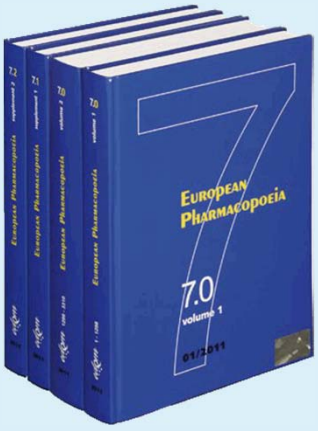
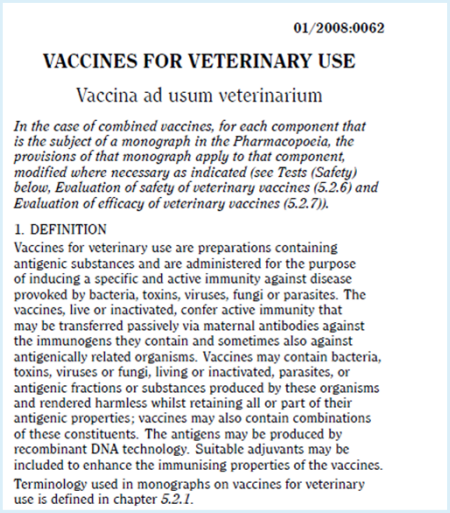
WHAT HAS CHANGED AND WHY?

Lukas Bruckner
Ph.Eur. expert group 15V

2011: Ph.Eur 7th edition



Texts on extraneous agents in Ph. Eur. chapters



01/2008:0062

VACCINES FOR VETERINARY USE

Vaccina ad usum veterinarium

In the case of combined vaccines, for each component that is the subject of a monograph in the Pharmacopoeia, the provisions of that monograph apply to that component, modified where necessary as indicated (see Tests (Safety) below, Evaluation of safety of veterinary vaccines (5.2.6) and Evaluation of efficacy of veterinary vaccines (5.2.7)).

1. DEFINITION

Vaccines for veterinary use are preparations containing antigenic substances and are administered for the purpose of inducing a specific and active immunity against disease provoked by bacteria, toxins, viruses, fungi or parasites. The vaccines, live or inactivated, confer active immunity that may be transferred passively via maternal antibodies against the immunogens they contain and sometimes also against antigenically related organisms. Vaccines may contain bacteria, toxins, viruses or fungi, living or inactivated, parasites, or antigenic fractions or substances produced by these organisms and rendered harmless whilst retaining all or part of their antigenic properties; vaccines may also contain combinations of these constituents. The antigens may be produced by recombinant DNA technology. Suitable adjuvants may be included to enhance the immunising properties of the vaccines.

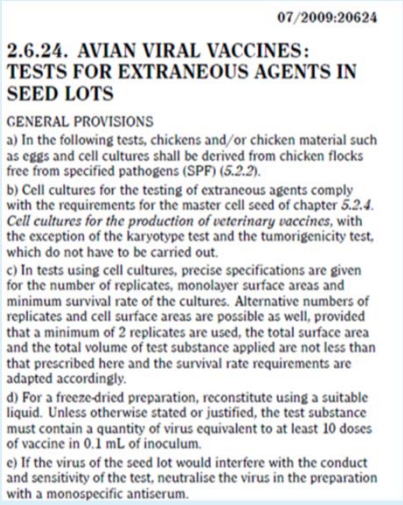
Terminology used in monographs on vaccines for veterinary use is defined in chapter *5.2.1*.

