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

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European Directorate for the Quality of Medicines & HealthCare | Direction européenne de la qualité du médicament & soins de santé

COUNCIL OF EUROPE
CONSEIL DE L'EUROPE

Ph. Eur. Training on Biologicals,

4-5 February 2020

- Vaccines for human use in the Ph. Eur.

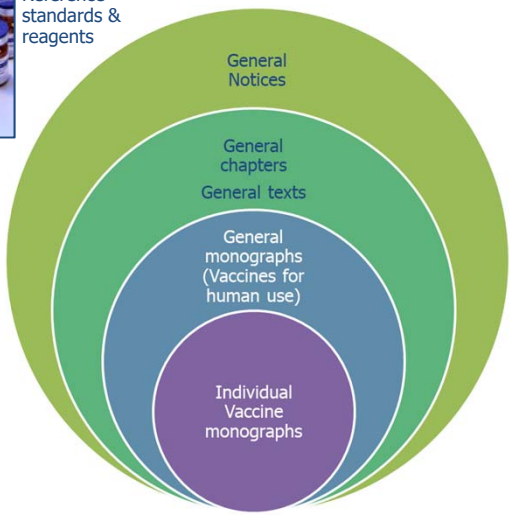
Outline

- Ph. Eur. standards for vaccines for human use
- General chapters supporting vaccine monographs
  (5.2.3, 2.6.16, 5.2.11, etc…)
- General monograph *Vaccines for human use* (0153)
- Individual vaccine monographs
- Technical Guide
- Adjuvants
- Reference standards supporting vaccine monographs
- Reduction of animal testing (3Rs)
- 3Rs achievements in the Ph. Eur. for vaccines - a case study
  (Suppression of ATT; aP vaccines: replacement of HIST)
- Coming next

Ph. Eur. standards for vaccines

Reference standards & reagents

- European Pharmacopoeia 10.1
  - European Pharmacopoeia 10.1
    - 00 Introduction
    - 01 General notices
    - 02 Methods of analysis
    - 03 Materials for containers and containers
    - 04 Reagents
    - 05 General Texts
    - 06 General Monographs
    - 07 Dosage forms
    - 08 Vaccines
    - 09 Immunosera
    - 10 Radiopharmaceutical preparations and st…
    - 11 Sutures
    - 12 Herbal drugs and herbal drug preparations
    - 13 Homoeopathic preparations
    - 14 Monographs A-C
    - 15 Monographs D-K

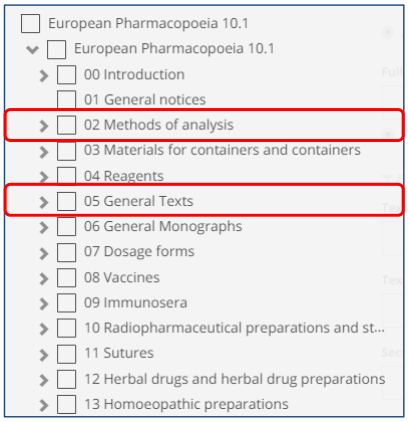


Technical guide for the elaboration of monographs on vaccines
(not part of Ph. Eur.)

General chapters supporting vaccine monographs

GC

- 2.4 Limit tests
- 2.5 Assays
- 2.6 Biological tests
- 2.7 Biological assays
- *5.1 General texts on microbiology**
- 5.2 General texts on biological products
- *5.3 Statistical analysis of results of biological assays and tests**



* Texts not specific to vaccines

General chapters supporting vaccine monographs

GC

1) General chapter on analytical methods

General methods	Chapter number
Assays	
Aluminium in adsorbed vaccines	2.5.13
Calcium in adsorbed vaccines	2.5.14
Phenol in immunosera and vaccines	2.5.15
Protein in polysaccharide vaccines	2.5.16
Nucleic acids in polysaccharide vaccines	2.5.17
Phosphorus in polysaccharide vaccines	2.5.18
O-acetyl in polysaccharide vaccines	2.5.19
Hexosamines in polysaccharide vaccines	2.5.20
Methylpentoses in polysaccharide vaccines	2.5.21
Uronic acids in polysaccharide vaccines	2.5.22
Sialic acid in polysaccharide vaccines	2.5.23
Ribose in polysaccharide vaccines	2.5.31
Limit tests	
Free formaldehyde	2.4.18

General methods	Chapter number
Biological tests	
Tests for extraneous agents in viral vaccines for human use	2.6.16
Test for neurovirulence of live virus vaccines	2.6.18
Residual pertussis toxin	2.6.33
Biological assays	
Assay of diphtheria vaccine (adsorbed)	2.7.6
Assay of pertussis vaccine (whole cell)	2.7.7
Assay of tetanus vaccine (adsorbed)	2.7.8
Assay of hepatitis A vaccine	2.7.14
Assay of hepatitis B vaccine (rDNA)	2.7.15
Assay of pertussis vaccine (acellular)	2.7.16
In vivo assay of poliomyelitis vaccine (inactivated)	2.7.20
Flocculation value (Lf) of diphtheria and tetanus toxins and toxoids (Ramon assay)	2.7.27
Immunonephelometry for vaccine component assay	2.7.35

General chapters supporting vaccine monographs

GC

2) General texts (substrates for propagation, carrier proteins, 3Rs…)

General texts	Chapter number
Chicken flocks free from specified pathogens for the production and quality control of vaccines	5.2.2
Cell substrates for the production of vaccines for human use	5.2.3
Carrier proteins for the production of conjugated polysaccharide vaccines for human use	5.2.11
Substitution of *in vivo* methods by *in vitro* methods for the quality control of vaccines	5.2.14

Microbiology

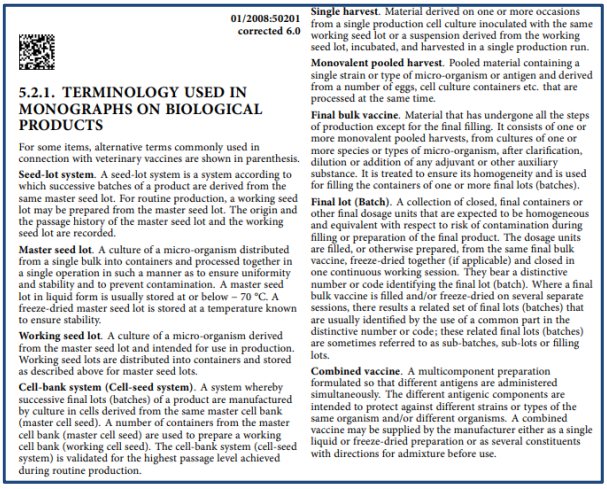
Efficacy of antimicrobial preservation — *2.4.18*

(specific acceptance criteria for vaccines in the General Monograph Vaccines for human use)

5.2.1 Terminology used in monographs on biological products

GC

- Seed-lot system (MSL/WSL)
- Cell-bank system (MCB/WCB)
- Production cell culture, Control cells
- Single/pooled harvest
- Vaccine final bulk/final lot
- Combined vaccine



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5.2.1. TERMINOLOGY USED IN MONOGRAPHS ON BIOLOGICAL PRODUCTS

For some items, alternative terms commonly used in connection with veterinary vaccines are shown in parenthesis.

