Alternatives to reduce the use of fish for development of new vaccines

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The Reference in Prevention for Animal Health

Hipra location

Hipra location

- No easy access to water/fish.
- R&D: collaboration with Research Centers
- Quality: collaboration with hatchery-provided centers

Hipra products

Main Biological products for Fish

LACTOCOCCOSIS

ICTHIOVAC® LG

ICTHIOVAC® LG, the first registered vaccine for rainbow trout with the exclusive adjuvant AQUAMUNE®.

PASTEURELLOSIS

ICTHIOVAC® PD

ICTHIOVAC® PD, recently registered in some EU countries for sea bream.

VIBRIOSIS

ICTHIOVAC® VR

ICTHIOVAC® VR, inactivated vaccine in suspension for immersion for turbot.

FLEXIBACTERIOSIS

ICTHIOVAC® TM

ICTHIOVAC® TM, inactivated vaccine in injectable suspension for turbot.

STREPTOCOCCOSIS

ICTHIOVAC® STR

ICHTIOVAC® STR, inactivated vaccine in injectable suspension for turbot.

Hipra products

- Mediterranean species, MUMS
- Continental species
- Fresh water species

Hipra challenges

- *Monograph no. 0062*: Vaccines for veterinary use
- General chapters
- 4 specific monographs applicable to fish vaccines

No. 1581: Vibriosis vaccine (inactivated) for salmonids.
No. 1580: Vibriosis (cold water) vaccine (inactivated) for salmonids.
No 1521: Furuncolosis vaccine (inactivated, oil adjuvanted injectable) for salmonids.
No 1950: Yersiniosis vaccine (inactivated) for salmonids.

- Evaluation of safety of veterinary vaccines (5.2.6)
- Evaluation of efficacy of veterimary vaccines (5.2.7)

Hipra challenges

- High number of fish used in trials/controls
- Lack of in vivo alternatives
- Need to improve batch potency tests

Hipra ideas

- Replacement: in vitro (antigen quantification) vs in vivo (challenge)
- Reduction: batches/year
- Refinement: serological methods vs challenge

Hipra ideas

Replacement: in vitro (antigen quantification) vs in vivo (challenge)

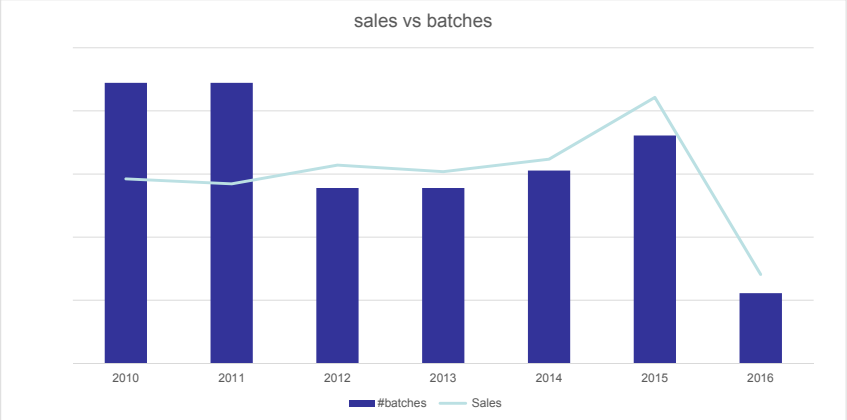
A possible *in vitro* batch potency test could be based on a validated ELISA procedure designed to quantify protective antigen in the final vaccine (by means of the interaction of antigen-antibody interactions (immunogenic epitopes recognition)).

Tests results should be expressed as Relative Potency (RP) in relation with a reference vaccine, analysed in conjunction with the sample, and proven to be efficacious in an *in vivo* test (challenge).

To consider the *in vitro* test as reliable as the *in vivo* test B2B consistency is also a must (GMP, validated manufacturing process, finished product test).

Hipra ideas

Reduction: batches/year



Hipra ideas

Refinement: serological methods vs challenge

If a good correlation between antibody response against a vaccinal antigen as measured by ELISA and protection in a challenge exist, serological method could be used.

Serological methods trigger less pain and suffering than a challenge, specially in the control group. Additionally, depending on the potency of the method, the number of animals used could be reduced.