0062 Vaccines for Veterinary Use

2-1-3-2-6. Absence of extraneous viruses. …

- … neutralising antibody to the virus of the seed lot … other methods are used to remove or neutralise the seed virus specifically.
- … at least the virus content of 10 doses of vaccine per 0.1 mL for avian vaccines and per milliliter for other vaccines.
- … inoculated onto cultures of at least 70 cm^2 of the required cell types … at any suitable stage of growth up to 70 per cent confluency. At least 1 monolayer of each type must be retained as a control. … monitored daily for a week. … freeze thawed 3 times, centrifuged … re-inoculated onto the same cell type … repeated twice.
  - Cytopathic and haemadsorbing agents are tested
  - immuno-fluorescence
- inoculated onto:
  - primary cells of the species of origin of the virus,
  - cells sensitive to viruses pathogenic for the species for which the vaccine is intended,
  - cells sensitive to pestiviruses.

Texts on extraneous agents in Ph. Eur. chapters



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2.6.24. AVIAN VIRAL VACCINES: TESTS FOR EXTRANEOUS AGENTS IN SEED LOTS

GENERAL PROVISIONS

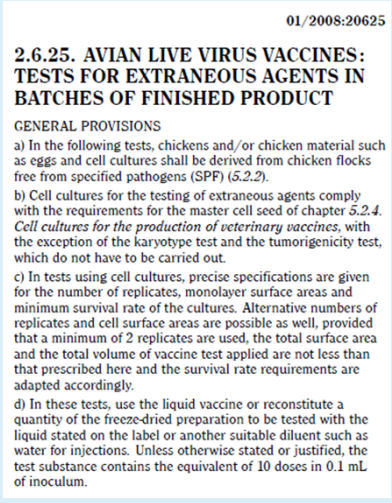
a) In the following tests, chickens and/or chicken material such as eggs and cell cultures shall be derived from chicken flocks free from specified pathogens (SPF) (*5.2.2*).

b) Cell cultures for the testing of extraneous agents comply with the requirements for the master cell seed of chapter *5.2.4. Cell cultures for the production of veterinary vaccines*, with the exception of the karyotype test and the tumorigenicity test, which do not have to be carried out.

c) In tests using cell cultures, precise specifications are given for the number of replicates, monolayer surface areas and minimum survival rate of the cultures. Alternative numbers of replicates and cell surface areas are possible as well, provided that a minimum of 2 replicates are used, the total surface area and the total volume of test substance applied are not less than that prescribed here and the survival rate requirements are adapted accordingly.

d) For a freeze-dried preparation, reconstitute using a suitable liquid. Unless otherwise stated or justified, the test substance must contain a quantity of virus equivalent to at least 10 doses of vaccine in 0.1 mL of inoculum.

e) If the virus of the seed lot would interfere with the conduct and sensitivity of the test, neutralise the virus in the preparation with a monospecific antiserum.



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2.6.25. AVIAN LIVE VIRUS VACCINES: TESTS FOR EXTRANEOUS AGENTS IN BATCHES OF FINISHED PRODUCT

GENERAL PROVISIONS

a) In the following tests, chickens and/or chicken material such as eggs and cell cultures shall be derived from chicken flocks free from specified pathogens (SPF) (*5.2.2*).

b) Cell cultures for the testing of extraneous agents comply with the requirements for the master cell seed of chapter *5.2.4. Cell cultures for the production of veterinary vaccines*, with the exception of the karyotype test and the tumorigenicity test, which do not have to be carried out.

c) In tests using cell cultures, precise specifications are given for the number of replicates, monolayer surface areas and minimum survival rate of the cultures. Alternative numbers of replicates and cell surface areas are possible as well, provided that a minimum of 2 replicates are used, the total surface area and the total volume of vaccine test applied are not less than that prescribed here and the survival rate requirements are adapted accordingly.

d) In these tests, use the liquid vaccine or reconstitute a quantity of the freeze-dried preparation to be tested with the liquid stated on the label or another suitable diluent such as water for injections. Unless otherwise stated or justified, the test substance contains the equivalent of 10 doses in 0.1 mL of inoculum.