Seed-lot system. A seed-lot system is a system according to which successive batches of a product are derived from the same master seed lot. For routine production, a working seed lot may be prepared from the master seed lot. The origin and the passage history of the master seed lot and the working seed lot are recorded.

Master seed lot. A culture of a micro-organism distributed from a single bulk into containers and processed together in a single operation in such a manner as to ensure uniformity and stability and to prevent contamination. A master seed lot in liquid form is usually stored at or below − 70 °C. A freeze-dried master seed lot is stored at a temperature known to ensure stability.

Working seed lot. A culture of a micro-organism derived from the master seed lot and intended for use in production. Working seed lots are distributed into containers and stored as described above for master seed lots.

Cell-bank system (Cell-seed system). A system whereby successive final lots (batches) of a product are manufactured by culture in cells derived from the same master cell bank (master cell seed). A number of containers from the master cell bank (master cell seed) are used to prepare a working cell bank (working cell seed). The cell-bank system (cell-seed system) is validated for the highest passage level achieved during routine production.

Single harvest. Material derived on one or more occasions from a single production cell culture inoculated with the same working seed lot or a suspension derived from the working seed lot, incubated, and harvested in a single production run.

Monovalent pooled harvest. Pooled material containing a single strain or type of micro-organism or antigen and derived from a number of eggs, cell culture containers etc. that are processed at the same time.

Final bulk vaccine. Material that has undergone all the steps of production except for the final filling. It consists of one or more monovalent pooled harvests, from cultures of one or more species or types of micro-organism, after clarification, dilution or addition of any adjuvant or other auxiliary substance. It is treated to ensure its homogeneity and is used for filling the containers of one or more final lots (batches).

Final lot (Batch). A collection of closed, final containers or other final dosage units that are expected to be homogeneous and equivalent with respect to risk of contamination during filling or preparation of the final product. The dosage units are filled, or otherwise prepared, from the same final bulk vaccine, freeze-dried together (if applicable) and closed in one continuous working session. They bear a distinctive number or code identifying the final lot (batch). Where a final bulk vaccine is filled and/or freeze-dried on several separate sessions, there results a related set of final lots (batches) that are usually identified by the use of a common part in the distinctive number or code; these related final lots (batches) are sometimes referred to as sub-batches, sub-lots or filling lots.

Combined vaccine. A multicomponent preparation formulated so that different antigens are administered simultaneously. The different antigenic components are intended to protect against different strains or types of the same organism and/or different organisms. A combined vaccine may be supplied by the manufacturer either as a single liquid or freeze-dried preparation or as several constituents with directions for admixture before use.

5.2.3 Cell substrates for the production of vaccines for human use

GC

- Scope: diploid cell lines and continuous cell lines used as cell substrates for the production of vaccines

- Requirements for cell substrates (cell seeds, MCB, WCB)
  - Cell-bank system
  - Media and substances of animal origin
  - Assessment of the suitability of cell seeds (based on source, history and characterisation)
  - Cell substrate stability
  - Testing strategy for extraneous agents (risk assessment)
  - Tumorigenicity
  - Residual host-cell DNA
  - Chromosomal characterisation

5.2.3 Cell substrates for the production of vaccines for human use

GC

Table 5.2.3.-1. – *Testing of cell lines*

Test	Cell seed	Master cell bank (MCB)	Working cell bank (WCB)	EOPC/ECB (Cells at or beyond the maximum population doubling level used for production)
1. IDENTITY AND PURITY				
Morphology	+	+	+	+
Identification	+	+	+	+
Karyotype (diploid cell lines)	+	+	+[1]	+[1]
Life span (diploid cell lines)	–	+	+	–
2. EXTRANEOUS AGENTS				
Bacterial and fungal contamination	–	+	+	–
Mycobacteria	–	+[2]	+[2]	–
Mycoplasmas	–	+	+	–
Spiroplasmas[3]	–	+	+	–
Electron microscopy	–	+[4]	–	+[4]
Tests for extraneous agents in cell cultures (with viable cells or equivalent cell lysate)	–	+	+	+
Tests in suckling mice and eggs	–	–	+[5]	+[5]
Test for specific viruses by NAT	–	+[6]	+[6]	+[6]
Test for viruses using broad molecular methods	+[7]	+[7]	+[7]	+[7]
Retroviruses	–	+[4]	–	+[4]
3. TUMORIGENICITY				
Tumorigenicity	+[8, 9]	–	–	+[8]

Testing methods for cell cultures:

- Identity and purity
- Extraneous agents
- Tumorigenicity

- Harmonised with WHO recommendations (WHO TRS 978 Annex 3 "Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterisation of cell banks").

2.6.16 Tests for extraneous agents in viral vaccines for human use

GC

- Applies to starting materials and substrates used for production and control of viral vaccines (virus seed lots, virus harvests, control cells/eggs)
- Panel of *in vivo* and *in vitro* methods
  - Cell culture methods
  - *In vivo* tests (suckling mice, fertilised eggs)
  - Molecular methods (for specific extraneous agent or broad virus detection)
- Testing strategy (package of suitable tests) to be built based on a risk assessment (package of tests able to detect different families of EAs that may infect the source of virus strains, capacity of the process to remove/inactivate viruses)
- List of tests must be adapted depending on the EAs that have the potential to contaminate the product

2.6.16 Tests for extraneous agents in viral vaccines for human use

GC

Table 2.6.16-1 Tests for extraneous agents at various production stages

	Virus seed lots	Virus harvests	Production of culture substrates	
			Control cells	Control eggs
Bacterial and fungal contamination	+	+	-	-
Mycoplasmas	+	+	-	-
Spiroplasmas[1]	+	-	-	-
Mycobacteria	+	+	-	-
Test in suckling mice[2]	+	-	-	-
Avian viruses[3]	+	+	-	-
Test for extraneous agents in cell cultures[4]	+	+	+	+
Insect viruses[5]	+	+	-	-
Test on control cells (microscopic examination)	-	-	+	-
Haemadsorbing viruses	-	-	+	-
Test on control eggs (haemagglutinating agents)	-	-	-	+
Avian leucosis viruses[6]	-	-	+	+
Test for specific viruses by NAT[7]	+	+	-	-
Test for viruses using broad molecular methods[8]	+	+	-	-

5.2.11 Carrier proteins for the production of conjugated PS vaccines

GC

- Aim: Harmonisation of quality profile of carrier proteins.
- Applies to all carrier proteins currently used in conjugated vaccines. *Revision needed whenever a new carrier protein is authorised.*
- Describes tests required for all carrier proteins.
- Describes characteristics for each type of carrier protein (diphtheria and tetanus toxoids, CRM 197, OMP and recombinant protein D).
- Production process for each type of carrier protein: production, concentration, purification, inactivation/treatment.

