combined Vibriosis/Furunculosis/Coldwater Vibriosis/*Moritella viscosa*/IPNV vaccine
8 IPN Virus vaccine
9 Pasteurella vaccine
10 Combined Pasteurella/Vibriosis vaccine
11 Vibriosis vaccine for cod
12 Shrimp Vibriosis vaccine
13 Warmwater *Vibrio* spp vaccine
14 SVC virus vaccine
15 *Lactococcus garvieae/Streptococcus iniae* vaccine
16 KHV vaccine
17 *Aeromonas hydrophila* vaccine
18 Carp Erythrodermatitis/Ulcer disease vaccine
19 *Piscirickettsia salmonis* vaccine
20 ISA virus vaccine
21 Gaffkaemia vaccine
22 *Flavobacterium psychrophilum* vaccine
23 Nodavirus vaccine
24 Pancreas disease virus vaccine
25 *Edwardsiella ictaluri* vaccine
26 *Streptococcus agalactiae* vaccine
27 PD vaccine

TOTAL = 27 +

-Major market in salmon
-Trout
-Expanding market in sea bass, sea bream, tilapia, turbot, halibut, yellowtail, cod etc
-Most whole cell killed vaccines
-Many now mult-ivalent

Important considerations for fish vaccination

* Fish species
* Status of the immune system
* Production cycle and life history
* Which diseases need to control?
* When do these diseases occur?
* Farming technology (handling, mechanisation)
* Environment (temperature, salinity)
* Stress factors, nutrition and cost benefit

Principals of Vaccination

1. Recommended only for healthy fish

Do not vaccinate sick or stressed fish

Principals of Vaccination

2. Fish should be deprived of food prior to vaccination- suffer less handling stress and respond better to anaesthetics

   The smaller the fish size and the higher the water temperature the smaller the required fasting interval

NO FOOD!

Principals of Vaccination

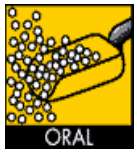
3. Disease free environment
4. MUST precede exposure to disease or transfer to a disease prone site by an appropriate time

LAG PHASE

Methods of Vaccine Delivery

* Most effective but need to anaesthetise and handle the fish

* Practical for mass vaccination of small fish only, does not work for all vaccines.



* Most suitable for mass vaccination but dosage uncertain and sometimes poor potency

Immersion Vaccination

Two application methods: **dip and bath**

Dip vaccination- more widely used

* Small fish immersed for very short duration (30 seconds) in a highly concentrated vaccine solution(1 part vaccine to 9 parts water
* **Bath vaccination**
* Larger fish are exposed for a longer period, usually one to several hours, in a lower concentration of vaccine

MUCOSAL SURFACES

- Suspended antigens **adsorbed by skin and gills**
- Specialised cells, such as **antibody-secreting cells, in the skin and gill epithelium are activated** and protect the fish when they are exposed to the live pathogen at a later stage
- Other cells in the epithelium of skin and gills, such as **antigen presenting cells (macrophages), also absorb vaccine antigens and transport them** to specialised tissues where the systemic immune response builds up

Dip Vaccination

* Rapid vaccination of large numbers of fish (up to 100kg of fish per litre of vaccine).
* Widely used for vaccination of fry from 1 to 5 g.
* Effective -relatively good protection.
* **Limitations**

-duration of immunity is not very long and a booster vaccination is required when the disease prevails over longer periods.

-impractical for larger fish due to cost-effectiveness and the stress that could be induced by vaccination.

-in fish smaller than 1g, the immune system might still be immature and, therefore, the vaccine efficacy may be reduced.

Bath Vaccination
eg large scale in sea bass in Europe

* Large groups of fish cut off from the rest in a cage
* A low dose of diluted anaesthetic is added.
* Air or oxygen is continuously pumped in to avoid anoxia

Injection Vaccination

* Intraperitoneal IP (in the abdominal cavity) or Intramuscular (IM) **IP most common**
* Potentially can cause stress - resulting from the handling and injection of the fish
* BUT no mortality associated with the vaccination process per se, although some weak fish may die due to the handling process.

Vaccination by Intraperitoneal Injection

Vaccine (usually 0.1ml to 0.2ml) is injected in the abdominal area of each anaesthetised fish (>50g). Now also micro vaccines (0.05ml).

Fish held ventral side up and the head away from the operator´s body.

The needle is inserted into the peritoneal cavity at a 45^{o} angle to a depth of approximately 0.5 cm.

Vaccination by Intraperitoneal Injection

Automatic injection guns are used.

A team of 4 people can vaccinate approx 5000 salmon per hour!

Fish are often graded at the same time.

Advantages of Injection Vaccination

- **Long duration of protection**, i.e., for over a year
- Multiple antigens can be combined in a single vaccine and, therefore, in a single administration.
- **Every fish** in the population has received the vaccine and at the **correct dose.** 10,000 fish per litre by IP (>25g)
- Injections are in general superior to any other vaccine application method; however, from a practical point of view, they can only be applied to fish of 10g or more (usually larger)
- Can include **adjuvants**

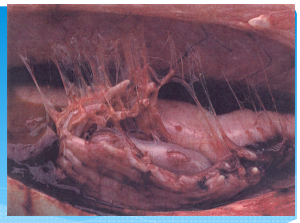
ADJUVANTS

- Stimulate non-specific defence mechanisms
- Enhance specific immune response
- Should have:
  - good efficiency
  - low side effects
  - easy handling

Short-term protection may be due to non-specific defence factors

- **Adjuvants**- result in release of interleukins which in turn result in non-specific activation of macrophages
- **Bacteria**-most bacterial fish pathogens are Gram-negative and therefore contain LPS intheir outer membrane. LPS increases phagocytic rates

ADHESIONS following IP vaccination with multivalent, oil adjuvanted vaccines



- Inflammatory response -> local and/or diffuse peritonitis with adhesions in internal organs and abdominal wall
- Invasion of fibroblasts, macrophages and lymphocytes
- Large number of melanomacrophages
- Can result in multiple granulomata
- Scoring scale from 0 to 6 based on macroscopic pathology findings ("the **Speilberg scale**"; Midtlyng et al. 1996a; annexed in EMA/CVMP/IWP/314550/2010) has gained wide acceptance.
- **At water temperatures of 10-12° C, the progression of injection-site reactions in Atlantic salmon may take 6-12 months**
- ≤ 2.5 can be considered acceptable
- Cause only slight reduction in growth rate when injection-site lesions are moderate but may increase beyond 10% among fish showing Speilberg scores of 3 or higher

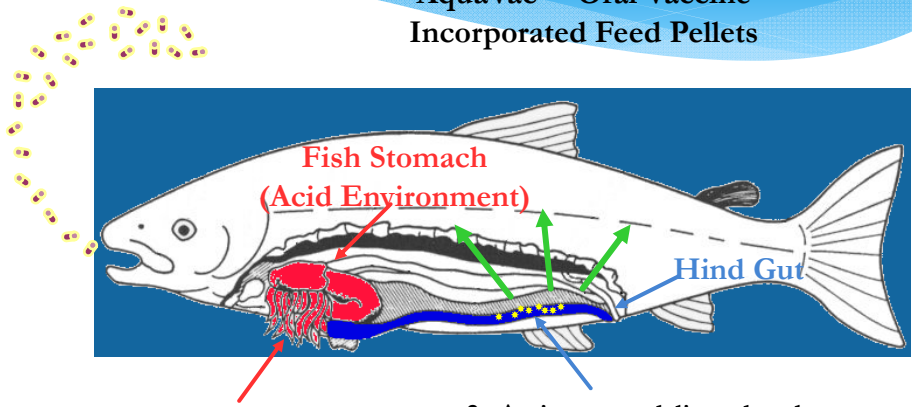
Oral Vaccination

- Most suitable for mass vaccination but dosage uncertain and sometimes poor potency
- Vaccine is either mixed with the feed, coated on top of the feed (top dressed) or bio-encapsulated
- Stability?
- Very few on the market and only as boosters