2.6.24/2.6.25 AVIAN LIVE VIRUS VACCINES: TESTS FOR EXTRANEOUS AGENTS

1. TEST FOR EXTRANEOUS AGENTS USING EMBRYONATED HENS' EGGS
   - *group 1: 0.2 mL into the allantoic cavity of each 9- to 11-day-old embryonated egg*
   - *group 2: 0.2 mL onto the chorio-allantoic membrane .....*
2. TEST IN CHICKEN KIDNEY CELLS
   - *Prepare 7monolayers of primary or secondary chick embryo fibroblasts ...*
3. TEST FOR AVIAN LEUCOSIS VIRUSES
4. TEST FOR AVIAN RETICULOENDOTHELIOSIS VIRUS
5. TEST FOR CHICKEN ANAEMIA VIRUS
6. TEST FOR EXTRANEOUS AGENTS USING CHICKS
   - *Inoculate each of at least 10 chicks with the equivalent of 100 doses of vaccine ...*

2.6.24/2.6.25 AVIAN LIVE VIRUS VACCINES: TESTS FOR EXTRANEOUS

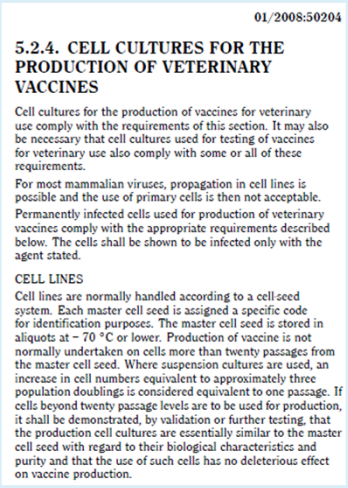
	Agent	Type of test
Standard tests	Avian adenoviruses, group 1	SN, EIA, AGP
	Avian encephalomyelitis virus	AGP, EIA
	Avian infectious bronchitis virus	EIA, HI
	Avian infectious laryngotracheitis virus	SN, EIA, IS
	Avian leucosis viruses	SN, EIA
	Avian nephritis virus	IS
	Avian orthoreoviruses	IS, EIA
	Avian reticuloendotheliosis virus	AGP, IS, EIA
	Chicken anaemia virus	IS, EIA, SN
	Egg drop syndrome virus	HI, EIA
	Avian infectious bursal disease virus	Serotype 1: AGP, EIA, SN Serotype 2: SN
	Influenza A virus	AGP, EIA, HI
	Marek's disease virus	AGP
	Newcastle disease virus	HI, EIA

2.6.24/2.6.25 AVIAN LIVE VIRUS VACCINES: TESTS FOR EXTRANEOUS

	Agent	Type of test
Standard tests	Turkey rhinotracheitis virus	EIA
	Salmonella pullorum	Agg
Additional tests for turkey extraneous agents	*Chlamydia* spp.	EIA
	Avian infectious haemorrhagic enteritis virus	AGP
	Avian paramyxovirus 3	HI
	Avian infectious bursal disease virus type 2	SN
Additional tests for duck extraneous agents	*Chlamydia* spp.	EIA
	Duck and goose parvoviruses	SN, EIA
	Duck enteritis virus	SN
	Duck hepatitis virus type I	SN
Additional tests for goose extraneous agents	Duck and goose parvovirus SN,	EIA
	Duck enteritis virus	SN
	Goose haemorrhagic polyomavirus	test in goslings shown ....

Lukas Bruckner, What has changed and why?
Training on the Management of Extraneous Agents in IVMPs, Strasbourg (F), 1 April 2020

Texts on extraneous agents in Ph. Eur. chapters



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5.2.4. CELL CULTURES FOR THE PRODUCTION OF VETERINARY VACCINES

Cell cultures for the production of vaccines for veterinary use comply with the requirements of this section. It may also be necessary that cell cultures used for testing of vaccines for veterinary use also comply with some or all of these requirements.

For most mammalian viruses, propagation in cell lines is possible and the use of primary cells is then not acceptable.

Permanently infected cells used for production of veterinary vaccines comply with the appropriate requirements described below. The cells shall be shown to be infected only with the agent stated.

