FISH VACCINES.
SUMMARY OF SPANISH SITUATION

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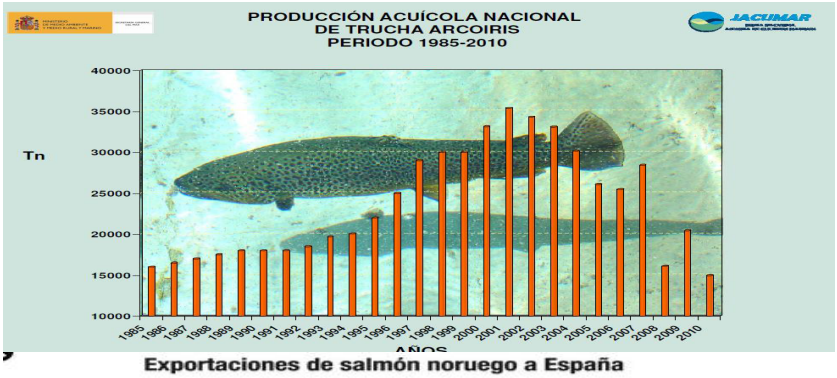
ACUACULTURE in SPAIN

Type of Aquaculture	Group-Specie	Tons (2014)
Marine	Fishes	46.883,64
	Crustaceans	158,12
	Molluscs	244.564,74
	Annelids	0,47
	Seaweed	3,44
	TOTAL	291.604,41
Continental	Fishes	14.118,6
	Crustaceans	6
	TOTAL	14.124,6

Species	Tons (2014)	
Mussel	241.478	(Ecologic 407)
Sea Bream	16.068	(Ecologic 11,36)- **42 % Marine Fish**
Rainbow Trout	14.009	(Ecologic 365)- **97% Continental Fish**
Sea bass	16.319	(Ecologic 163,83)- **33% Marine Fish**
Turbot	**7.891**	**(2nd producer in the world, Chile 1st)**
Red tuna	3.966	

(MAGRAMA, JACUMAR)

5.025 Fish farms: 163 continental and 4.862 marine



Vaccines authorised in Spain (EU procedures)

Reg. Nº	Name	MAH	Auth Date	Active substance	Fish Specie
1658 ESP	**AquaVac ERM INMERSION**	MSD	2005	***Yersinia Ruckeri***	**Trout**
1688 ESP	**AquaVac ERM ORAL**	MSD	2006	***Yersinia Ruckeri***	**Trout**
1687 ESP	**AQUAVAC FNM**	MSD	2006	***Aeromonas salmonicida***	**Atlantic Salmon**
2054 ESP	**AQUAVAC RELERA**	MSD	2009	***Yersinia Ruckeri***	**Trout**
1712 ESP	**AQUAVAC VIBRIO INMERSION E INYECCION**	MSD	2006	***Vibrio anguillarum and ordalli***	**Trout**
1708 ESP	**AQUAVAC VIBRIO ORAL**	MSD	2006	***Vibrio anguillarum and ordalli***	**Trout**
Decentralized	**ALPHA DIP Vib**	PharmaQ (ZOETIS)	2016	***Vibrio anguillarum***	**Sea bass**
Centralized	**CLYNAV**	ELANCO	2016	**Plasmid DNA SAV**	**Atlantic Salmon**

Field trials and possible future procedures:

- ***Photobacterium damselae*** **and/or** ***Vibrio anguillarum*** **for sea bass and/or sea bream.**

Vaccines authorised in Spain (National procedures)

Reg. Nº	Name	MAH	Auth Date	Active substance	Fish Specie
1642 ESP	**ICTHIOVAC-LG LACTOCOCOSIS TRUCHA**	L. HIPRA, S.A.	2005	***Lactococcus garvieae.***	**Trout**
2949 ESP	**YERSYVAC**	L. SYVA, S.A.U.	1995	***Yersinia Ruckeri***	**Trout**
1466 ESP	**ICTHIOVAC-PD PASTEURELOSIS DORADA**	L. HIPRA, S.A.	2002	***Photobacterium damselae***	**Sea bream**
1465 ESP	**ICTHIOVAC-STR ESTREPTOCOCOSIS RODABALLO**	L. HIPRA, S.A.	2002	***Streptococcus parauberis***	**Turbot**
1691 ESP	**ICTHIOVAC-TM TENACIBACULOSIS RODABALLO**	L. HIPRA, S.A.	2006	***Tenacibaculum maritimum***	**Turbot**
1467 ESP	**ICTHIOVAC-VR VIBRIOSIS RODABALLO**	L. HIPRA, S.A.	2002 Line ext 2016	***Vibrio anguillarum***	**Turbot and sea bass**