- When antigens are to be incorporated in feed, the **heat sensitivity** of the antigen has to be considered
- When vaccines are used as top dressing in feed, a coating agent is usually applied, either to **prevent leaching** of the antigen from the pellets or to **prevent breakdown** of the antigen in the acidic environment of the stomach

AquaVac™ Antigen Protection System

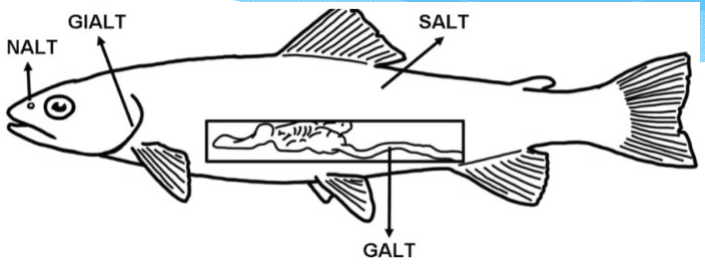
AquaVac™ Oral Vaccine Incorporated Feed Pellets



1. In the acid environment of the fish stomach, the feed pellets are digested. The Antigens themselves are protected by the APV and pass through intact.
2. Antigens are delivered to the area of the hind gut where they are absorbed and activate an effective immune response in the fish.

Antigens reach intra-epithelial macrophages that show antigen presenting function
GALT contains lymphoid cells such as macrophages, granulocytes and plasma cells

Mucosa-associated lymphoid tissues (MALT)



GALT: gut-associated lymphoid tissue; SALT: skin-associated lymphoid tissue; GIALT: gill-associated lymphoid tissue; NALT: nasopharynx-associated lymphoid tissue.
Irene Salinas, Biology (Basel). 2015 Sep; 4(3): 525–539.

Vaccine Efficacy

Three criteria used:

- Rate at which protection is achieved
- The final degree of protection (RPS)
- Duration of immunity

RPS=1 -%vaccinate mortalities X 100
% control mortalities

NC3Rs
-Need alternative methods

- The speed of the immune response is temperature dependant
- It usually takes several weeks or months (depending on the temperature) before good immune protection is developed as a result of vaccination
- Important not to stress the fish in the weeks following vaccination as stress is known to suppress the immune system

Vaccination Strategies

* Disease status/degree of risk
* Size of fish
* Temperature

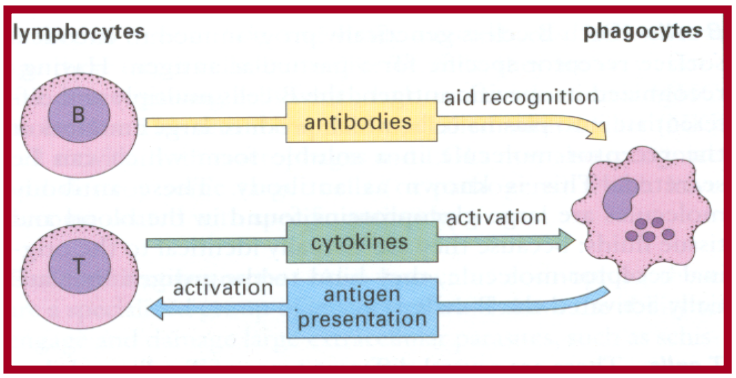
-The effect of temperature can be put to good use during vaccination eg by increasing water temperature an effective antibody response with long term memory can be achieved which would not occur at low temperatures for salmonids

- Fish can be successfully vaccinated in the **autumn with the acquired immunity surviving the drop in temperature over the winter** and it is then effective the following spring when water temperatures increase as do the number of disease outbreaks

The immune response of fish

Adaptive immunity

Innate immunity



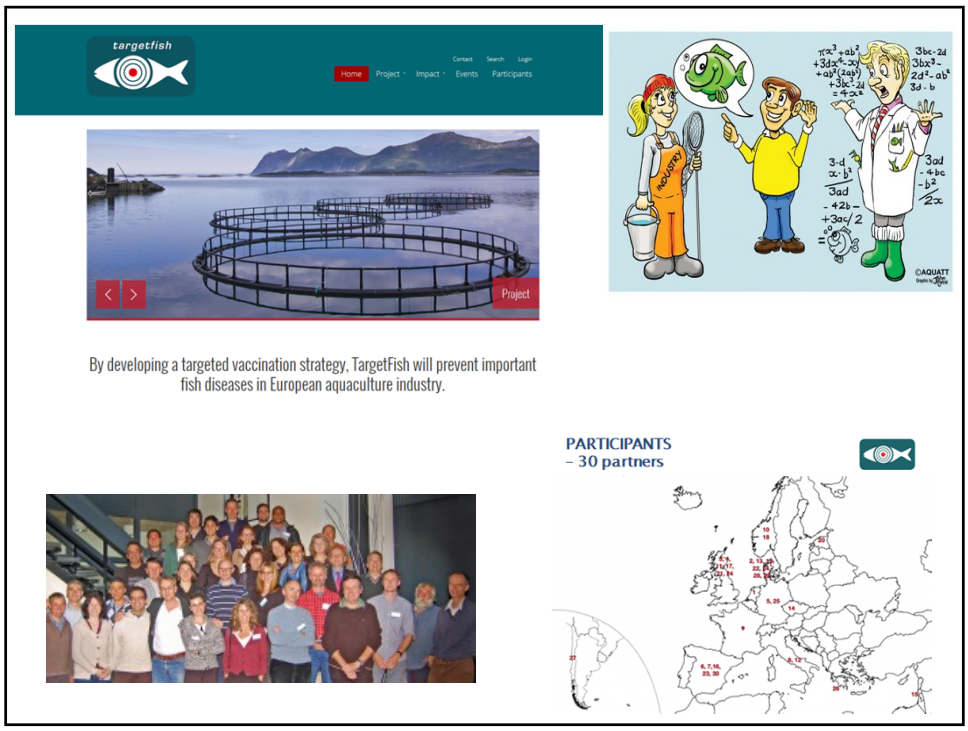
Measuring Immune Response

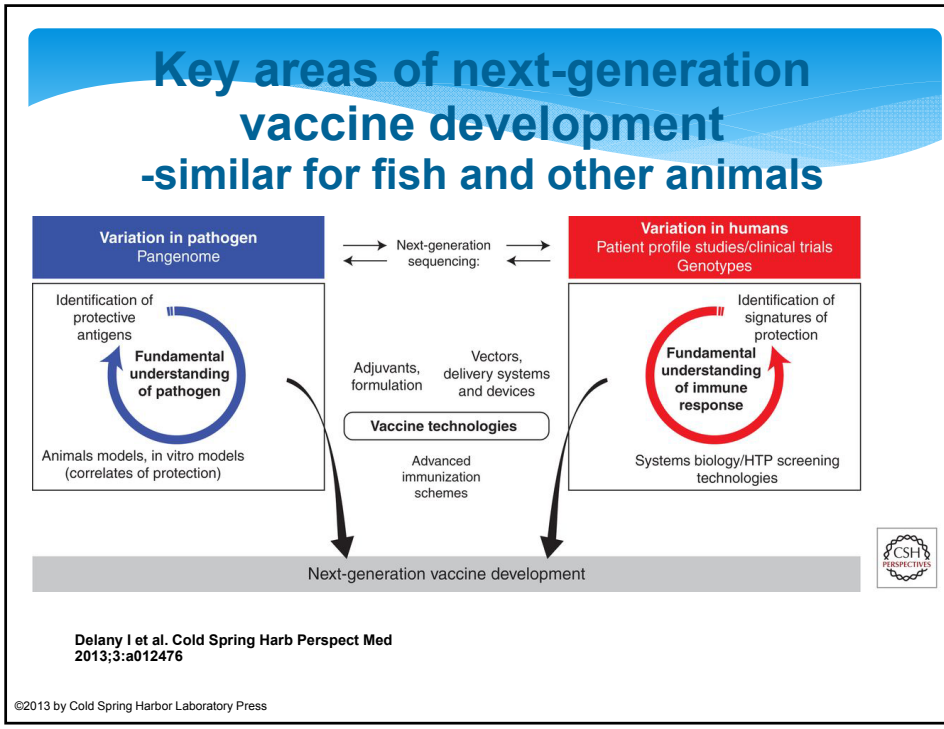
* Blood parameters
* Identification of cell types and how these populations change
* Simple functional assays *e.g.* ELISA