General chapters supporting vaccine monographs

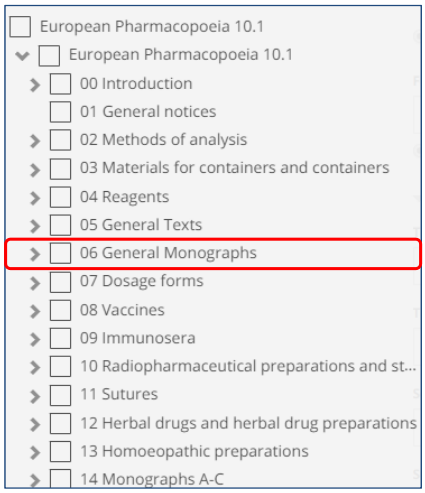
GC

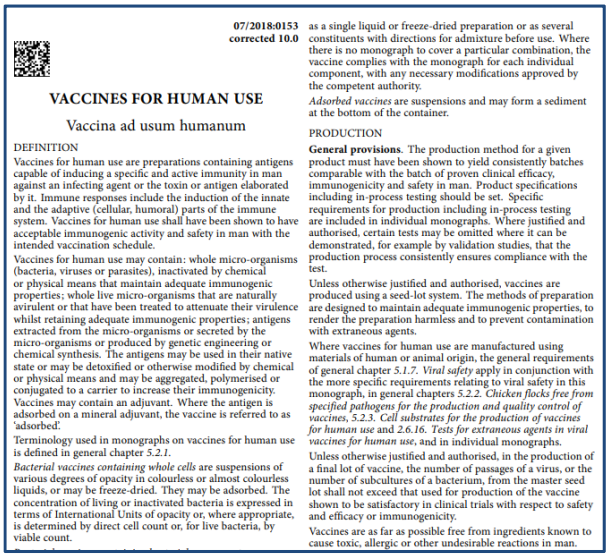
Can NGS methods be used for broad detection of viruses in virus seed lots / virus harvests, as per general chapter 2.6.16?

Yes. The use of NGS methods as part of the strategy to control extraneous agents is now foreseen in chapter 2.6.16.

General monograph *Vaccines for human use* (0153)

GM





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VACCINES FOR HUMAN USE

Vaccina ad usum humanum

DEFINITION

Vaccines for human use are preparations containing antigens capable of inducing a specific and active immunity in man against an infecting agent or the toxin or antigen elaborated by it. Immune responses include the induction of the innate and the adaptive (cellular, humoral) parts of the immune system. Vaccines for human use shall have been shown to have acceptable immunogenic activity and safety in man with the intended vaccination schedule.

Vaccines for human use may contain: whole micro-organisms (bacteria, viruses or parasites), inactivated by chemical or physical means that maintain adequate immunogenic properties; whole live micro-organisms that are naturally avirulent or that have been treated to attenuate their virulence whilst retaining adequate immunogenic properties; antigens extracted from the micro-organisms or secreted by the micro-organisms or produced by genetic engineering or chemical synthesis. The antigens may be used in their native state or may be detoxified or otherwise modified by chemical or physical means and may be aggregated, polymerised or conjugated to a carrier to increase their immunogenicity. Vaccines may contain an adjuvant. Where the antigen is adsorbed on a mineral adjuvant, the vaccine is referred to as 'adsorbed'.

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

Bacterial vaccines containing whole cells are suspensions of various degrees of opacity in colourless or almost colourless liquids, or may be freeze-dried. They may be adsorbed. The concentration of living or inactivated bacteria is expressed in terms of International Units of opacity or, where appropriate, is determined by direct cell count or, for live bacteria, by viable count.

as a single liquid or freeze-dried preparation or as several constituents with directions for admixture before use. Where there is no monograph to cover a particular combination, the vaccine complies with the monograph for each individual component, with any necessary modifications approved by the competent authority.

Adsorbed vaccines are suspensions and may form a sediment at the bottom of the container.

PRODUCTION

General provisions. The production method for a given product must have been shown to yield consistently batches comparable with the batch of proven clinical efficacy, immunogenicity and safety in man. Product specifications including in-process testing should be set. Specific requirements for production including in-process testing are included in individual monographs. Where justified and authorised, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test.

Unless otherwise justified and authorised, vaccines are produced using a seed-lot system. The methods of preparation are designed to maintain adequate immunogenic properties, to render the preparation harmless and to prevent contamination with extraneous agents.

Where vaccines for human use are manufactured using materials of human or animal origin, the general requirements of general chapter *5.1.7. Viral safety* apply in conjunction with the more specific requirements relating to viral safety in this monograph, in general chapters *5.2.2. Chicken flocks free from specified pathogens for the production and quality control of vaccines*, *5.2.3. Cell substrates for the production of vaccines for human use* and *2.6.16. Tests for extraneous agents in viral vaccines for human use*, and in individual monographs.

Unless otherwise justified and authorised, in the production of a final lot of vaccine, the number of passages of a virus, or the number of subcultures of a bacterium, from the master seed lot shall not exceed that used for production of the vaccine shown to be satisfactory in clinical trials with respect to safety and efficacy or immunogenicity.

Vaccines are as far as possible free from ingredients known to cause toxic, allergic or other undesirable reactions in man.

General monograph *Vaccines for human use* (0153)

GM

Pharmacopoeial requirements for vaccines:

→ Given in general monograph *Vaccines for Human Use* and individual vaccine monographs.

General Monograph *Vaccines for Human Use* :

- Provisions apply to all vaccines, including those for which there is no individual monograph.
- Essential requirements which supplement and expand on requirements contained in the monographs for specific vaccines.
- Requirements usually not repeated in individual monographs.