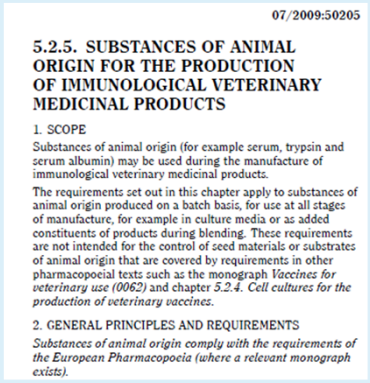
CELL LINES

Cell lines are normally handled according to a cell-seed system. Each master cell seed is assigned a specific code for identification purposes. The master cell seed is stored in aliquots at − 70 °C or lower. Production of vaccine is not normally undertaken on cells more than twenty passages from the master cell seed. Where suspension cultures are used, an increase in cell numbers equivalent to approximately three population doublings is considered equivalent to one passage. If cells beyond twenty passage levels are to be used for production, it shall be demonstrated, by validation or further testing, that the production cell cultures are essentially similar to the master cell seed with regard to their biological characteristics and purity and that the use of such cells has no deleterious effect on vaccine production.

5.2.4 CELL CULTURES FOR THE PRODUCTION OF VETERINARY VACCINES

- Absence of contaminating viruses.
  - *The cells must not be contaminated by viruses; suitably sensitive tests, including those prescribed below, are carried out. The monolayers tested shall have an area of at least 70 cm^2, ... maintained ... for a total of at least 28 days. Subcultures are made at 7-day intervals, ...*
- Detection of cytopathic viruses.
  - *Two monolayers of at least 6 cm^2 each are stained with an appropriate cytological stain. ... examined for any inclusion bodies, abnormal numbers of giant cells or any other lesion indicative of a cellular abnormality ...*
- Detection of haemadsorbent viruses.
  - *Monolayers totalling at least 70 cm^2 are washed several times with an appropriate buffer and a sufficient volume of a suspension of suitable red blood cells added to cover the surface of the monolayer evenly. After different incubation times cells are examined for the presence of haemadsorption.*
- Detection of specified viruses.
  - *Tests are carried out for freedom from contaminants specific for the species of origin of the cell line and for the species for which the product is intended.*
- Tests in other cell cultures.
  - *Monolayers totalling at least 140 cm^2 are required. ...*

Texts on extraneous agents in Ph. Eur. chapters



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5.2.5. SUBSTANCES OF ANIMAL ORIGIN FOR THE PRODUCTION OF IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

1. SCOPE

Substances of animal origin (for example serum, trypsin and serum albumin) may be used during the manufacture of immunological veterinary medicinal products.

The requirements set out in this chapter apply to substances of animal origin produced on a batch basis, for use at all stages of manufacture, for example in culture media or as added constituents of products during blending. These requirements are not intended for the control of seed materials or substrates of animal origin that are covered by requirements in other pharmacopoeial texts such as the monograph *Vaccines for veterinary use (0062)* and chapter *5.2.4. Cell cultures for the production of veterinary vaccines.*

2. GENERAL PRINCIPLES AND REQUIREMENTS

Substances of animal origin comply with the requirements of the European Pharmacopoeia (where a relevant monograph exists).

5.2.5 SUBSTANCES OF ANIMAL ORIGIN FOR THE PRODUCTION OF IMMUNOLOGICAL VETERINARY MEDICINAL PRODUCTS

- Freedom from living extraneous viruses.
  - *A sample from each batch of the substance is tested for extraneous viruses by general and specific tests. These tests are validated with respect to sensitivity and specificity for detection of a suitable range of potential extraneous viruses. Suitably sensitive cell cultures are used for the tests for extraneous viruses ….*
  - *General test. The inoculated cell cultures are observed regularly for 21 days for cytopathic effects. … and a proportion is tested for haemadsorbing agents.*
  - *Specific tests. … The specific viruses to be tested for are potential extraneous viruses that are identified through the risk assessment and that would not be detected by the general test. A test for pestiviruses …*