  -Easy to measure IgM by ELISA **but not** IgT (mucosal antibody)
* Gene expression (*e.g.* cytokines, IgT)

Fish Vaccines

* Increase in commercially available vaccines
* **BUT** there are still diseases where no are vaccines available
* **AND** some existing vaccine do not perform well





Delany I et al. Cold Spring Harb Perspect Med 2013;3:a012476

Still Many Future Challenges for Vaccine Development

Inactivated Vaccines

* Improvements in efficacy (ID of antigens) and safety; improved adjuvants, oral administration

Intracellular bacteria and viruses

* Live attenuated vaccines, oral administration

Parasites and fungi

* Pathogenesis, immune response, ID of antigens

GENERAL

* Fish used in challenge testing (genetic background, previous exposure *in vitro* tests), licencing, DNA, DIVA, production without having isolated the pathogen
* This includes alternative methods for testing vaccines *e.g. in vitro* methods

EDQM/European Pharmacopoeia: assuring the quality of medicines

EDQM Symposium on Challenges of Quality Requirements for Fish Vaccines

OSLO 10-11 May 2016

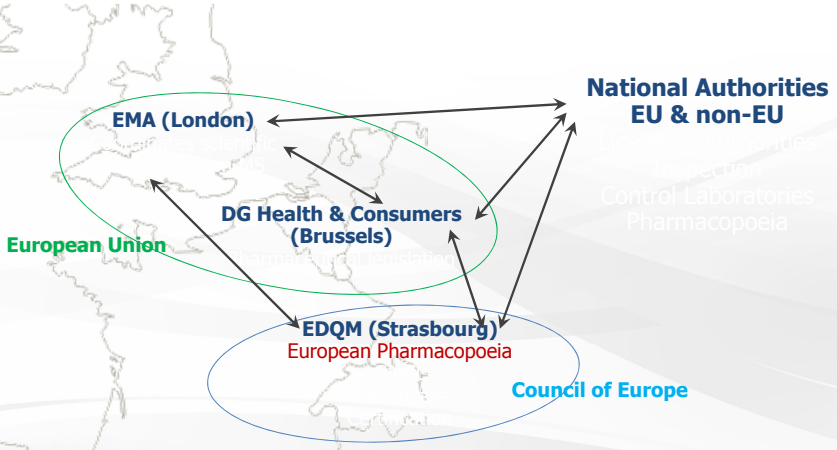
Mrs Catherine LANG

Scientific Officer, responsible for Group 15V
European Pharmacopoeia Department,
EDQM, Council of Europe
catherine.lang@edqm.eu

Place of the Ph. Eur. in the European regulatory network

- Lays down common, compulsory quality standards for all medicinal products in Europe. Mandatory on the same date in 37 states (CoE) and the European Union;
- The Ph. Eur. is legally binding. The legislation also includes a mechanism to provide the pharmacopoeia authority with information on the quality of products on the market;
- The European Pharmacopoeia needs to keep pace
  - with the regulatory needs of licensing, control and inspection authorities in the public health area,
  - with industrial constraints,
  - with technological and scientific advances.

Relationship with European Regulators: A strength of the Ph. Eur.!



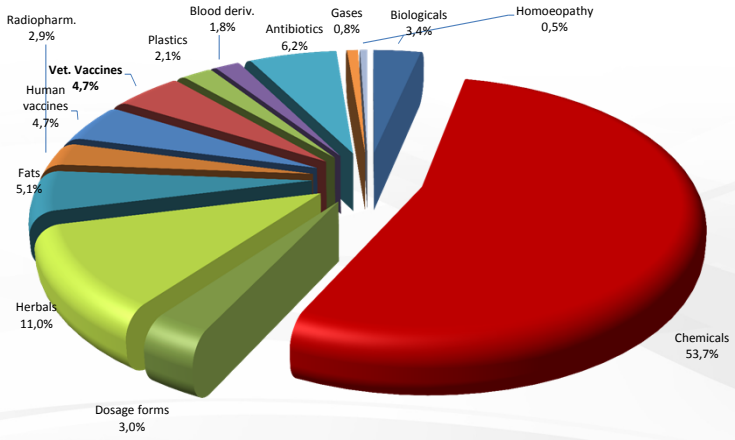
The Ph.Eur. - one decision body …

https://www.edqm.eu/en/EDQM-history-93.html

The Ph. Eur. Commission:

- 3 sessions per year
- 38 delegations
  (37 member states + EU)
  of up to three representatives
- All technical decisions **by consensus**
- Observers welcome!

… and more than 70 groups/800 experts coordinated by the European Pharmacopoeia …



… going into one direction: The Ph. Eur. ➔ A success story!

- A unique example of an efficient collaborative process:
  37 national secretaries contributing resources to this collaborative process rather than developing national standards (2 member states interested in one topic ➔ added on the Ph. Eur. work programme)
- Opportunities:
  - saving of resources
  - no subsequent need to harmonise national positions
- Concrete outcomes ➔ More than 2200 monographs and 340 general chapters adopted

The European Pharmacopoeia: a transparent process

- All revised and new texts published online in Pharmeuropa (the European pharmacopoeial forum, free access) for public enquiry
- Work programme available on EDQM website
- Style guide and technical guides freely available and downloadable on EDQM website
- Knowledge database (free access) ➔ useful information
- Organisation of hearings of interested parties

Ph. Eur. – General organisation

- Introduction
- General notices
- General chapters
- General monographs
- Individual monographs

- apply to all monographs and other texts of the Ph. Eur.
- instructions to understand texts, conventional expressions
- **essential reading before starting to use monographs**

Ph. Eur. texts (cont'd)

standard analytical methods
general requirements for equipment e.g.:

- 2.6 Biological tests
  - 2.6.1 Sterility
  - 2.6.7 Mycolplasmas
  - 2.6.8 Pyrogens
  - …
  - 2.6.24 & 2.6.25
- 5.1 General texts on microbiology
- etc

General chapters

Individual monographs

General monographs

Reference standards

- Editorial convenience: avoid repeating standard methods in each monograph
- Provide standard methods that can be used where there is no monograph
- Give general requirements for equipment, equipment verification
- Not mandatory *per se*
- When referred to in a monograph, they become part of the standard

Ph. Eur. texts

classes of substances, dosage forms

- *Substances for pharmaceutical use* (2034)
- *Pharmaceutical preparations* (2619)
- *Vaccines for veterinary use* (0062)
- *Immunosera for veterinary use* (0030)

General chapters

Individual monographs

General monographs

Reference standards

- Quality aspects that cannot be dealt with in each individual monograph
- Quality aspects that are common to a class of products
- Classes defined by different criteria: production method, origin, risk factors
- General monographs apply to all substances and preparations within the scope of the **DEFINITION section of the general monograph**, except where a preamble limits its application

General monographs

- General monographs are ALL mandatory and apply to ALL substances and preparations **within the scope of the Definition section** of the general monograph
- No cross-reference in individual monographs: ***Check in the Introduction & Definition which monograph applies!***

04/2013:0062

VACCINES FOR VETERINARY USE

Vaccina ad usum veterinarium

01/2008:0030

IMMUNOSERA FOR VETERINARY USE

Immunosera ad usum veterinarium

PHARMACEUTICAL PREPARATIONS

SUBSTANCES FOR PHARMACEUTICAL USE

Ph. Eur. texts (cont'd)

- based on approved specification(s) backed up by batch data
- specifications for drug substance or finished products
- analytical procedures and acceptance criteria to demonstrate that the substance meets required quality standards

General chapters

Individual monographs

General monographs

Reference standards

DEFINITION: defines the scope of the monograph – suitable strain

PRODUCTION: safety and Immunogenicity, antimicrobial preservatives, stability.