General monograph *Vaccines for human use* (0153)

GM

- *"The production method for a given product must have been shown to yield consistently batches comparable with the batch of proven clinical efficacy, immunogenicity and safety in man. Product specifications including in-process testing should be set. Specific requirements for production including in-process testing are included in individual monographs. Where justified and authorised, certain tests may be omitted where it can be demonstrated, for example by validation studies, that the production process consistently ensures compliance with the test."*

- Consistency of production process: batches must be comparable to batches of proven safety and efficacy
- Omission of tests is possible when consistency is demonstrated
  - validation
  - agreement by the competent authority

General monograph *Vaccines for human use* (0153)

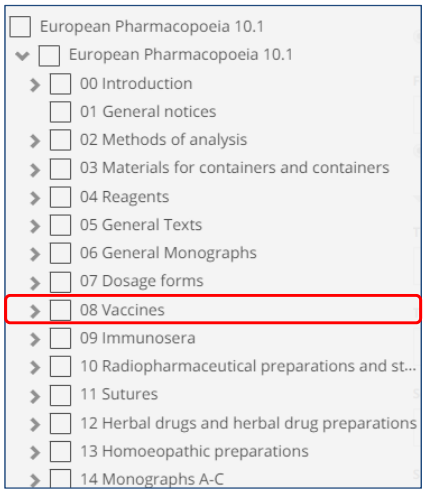
GM

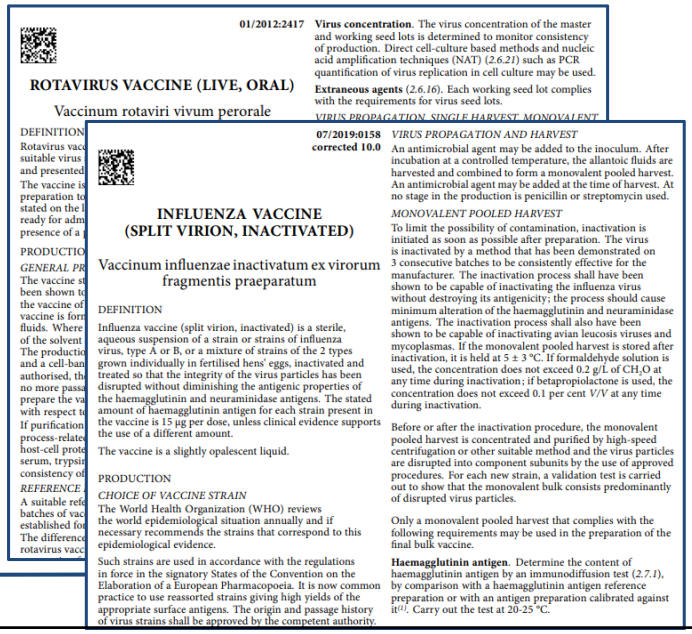
" *Consistency of production is an important feature of vaccine production. Monographs on vaccines for human use give limits for various tests carried out during production and on the final lot. […] While compliance with these limits is required, it is not necessarily sufficient to ensure consistency of production for a given vaccine. For relevant tests, the manufacturer must therefore define for each product a suitable action or release limit or limits to be applied in view of the results found for batches tested clinically and those used to demonstrate consistency of production. These limits may subsequently be refined on a statistical basis in light of production data."*

- Consistency of production important feature
- Compliance to the Tests described in monographs (during production or on the final lot) is not necessarily sufficient to ensure consistency of production
- The manufacturer must define suitable action or release limit(s) for relevant tests

Individual vaccine monographs

IM





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ROTAVIRUS VACCINE (LIVE, ORAL)

Vaccinum rotaviri vivum perorale

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INFLUENZA VACCINE (SPLIT VIRION, INACTIVATED)

Vaccinum influenzae inactivatum ex virorum fragmentis praeparatum

DEFINITION

Influenza vaccine (split virion, inactivated) is a sterile, aqueous suspension of a strain or strains of influenza virus, type A or B, or a mixture of strains of the 2 types grown individually in fertilised hens' eggs, inactivated and treated so that the integrity of the virus particles has been disrupted without diminishing the antigenic properties of the haemagglutinin and neuraminidase antigens. The stated amount of haemagglutinin antigen for each strain present in the vaccine is 15 µg per dose, unless clinical evidence supports the use of a different amount.

The vaccine is a slightly opalescent liquid.

PRODUCTION

CHOICE OF VACCINE STRAIN

The World Health Organization (WHO) reviews the world epidemiological situation annually and if necessary recommends the strains that correspond to this epidemiological evidence.

Such strains are used in accordance with the regulations in force in the signatory States of the Convention on the Elaboration of a European Pharmacopoeia. It is now common practice to use reassorted strains giving high yields of the appropriate surface antigens. The origin and passage history of virus strains shall be approved by the competent authority.

VIRUS PROPAGATION AND HARVEST

An antimicrobial agent may be added to the inoculum. After incubation at a controlled temperature, the allantoic fluids are harvested and combined to form a monovalent pooled harvest. An antimicrobial agent may be added at the time of harvest. At no stage in the production is penicillin or streptomycin used.

MONOVALENT POOLED HARVEST

To limit the possibility of contamination, inactivation is initiated as soon as possible after preparation. The virus is inactivated by a method that has been demonstrated on 3 consecutive batches to be consistently effective for the manufacturer. The inactivation process shall have been shown to be capable of inactivating the influenza virus without destroying its antigenicity; the process should cause minimum alteration of the haemagglutinin and neuraminidase antigens. The inactivation process shall also have been shown to be capable of inactivating avian leucosis viruses and mycoplasmas. If the monovalent pooled harvest is stored after inactivation, it is held at 5 ± 3 °C. If formaldehyde solution is used, the concentration does not exceed 0.2 g/L of CH_2O at any time during inactivation; if betapropiolactone is used, the concentration does not exceed 0.1 per cent *V/V* at any time during inactivation.

Before or after the inactivation procedure, the monovalent pooled harvest is concentrated and purified by high-speed centrifugation or other suitable method and the virus particles are disrupted into component subunits by the use of approved procedures. For each new strain, a validation test is carried out to show that the monovalent bulk consists predominantly of disrupted virus particles.

Only a monovalent pooled harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Haemagglutinin antigen. Determine the content of haemagglutinin antigen by an immunodiffusion test (*2.7.1*), by comparison with a haemagglutinin antigen reference preparation or with an antigen preparation calibrated against it[1]. Carry out the test at 20-25 °C.

Virus concentration. The virus concentration of the master and working seed lots is determined to monitor consistency of production. Direct cell-culture based methods and nucleic acid amplification techniques (NAT) (*2.6.21*) such as PCR quantification of virus replication in cell culture may be used.

Extraneous agents (*2.6.16*). Each working seed lot complies with the requirements for virus seed lots.

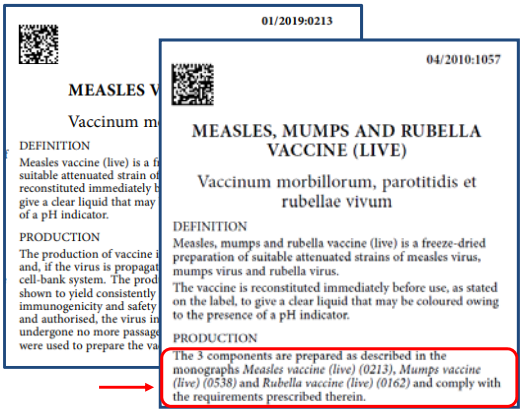
Individual vaccine monographs

IM

Monographs are elaborated for:

- Single type vaccines (e.g. Measles vaccine)
- Combined vaccines (e.g. MMR vaccine)

→*A combined vaccine must also comply with the individual monographs for each valence of the vaccine.*



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MEASLES V

Vaccinum m

DEFINITION

Measles vaccine (live) is a f
suitable attenuated strain o
reconstituted immediately b
give a clear liquid that may
of a pH indicator.