The serological method should be able to distinguish substandard batches (close dose-response correlation).

Challenge the challenge: development of new potency tests for multivalent fish vaccines

Presenter: M.C.W. van Hulten
Global Regulatory Affairs Biologicals, MSD Animal Health

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Batch potency test development

- Batch potency test development is needed as part of the documentation for Market Authorisation (MA) applications.
- 3R considerations should be taken into account during the product development and registration phase;
  post MA the approved batch potency tests needs to be performed for each batch release.
- During product registration the batch potency test will be subject to evaluation by medical authorities; development of novel/non-standard tests create a potential risk for the applicant.

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Batch potency test development

Development of robust and discriminative potency tests for inactivated vaccines is a major challenge during product development

- Complicated by vaccines with multiple components
- Complicated by presence of multiple serotypes of the same agent

Development of potency test in line with 3Rs:

1. "In vitro" test is preferred test
2. Test based on serology
3. Test based on challenge

Batch potency tests needs to comply with monographs for specific vaccines whenever available.

3

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Batch potency test development and monographs
Current situation

- For inactivated oil adjuvant injectable for salmonids:
  - Ph.Eur. 1521: Furunculosis vaccine
  - Ph.Eur. 1580: Vibriosis (cold water) vaccine
  - Ph.Eur. 1581: Vibriosis vaccine

- The monographs are per bacteria while most vaccines are multivalent and may include multiple serotypes of the same agents. What is possible for a monovalent vaccine may not be possible for a multivalent vaccine.

- For the potency test (section 2-3-1) all these existing fish monographs have the following requirements:
  - Use of animals and only target animals
    - Either a challenge that must result in mortality using at least 2 x 30 fish (test and control)
    - Or a validated serological test (example using at least 25+10 fish (test and control)).

4

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Batch potency test development and monographs
Current situation

The existing monographs limit 3R possibilities; only serology tests allowed to replace the test by challenge

- Wording used: …… an alternative validated method based on antibody response may be used, …….
- Other monographs allow development of alternative tests. Wording used: …… an alternative validated method is used, …….

Fish monographs:

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.

5

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Batch potency test development, no monographs

- There are no monographs for Pancreas disease, Infectious pancreatic necrosis and *Moritella viscosa*; then what?
- Example from recently approved 7 component salmon vaccine from MSD Animal Health.

Antigen	Monograph
Salmon pancreas disease virus (SPDV)	No
Infectious pancreatic necrosis virus (IPNV)	No
Aeromonas salmonicida	Ph. Eur. 1521
Vibrio salmonicida	Ph. Eur. 1580
Vibrio anguillarum serotype O1	Ph. Eur. 1581
Vibrio anguillarum serotype O2a	Ph. Eur. 1581
Moritella viscosa	No

6

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Batch potency test development
MSD Animal Health examples

Solutions developed in presence and absence of monographs for multivalent vaccine:

- In absence of a monograph:
  - Pancreas disease: challenge based, but endpoint adjusted to prior to clinical disease/mortality (animal welfare refinement)
  - IPN: *In vitro* test (antigenic mass); no animals used
  - *Moritella viscosa*: former challenge and mortality based potency test replaced with serology (replaced, reduced and refined)
- In presence of a monograph:
  - *Aeromonas salmonicida*: former challenge and mortality based potency test replaced with serology (replaced, reduced and refined)

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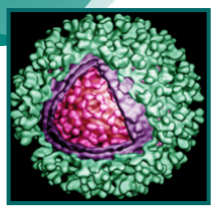
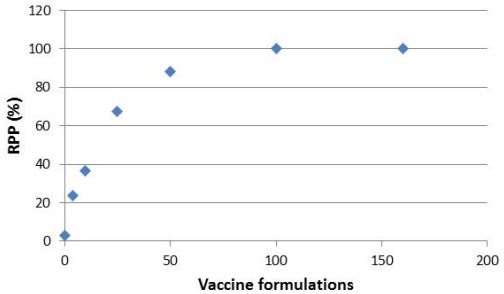
Batch potency test development - PD
MSD Animal Health examples