Also authorized under Article 8 of Directive (special national authorization): vaccine against nodavirus (sea bass), manufactured by PHARMAQ- possible future DC procedure
(This vaccine was first authorised in Greece as an autogenous vaccine)

For all of them (EU and National) : in vivo assay for immunogenicity and except DNA vaccine, in vivo challenge for batch potency test

Other vaccines for fish:

AUTOGENOUS FISH VACCINES

DIRECTIVE 2001/82/EC . Article 3. 2: Inactivated immunological veterinary medicinal products which are manufactured from pathogens and antigens obtained from an animal or animals from a holding and used for the treatment of that animal or the animals of that holding in the same locality

CMDV group of autogenous vaccines

In SPAIN All are bacterial and inactivated, monovalent or bivalent

Active susbtances:

- ***Yersinia ruckery***
- ***Lactocossus garviae***
- ***Aeromonas spp. (salmonicida)***
- ***V. anguillarum***
- ***Philasterides dicentrarchi***
- ***Pasteurella pisicida***
- ***Vibrio fluvialis***
- ***Tenacibaculum* spp *(maritimum)***

Fish species: **Trout, sea bass, sea bream and sole**

- **Total doses:** With the numbers that we have, during 2012 and 2013, each year approximately **35.000 Litres (aprox 22 million of doses of autogenous vaccines for fishes)**
- **During 2015, Commercial fish vaccines in Spain: Total= 1.494,5 L**

Other biologicals

Bacteriophages for bacterial diseases treatment in fishes

Evaluate the effect of phages use in environmental bacterial ecology
Evaluar el uso de fagos en la ecología bacteriana ambiental
Avaliar o efeito de fagos na ecologia bacteriana ambiental

AZTI y Biopolis S.L. (Spain), Aveiro University and Acuicultura Aguacircia (Portugal)

Use of Probiotics and Prebiotics in Aquaculture

BACTOCELL®: the First Probiotic Authorized for use in Aquaculture in the European Union (lactic acid bacteria strain *Pediococcus acidilactici* CNCM MA18/5M), for use as a zootechnical feed additive in **salmonids and shrimp**

Review of immune stimulator substances/agents that are susceptible of being used as feed additives: mode of action and identification of end-points for efficacy assessment

Institut de Recerca i Tecnologia Agroalimentàries (IRTA)-SPAIN . Joaquim Brufau, Enric Esteve, Joan Tarradas

EFSA Supporting publication 2015:EN-905

Problems of the market:

- **High number of doses of autogenous vaccines in fish** *(is Spanish example the same in other countries?).*
- **Not high number of industrial vaccines authorized** *(but now increasing-at least in Spain).*
- **Why is increasing the use of biological treatments (and research) in fishes?:**
- **- Because antimicrobials use in fish farms is decreasing?**
- **About the authorization of new commercial fish vaccines**
- **- MUMS guideline** EMA/CVMP/IWP/123243/2006 Rev.3 *(including reduction of taxes) :*
- ***(and in the revised Rev3 Immuno MUMs all fishes except salmon are minor species)***
- **- EMA guideline for assessment of safety and efficacy of fish vaccines**
- ***Could be a general Ph. Eur text for fish vaccines an aid for the development of future vaccines?***
- **The specific monographs in Ph Eur are only for salmonids:**
  - **01/2015:1521 Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids**
  - **04/2013: 1580 Vibriosis cold-water vaccine inactivated for salmonids**
  - **04/2013: 1581 Vibriosis vaccine inactivated for salmonids**
  - **04/2013: 1950 Yersiniosis vaccine inactivated for salmonids**
- **Is there a need for other specific fish monographs?**

Questions/ Answer during assessments:

- When tests are described in a Ph. Eur. monograph with a particular fish species (Salmonids), is it possible for manufacturers to "adapt" the test for another fish species (for ex. Vibrio anguillarum for Salmonids to sea bass/Sea bream ; batch potency tests?

