General Overview of Fish Vaccination

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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

Norwegian Salmon Production,
Use of Pure Antibiotics and the Effect of Vaccines

Antibiotic usage has reduced by 99.5%

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

Atlantic salmon farm sites

SCOTTISH SALMON PRODUCTION 1989-2013
Value

* Largest producer in EU
* Largest food export
* Worth £540M, retail value >£1billion
* 1 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

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**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

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccines</th>
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<tbody>
<tr>
<td>1982</td>
<td>1 Enteric Redmouth (ERM) vaccine</td>
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<tr>
<td></td>
<td>2 Vibrio anguillarum vaccine</td>
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<td></td>
<td>3 Furunculosis vaccine</td>
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<td>4 Vibrio salmonicida vaccine</td>
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<td>5 Combined Vibriosis/Furunculosis vaccine</td>
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<tr>
<td></td>
<td>6 Combined Vibriosis/Coldwater Vibriosis/Moritella viscosa vaccine</td>
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<tr>
<td>2016</td>
<td>1 Enteric Redmouth (ERM) vaccine</td>
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<td></td>
<td>2 Vibrio anguillarum vaccine</td>
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<td>3 Furunculosis vaccine</td>
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<td>6 Combined Vibriosis/Coldwater Vibriosis/Moritella viscosa vaccine</td>
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<td>7 Combined Vibriosis/Coldwater Vibriosis/Moritella viscosa/IPNV vaccine</td>
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<td>8 IPN Virus vaccine</td>
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<td></td>
<td>9 Pasteurella vaccine</td>
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<td>10 Combined Pasteurella/Vibriosis vaccine</td>
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<td>11 Vibriosis vaccine for cod</td>
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<td>12 Shrimp Vibriosis vaccine</td>
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<td>13 Warmwater Vibriosis agg vaccine</td>
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<td>14 SVC virus vaccine</td>
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<td>15 Lactococcus garvieae/Streptococcus iniae</td>
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<td></td>
<td>16 Aeromonas hydrophila vaccine</td>
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<td>17 Aphanizomenon azollae</td>
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<td></td>
<td>18 Aeromonas hydrophila vaccine</td>
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<td>19 Aphanizomenon azollae</td>
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<td>20 ALC virus vaccine</td>
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<td>21 Gaffkaemia vaccine</td>
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<td>22 Flavobacterium psychrophilum</td>
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<td>23 Flavobacterium psychrophilum</td>
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<td></td>
<td>24 Pancreas disease virus vaccine</td>
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<td></td>
<td>25 Pseudomonas turbinatalvae</td>
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<tr>
<td></td>
<td>26 Pancreas disease virus vaccine</td>
</tr>
<tr>
<td></td>
<td>27 PD vaccine</td>
</tr>
</tbody>
</table>

**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 multivalent

### 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**

- 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) 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° 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 in their 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
**Vaccine Efficacy**

**Three criteria used:**
- Rate at which protection is achieved
- The final degree of protection (RPS)
- Duration of immunity

\[ \text{RPS} = 1 - \frac{\% \text{ vaccinate mortalities}}{\% \text{ control mortalities}} \times 100 \]

- The speed of the immune response is temperature dependent
- 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
Optimum strategy for vaccination is determined by:

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

- The effect of temperature can be put to good use during vaccination. 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.

Vaccination Strategies

- Innate immunity
- Adaptive immunity

The immune response of fish
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
Key areas of next-generation vaccine development - similar for fish and other animals

- Variation in pathogen Pathogenome
  - Identification of protective antigens
  - Fundamental understanding of pathogen
  - Animals models, in vitro models (correlates of protection)

- Next-generation sequencing

- Variation in humans
  - Patient profile studies/clinical trials Genotypes
  - Identification of signatures of protection
  - Fundamental understanding of immune response
  - Systems biology/HTP screening technologies

- Vaccine technologies
  - Adjuvants, formulation
  - Vectors, delivery systems and devices
  - Advanced immunization schemes

- Next-generation vaccine development


©2013 by Cold Spring Harbor Laboratory Press
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

Thank you!
EDQM/European Pharmacopoeia:
assuring the quality of medicines

EDQM Symposium on Challenges of Quality Requirements for Fish Vaccines
OSLO 10-11 May 2016

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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 ...