Pancreas disease (caused by SPDV)

- Potency test based on challenge:
  - Robust challenge model: initial read-out on reduction of clinical disease
  - Refinement: read-out based on reduction in viremia
    - Read-out before clinical signs occur
    - Robust system: less fish are needed
    - System optimized to obtain dose response in the potency test:



Alphavirus, enveloped virus particle



Release requirement:
-> only efficacious batches are released

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Batch potency test development - IPNV
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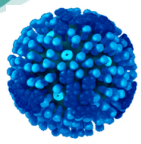
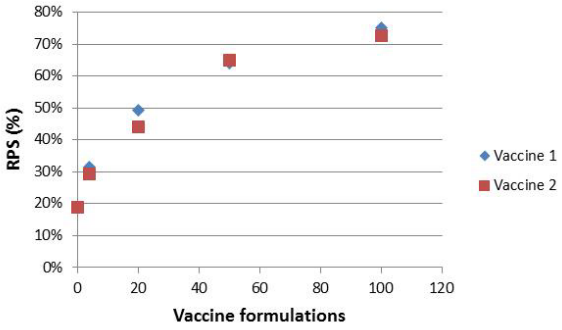
Infectious pancreatic necrosis (caused by IPNV)

- Test based on challenge
  - Dose response study
    - Smoltification of fish, co-habitation challenge
    - Non-specific immune response is induced with mock vaccine
    - There is a tank to tank variation > duplications needed
    - Model is suitable for demonstration of efficacy, but not suitable as batch potency test



Aquabirnavirus, non-enveloped virus particle



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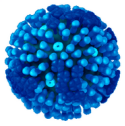
Batch potency test development - IPNV
MSD Animal Health examples

Infectious pancreatic necrosis (caused by IPNV)



- Efficacy test with challenge not robust > not suited for potency
- Solution: *In vitro* test development:
  - Very stable virus particle > can be recovered from vaccine formulation
  - Many monoclonal antibodies available > robust antigenic mass test
  - Dose response correlation efficacy and antigenic mass established

Formulation	U/dose
100%	2,72
50%	1,25
20%	0,52

Release requirement:
-> only efficacious batches are released

- Advantages *in vitro* potency test :
  - No animals used
  - As no animals are used: less test variation
  - Fast and robust test

10

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Batch potency test development - *A. salm*
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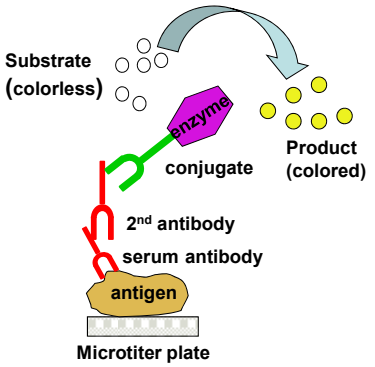
Furunculosis (caused by *Aeromonas salmonicida*)

- Replacement of challenge test by test based on serology in line with Ph. Eur. 1521
  - Optimization ELISA test to reduce background, increase specificity and precision and to have a robust test:
    - Microtiter plates
    - Coating antigen and concentration
    - Blocking
    - Selection of high affinity antibodies (2nd antibody, conjugate)
    - Substrate
    - Dilution buffer, dilutions, incubation time and -temperatures, washing
    - Validated calculation programme
  - Optimization of the test in Salmon:
    - Optimal temperature after vaccination
    - Optimum time point after vaccination



11

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Batch potency test development - A. salm
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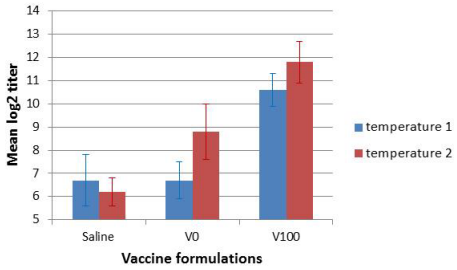
Determine optimal temperature for vaccination:

- Batches with or without *A.salmonicida* antigen were used or saline
- Vaccination was performed at different temperatures



Conclusion:

- At both temperature serology can discriminate batches
- Vaccination at temperature 1 is better, as temperature 2 results in higher test variation and higher background reaction