MANUFACTURER'S TESTS: Batch potency test, inactivation test

BATCH TESTS: Identification (alternative tests may be used), Sterility/Bacteria and fungi, Potency (must comply if tested)

STORAGE & LABELLING: Items necessary for use of the monograph

Ph. Eur. reference standards

Ph. Eur. Chapter 5.12 *Reference standards*

- Established specifically for use in monographs or general chapters of the Ph. Eur., as prescribed in the methods given
- **Chemical Reference Standards** (CRSs) and **Biological Reference Preparations (BRPs**)

Take home messages

- The Ph. Eur. texts are mandatory but not set in stone.
- They are routinely updated to reflect the state-of-the-art thanks to YOUR input (National Authorities, manufacturers, Experts in Ph Eur Groups) – confidentiality of data is taken into account.
- The decisions are taken by one decision body by consensus.
- Nominations of experts worldwide
- The elaboration/revision process is transparent (knowledge database, work programme, guides) and includes public enquiries (hearing before Pharmeuropa if major change, Pharmeuropa)

Thank you for your attention

European Directorate for the Quality of Medicines & HealthCare (EDQM)

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

How to use the European Pharmacopoeia texts for the control of fish vaccines
&
How to collaborate to the work of the Ph. Eur. Group of Experts 15V

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Flexibility in the Ph.Eur. -Alternative methods

- Ph. Eur. tests are reference methods, essential in cases of dispute.
- Compliance is required, but alternative methods may be used as long as they lead to the same pass/fail result.
- It is the responsibility of the user to demonstrate their suitability. Approval of the competent authority is necessary in many cases.

Flexibility in the Ph.Eur. – Animal welfare

- Reference to the *European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of the Council of Europe (1986)*
- Minimise the use of animals
- Consistency of production (latest version in Supplement 8.2)

A Guide Through The Different Sections

Supplement and Version date

English (or French) title

Latin subtitle

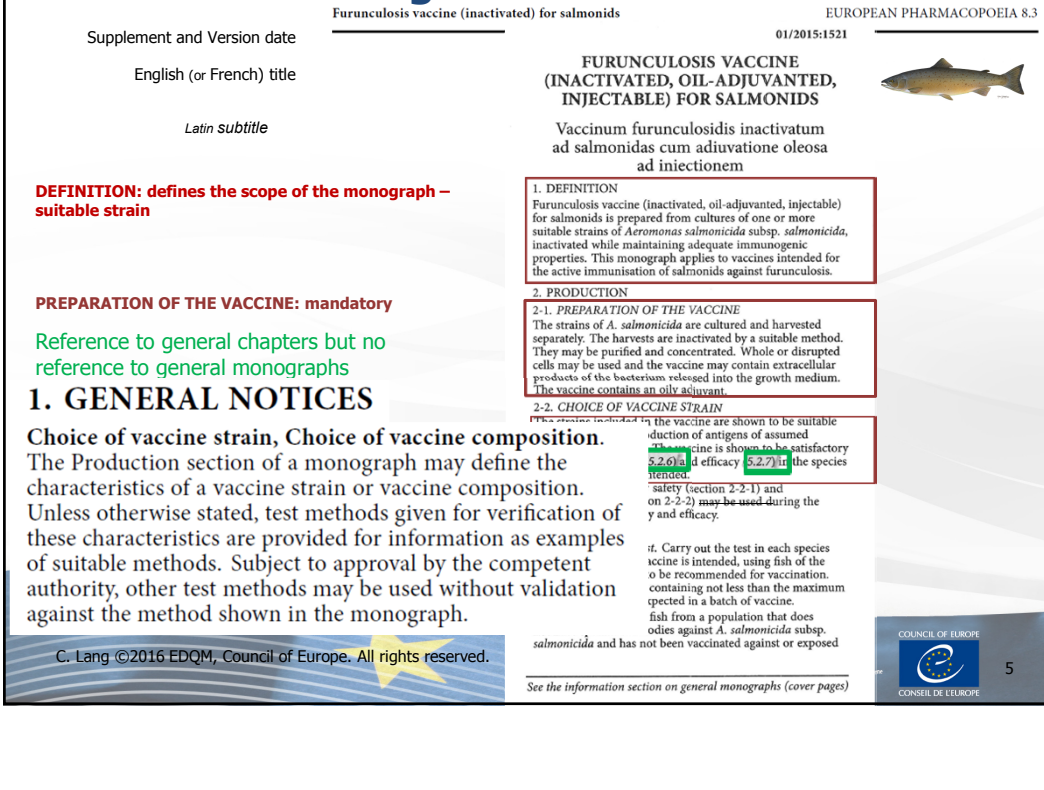
DEFINITION: defines the scope of the monograph – suitable strain

PREPARATION OF THE VACCINE: mandatory

Reference to general chapters but no reference to general monographs

1. GENERAL NOTICES

Choice of vaccine strain, Choice of vaccine composition. The Production section of a monograph may define the characteristics of a vaccine strain or vaccine composition. Unless otherwise stated, test methods given for verification of these characteristics are provided for information as examples of suitable methods. Subject to approval by the competent authority, other test methods may be used without validation against the method shown in the monograph.



Furunculosis vaccine (inactivated) for salmonids EUROPEAN PHARMACOPOEIA 8.3

01/2015:1521

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

Vaccinum furunculosidis inactivatum ad salmonidas cum adiuvatione oleosa ad iniectionem

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*

See the information section on general monographs (cover pages)

A Guide Through The Different Sections (cont.)

Safety: Mandatory

Immunogenicity: Mandatory

Residual live virus: The verification of the inactivation is mandatory

EUROPEAN PHARMACOPOEIA 8.0 Rabies vaccine (inactivated) for veterinary use

2-3-1. **Safety**. Carry out the test for each route and method of administration 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.

2-3-2. **Immunogenicity**. Each test is carried out for each route and method of administration to be recommended, using in each case animals of the minimum age to be recommended for vaccination. The vaccine administered to each animal is of minimum potency.

2-4-1. **Residual live virus**. The test for residual live virus is carried out by inoculation of the inactivated virus into the same type of cell culture as that used in the production of the vaccine or a cell culture shown to be at least as sensitive. The quantity of inactivated virus harvest used is equivalent to not less than 25 doses of the vaccine. After incubation for 4 days, a subculture is made using trypsinised cells; after incubation for a further 4 days, the cultures are examined for residual live rabies virus by an immunofluorescence test. The inactivated virus harvest complies with the test if no live virus is detected.

2-4-2. **Antigen content of the harvest**. The content of rabies virus glycoprotein is determined by a suitable immunochemical method (2.7.1). The content is within the limits approved for the particular preparation.

2-4-3. **Antigen content of the pooled harvest**. The quantity of rabies virus glycoprotein per dose, determined by a suitable

2-4-4. **Batch potency test**. It is not necessary to carry out the potency test (section 3-4) 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 described under Potency. In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, such an alternative validated method should preferably be used for routine testing. The following serological assay has been shown to be suitable[6] and may be used provided the test for antigen content of the pooled harvest (section 2-4-3) has been carried out with satisfactory results.

3. BATCH TESTS

3-1. **Identification**. Administered to animals that do not have antibodies against rabies virus, the vaccine stimulates the production of such antibodies.

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 virus**. Carry out the test using a pool of the contents of 5 containers.

For vaccines which do not contain an adjuvant, carry out a suitable amplification test for residual live virus using the same type of cell culture as that used in the production of the vaccine or a cell culture shown to be at least as sensitive. The vaccine complies with the test if no live virus is detected.