- *From our experience, it is possible (at least vibriosis) for sea bass and sea bream.*
- *Batch potency performed by challenge*

- For a vaccine intended for two or more different species, how to perform batch potency test?

- *In a recent example ("line extension" of a vaccine -batch potency: challenge), finally it was agreed to perform batch potency by challenge only in one fish specie*
- *Proposal: if it is possible to develop a batch potency test without challenge (by an in vitro test), this problem could be solved*

- *Is water for injection quality needed for intraperitoneal route in fishes?*

- *In this example (line extension-bath immersion). In line with actual EU legislation and guidelines, the quality of the water was changed form "highly purified" to "water for injection"*
- *My opinion: Good argumentation from the company, and to be taken into account for future discussions (it can also help the development of future fish vaccines)*

- In which extent safety and efficacy results from one fish species can be applicable to other (trout to salmon could be, but from trout/salmon to sea bream?)

- *Not in the present fish monographs, but included in MUMS guideline EMA*
- *Proposal: To be taken into account also to help the development and authorisation of future vaccines. Possible in a general text for fish vaccines in the Ph. Eur?*

Other questions:

- How can the Ph. Eur. better address the needs of its users considering the current regulatory environment in Europe?
- A general monograph dedicated to fish vaccines could help?

- *Proposal of general monograph for fish vaccines*
  (- also taking into account DNA fish vaccines?.)

- Is there a need for new or revised monographs?:
- Proposal
- *Vibrio anguilarum for sea bass and/or sea bream (revised)*
- *Possible future Photobacterium damsellae for sea bass/ sea bream (new)?*

- How to facilitate availability of fish vaccines?
- *Proposals:*
- *To include MUMS principles in the general monograph for fish vaccines?.*
- *Possible extrapolations from safety/efficacy data of one fish specie to others?*
- *Development of "in vitro" potency test*


THANK YOU

Veterinary Medicines Directorate

Quality control testing of fish vaccines, 3Rs issues and development of in vitro tests

Dr Rory Cooney
Biologicals Assessment Team, VMD, UK

Outline of presentation

- Overview of availability of fish vaccines in the UK
- QC testing of veterinary vaccines
- Regulatory requirements for batch potency testing
- Estimation of number of fish used in QC testing
- Principles of validation of potency tests
  - Virulent challenge
  - Serology
  - In vitro methods
- 3Rs perspective
- Experience in replacing challenge tests for some mammalian vaccines

Overview of availability of fish vaccines in the UK

Authorised vaccines:

- Currently 12 fish vaccines authorised in the UK
  - 9 MRP
  - 3 National
  - (1 EUCE)*
- Zoetis (Pharmaq), MSD (Intervet), Elanco (Novartis)
- Includes inactivated vaccines against *Aeromonas salmonicida*, infectious pancreatic necrosis virus (IPNV), *Yersinia ruckeri*, salmon pancreas disease virus, *Listonella anguillarum*, *Moritella viscosa*
- For use in trout or Atlantic salmon

Overview of availability of fish vaccines in the UK

Autogenous vaccines:

- Currently 2 manufacturers authorised to produce autogenous fish vaccines and includes vaccines for use in:
  - salmon against *A. salmonicida* and *Y. ruckeri*
  - salmon, cod, trout, halibut, turbot & barramundi against vibriosis, cataracts caused by *Pseudomonas anguilloseptica*, rainbow trout fry syndrome, enteric red-mouth disease, gill disease, furunculosis and ulcerations, fungal infections due to Saprolegnia, streptococcal and other Gram+ cocci infections of farmed fish
  - a range of exotic species including display fish in zoological collections (not intended for food consumption) against systemic infections and yersiniosis
- No potency test for autogenous vaccines but require a batch safety test conducted with a double dose of vaccine in groups of up to 60 fish carried out on farm site

Quality Control Testing of Veterinary Vaccines

- Biological nature of immunological veterinary medicinal products (IVMPs) leads to some unavoidable batch to batch variation in production
- Consequently, production requires the routine use of QC tests to monitor production consistency and to ensure comparability of the quality attributes between commercial batches and those batches originally found to safe and efficacious in clinical studies
- QC tests for manufacturing control and batch release can involve the use of large numbers of animals