12

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Batch potency test development - *A. salm*
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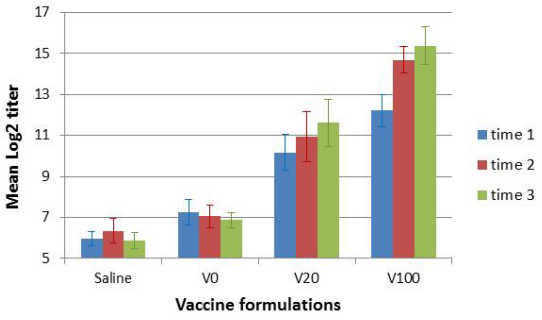
Determine optimum time point of sampling:

- Batches containing graded *A.salmonicida* antigen were used
- Serological samples taken at three different time point post vaccination



- All sampling points can discriminate 100% batches from sub-potent batches
- Difference between 100% and sub-potent batches increases over time
- First time point was below the minimum of 500 degree days as stated in the updated monograph

Time point chosen aligned with serological test developed for *M.viscosa*

13

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Batch potency test development - *A.salm*
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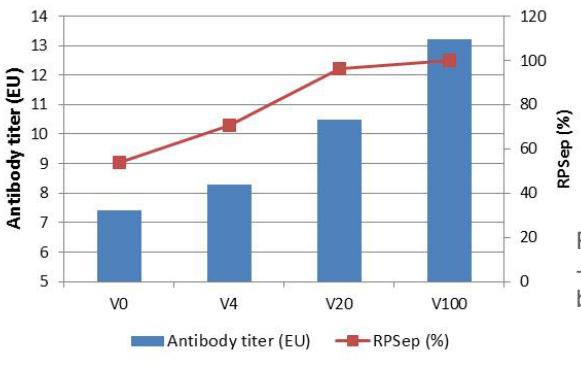
Furunculosis (caused by *Aeromonas salmonicida*)

- Challenge test according to Ph. Eur. 1521 replaced by serology according to Ph. Eur. 1521.
  - ELISA method with high specificity
  - Dose response study correlation efficacy and serology



Release requirement:
-> only efficacious batches are released

14

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Batch potency test development – serological tests

Conclusion on serological test development for bacterial components:

- Large advantage over test by challenge:
  - No infection induced in test animals – major animal welfare improvement
  - More robust since not affected by variations in disease development
  - Less fish needed
  - More than one vaccine component can be read from the same set of antisera
- Difficulties:
  - Lots of optimization needed on ELISA methods development and immunization strategy
  - Requires system to safeguard consistency between batches of fish (no such thing as SPF fish breeds)

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Challenge the challenge

7 component vaccine:

- IPNV: *in vitro* test
- *A.salm* and *M.visc*: serological tests > sera from the same fish are used > further reduction in number of fish used
- SPDV: refinement of challenge read-out
- Vibrio components: development alternatives under investigation (within monograph limitations)

Overall conclusions and discussion

MSD-AH perspective on potency test development:

- In line with 3Rs:
  - 1: in vitro test
  - 2: serological test
  - 3: challenge test, decrease impact on fish

All vaccine producers will have a motivation to develop potency tests according to 3R

- Animal welfare
- Fewer live phase variables > more robust test
- Often less time consuming test
- Cost reduction

Are the existing very specific fish monographs limiting the 3R possibilities?

17

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Acknowledgements

- Aslaug Ness
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Experiences from development of new methods for *in vitro* batch potency testing

Anette Kilander

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PHARMAQ AN INNOVATION SUCCESS STORY

- Leading global provider of **vaccines** and **therapeutics** to the aquaculture industry
- A significant amount of the revenue go back to R&D and innovation
- **>25 years** of dedicated R&D efforts
- **International presence** with operations in Norway, Chile, Turkey, Spain, UK, Vietnam, Hong Kong and Central America
- Earlier owned by a Permira Funds and PHARMAQ employees
- November 2015 Zoetis acquired PHARMAQ
- Zoetis is the leading pharmaceutical company within Animal Health