PRODUCTION

The production of vaccine i
and, if the virus is propagat
cell-bank system. The prod
shown to yield consistently
immunogenicity and safety
and authorised, the virus in
undergone no more passag
were used to prepare the va

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MEASLES, MUMPS AND RUBELLA VACCINE (LIVE)

Vaccinum morbillorum, parotitidis et rubellae vivum

DEFINITION

Measles, mumps and rubella vaccine (live) is a freeze-dried preparation of suitable attenuated strains of measles virus, mumps virus and rubella virus.

The vaccine is reconstituted immediately before use, as stated on the label, to give a clear liquid that may be coloured owing to the presence of a pH indicator.

PRODUCTION

The 3 components are prepared as described in the monographs *Measles vaccine (live) (0213)*, *Mumps vaccine (live) (0538)* and *Rubella vaccine (live) (0162)* and comply with the requirements prescribed therein.

The quality standards attained by vaccines already on the market are taken into consideration during the elaboration of a new monograph.

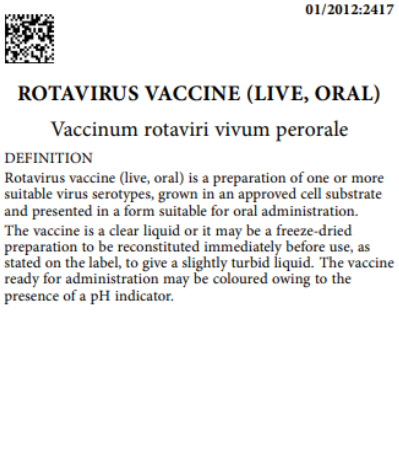
Individual vaccine monographs – structure

IM

DEFINITION

- Defines the scope of the monograph and its applicability to products on the market, briefly states the product composition;

→ Monograph sets the official standard for all products covered by this definition.



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ROTAVIRUS VACCINE (LIVE, ORAL)

Vaccinum rotaviri vivum perorale

DEFINITION

Rotavirus vaccine (live, oral) is a preparation of one or more suitable virus serotypes, grown in an approved cell substrate and presented in a form suitable for oral administration.

The vaccine is a clear liquid or it may be a freeze-dried preparation to be reconstituted immediately before use, as stated on the label, to give a slightly turbid liquid. The vaccine ready for administration may be coloured owing to the presence of a pH indicator.

Individual vaccine monographs – structure

IM

PRODUCTION

- Describes essential features of the manufacturing process, prior to and including batch release.
- Generally follows the chronological order of vaccine production:

❑ General Provisions
❑ Substrate for propagation
❑ Seed lots
❑ Propagation and harvest of the virus, or Culture and harvest of bacteria
❑ Purification and Inactivation (inactivated vaccines)
❑ Final bulk vaccine
❑ Final lot

- Section primarily addressed to manufacturers.

Individual vaccine monographs – structure

IM

PRODUCTION

- Points to be addressed for vaccine production; tests to be conducted during product development, routinely on intermediates and on each vaccine batch.

❑ *GENERAL PROVISIONS*

- General requirements for the manufacturing process. May relate e.g. to source materials, to the manufacturing process itself and to its validation and control, as well as in-process requirements which enable demonstration of manufacturing consistency.

❑ *REFERENCE PREPARATION*

PRODUCTION

GENERAL PROVISIONS

The vaccine strains and the production method shall have been shown to yield consistently vaccines comparable with the vaccine of proven clinical efficacy and safety in man. The vaccine is formulated so as to avoid inactivation by gastric fluids. Where the vaccine is freeze-dried, the antacid capacity of the solvent and its stability are established.

The production of vaccine is based on a virus seed-lot system and a cell-bank system. Unless otherwise justified and authorised, the virus in the final vaccine shall have undergone no more passages from the master seed lot than were used to prepare the vaccine shown in clinical studies to be satisfactory with respect to safety and efficacy.

If purification steps are present, the reduction of selected process-related impurities and residuals such as residual host-cell proteins, residual cellular DNA, endotoxins, bovine serum, trypsin, and antibiotics is monitored to establish consistency of the purification process.

REFERENCE PREPARATION

A suitable reference preparation that is representative of batches of vaccine shown to be effective in clinical trials is established for use in tests to determine virus concentration. The differences in the composition and characteristics of rotavirus vaccines mean that there will be a specific reference preparation for each one.

Individual vaccine monographs – structure

IM

PRODUCTION

❑ *SUBSTRATE FOR PROPAGATION*

- Culture media for bacterial vaccines, or substrates for virus propagation: additional requirements may be given (*to be read in conjunction with general monograph 0153*)
- Cell substrates: requirements for cell lines are given in chapter 5.2.3; requirements for primary cells in the individual monograph
- SPF eggs: requirements are given in chapter 5.2.2

❑ *SEED LOTS*

- Requirements for seed lots: identification, test for extraneous agents for viral vaccines, test for contaminants for bacterial vaccines, possibly test for virulence, etc.

SUBSTRATE FOR VIRUS PROPAGATION

The virus is propagated in a suitable cell line (*5.2.3*).

VIRUS SEED LOTS

The strain(s) of rotavirus used shall be identified by historical records that include information on the origin of each strain and its subsequent manipulation including the method of attenuation, whether the strains have been biologically cloned prior to generation of the master seed lot, genetic sequence information, the phenotypic and genotypic stability of the master and working seed lots when passaged up to the single harvest level, and the passage level at which attenuation for humans was demonstrated by clinical trials. Virus seed lots are stored at temperatures below – 20 °C if freeze-dried, or below – 60 °C if not freeze-dried.

Only a seed lot that complies with the following requirements may be used for virus propagation.

Identification. The master and working seed lots are shown to be of the required rotavirus type by an immunological assay using specific antibodies or by a molecular identity test such as polyacrylamide gel electrophoresis of RNA, RNA/RNA hybridisation, or restriction-enzyme mapping of genetic sequences of polymerase chain reaction (PCR)-amplified VP7 gene segments.

Virus concentration. The virus concentration of the master and working seed lots is determined to monitor consistency of production. Direct cell-culture based methods and nucleic acid amplification techniques (NAT) (*2.6.21*) such as PCR quantification of virus replication in cell culture may be used.

Extraneous agents (*2.6.16*). Each working seed lot complies with the requirements for virus seed lots.

Individual vaccine monographs – structure

IM

PRODUCTION

❑ *PROPAGATION AND HARVEST (viruses), CULTURE AND HARVEST (bacteria)*

- Purity of the harvest before purification, testing for contaminants;
- For viral vaccines, requirement for control cells from the production cell culture to comply with an identification test and with the requirements for extraneous agents (chapter 2.6.16).