Regulatory requirements for batch potency test

- Mainly European Pharmacopoeia requirements:
  - Ph. Eur. 0062: Vaccines for veterinary use
  - Ph. Eur. 1521: Furunculosis vaccine (inactivated, oil-adjuvanted, injectable) for salmonids
  - Ph. Eur. 1580: Vibriosis (cold-water) vaccine (inactivated) for salmonids
  - Ph. Eur. 1581: Vibriosis vaccine (inactivated for salmonids)
  - Ph. Eur. 1950: Yersiniosis vaccine (inactivated) for salmonids
- Specific monographs describe classical testing method based on vaccination and challenge with mortality as end-point but allow for alternative test method
- Directive 2001/82/EC as amended requires that a control of the batch titre or potency is carried out on the finished product

3Rs and legislative framework

- A framework of principles for humane animal research
  - Reduction: methods which minimise the number of animals in an experiment
  - Refinement: methods which minimise suffering and improve animal welfare
  - Replacement: methods which avoid use of animals
- Directive 2009/9/EC amending Directive 2001/82/EC:
  - "Member States shall ensure that all experiments on animals are conducted in accordance with Council Directive 86/609/EEC"
  - Minimum requirements that a product must meet are those laid down in the relevant Ph. Eur. monographs
- Directive 2010/63/EU replaces Directive 86/609/EEC on the protection of animals used for scientific purposes
  - Came into full effect on 1 January 2013

Directive 2010/63/EU on the protection of animals used for scientific purposes

Article 13

Choice of methods

1. Without prejudice to national legislation prohibiting certain types of methods, Member States shall ensure that a procedure is not carried out if another method or testing strategy for obtaining the result sought, not entailing the use of a live animal, is recognised under the legislation of the Union.

2. In choosing between procedures, those which to the greatest extent meet the following requirements shall be selected:

(a) use the minimum number of animals;

(b) involve animals with the lowest capacity to experience pain, suffering, distress or lasting harm;

(c) cause the least pain, suffering, distress or lasting harm;

and are most likely to provide satisfactory results.

3. Death as the end-point of a procedure shall be avoided as far as possible and replaced by early and humane end-points. Where death as the end-point is unavoidable, the procedure shall be designed so as to:

(a) result in the deaths of as few animals as possible; and

(b) reduce the duration and intensity of suffering to the animal to the minimum possible and, as far as possible, ensure a painless death.

Estimating numbers of animals used in QC testing

- Official batch release of IVMPS in the UK is conducted by the Veterinary Medicines Directorate (VMD)
- VMD periodically reviews the use of animals in the QC testing of all batches of IVMPS released via the UK:
  - https://www.gov.uk/government/publications/animal-usage-in-qc-tests-for-batch-release-of-immunological-products
  - Collates data in the marketing authorisation dossiers with data of all batches released via the UK
- Poster presentation of conference:
  - Animal use in the quality control testing of fish vaccines
  - Data from all batches released via the UK between 2007 and 2014

QC testing of fish vaccines: 2007-2014

- Animal use in the QC tests for the batch release of fish vaccines via the UK:

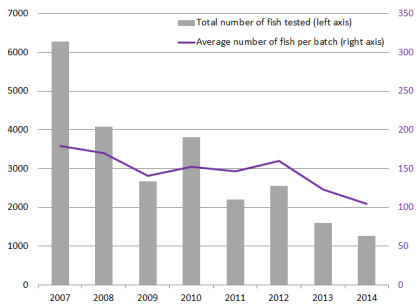
	2007	2008	2009	2010	2011	2012	2013	2014
Number of batches released	35	24	19	25	15	16	13	12
Total number of fish tested	6280	4080	2680	3820	2200	2560	1600	1260
Average number of fish per batch	179	170	141	153	147	160	123	105

- A total of 159 batches of 14 authorised vaccines for use in trout and salmon were released via the UK
  - Whilst a relatively small number of batches – large numbers of individual fish
  - Total number of fish used was 6% of total number of all animals used in QC testing of IVMPs but total number of fish vaccine batches released was only 1% of total number released
  - Average number of fish used per batch released was 160 compared to average of 36 animals for all IMVPs