For vaccines that contain an adjuvant, inject intracerebrally into each of not fewer than 10 mice, each weighing 11-15 g, 0.03 mL of a pool of at least 5 times the smallest stated dose. To avoid interference from any antimicrobial preservative

[6] E. Kramer et al. Collaborative Study for Validation of a Serological Potency Assay for Rabies Vaccines Inactivated) for Veterinary Use. *Pharmeur Bio Sci Note* 2010(2):97-115.
E. Kramer et al. The rapid fluorescent focus inhibition test is a suitable method for batch potency testing of inactivated rabies vaccines. *Biologicals* 2009;37:119-126.

General Notices (1) apply to all monographs and other texts 1009

Batch potency test: The verification of the potency is mandatory.
The model proposed is given as an example of satisfactory method. A validation by the manufacturer for the particular product is necessary. The test used must be able to detect sub-potent vaccines.

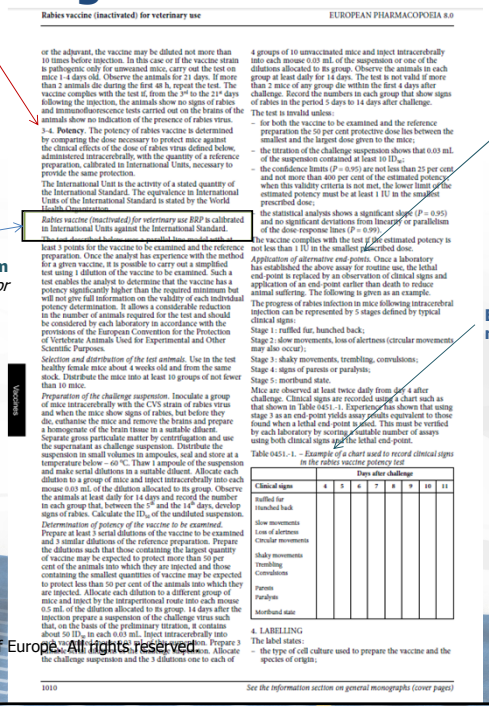
Reference standard available from EDQM:
Rabies vaccine (inactivated) for veterinary use BRP
Check General Chapter 5.12

BATCH TESTS: Mandatory. Apply throughout shelf-life. The tests are not necessarily carried out on each batch for batch release.
Identification: must comply if tested; alternative test may be used.
Bacteria and fungi: must comply if tested, e.g. parametric release may be applied.
Residual live virus: must comply if tested – can be tested upstream.

A Guide Through The Different Sections (cont.)

Potency: must comply if tested. The detailed test is given as an example of a suitable method. The method used may be the method developed by the manufacturer during the development of the vaccine subject to agreement by the Competent Authority.

Reference standard available from EDQM: *Rabies vaccine (inactivated) for veterinary use BRP*



Rabies vaccine (inactivated) for veterinary use — EUROPEAN PHARMACOPOEIA 8.0

or the adjuvant, the vaccine may be diluted not more than 10 times before injection. In this case or if the vaccine strain is pathogenic only for unweaned mice, carry out the test on mice 1-4 days old. Observe the animals for 21 days. If more than 2 animals die during the first 48 h, repeat the test. The vaccine complies with the test if, from the 3rd to the 21st days following the injection, the animals show no signs of rabies and immunofluorescence tests carried out on the brains of the animals show no indication of the presence of rabies virus.

3-4. **Potency**. The potency of rabies vaccine is determined by comparing the dose necessary to protect mice against the clinical effects of the dose of rabies virus defined below, administered intracerebrally, with the quantity of a reference preparation, calibrated in International Units, necessary to provide the same protection.

The International Unit is the activity of a stated quantity of the International Standard. The equivalence in International Units of the International Standard is stated by the World Health Organisation.

Rabies vaccine (inactivated) for veterinary use BRP is calibrated in International Units against the International Standard.

[illegible] at least 3 points for the vaccine to be examined and the reference preparation. Once the analyst has experience with the method for a given vaccine, it is possible to carry out a simplified test using 1 dilution of the vaccine to be examined. Such a test enables the analyst to determine that the vaccine has a potency significantly higher than the required minimum but will not give full information on the validity of each individual potency determination. It allows a considerable reduction in the number of animals required for the test and should be considered by each laboratory in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

Selection and distribution of the test animals. Use in the test healthy female mice about 4 weeks old and from the same stock. Distribute the mice into at least 10 groups of not fewer than 10 mice.

Preparation of the challenge suspension. Inoculate a group of mice intracerebrally with the CVS strain of rabies virus and when the mice show signs of rabies, but before they die, euthanise the mice and remove the brains and prepare a homogenate of the brain tissue in a suitable diluent. Separate gross particulate matter by centrifugation and use the supernatant as challenge suspension. Distribute the suspension in small volumes in ampoules, seal and store at a temperature below – 60 °C. Thaw 1 ampoule of the suspension and make serial dilutions in a suitable diluent. Allocate each dilution to a group of mice and inject intracerebrally into each mouse 0.03 mL of the dilution allocated to its group. Observe the animals at least daily for 14 days and record the number in each group that, between the 5th and the 14th days, develop signs of rabies. Calculate the ID_{50} of the undiluted suspension.

Determination of potency of the vaccine to be examined. Prepare at least 3 serial dilutions of the vaccine to be examined and 3 similar dilutions of the reference preparation. Prepare the dilutions such that those containing the largest quantity of vaccine may be expected to protect more than 50 per cent of the animals into which they are injected and those containing the smallest quantities of vaccine may be expected to protect less than 50 per cent of the animals into which they are injected. Allocate each dilution to a different group of mice and inject by the intraperitoneal route into each mouse 0.5 mL of the dilution allocated to its group. 14 days after the injection prepare a suspension of the challenge virus such that, on the basis of the preliminary titration, it contains about 50 ID_{50} in each 0.03 mL. Inject intracerebrally into each mouse [illegible] Allocate the challenge suspension and the 3 dilutions one to each of 4 groups of 10 unvaccinated mice and inject intracerebrally into each mouse 0.03 mL of the suspension or one of the dilutions allocated to its group. Observe the animals in each group at least daily for 14 days. The test is not valid if more than 2 mice of any group die within the first 4 days after challenge. Record the numbers in each group that show signs of rabies in the period 5 days to 14 days after challenge.

The test is invalid unless:

- for both the vaccine to be examined and the reference preparation the 50 per cent protective dose lies between the smallest and the largest dose given to the mice;
- the titration of the challenge suspension shows that 0.03 mL of the suspension contained at least 10 ID_{50};
- the confidence limits ($P = 0.95$) are not less than 25 per cent and not more than 400 per cent of the estimated potency; when this validity criteria is not met, the lower limit of the estimated potency must be at least 1 IU in the smallest prescribed dose;
- the statistical analysis shows a significant slope ($P = 0.95$) and no significant deviations from linearity or parallelism of the dose-response lines ($P = 0.99$).

The vaccine complies with the test if the estimated potency is not less than 1 IU in the smallest prescribed dose.

Application of alternative end-points. Once a laboratory has established the above assay for routine use, the lethal end-point is replaced by an observation of clinical signs and application of an end-point earlier than death to reduce animal suffering. The following is given as an example.

The progress of rabies infection in mice following intracerebral injection can be represented by 5 stages defined by typical clinical signs:

Stage 1: ruffled fur, hunched back;
Stage 2: slow movements, loss of alertness (circular movements may also occur);
Stage 3: shaky movements, trembling, convulsions;
Stage 4: signs of paresis or paralysis;
Stage 5: moribund state.

Mice are observed at least twice daily from day 4 after challenge. Clinical signs are recorded using a chart such as that shown in Table 0451.-1. Experience has shown that using stage 3 as an end-point yields assay results equivalent to those found when a lethal end-point is used. This must be verified by each laboratory by scoring a suitable number of assays using both clinical signs and the lethal end-point.