Specific Monographs for Veterinary Vaccines

- Inactivated viral vaccines for pigs
  - *Extraneous agents.*
    - On the pigs used for the safety test carry out tests for antibodies. The vaccine complies with the test if it does not stimulate the formation of antibodies, other than those against the vaccine virus, against viruses pathogenic for pigs or against viruses that could interfere with the diagnosis of infectious diseases of pigs (including the viruses of the pestivirus group).
- Inactivated viral vaccines for chicken
  - *Extraneous agents.*
    - Use the chickens from the safety test. 21 days after injection of the double dose of vaccine, inject 1 dose by the same route into each chicken. Collect serum samples from each chicken 2 weeks later and carry out tests for antibodies against the following agents ... : avian encephalomyelitis virus, avian leucosis viruses, egg-drop syndrome virus, avian infectious bursal disease virus, avian infectious laryngotracheitis virus, influenza A virus, Marek's disease virus, Newcastle disease virus. ....

Specific Monographs for Veterinary Vaccines

- Live viral vaccines for Mammals
  - *Extraneous agents.*
    - Neutralise the vaccine virus with a suitable monospecific antiserum against the vaccine virus and inoculate into cell cultures known for their susceptibility to viruses pathogenic for the target species. Carry out at least one passage and maintain the cultures for 14 days. The vaccine complies with the test if no cytopathic effect develops and there is no sign of the presence of haemadsorbing agents.
      (Carry out a specific test for pestiviruses.)
- Avian Live viral vaccines
  - *Extraneous agents.*
    - The vaccine complies with the tests for extraneous agents in batches of finished product (2.6.25).

Limitations of the approach in handling extraneous agents

- No harmonized approach, requirements scattered over several texts
- Testing requirements are different from species to species
  - *Very precise for avian vaccines*
    - List of agents to be tested for
  - *General approach for vaccines for mammals*
  - *No policy for vaccines for fish*
- Molecular methods are neglected
- Focus on laboratory testing only
- Consequences of good manufacturing are not taken in account

Limitations of the approach to handle extraneous agents

- Tests in tissue culture, embryonated eggs (or animals)
  - *Detailed description of the test to be performed*
    - Number and size of test replicates
    - Volume of inoculate
    - Incubation time and temperature
    - Test evaluation: macroscopic and microscopic examination, staining of cultures, cytopathic effects, hemadsorption, immunofluorescence …
  - *No positive controls*
  - *Suitability of the test justified by historic experience*

Harmonization

- All requirements in one chapter

5.2.5 Management of extraneous agents in immunological veterinary medicinal products

5.2.5 Management of extraneous agents in immunological veterinary medicinal products

- Applies to all substances: seed material, material used during production, etc
- Risk management
- Control measures
  - *Starting material*
  - *Control measures during production*
  - *Methods for detection of extraneous agents*
    - 2.6.1 Sterility
    - 2.6.21 Nucleic acid amplification techniques
    - 2.6.7 Mycoplasmas
    - 2.6.37 Principles for the detection of extraneous viruses in immunological veterinary medicinal products using culture methods
- ANNEX I: LIST OF EXTRANEOUS AGENTS TO BE CONSIDERED FOR THE RISK ASSESSMENT

THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

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Extraneous agents testing in the European Pharmacopoeia (Ph. Eur.) for IVMPs