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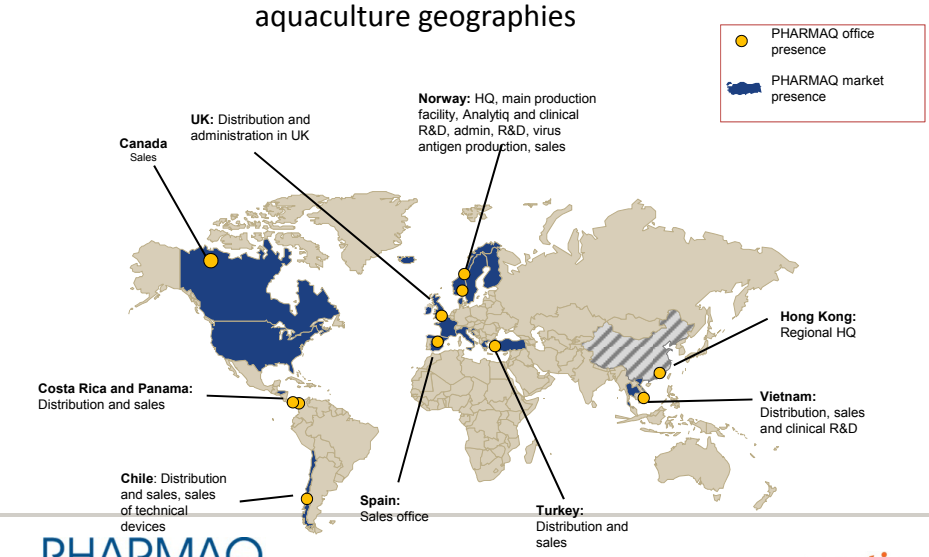
High market shares and strong geographical footprint across key aquaculture geographies



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Note: Refers to estimated 2015 market shares

R&D in Norway is responsible for developing vaccines in all markets where PHARMAQ is present.

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PHARMAQ vaccines

- Multivalent vaccines, both bacterial and viral antigens
- Water-in-oil, inactivated and live vaccines
- Current *in vivo* batch potency tests/batch release tests are performed in fish (the vaccine target species)
- Batch potency is measured by mortality rate

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PHARMAQ vaccines

New *in vitro* batch potency method for PHARMAQ vaccines:

- Performed on broken vaccine (or in-put water phase) with a mixture of both bacterial and viral antigens present
- Technically challenging to measure one antigen in a complex matrix
- No reference laboratories that can supply common standards for fish patogens

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PHARMAQ vaccines

New products:

- additional antigens to excisting products
- new antigens

- The same antigens are often used in different products for different markets
- The products are custom made for each market depending on the species and disease situation

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PHARMAQ vaccines

- For many of the PHARMAQ vaccines it is necessary to fulfill the requirements from several authorities for the new alternative potency tests
- The authorities may have different key focus
  - Animal welfare
  - Discriminating power of the PhEur Monograph method

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3R and the Vaccine producer

- 3R incentive
  - Refine, Replace and Reduce

- PHARMAQ incentive:
  - Reduce vaccine batch release time
    (Potentially up to 2-3 months)
  - Reduce cost of batch potency testing
  - Reduce the use of fish in batch release trials

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Implementation of new alternative BP tests

New antigens

- Aim for *in vitro* batch potency tests for all new antigens
- Aqua culture is still in its early phase
- For new emerging diseases in aqua culture, the antigens may not be well characterized before the vaccine development
- Requirements for quantification as well as a functionality test will be difficult to fulfill, as a result it is likely that the new product will have an *in vivo* potency test at the MA application

Ref. from the draft on new guidance

"If a single method is used, it should preferably measure the content and integrity of the antigen by targeting epitope(s) relevant to the protection offered by the vaccine."