VIRUS PROPAGATION, SINGLE HARVEST, MONOVALENT POOLED HARVEST

All processing of the cell bank and subsequent cell cultures is done under aseptic conditions in an area where no other cells are being handled. Suitable animal (but not human) serum may be used in the culture media, but the final medium for maintaining cell growth during virus multiplication does not contain animal serum. Serum and trypsin used in the preparation of cell suspensions and culture media are shown to be free from extraneous agents. The cell culture medium may contain a pH indicator such as phenol red and suitable antibiotics at the lowest effective concentration. It is preferable to have a substrate free from antibiotics during production.

[...]

VIRUS PROPAGATION AND SINGLE HARVEST

On the day of inoculation with the virus working seed lot or stored virus intermediate culture, cell cultures employed for vaccine production are set aside as uninfected cell cultures (control cells). If bioreactor technology is used, the size and handling of the cell sample to be examined is approved by the competent authority. The virus suspensions are harvested at a time appropriate to the strain of virus being used.

Only a single virus harvest that complies with the following requirements may be used for further processing.

Bacterial and fungal contamination. Each single virus harvest complies with the test for sterility (*2.6.1*), carried out using 10 mL for each medium.

Control cells. The control cells of the production cell culture from which each single harvest is derived comply with a test for identity and with the requirements for extraneous agents (*2.6.16*).

[...]

Individual vaccine monographs – structure

IM

PRODUCTION

❑ *PURIFICATION AND INACTIVATION (for inactivated vaccines)*

- Concentration of micro-organisms or the antigen content;
- Host-cell DNA (if production involves continuous cell lines);
- The absence of residual infectious virus is verified through the evaluation of the consistency of the inactivation process, as well as testing during the course, and at the final stage, of inactivation.

Example of Hepatitis A vaccine

INACTIVATION AND INACTIVATED HARVEST

Several purified harvests may be pooled before inactivation. In order to avoid interference with the inactivation process, virus aggregation must be prevented or aggregates must be removed immediately before and/or during the inactivation process. The virus suspension is inactivated by a validated method; the method shall have been shown to be consistently capable of inactivating hepatitis A virus without destroying the antigenic and immunogenic activity; for each inactivation procedure, an inactivation curve is plotted representing residual live virus concentration measured at not fewer than 3 points in time (for example, on days 0, 1 and 2 of the inactivation process). If formaldehyde is used for inactivation, the presence of excess free formaldehyde is verified at the end of the inactivation process.

Only an inactivated harvest that complies with the following requirements may be used in the preparation of the final bulk vaccine.

Inactivation. Carry out an amplification test for residual infectious hepatitis A virus by inoculating a quantity of the inactivated harvest equivalent to 5 per cent of the batch or, if the harvest contains the equivalent of 30 000 doses or more, not less than 1500 doses of vaccine into cell cultures of the same type as those used for production of the vaccine; incubate for a total of not less than 70 days making not fewer than one passage of cells within that period. At the end of the incubation period, carry out a test of suitable sensitivity for residual infectious virus. No evidence of hepatitis A virus multiplication is found in the samples taken at the end of the inactivation process. Use infectious virus inocula concurrently as positive controls to demonstrate cellular susceptibility and absence of interference.

Individual vaccine monographs – structure

IM

PRODUCTION

❑ *FINAL BULK VACCINE*

- Amount of antimicrobial preservative (if applicable)
- Test for sterility/bacterial and fungal contamination (2.6.1)
- *Only a final bulk that complies with the requirements of this section may be used in the preparation of the final lot.*

❑ *FINAL LOT*

= final bulk vaccine aseptically distributed into sterile tamper-evident containers

- *Only a final lot that complies with each of tests described under Identification, Tests and Assay (and any additional requirements under Final lot) may be released.*

FINAL BULK VACCINE

The final bulk vaccine is prepared from one or more satisfactory purified monovalent harvests and may contain more than one virus type. Suitable stabilisers may be added. Only a final bulk vaccine that complies with the following requirement may be used in the preparation of the final lot.

Bacterial and fungal contamination. The final bulk vaccine complies with the test for sterility (*2.6.1*), carried out using 10 mL for each medium.

FINAL LOT

The final bulk vaccine is distributed aseptically into sterile containers and may be freeze-dried to a moisture content shown to be favourable to the stability of the vaccine. The containers are then closed so as to avoid contamination and the introduction of moisture.

An approved minimum virus concentration for release of the product is established for each virus type to ensure, in light of stability data, that the minimum concentration stated on the label will be present at the end of the period of validity.

For freeze-dried vaccines, tests for identity, pH, volume, sterility and content of key components are carried out on the solvent.

Only a final lot that complies with the following requirement for thermal stability and is satisfactory with respect to each of the requirements given below under Identification, Tests and Assay may be released for use.

[...]

Individual vaccine monographs

IM

IDENTIFICATION

- Describes how to identify the product.
- The main product characteristic is usually checked by appropriate methods such as the assay method to identify the vaccine, or recognition of the antigens in the vaccine by specific antibodies, etc…

IDENTIFICATION

The vaccine is shown to contain rotavirus of each type stated on the label by an immunological assay using specific antibodies or by a molecular identity test. If PCR is used for the assay, this may serve as the identity test.

TESTS

- Series of batch tests with limits, e.g. the content of antimicrobial preservatives, aluminium, free formaldehyde, BSA, ovalbumin, water, a test for inactivation, for toxicity, sterility, pyrogens or bacterial endotoxins
- The product should comply throughout its shelf life.

TESTS

Bacterial and fungal contamination. The vaccine complies with the test for sterility (*2.6.1*).

Water (*2.5.12*): maximum 3.0 per cent for each final lot of freeze-dried vaccine.

Individual vaccine monographs

IM

ASSAY or LIVE VIRUS/BACTERIA CONCENTRATION or PS CONTENT

- Potency test (in individual monograph or in separate chapter)
- Aims at determining the capacity of the vaccine to induce the formation of specific antibodies against the pathogen, or to titrate the infective virus/live bacteria/antibodies against toxoids, or to determine the content of an antigen which is relevant to measure the efficacy of the vaccine, or to assess the protection of a vaccine, etc…
- Statistical methods for calculation of results in chapter 5.3 *Statistical analysis of results of biological assays and tests.*

ASSAY

The assay of rotavirus vaccine is carried out by inoculation of suitable cell cultures with dilutions of the vaccine and evaluation of the rotavirus concentration, either by visualisation of infected areas of a cell monolayer or by comparison of the capacity of the vaccine to produce viral RNA following infection of cells with the corresponding capacity of an approved reference preparation.

For the assay based on visualisation of infected areas of a cell monolayer, titrate the vaccine for infective virus using at least 3 separate containers. Titrate the contents of 1 container of an appropriate virus reference preparation in triplicate to validate each assay. If the vaccine contains more than 1 rotavirus type, titrate each type separately using a method of suitable specificity. The virus concentration of the reference preparation is monitored using a control chart and a titre is established on a historical basis by each laboratory.