Table 0451.-1. – *Example of a chart used to record clinical signs in the rabies vaccine potency test*

Clinical signs	Days after challenge							
	4	5	6	7	8	9	10	11
Ruffled fur Hunched back								
Slow movements Loss of alertness Circular movements								
Shaky movements Trembling Convulsions								
Paresis Paralysis								
Moribund state								

4. LABELLING

The label states:

- the type of cell culture used to prepare the vaccine and the species of origin;

1010 — *See the information section on general monographs (cover pages)*

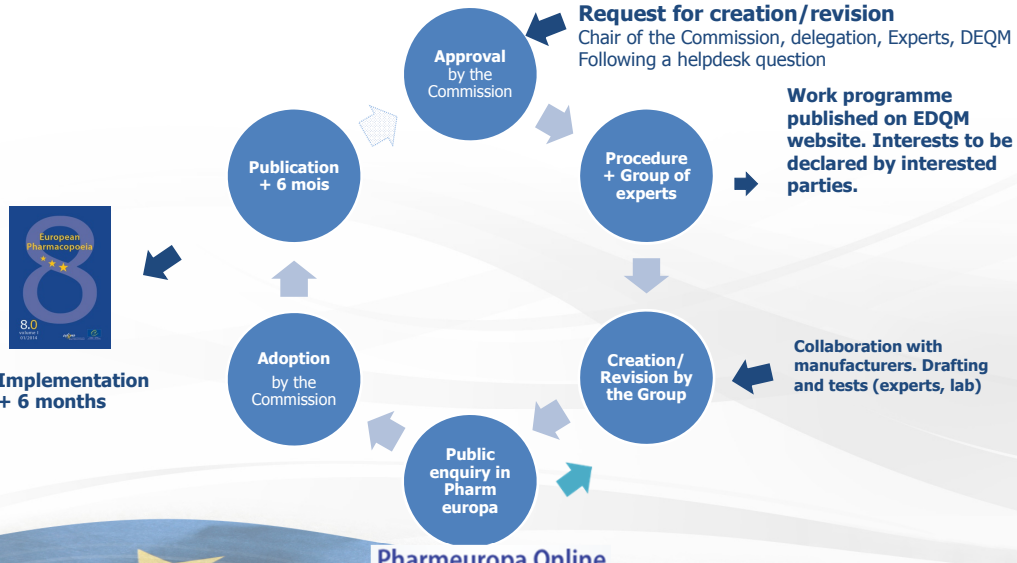
Humane end-points advisory

Example of a chart used to record clinical signs

Storage: a section exist only if what is in the general monopgraph is not sufficient. This section is advisory

Labelling: Items necessary for use of the monograph are mandatory, others are advisory.
Labelling requirements are decided during licensing.

Creation or revision of a text




Pharmeuropa
Deadline: 30.06.2016
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Reference: PA/PH/Exp. 15/T (15) 16 ANP
NOTE ON THE GENERAL CHAPTER
5.2.14. SUBSTITUTION OF IN VIVO METHOD(S) BY IN VITRO METHOD(S) FOR THE QUALITY CONTROL OF VACCINES
PURPOSE
CONTEXT
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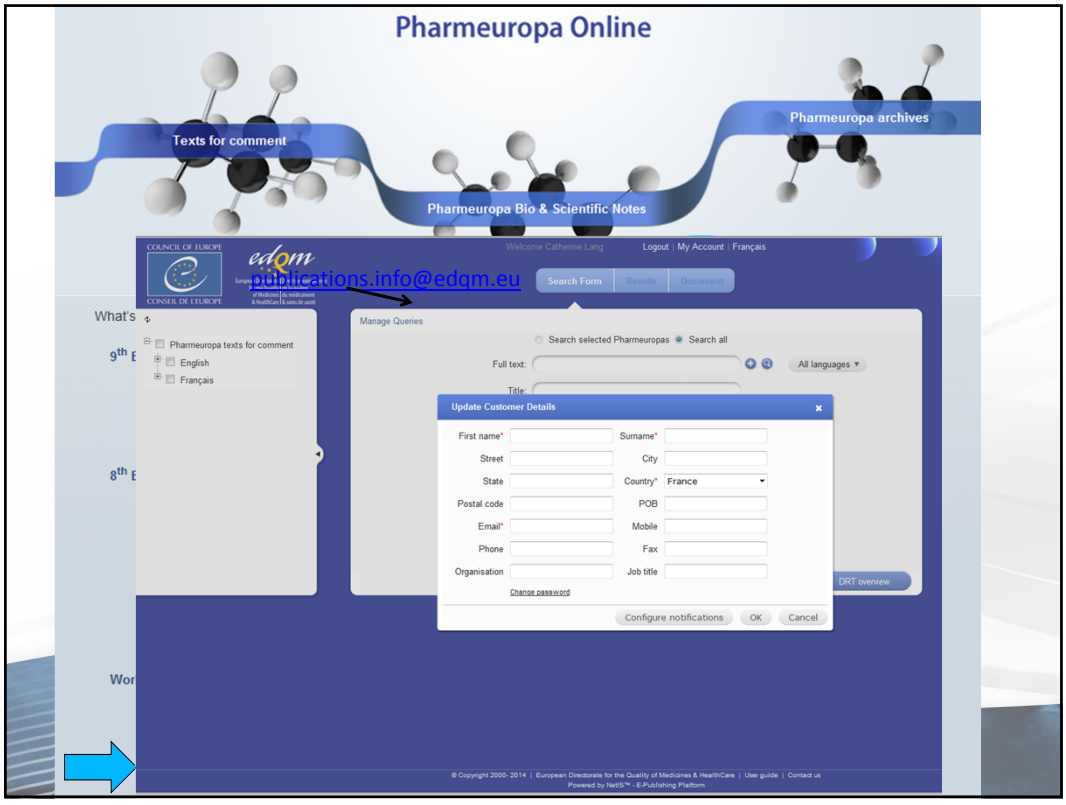
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The Standard Terms database contains terms and definitions to describe pharmaceutical dose forms, routes and methods of administration, containers, closures and administration devices and patient-friendly terms.

Search Standard Terms Database

Meiclass

Database on the legal classification of medicines as regards their supply: conditions and details of prescription status.

Search Meiclass

Search Database online | *Knowledge Database*

Detailed view of Vaccinum furunculosidis inactivatum ad salmonidas cum adiuvatione oleosa ad inie.

Status	In use
Monograph Number	01521
English Name	Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids
French Name	Vaccin inactivé, injectable, à adjuvant huileux, de la furonculose pour salmonid
Latin Name	Vaccinum furunculosidis inactivatum ad salmonidas cum adiuvatione oleosa ad inie
Pinyin Name	
Chinese Name	
Pharmeuropa	26.3
Published in English Supplement	8.3
Published in French Supplement	8.3
On-going	Identification
State of work	4 - DEF
Pharmeuropa	26.3
Description	
Chromatogram	Not available
Additional information	Not available
History	View history
Interchangeable (ICH_Q4B)	NO
International Harmonisation chapter 5.8	NO
Reference standards	
Trade Names	To be used in test(s) / Brand Name
CEP	

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- status: « in use » / « elaboration »
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Participate in the work programme http://www.edqm.eu/en/European-Pharmacopoeia-participate-54.html

Take home messages

- Read the General Notices first.
- Follow the structure of the Ph. Eur. texts and keep up to date.
- Follow our work via the knowledge database and our website.
- Work with us to improve the Ph. Eur. Texts:
  - Participate to the public enquiry in Pharmeuropa,
  - contact us via the helpdesk
  - give your opinion, share your data in our Reader's tribune,

Thank you for your attention

European Directorate for the Quality of Medicines & HealthCare (EDQM)

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SPECIAL FOCUS ON PH. EUR. MONOGRAPHS FOR FISH VACCINES

EDQM international symposium – OSLO - 10/05/2016

Céline Lorteau
DVM - French Agency for Veterinary Medicinal Products
Chair Group 15V Ph. Eur.