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Implementation of new alternative BP tests

Antigens in existing products

- prioritize to replace tests for all antigens in one product
- will replace potency methods for antigens which are part of vaccines produced for many years with well documented vaccine effect and safety

- Requirements from regulatory authorities
  - The EU countries have come a long way, the rest of the world is still in early phase regarding 3R. At present no clear requirements for new alternative test in countries outside Europe. Important to fulfill the requirements from several authorities
  - Very few monoghraphs in PhEur, only for salmonid not for other species f eks seabass in the Medittereanean region

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3R and PHARMAQ experiences

PHARMAQ's conclusion after evaluating serology studies for bacterial antigens in multivalent vaccines (*e.g Moritella viscosa and Aeromonas salmonicida)*

Serology as an alternative potency method:

-Has potential to work as an alternative test for some of the antigens

-For other antigens and depending on vaccine content, low and unspecific antibody responses can be limiting factors

-high method variation and low presicion

-still *in vivo* test with animal use

-long test period

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In vitro batch potency methods

Important criteria for new alternative methods:

- no laboratory animals
- capacity to reveal sub potent batches
- method with high reproducibility, resolution and precision
- Same testing strategy and analytical methods for all (most) bacterial and viral vaccine components
- Advantage if the new batch potency assay also can be used for stability testing. However the new assay may not be suitable for stability testing to measure stability indicating parameters

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Alternative batch potency

Control tests on the finished product

1. **Quantification of the active substance**

   Batch potency Directive 2001/82/EC

   Current: «A quantification of the active substance shall be carried out on each batch to s show that each batch will contain the appropriate potency or titre to ensure its safety and efficacy»

   Previously: «The assay of biological activity of the active substance(s) shall be carried out either on a representative sample from the production batch or in a number of dosage- units analysed individually.»

2. **Control of the adjuvant**
3. **Consistency of production**

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Alternative batch potency

The consistency approach for the routine release of vaccines are based upon:

- the principle that the quality of vaccines is a consequence of a quality system and of consistent production of batches with similar characteristics to those batches that have been shown to be safe and effective in humans or the target species.
- Requires: consistent production, tight in-process control, strict application of GMP and Quality Assurance

Batch release is then based on equivalence with the reference batch

In vitro test do not have to provide the same information as the *in vivo* tests

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Alternative batch potency

Requirements from guidelines:

- Analyse and demonstrate a correlation between the quantity of the antigen(s) and the ability of the vaccine to protect.
- Analyse and demonstrate an ability of the method to detect sub-standard batches containing less active substance than standard batches

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Challenges - Replacement of in vivo assays for antigens in excisting products

- Link between old *in vivo* assay and new *in vitro* assay
  - -correlation may be difficult due to low discriminating power and/or high variability of the *in vivo* assay
- Link between the new *in vitro* assay and the ability of the vaccine to protect. To obtain correlation data and depending on the discriminating power of current potency assay, it may require:
  - -establishment of new challenge models
  - -new representative fish trials (dose response, different antigen and production batches etc)
  - Results in a significant additional use of animals

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Challenges - Replacement of *in vivo* assays for antigens in excisting products

Possible way forward:

- Quantifying the antigen using an antibody based analytical method then **quantity reflects the antigenicity.** Note, the antigen and/or epitope(s) relevant to vaccine protection are often not fully characterized for fish bacteria
- **Monitor consistency during production**: The **new *in vitro* assay can be used as additional IPC test** during the whole production chain e.g:
  - incativated antigen, antigen in vaccine input water phase
  - vaccine finished product , stored vaccine- stability testing
- **Aim for one *in vitro* batch potency test, depending on the integrity of the antigen more than one *in vitro* assay will be developed**

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Example of an *in vitro* batch potency test in use at PHARMAQ

- An *in vitro* batch potency test for a viral component, IPNV. Which is part of two of PHARMAQ's multivalent vaccines for Norway and UK
- The *in vitro* batch potency test has been in use since 2004 for a product with MA
- Relative potency test with a reference vaccine and a test using a standard item
- *In vitro* assay is used for antigen quantification, potency and stability test
- Control of the adjuvant with additional tests

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Summary/Discussion

- Interpretation of guidelines

  E.g quantification of active substance or in practice: requirement to measure antigenicity
- Vaccine producers work globally and need consensus guidelines from the authorities in the different countries, also outside of Europe
- Generalized monographs to include more fish species?
- Old antigens in well known products, replacement of in vivo test
  - Demonstrate correlation to efficacy

  Or: Two sets of BPT (*in vitro and in vivo*) at submission and transition to in vitro only. Document data from both tests in a transition period

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