Calculate the individual virus concentration for each container of vaccine and for each replicate of the reference preparation as well as the corresponding combined virus concentrations, using the usual statistical methods (for example, *5.3*).

The assay is not valid if:

- the confidence interval ($P = 0.95$) of the estimated virus concentration of the reference preparation for the 3 replicates combined is greater than $\pm$ 0.3 $\log_{10}$ $CCID_{50}$ (or an equivalent value expressed with a unit suitable for the method used for the assay);
- the virus concentration of the reference preparation differs by more than 0.5 $\log_{10}$ $CCID_{50}$ (or an equivalent value expressed with a unit suitable for the method used for the assay) from the established value. [...]

Individual vaccine monographs

IM

STORAGE

- *Section given for information*.
- Information on storage conditions.
- Unless otherwise indicated, the storage of vaccines is expected to conform to that described in the general monograph *Vaccines for human use*

LABELLING

- *Section given for information*.
- Labelling requirements specific to the product.
- Supplementary to the labelling statements of the general monograph *Vaccines for human use*.

STORAGE

Store protected from light. Unless otherwise stated, the storage temperature is 5 ± 3 °C; liquid adsorbed vaccines must not be allowed to freeze.

LABELLING

The label states:

- the name of the preparation;
- a reference identifying the final lot;
- the recommended human dose and route of administration;
- the storage conditions;
- the expiry date;
- the name and amount of any antimicrobial preservative;
- the name of any antibiotic, adjuvant, flavour or stabiliser present in the vaccine;
- where applicable, that the vaccine is adsorbed;
- the name of any constituent that may cause adverse reactions and any contra-indications to the use of the vaccine;

General Monograph 0153

No STORAGE section

Individual Monograph Rotavirus vaccine

LABELLING

The label states:

- the type or types of rotavirus contained in the vaccine;
- the minimum amount of each type of virus contained in 1 single human dose;
- the cell substrate used for the preparation of the vaccine.

Adjuvants

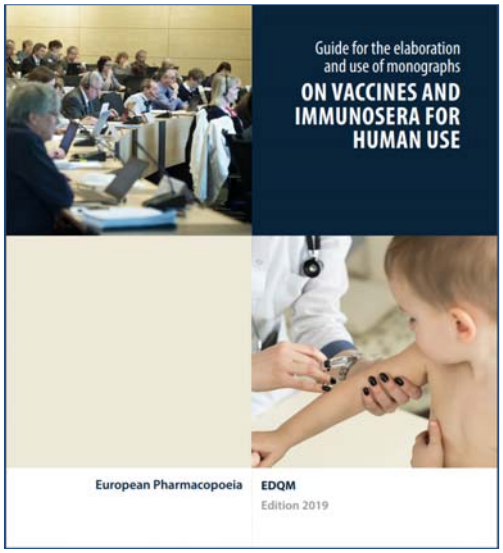
IM

- Individual monographs for vaccine adjuvants:
  - Aluminium salts:
    - Aluminium hydroxide, hydrated, for adsorption (1664),
    - *Aluminium phosphate, hydrated, for adsorption (3065) [monograph under development]*
  - Other adjuvants:
    - 3-o-desacyl-4'-monophosphoryl lipid A (2537) ("MPL")
    - Squalene (2805)

Technical Guide (Vaccine monographs)

- Guidance to experts on the information to be included in vaccine monographs, and guide to harmonise the style of the different monographs;
- Useful information to help users better understand the requirements and structure of these monographs;
- New Edition 2019.

Access to Technical guide here.



Reference standards supporting vaccine monographs

Common - reference standards - critical reagents

- Physico-chemical tests → Chemical Reference Substances (CRS)
- Biological assays → Biological Reference Preparations (BRP), Biological Reference Reagents (BRR)

Ph. Eur. Reference standards for vaccines are:

- Working standards for use in conjunction with the Ph. Eur. texts
- Officially adopted by the Ph. Eur. Commission
- Calibrated against the corresponding WHO IS (when existing)
- Assigned with a content in IU (as appropriate)

Examples: Diphtheria toxin BRP, Varicella vaccine (live) BRP, Bordetella pertussis mouse antiserum BRP

Individual vaccine monographs

IM

Which of the following apply to combined hepatitis A (inactivated) and hepatitis B (rDNA) vaccine?

- ○ Individual vaccine monograph(s)
- ○ General Notices
- ○ General monograph vaccines for human use
- ○ 2.7.15 Assay of hepatitis B vaccine (rDNA)

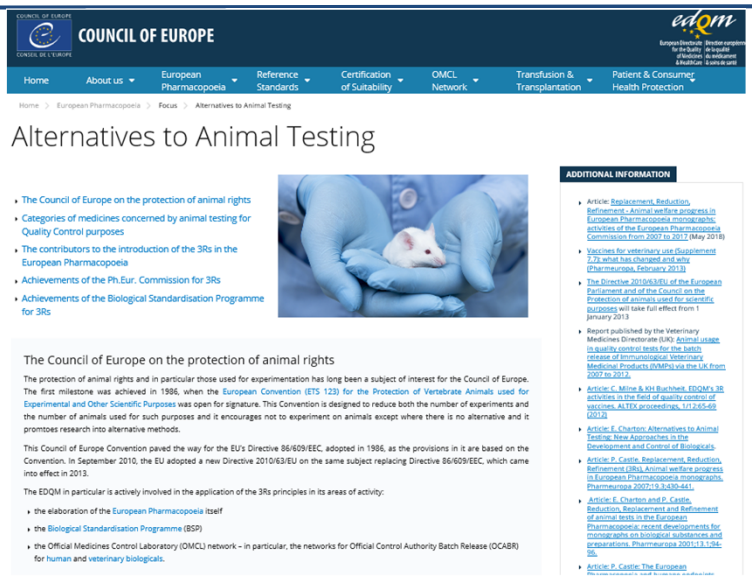
Individual vaccine monographs

IM

Which of the following apply to combined hepatitis A (inactivated) and hepatitis B (rDNA) vaccine?

- Individual vaccine monograph(s). *Monograph 1526 for the combination AND individual monographs for each valence.*
- General Notices
- General monograph vaccines for human use
- 2.7.15 Assay of hepatitis B vaccine (rDNA)

Alternatives to Animal Testing at EDQM/Ph. Eur.



Why 3R Alternatives?

- Ethical concerns
- Legal obligations
- Variability of *in vivo* results
- Costs
- Advances in analytics and production
- Public pressure

Application of 3Rs is of high importance for EDQM/Ph. Eur.