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) usefultful information
- Organisation of hearings of interested parties

Ph. Eur. – General organisation

- 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

• 2.6 Biological tests
  • 2.6.1 Sterility
  • 2.6.7 Mycoplasmas
  • 2.6.8 Pyrogens
  • 2.6.24 & 2.6.25
• 5.1 General texts on microbiology
  • etc

General chapters

• 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

Reference standards

Individual monographs

General monographs

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Ph. Eur. texts

classes of substances, dosage forms

• Substances for pharmaceutical use (2034)
• Pharmaceutical preparations (2619)
• Vaccines for veterinary use (0062)
• Immunsera 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

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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!

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

### 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

- 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)
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

EDQM Symposium on Challenges of Quality Requirements for Fish Vaccines

OSLO 10-11 May 2016

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Ph. Eur. – General organisation

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

Safety: Mandatory

Immunogenicity: Mandatory

Residual live virus: The verification of the inactivation is mandatory

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 BVP
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.)

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

Reference chart used to record clinical signs

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.

Humane end-points advisory

Example of a chart used to record clinical signs

Storage: a section exist only if what is in the general monograph 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

Request for creation/revision
Chair of the Commission, delegation, Experts, DEQM
Following a helpdesk question

Work programme published on EDQM website. Interests to be declared by interested parties.

Collaboration with manufacturers. Drafting and tests (experts, lab)

Pharneupedia Online

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5.2.14. SUBSTITUTION OF IN VIVO METHOD(S) BY IN VITRO METHOD(S) FOR THE QUALITY CONTROL OF VACCINES.

The purpose of this section is to facilitate the implementation of in vitro methods as substitutes for in vivo methods, when these are not desired or are not appropriate for reasons unrelated to the safety of an in vivo method. This section will not discuss the details of assay validation as such, since these principles are described elsewhere.

The general chapter applies primarily to human and veterinary vaccines; however, the principles described may also apply to other biologics such as sera.

The text methods used for quality control of vaccines are intended to ensure production consistency and to ensure comparability of the quality attributes between different batches and the batches originally found to be safe and effective in clinical studies or in the target species for veterinary vaccines.

While in vivo tests for safety and efficacy are described, recent advances in in vitro methods of testing have contributed to reducing the number of animals used for vaccine testing. These methods include the use of cell culture systems and the use of recombinant proteins.

In addition to the benefits resulting from the substitution of in vivo methods by in vitro methods, the European Pharmacopoeia (Ph. Eur.) Commission has contributed to the reduction of animal usage whenever possible in Pharmacopoeial testing. Under the convention, those associated with this work are encouraged to develop and implement in vitro procedures and the General Notice of the Ph. Eur. supports the introduction of in vitro methods described in Pharmacopoeial monographs.
Knowledge Database

- **status**: « in use »
- Work in progress and why
- View history
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)

How to contact the EDQM?

Information and orders via the Internet:
www.edqm.eu

Questions must be submitted through the HELPDESK, which is accessible on the EDQM Internet site:
www.edqm.eu/help

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Fax: +33 (0)3 88 41 27 71*

*Please add the 0 when calling from outside France

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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

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

General texts

OF BROADER APPLICATION
PROVIDE GENERAL GUIDANCE
AVOID REPEITION 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
• .....

anses
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
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 additional, unless indicated

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
• …
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

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
• …
EXAMPLE (1/2)

VIBRIONS VACCINE (INACTIVATED) FOR SALMONIDS

1. **Definition**
   - Vibrios vaccine (inactivated) for salmonids is prepared from cultures of one or more suitable species of vibrios, such as *Vibrio anguillarum* (ichthyic vibriosis), inactivated while maintaining adequate immunogenic properties. The vaccine may also include *Vibrio cholerae*. This monograph applies to vaccines intended for administration by intraperitoneal or intramuscular injection for the active immunization of salmonids against vibriosis.