QC testing of fish vaccines: 2007-2014

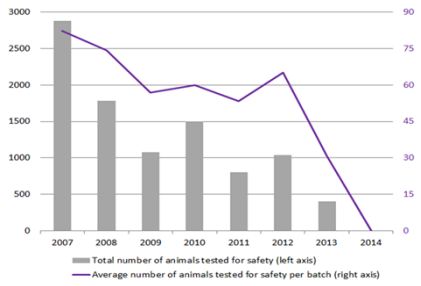
- All the vaccines were inactivated, many were multi-valent, requiring the use of relatively large numbers of fish to assess potency
- Between 60 and 260 fish were tested for safety and potency per batch released (not including any retesting that may have taken place)
- Other than in vitro ELISA test for infectious pancreatic necrosis virus (IPNV) vaccine component all of the potency tests used were in vivo challenge

Fish used in QC tests: 2007 - 2014



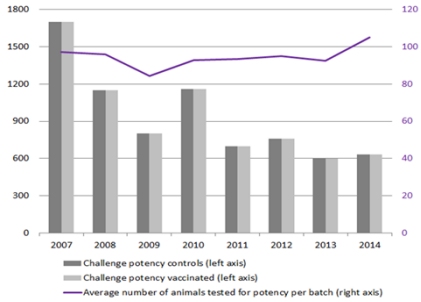
- 24,480 fish were used in safety (9,480) and potency (15,000) testing
- 80% decrease in the total number of fish used
- 66% decrease in the number of batches released
- **Average number of fish used per batch released decreased by 42%**

Target animal batch safety test (TABST)



- TABST was responsible for around 40% of all fish used in batch testing between 2007 - 2012
- Deletion of the TABST from the Ph. Eur. from 2013 resulted in a significant decrease in the use of fish in QC testing
- **Decrease of about 1,000 fish per year based on 2012 figure**

Potency testing



- **But no real difference in the average number of fish per batch released**
- The decrease in the total number of fish used was mostly due to the decrease in number of batches released over the period
- Also some evidence that larger batch sizes were being manufactured and released for some vaccines

Principles of validation of potency tests

- Aim to ensure that all batches released on the market are as safe and efficacious as those used in safety and efficacy studies
- Validation:
  - Precision (repeatability, intermediate precision, reproducibility)
  - Dose response
  - Detect sub-standard batches of vaccine
- Reflection paper on control of the active substance in the finished product for immunological veterinary medicinal products (IVMPs) (EMA/CVMP/IWP/582970/2009)

Potency test: virulent challenge

- Confirms efficacy of the batch at time of release
- Uses large numbers of animals
- Control animals are not protected and are expected to exhibit signs of disease or die (humane end points)
- Generally vaccine must meet “threshold”
  - e.g. protection of 90% of vaccinated animals
  - usually no indication of how much the threshold is exceeded
  - potential problem for interpretation of stability studies

Potency test: serology

- Widely used for mammalian species but so far not adopted for fish:
  - Why not?
  - Peculiarities of fish immune response?
- A lot of R&D is being carried out:

Vaccine. 2013 Jan 21;31(5):791-6. doi: 10.1016/j.vaccine.2012.11.069. Epub 2012 Dec 7.

Antibody responses correlate with antigen dose and in vivo protection for oil-adjuvanted, experimental furunculosis (Aeromonas salmonicida subsp. salmonicida) vaccines in Atlantic salmon (Salmo salar L.) and can be used for batch potency testing of vaccines.

Romstad AB[1], Reitan LJ, Midtlyng P, Gravningen K, Evensen Ø.

Biologicals. 2012 Jan;40(1):67-71. doi: 10.1016/j.biologicals.2011.09.011. Epub 2011 Oct 14.

Development of an antibody ELISA for potency testing of furunculosis (Aeromonas salmonicida subsp salmonicida) vaccines in Atlantic salmon (Salmo salar L).

Romstad AB[1], Reitan LJ, Midtlyng P, Gravningen K, Evensen O.