The overall new approach

EDQM training on the Management of Extraneous Agents in IVMPs

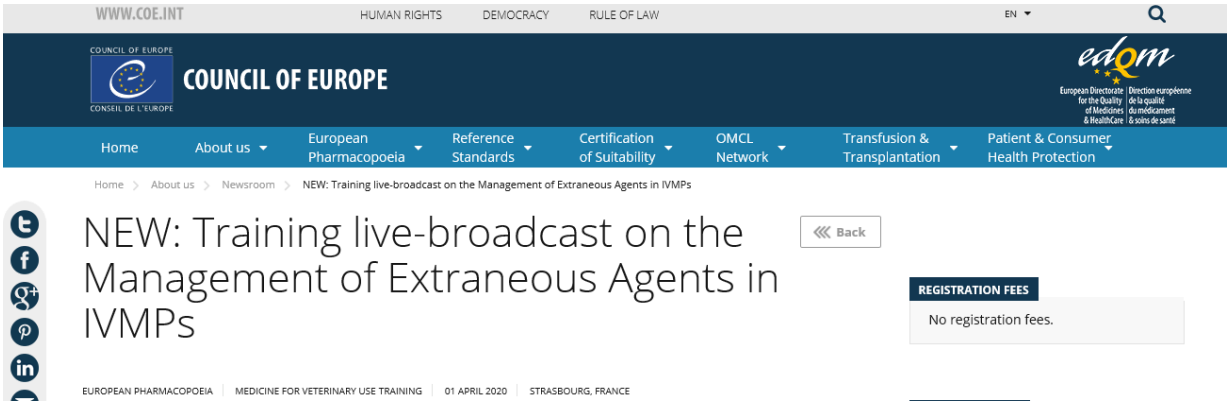
April 1-2, 2020 EDQM, Strasbourg

Catherine LANG, EDQM, Group 15V Secretary

STRUCTURE

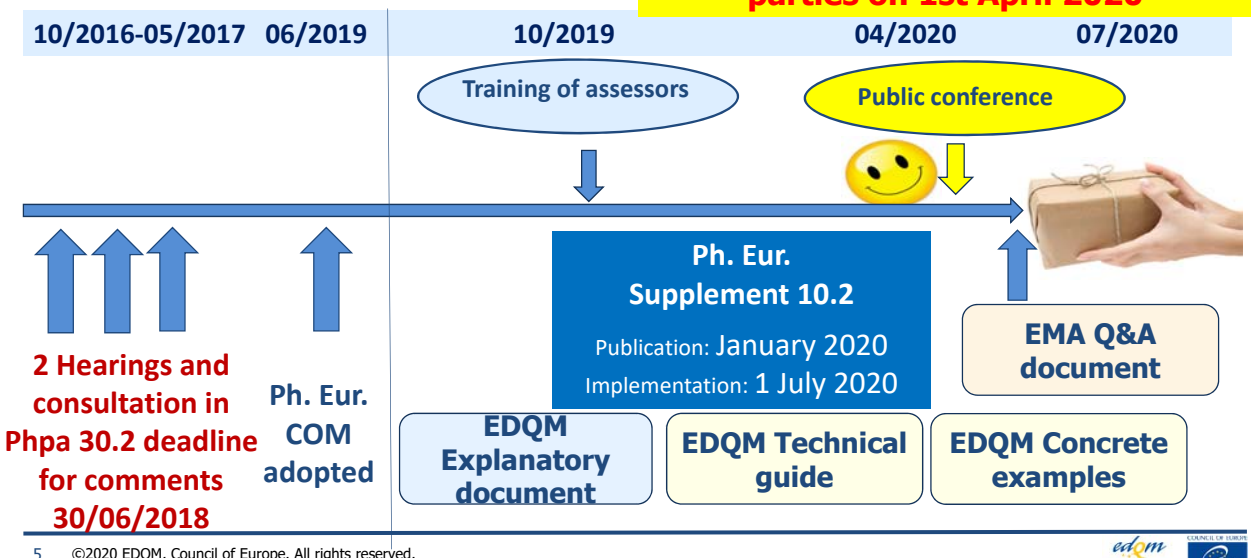
- Revision of the extraneous agents testing approach
  - Where are we now and what are the next steps?
  - Aim and drivers
  - Impact on the Ph. Eur. texts
  - New approach brings opportunities and benefits

Training replaced by a GoToWebinar on 1st April 2020



Where are we now and next steps

EDQM training for all interested parties on 1st April 2020



EA testing adopted 06/2019; publication 01/2020; implementation 07/2020

Revision of the extraneous agents testing approach:

- **43 Ph. Eur. texts** involved in the revision
- Requirements collated and harmonised, compiled in chapter **5.2.5 "Management of extraneous agents in immunological veterinary products"**
- **Risk assessment** + list of extraneous agents (avian revised list + EMA IWP list as is)
- **Open to any fit-for-purpose method**

OTHER CHANGES (out of scope of the training)

- **Deletion of the test for Specified Extraneous Agents in some individual monographs** (batch test using 2 animals (pigs, cattle, rabbits))
- To continue to allow the **use of antibiotics** during vaccine production, but **request justification for such use** (general monograph 0062 – Vaccines for veterinary use).
- **To allow identification of live vaccines by any suitable method** instead of using an immunostaining/neutralisation test in cultures - cells or SPF eggs - with a monospecific antiserum or monoclonal antibodies.

Impact on the Ph. Eur. texts: EA testing

Deleted texts

- **2.6.24. AVIAN VIRAL VACCINES: TESTS FOR EXTRANEOUS AGENTS IN SEED LOTS**
- **2.6.25. AVIAN LIVE VIRUS VACCINES: TESTS FOR EXTRANEOUS AGENTS IN BATCHES OF FINISHED PRODUCT**

These chapters, which contain detailed protocols for testing extraneous agents, will be suppressed from the Ph. Eur. **as of 1st of July 2020**.

➔ current methods still acceptable provided they fulfill the new requirements (see new chapter 2.6.37)

Still accessible in Ph. Eur. archives

https://pheur.edqm.eu/app/arch/search/

Impact on the Ph. Eur. texts - Revised texts

General chapter 5.2.5 (Core chapter)

"Substances of animal origin for the production of IVMPs" **now**

"Management of extraneous agents in immunological veterinary products"

- Risk management: already described but only for substances of animal origin (e.g. Trypsin)
- Compilation of all existing requirements, collated and harmonised from other chapters and monographs
- The scope modified to cover the whole production process

Raw materials

Final product

- Includes **ANNEX I**: List of extraneous agents to be considered for the risk assessment: *consolidates the list of avian agents already published in the Ph. Eur., includes the list of mammalian and fish agents which are currently outlined in the EMA guideline on requirements for the production and control of IVMPs (EMA/CVMP/IWP/206555/2010-Rev.1).*

NEW

Chapter 5.2.5 Annex I

ANNEX I: LIST OF EXTRANEOUS AGENTS TO BE CONSIDERED FOR THE RISK ASSESSMENT

AVIAN (Poultry) - main list	
Viral agents	**Bacterial agents**
Atadenovirus (group III avian adenoviruses)	*Avibacterium (Haemophilus) paragallinarum*
Aviadenoviruses	*Chlamydia* spp.
Avian encephalomyelitis virus	*Mycobacterium avium*
Avian leucosis virus (excluding endogenous type)	*Salmonella* Pullorum

AVIAN (additional list for Chicken)	
Viral agents	**Bacterial agents**
Avian infectious bronchitis virus	
Chicken anemia virus (CAV)	
Gallid herpesvirus type I	

AVIAN (additional list for Duck)	
Viral agents	**Bacterial agents**
Duck and goose parvoviruses	
Duck enteritis virus	
Duck hepatitis B virus (DHBV)	
Duck hepatitis virus type 1	

AVIAN (additional list for Goose)	
Viral agents	**Bacterial agents**

AVIAN (additional list for Turkey)	
Viral agents	**Bacterial agents**

AVIAN (additional list for Pigeon)	
Viral agents	**Bacterial agents**

BOVINE	
Viral agents	**Bacterial agents**
Akabane virus	*Brucella* spp.
Alcelaphine herpesvirus	*Chlamydia* spp.