CONTENT

RECALL OF THE Ph. Eur. TEXTS APPLICABLE

COMPLEX INTERACTIONS BETWEEN TEXTS

A PRACTICAL EXAMPLE
VIBRIOSIS MONOGRAPH + MONOGRAPH 62

CONCLUSION

PH. EUR. TEXTS APPLICABLE TO FISH VACCINES (1/2)

Specific monographs

FURUNCULOSIS VACCINE (INACTIVATED, OIL-ADJUVANTED, INJECTABLE) FOR SALMONIDS – 1521

VIBRIOSIS (COLD-WATER) VACCINE (INACTIVATED) FOR SALMONIDS – 1580

VIBRIOSIS VACCINE (INACTIVATED) FOR SALMONIDS – 1581

YERSINIOSIS VACCINE (INACTIVATED) FOR SALMONIDS - 1950

4 BACTERIAL INACTIVATED VACCINES

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PH. EUR. TEXTS APPLICABLE TO FISH VACCINES (2/2)

General texts

OF BROADER APPLICATION
PROVIDE GENERAL GUIDANCE
AVOID REPETITION OF REQUIREMENTS

GENERAL NOTICES

VACCINES FOR VETERINARY USE - 0062

GENERAL CHAPTERS

- 2.6.1: Sterility
- 5.1.1: Methods of preparation of sterile products
- 5.1.3: Efficacy of antimicrobial preservation
- 5.2.5: Substances of animal origin for the production of vaccines for veterinary use
- 5.2.6/5.2.7: Evaluation of safety/efficacy of vet. vaccines and immunosera
- ….

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COMPLEX INTERACTIONS BETWEEN TEXTS (1/2)

How to use the Eur.Ph. ?

1- Read the General Notices

2 – Read the General Monograph

3 – Read the Specific Monograph

Applying at the same time the general chapters cited in the monographs

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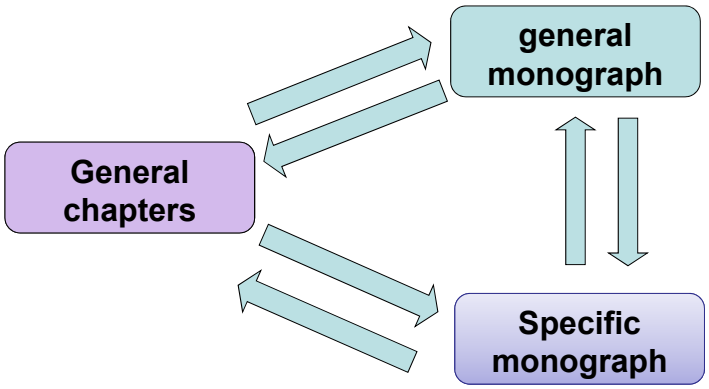
COMPLEX INTERACTIONS BETWEEN TEXTS (2/2)

GENERAL NOTICES



Guide for the elaboration and use of the monographs (2009)

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KEEP IN MIND

Scope

the texts have been written for/based on products falling into the scope

→ requirements applicable within the scope
(i.e. any fish vaccine complies to general monograph 62)

→ requirements may become unsuitable for a product out of scope
(i.e. a vibrosis vaccine for sea bass is out of scope of mono 1581)

Interactions between texts

The requirements from general texts and specific monographs are additionnal, unless indicated

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PH. EUR. TEXTS APPLICABLE TO FISH VACCINES (1/3)

General texts

04/2013:0062

VACCINES FOR VETERINARY USE

Vaccina ad usum veterinarium

GENERAL RULES APPLICABLE TO ALL THE VET. VACCINES

- Quality of the starting materials
- Production of the antigen in a seed lot system
- Inactivation process and control
- How to perform the developmental safety and efficacy tests
- …

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PH. EUR. TEXTS APPLICABLE TO FISH VACCINES (2/3)

General texts

General chapters

GENERAL RULES BROADLY APPLICABLE WITHIN THE Ph. Eur.

- 2.6.1: Sterility
- 5.1.1: Methods of preparation of sterile products
- 5.1.3: Efficacy of antimicrobial preservation
- 5.2.5: Substances of animal origin for the production of vaccines for veterinary use
- 5.2.6: Evaluation of safety of vet. vaccines and immunosera
- 5.2.7: Evaluation of efficacy of vet. vaccines and immunosera

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PH. EUR. TEXTS APPLICABLE TO FISH VACCINES (3/3)

Specific monographs

PROVIDE SPECIFIC INFORMATION:

- Target species, vaccine strains…
- The minimum level of protection (potency/immunogenicity)
- Example for batch potency testing
- Any specific test, i.e. safety characteristics of live vaccine strains
- …

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EXAMPLE (1/2)

04/2013:1581

VIBRIOSIS VACCINE (INACTIVATED) FOR SALMONIDS

Vaccinum vibriosidis inactivatum ad salmonidas

1. DEFINITION

Vibriosis vaccine (inactivated) for salmonids is prepared from cultures of one or more suitable strains or serovars of *Listonella anguillarum (Vibrio anguillarum)*, inactivated while maintaining adequate immunogenic properties; the vaccine may also include *Vibrio ordalii*. This monograph applies to vaccines intended for administration by injection or immersion for the active immunisation of salmonids against vibriosis.

2. PRODUCTION

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

The strains of *L. anguillarum* and *V. ordalii* 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.

2-2. **CHOICE OF VACCINE COMPOSITION**

The strains of *L. anguillarum* and *V. ordalii* used are shown to be suitable with respect to production of antigens of assumed protective 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 tests*. Safety is tested using test 2-2-1-1-1, test 2-2-1-1-2, or both, depending on the recommendations for use.

Carry out the test in each species of fish for which the vaccine is intended, using 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. The test is carried out in the conditions to be recommended for use of the vaccine with a water temperature not less than 10 °C.

2-2-1-1-1. *Vaccines intended for administration by injection*. Use not fewer than 50 fish from a population that does not have specific antibodies against *L. anguillarum* or where applicable *V. ordalii* and has not been vaccinated against or exposed to vibriosis. Administer to each fish by the intraperitoneal route 1 dose of the vaccine. Observe the fish at least daily for 21 days.

The test is not valid if more than 6 per cent of the fish die from causes not attributable to the vaccine. The vaccine complies with the test if no fish shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-2-1-1-2. *Vaccines intended for administration by immersion*. Use not fewer than 50 fish from a population that does not have specific antibodies against *L. anguillarum* or where applicable *V. ordalii* and has not been vaccinated against or exposed to vibriosis. Prepare an immersion bath at twice the concentration to be recommended. Bathe the fish for twice the time to be recommended. Observe the fish at least daily for 21 days.

The test is not valid if more than 6 per cent of the fish die from causes not attributable to the vaccine. The vaccine complies with the test if no fish shows abnormal local or systemic reactions or dies from causes attributable to the vaccine.

2-2-1-2. *Field studies*. Safety is demonstrated in addition in field trials by administering the dose to be recommended to a sufficient number of fish distributed in not fewer than 2 sets of premises.

The vaccine complies with the test if no fish shows abnormal reactions or dies from causes attributable to the vaccine.

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EXAMPLE (2/2)

2-2-2. **Immunogenicity**. Carry out a separate test for each fish species and each serovar included in the vaccine, according to a protocol defining water source, water flow and temperature limits, and preparation of a standardised challenge. Each test is carried out for each route and method of administration to be recommended. The vaccine administered to each fish is of minimum potency.

Use for the test not fewer than 60 fish of the minimum body mass to be recommended for vaccination, from a population that does not have specific antibodies against *L. anguillarum* or where applicable *V. ordalii* and has not been vaccinated against or exposed to vibriosis. Vaccinate not fewer than 30 fish according to the instructions for use. Perform mock vaccination on a control group of not fewer than 30 fish; mark vaccinated and control fish for identification. Keep all the fish in the same tank or mix equal numbers of controls and vaccinates in each tank if more than 1 tank is used. Where justified and when fish cannot be marked, non-marked fish may be used. Vaccinates and controls may then be kept in the same tank but physically separated (for example by fishing nets). Challenge each fish at a fixed interval after vaccination, corresponding to the onset of immunity claimed, by a suitable route with a sufficient quantity of cultures of *L. anguillarum* or *V. ordalii* whose virulence has been verified. Observe the fish at least daily until at least 60 per cent specific mortality is reached in the control group. Plot for both vaccinates and controls a curve of specific mortality against time from challenge and determine by interpolation the time corresponding to 60 per cent specific mortality in controls.