Serological methods for batch potency testing of vaccines to replace current challenge testing with *Aeromonas salmonicida* subsp. *salmonicida* in salmon. Institute of Aquaculture, University of Stirling. VM0140:

Potency test: serology

- Limitation of serological methods in general
  - Animal responses are variable so validation needs to cover all aspects of the test, including in vivo phase
  - Poor precision – large confidence intervals make it difficult to reliably detect a sub-standard batch

Potency test: in vitro methods

- Move away from using animals completely
  - e.g. quantification of antigen
- Only used to limited extent for fish vaccines
  - e.g. IPNV
- Which antigen?
  - Protective antigen or at least predictive of protection?
- Many fish vaccines contain adjuvants
  - May interfere with the test methods
  - Need to consider the contribution that adjuvants make to efficacy
  - At least need to quantify separately from antigen

Potency test: in vitro methods

- New technology gives scope for developing in vitro QC tests as the vaccine is developed
  - e.g. DNA vaccines
  - No adjuvant
  - Potency can be measured with fish-free method
  - Might use a quantitative test to directly quantify the DNA
  - Might use a qualitative test of protein expression in a suitable cell line to ensure functionality
  - Step forward in vitro testing and fish welfare
- Announcement by EMA's CVMP in April of adoption by majority a positive opinion for an initial marketing authorisation application for CLYNAV, from Elanco Europe Limited, a new plasmid DNA vaccine for the active immunisation of Atlantic salmon against pancreas disease caused by salmonid alphavirus subtype 3.

Potency tests for fish vaccines

- From 3Rs perspective, ultimate aim should be to replace all in vivo tests with in vitro methods. At the very least aim to replace challenge tests with more welfare-friendly methods.
- Little experience actually doing this for fish vaccines
  - Developing and validating alternative methods is time consuming and costly
  - Has been little incentive to develop alternative methods when challenge methods are described in Ph. Eur. Even when there is no monograph for a particular vaccine a challenge test is often chosen as the quickest and easiest method to develop
  - However, once developed, alternative methods can be cheaper, more precise and often quicker to do, thereby facilitating the prompt release of batches of vaccine

Experience from mammalian vaccines

- Experience in replacing challenge tests for some mammalian vaccines:
  - Leptospira vaccines – antigen quantification
  - Rabies vaccines – serology combined with in-process antigen quantification.

Conclusions

- *In vivo* potency tests tend to have a number of inherent problems that make them difficult to validate and present problems in interpretation
- The development of alternative *in vitro* methods to control the quality of veterinary medicines is encouraged, or even required, by the latest legislation
- Currently not possible to recommend general solutions – manufacturers encouraged to discuss with regulators

Acknowledgements

- Ralph Woodland for his invaluable assistance with preparation of the presentation
- Anna Tout for review of the use of animals in the QC testing of all batches of IVMPS released via the UK

Statens legemiddelverk
Norwegian Medicines Agency

New tests for old vaccine antigens

Experience from assessment of national products

Ane M. Kvingedal

Outline

- Fish vaccines in Norway
  Oil adjuvanted multivalent salmon vaccines
- Alternative batch potency tests
  - Serological tests
  - Quantitative tests

Fish vaccines in Norway

- Authorised

  11 salmon vaccines
  (inactivated oil adjuvanted multivalent vaccines and fall-out products)
  2 vaccines for rainbow trout
  2 vaccines for cod

- Exempt from MA/Autogenous

  Vaccines for salmon, rainbow trout and lumpfish
  (ISA, yersiniosis, vibriosis, atypical furunculosis, *Flavobacterium psychrophilum*, *Pseudomonas fluorescens*)

Oil adjuvanted multivalent vaccines for salmon

- Used in Norway for > 15 years
- Vaccines against 5 diseases most common

  2015: ~ 75 % of total sale (doses of oil adjuvanted vaccines for salmon)
- Products from 3 manufacturers

  Pharmaq (Zoetis): Alpha Ject 6-2, Alpha Ject Micro 6
  MSD Animal Health: Norvax Compact 6, Norvax Minova 6
  Elanco Animal Health: Pentium Forte Plus
- Marketing autorisations: 2003 – 2010

Oil adjuvanted multivalent vaccines against 6 antigens (5 diseases)

	Ph.Eur. Monograph
Aeromonas salmonicida (furunculosis)	1521
Vibrio salmonicida (cold water vibriosis)	1580
Listonella anguillarum serotype O1 and O2a (classical vibriosis)	1581
Moritella viscosa (winter ulcer)	No (1)
IPNV* (Infectious Pancreatic Necrosis) (*or rVP2)	No (2)