2. **INDICATIONS**
   - This vaccine is indicated for the active immunization of salmonids against vibriosis.

3. **PREPARATION OF VACCINE**
   - The vaccine consists of cultures of viable *Vibrio cholerae* or *Vibrio anguillarum* that are harvested and concentrated separately. The cultures are harvested by a suitable method. They may be purified and concentrated. Whole or disrupted cells may be used. The vaccine may be used in the form of a suspension suitable for inactivation of the vaccine against slides or vials of the vaccine that may be stored for use over a period of time.

4. **STORAGE OF VACCINE**
   - The vaccine should be stored in a refrigerator at temperatures between 2°C and 8°C.

5. **ADMINISTRATION**
   - The vaccine may be administered by intraperitoneal or intramuscular injection. For fish weighing less than 500 grams, the injection dose is approximately 50 microliters. For fish weighing over 500 grams, the injection dose is approximately 50 microliters per 100 grams of body weight.

6. **SAFETY**
   - Safety is tested using the test described in Section 2.2.2 on whole fish, depending on the recommendations for use.

- **Carry out the test in each species of fish for which the vaccine is intended, using the fish of the appropriate size and weight as recommended for vaccination. Use a batch of vaccine containing not less than the minimum potency that may be expected in a batch of vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section.**

7. **IMMUNITY**
   - The test is carried out at least 10 days after the last dose of the vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section. The test is carried out at least 10 days after the last dose of the vaccine.

8. **Field studies**
   - Safety is demonstrated in field trials by administering the dose to be recommended in a sufficient number of fish within 2 weeks of the first dose of the vaccine.

9. **Vaccine compliance with the test if no fish shows abnormal reactions or deaths from causes attributable to the vaccine.

EXAMPLE (2/2)

2.2.2 **Immunogenicity**
   - Carry out a separate test for each fish species to be vaccinated in the vaccine, according to a protocol defined by the manufacturer, taking into account the optimal conditions of vaccination and the species, and the duration of the test. The vaccine is administered to each fish of the appropriate size and weight as recommended for vaccination. Use a batch of vaccine containing not less than the minimum potency that may be expected in a batch of vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section.

- **Carry out the test in each species of fish for which the vaccine is intended, using the fish of the appropriate size and weight as recommended for vaccination. Use a batch of vaccine containing not less than the minimum potency that may be expected in a batch of vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section.**

2.2.3 **Requirements for the test**
   - The test is carried out at least 10 days after the last dose of the vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section. The test is carried out at least 10 days after the last dose of the vaccine.

2.2.4 **Immunogenicity**
   - The test is carried out at least 10 days after the last dose of the vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section. The test is carried out at least 10 days after the last dose of the vaccine.

2.2.5 **References**
   - The test is carried out at least 10 days after the last dose of the vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section. The test is carried out at least 10 days after the last dose of the vaccine.

3. **REFERENCES**
   - The test is carried out at least 10 days after the last dose of the vaccine. The test is carried out in the conditions recommended for use of the vaccine under the conditions stated in this section. The test is carried out at least 10 days after the last dose of the vaccine.

4. **LABELING**
   - The label states information on the time needed for the development of immunity after vaccination under the conditions of use, corresponding to the recommended use.
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.

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.
2-1-3-1-1. 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.

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.
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...

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
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 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)

3-4. 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

CONCLUSION
Few monographs for fish vaccines (4)
Only for bacterial inactivated vaccines
Numerous fish used / trial
Limited knowledge & availability of in vitro alternatives
Batch potency test to improve (3Rs)

→ Symposium
THANK YOU FOR YOUR ATTENTION!

Picture Christopher Swann / Biosphoto