The test is not valid if the specific mortality is less than 60 per cent in the control group 21 days after the 1st death in the fish. Read from the curve for vaccinates the mortality (M) at the time corresponding to 60 per cent mortality in controls. Calculate the relative percentage survival (RPS) using the following expression:

$$\left(1 - \frac{M}{60}\right) \times 100$$

The vaccine complies with the test if the RPS is not less than 60 per cent for vaccines administered by immersion and 75 per cent for vaccines administered by injection.

2-3. **MANUFACTURER'S TESTS**

2-3-1. **Batch potency test**. The potency test (section 3-3) may be carried out for each batch of vaccine, using fish of one of the species for which the vaccine is intended. Where the test is not carried out, an alternative validated method based on antibody response may be 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. The following test may be used.

Use not fewer than 35 fish from a population that does not have specific antibodies against *L. anguillarum* included in the vaccine and where applicable against *V. ordalii*, and that are within specified limits for body mass. Carry out the test at a defined temperature. Inject into each of not fewer than 25 fish 1 dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than 10 fish. Collect blood samples at a defined time after vaccination. Determine for each sample the level of specific antibodies against *L. anguillarum* included in the vaccine and where applicable against *V. ordalii*, by a suitable immunochemical method (2.7.1). The test is not valid if the control group shows antibodies against *L. anguillarum* or, where applicable, against *V. ordalii*. The vaccine complies with the test if the mean level of antibodies in the vaccinates is not significantly lower than that found for a batch that gave satisfactory results in the test described under Potency.

3. BATCH TESTS

3-1. **Identification**. When injected into fish that do not have specific antibodies against *L. anguillarum* and, where applicable, *V. ordalii*, the vaccine stimulates the production of such antibodies.

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 mentioned under Immunogenicity (section 2-2-2) when administered by a recommended route and method.

4. LABELLING

The label states information on the time needed for the development of immunity after vaccination under the range of conditions corresponding to the recommended use.

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MONO 1581 - VIBRIOSIS

1. DEFINITION → SCOPE

Vibriosis vaccine (inactivated) for salmonids is prepared from cultures of **one or more suitable strains** or serovars of ***Listonella anguillarum*** *(Vibrio anguillarum)*, **inactivated** while maintaining adequate immunogenic properties; the vaccine may also include ***Vibrio ordalii***.

This monograph applies to vaccines intended for administration by **injection or immersion** for the **active immunisation** of **salmonids** against vibriosis.

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MONOGRAPH 1581 - MONOGRAPH 0062

2. PRODUCTION
2-1. PREPARATION OF THE VACCINE

The strains of *L. anguillarum* and *V. ordalii* 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.

2. PRODUCTION
2-1. PREPARATION OF THE VACCINE

2-1-1. Substrates for production
[…] Reference to 5.2.2 & 5.2.4

2-1-2. Media used for seed culture preparation and for production
[…] Reference to 5.2.5

2-1-3. Seed lots
2-1-3-1. *Bacterial seed lots*
2-1-3-1-1. General requirements. The genus and species […]
2-1-3-1-2. Propagation. The minimum and maximum number of subcultures of each master seed lot […]
2-1-3-1-3. Identity and purity. Each master seed lot is shown to contain only the species and strain stated […]

2-1-4. Inactivation

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MONOGRAPH 0062 – PRODUCTION

2-1-3-1-1. General requirements. The **genus** and **species** (and varieties where appropriate) of the bacteria used in the vaccine are stated. Bacteria used in manufacture are handled in a **seed-lot system** wherever possible. Each master seed lot is tested as described below. A record of the **origin**, **date of isolation**, **passage history** (including purification and characterisation procedures) and **storage** conditions is maintained for each master seed lot. Each master seed lot is assigned a **specific code for identification** purposes.

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MONOGRAPH 0062 – PRODUCTION

2-1-3-1-3. Identity and purity. Each master seed lot is shown to contain **only the species and strain of bacterium stated**.
A brief description of the method of identifying each strain by **biochemical, serological and morphological characteristics** and distinguishing it as far as possible from related strains is recorded, as is also the method of determining the **purity of the strain**. If the master seed lot is shown to contain living organisms of any kind other than the species and strain stated, then it is unsuitable for vaccine production.

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VIBROSIS – PRODUCTION (EXTRACTS)

2. PRODUCTION
2-2. CHOICE OF VACCINE COMPOSITION

Gives the level of efficacy expected – part of the efficacy demonstration

2-2-2. Immunogenicity. Carry out a separate test for each fish species and each serovar included in the vaccine, […]for each route and method of administration to be recommended.
[…] **Vaccinate** not fewer than **30 fish** according to the instructions for use. Perform mock vaccination on a **control group** of not fewer than **30 fish**; […] **Challenge** each fish at a fixed interval after vaccination, corresponding to the onset of immunity claimed, by a suitable route with a sufficient quantity of cultures of *L. anguillarum* or *V. ordalii* whose virulence has been verified. Observe the fish at least daily until at least 60 per cent specific mortality is reached in the control group. […]← protocol
The test is not valid if […]. ← validity criteria
The vaccine complies with the test if **the RPS is not less than 60 % for vaccines administered by immersion and 75 % for vaccines administered by injection**.
← Acceptance limit

Beside this, the general monograph 0062 and chapter 5.2.7. provide details on administration routes, how to demonstrate onset and duration of immunity…

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VIBRIOSIS – PRODUCTION & IN-PROCESS TESTING

2. PRODUCTION
2-3. MANUFACTURER'S TESTS
2-3-1. Batch potency test. The potency test (section 3-3) may be carried out for each batch of vaccine […]. Where the test is not carried out, an **alternative validated method** based on antibody response may be 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. The following test may be used.
Use […] fish from a population that does not have specific antibodies […]. Inject into each of not fewer than 25 fish 1 dose of vaccine, according to the instructions for use. Perform mock vaccination on a control group of not fewer than 10 fish. Collect blood samples at a defined time after vaccination. Determine for each sample the level of specific antibodies against *L. anguillarum* included in the vaccine and where applicable against *V. ordalii*, by a suitable immunochemical method (2.7.1). The test is not valid […] The vaccine complies with the test if the mean level of antibodies in the vaccinates is not significantly lower than that found for a batch that gave satisfactory results in the test described under Potency.

Scope of 3Rs:
Reduction
Refining
Replacing

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MONO 1581 - MONO 62

3. BATCH TESTS

3-1. Identification. (soon revised to:) 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 mentioned under Immunogenicity (section 2-2-2) when administered by a recommended route and method. (Usually replaced by a manufacturer's test by serology or challenge)

3. BATCH TESTS (soon revised to:)

3-1. Identification […]

3-2. Physical tests […]

3-3. Chemical tests […]

3-4. pH. […]

3-5. Water. […]

3-6. Formaldehyde […]

3-7. Phenol (2.5.15) […]

3-8. Bacteria and fungi […]

3-9. Extraneous agents […]

3-10. Residual live virus /bacteria and/or detoxification testing […]

3-11. Mycoplasmas (2.6.7) […]

3-12. Potency […]

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CONCLUSION

Few monographs for fish vaccines (4)

Only for bacterial inactivated vaccines

Numerous fish used / trial

Limited knowlege & availablility of *in vitro* alternatives

Batch potency test to improve (3Rs)

→ **Symposium**

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THANK YOU FOR YOUR ATTENTION!
Picture Christopher Swann / Biosphoto

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