1) Draft monograph (2004) not finalized
2) No reliable challenge model for routine use

Batch potency testing

- Challenge tests (Ph.Eur. Mono.) or similar for all bacterial antigens, situation stable until 2012
- New requirements for new MA applications:
  - Data on suitability of the Ph.Eur. monograph tests
  - Valid *M.viscosa* test (ability to detect sub-potent batches)
- Data from potency testing of experimental vaccines varying in antigen content (one antigen varied, all other kept at standard level): Vaccines with antigen content < 10% could pass the RPS requirements

Oil adjuvanted multivalent vaccines
- production and characteristics

- Standard formulation: aiming for high antigen content, but limited by side effects caused by bacterial antigens
  - Antigen < 10% likely sub-potent, reduced efficacy in field (?)
- Emulsion (water in oil)
  – quality problems related to emulsion instability
- Production
  - bacterial antigens (fermentation, inactivation, processing) IPC: purity, inact., yield
  - viral antigens (cell culture, harvest, inactivation, processing)
  - formulated based on antigen mass (e.g. cell count or quantification by ELISA)

  Final product tests: Emulsion characteristics/stability, potency test in target animal

Oil adjuvanted multivalent vaccines

- Safe and efficacious based on field experience
  - consistent vaccine production?
- What requirements should we set for alternative batch potency tests?

Serological tests

- Development and validation of antibody quantification assay, e.g. ELISA
- Dose response experiments with vaccines containing varying amounts of antigen (Limited number of experiments)
  – correlation between antigen dose and vaccine potency,
  – correlation between antigen dose and antibody response
- Establishment of release requirement
  (Calculated based on test results from a number of vaccine batches)

Serological tests
– prediction of vaccine-induced protection?

Aeromonas salmonicida

- Several known protective antigens - A-layer protein important
- Possible to demonstrate specificity of the vaccine-induced antibody response is relevant for protection
  antibodies against A-layer (Western blot analysis)

Moritella viscosa

- Situation less clear regarding protective antigens

Serological tests

- Protective antigens – unknown or known
  - should this affect documentation requirements?
- Consistent production of vaccine antigen always important
  (correlation between test and undectectable factors)
  - but more or less?
- Documentation requirements applied for *M. viscosa* test and *A. salmonicida* test were principally the same
  (Aquavac PD7 assessment)

Serological tests

The tests for *A. salmonicida* and *M. viscosa* - considerations

- Better than the previous challenge tests
- Not ideal as fish is used and discriminating capacity limited
- Sufficient for intended use in batch potency testing of well-known antigens in oil adjuvanted multivalent vaccines

In vitro batch potency test

(Quantification of active substance)

- Used for IPNV (ELISA quantification of VP2 dominant protective antigen)
- For bacterial antigens in oil adjuvanted multivalent vaccines?

  - Requirements (From «Reflection paper on control of active substance…):
  «…select and justify the antigen(s) to be measured.»
  If not protective: satisfactory correlation with protective antigens (potency)

- *A. salmonicida* A-layer protein
- *M. viscosa* ?

In vitro batch potency test

- antigen(s) to be measured

Possible starting point

- Investigate serological response in vaccinated fish by use of Western blot analysis
- Candidate antigen(s) – some characterisation

  (LPS, protein, location)

- Generate specific antibodies against these for use in assay
  (can also be used for IPCs and analysis of production consistency)

In vitro batch potency test
- correlations

- Dose response experiments with vaccines containing varying amounts of antigen (Limited number of experiments)
  – correlation between antigen dose and vaccine potency
  – correlation with results from new quantification assay
- Investigate relationship between antigen mass (as determined for vaccine formulation, e.g. cell count)
  and antigen quantity determined with new assay
  Relationship ~ constant or varying between antigen batches?

New tests for old vaccine antigens
- possibilities and challenges

- Well-known products, current challenge tests less suitable,
  new test does not have to be ideal, but «fit for purpose»
- Incomplete scientific knowledge of protective antigens
  and immune mechanisms
- Basic research needed to obtain supportive data relevant for product
- Special competence needed (fish immune response, analytical methods)
- Documentation requirements may be difficult to set