OVINE/CAPRINE

PORCINE

EQUINE

CANINE

FELINE

RABBIT

RODENT (MOUSE)

RODENT (HAMSTER)

RODENT (RAT)

PRIMATES (VERO CELL)	
Viral agents	**Bacterial agents**
Bovine viral diarrhoea virus	
Endogenous retrovirus (replication competent)	
Herpesvirus	
Reovirus	
Simian virus 5	
Simian virus 40	

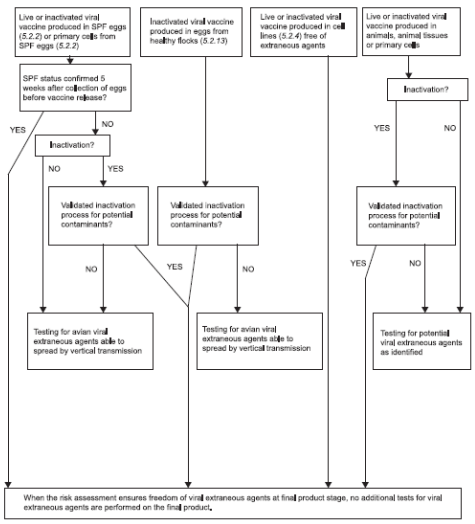
SALMONIDS	
Viral agents	**Bacterial agents**
Infectious haematopoietic necrosis virus (IHNV)	*Aeromonas salmonicida*

FINFISH	
Viral agents	**Bacterial agents**
Betanodavirus	*Aeromonas salmonicida*
	Edwardsiella ictaluri

- Non-exhaustive: any new agent should be tested even if not on the list yet
- Regularly reviewed + Requests for revision supported by data

Chapter 5.2.5 Annex II

ANNEX II: TESTING STRATEGY - EXAMPLE OF A DECISION TREE



gives an example of a **testing strategy decision tree** for substrates which is irrespective of the target and sources species

For more information, please consult the revised version of the **technical guide** (available around 8th April) and in the **explanatory document "What has changed and Why"**

More examples to follow (EDQM training)

Impact on the Ph. Eur. texts - Revised texts

General chapter 5.2.5 new section 4-3 "Methods of detection of extraneous agents"

- Allows the use of **new technologies**, particularly *in vitro* methods such as molecular techniques
- States general prerequisites for suitable methods including, e.g. specification for **positive and negative controls**.
- Focuses on specific information regarding testing
- References new general chapter **2.6.37**. "**Principles for the detection of extraneous viruses in IVMPs using culture methods.**"

*General principles and examples of parameters to be taken into account to use **fit-for-purpose methods*** (manufacturers have to check that the method is able to detect what they are looking for)

NEW

Impact on the Ph. Eur. texts - Revised texts

0062 - VACCINES FOR VETERINARY USE

5.2.4. CELL CULTURES FOR THE PRODUCTION OF VACCINES FOR VETERINARY USE

0030 - IMMUNOSERA FOR VETERINARY USE

Rearrangements in view of the new chapter 2.6.37 and the revised chapter 5.2.5.

+ Thirty-eight individual monographs

To know more: see EDQM press release

New Approach brings opportunities & benefits

- New approach based on risk assessment allows reduction in testing during manufacture and deletion of unnecessary tests for EAs on final product
- Comprehensive requirements for EA testing are centralised, Ph. Eur. texts now cover all species, this brings more clarity (no duplication, no discrepancies)
- Flexibility to choose methods for specific EA testing – fit-for-purpose sensitive techniques reflecting progress in science
- Methods no longer described in detail, building in flexibility of approach and allowing tailoring to individual product needs
- Use of state-of-the-art methods results in reduction of *in vivo* testing (decreased reliance on less robust methods)
- Coordinated Ph. Eur./EU approach, important also in the context of VICH
- Reduction in costs per batch

Huge thanks to everyone involved

- Experts of Group 15V and its Chairs, Céline Lorteau, Lukas Bruckner, Prof. Person
- EDQM team in charge of the secretary to Group 15V