Classic 3Rs - Russell and Burch (1959)

- Replace, Reduce, Refine

Plus important 4th R

- Remove

Application is case specific – all are used

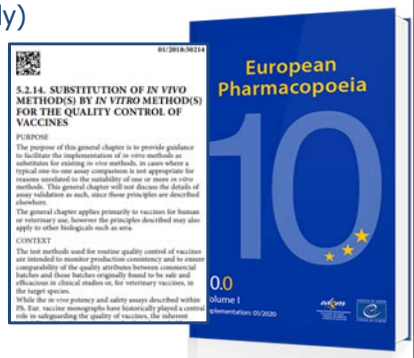
3Rs in pharmacopoeial testing

- The General Notices foster and enable the application of 3Rs
  - Encourage reduction of animal usage, allow use of alternative methods and consistency approach
- Requirements in General monograph *Vaccines for human use* on reducing animal numbers and suffering
  - "*In accordance with the […] European Convention* […], tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm*".
- Individual vaccine monographs encourage the use of alternative 3Rs methods, humane endpoints
  - The detailed protocol of a validated 3Rs method may be provided as an example, where available (e.g. Assay of hepatitis A vaccine (2.7.14), Residual pertussis toxin (2.6.33))
- Guidance in chapter 5.2.12 on concept of "substitution" of animal tests for QC of vaccines

5.2.12 Substitution of in vivo methods for the QC of vaccines

- The introduction of in vitro methods to replace in vivo methods often prevented due to the properties of in vivo methods (e.g. variability, validation of in vivo methods, product attributes assessed differently)
- Demonstration of equivalence may not only be problematic, but also of limited relevance

  → New general chapter 5.2.14



Aim: facilitate the transition from *in vivo* to *in vitro* methods

5.2.12 Substitution of in vivo methods for the QC of vaccines

- Chapter 5.2.14 provides guidance on how to introduce alternative *in vitro* methods, where a head-to-head comparison is not possible
- Envisages the possibility that the validity of the *in vitro* method be demonstrated without such head-to-head comparison (concept of "substitution") and discusses alternative approaches for replacement
- Focus on the scientific rationale behind the in vitro methods, relative to what is provided with current in vivo methods

Recent 3Rs achievement in the Ph. Eur. for vaccines

- 2 case studies

- Suppression of ATT
  - An example of Remove (4th R) impacting multiple products, and convergence towards global acceptance
- Replacement of HIST & review of toxicity testing requirements for acellular Pertussis vaccines
  - An example of Replacing an animal test and Removing outdated requirements

Abnormal Toxicity Test (ATT)

= General Safety Test (US), Innocuity Test (WHO)

- Principle: inject batches of product into guinea pigs/mice. A batch passes the test if no animal shows any sign of illness, or dies within a defined timeframe
  → Animal suffering

- Considerable usage of animals: e.g. for vaccines, 5 mice and 2 guinea pigs for each batch

- One of the most controversial animal tests in the Ph. Eur.
  → *Priority target for 3Rs!*

Relevance of ATT

- Safety tests in mice and guinea pigs date back to the early 1900s
  - detection of toxic levels of phenol in sera (mice)
  - detection of contamination with tetanus toxin & spores in sera (guinea pigs)
- In 1940s both tests were combined to become a general safety test. ATT largely unchanged since then, despite evolution of analytical techniques, manufacturing processes
- Retrospective analysis concluded: the ATT is neither specific, reproducible, reliable, nor suitable for the intended purpose (*Duchow et al, 1994*)
  - More relevant tests used for testing phenols, toxins
- Deletion as a routine batch release test from >80 monographs in 1998
  - Moved to Production section (development test)
- Use of GMP and stringent QC measures to prevent contamination also puts in question the relevance of the ATT

Converging regulatory agreement on ATT

European Partnership for Alternative Approaches to Animal Testing (EPAA)

- Discussed the ATT issue in depth in a Workshop in 2016 with global stakeholders
- Workshop included review of case studies and data
- Conclusion: the ATT lacks scientific relevance and its omission does not compromise the safety of biologics. *Consensus to strive for deletion of the ATT from regulatory requirements*
- Deletion of ATT should be addressed at a global level
  - A harmonised approach by all regulators across the globe is important for a real and effective deletion

Suppression of ATT from the Ph. Eur. … and beyond!

Ph. Eur.

- Based on this review, the Ph. Eur. Commission decided to embark on the deletion of ATT from 49 monographs
- A detailed evaluation was conducted for each monograph
- Decision to suppress the ATT (Nov 2017)
  - Revised monographs omitting the ATT published in Supplement 9.6 (July 2018)
  - Simultaneous suppression of chapter *2.6.9 Abnormal toxicity*, no longer referenced in any monograph

No More ATT in the Ph. Eur.!
A MAJOR STEP FORWARD FOR 3Rs

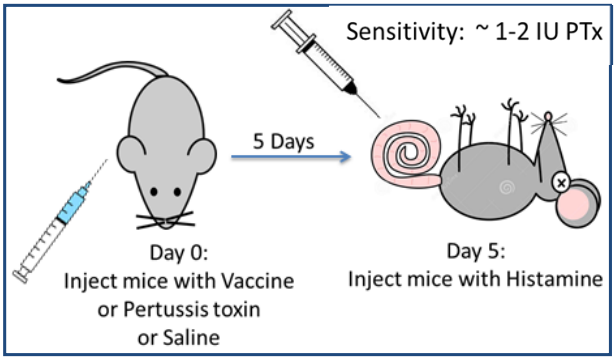
WHO

- WHO's ECBS recommendation to discontinue ATT in guidelines on vaccines and biologicals (Nov 2018) → A further step towards global acceptance

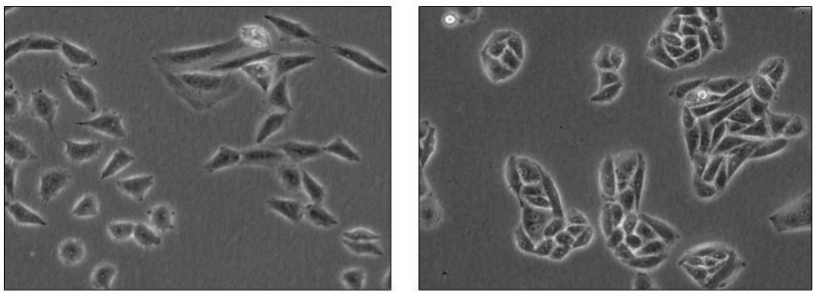
Histamine Sensitisation Test (HIST)

- HIST is used as a release test for acellular Pertussis (aP) vaccines (*test for residual pertussis toxin and irreversibility of toxoid*)
- Large number of animals, high variability, several versions of HIST are required in different regions
- End-point = death, 24h after injection of histamine
- High distress of animals

→ *Replacement considered a priority!*



Replacement of HIST: CHO Cell Clustering Assay



Source: R. Isbrucker, Health Canada

Control (no PTx) + PTx (48 hrs)

Sensitivity: ~ 0.006 IU PTx

- Induction of clusters in non-confluent CHO cell cultures by active pertussis toxin
- Standardised method validated in a collaborative study (*cf. Isbrucker R, Daas A, Wagner L et al. Transferability study of CHO cell clustering assays for monitoring of pertussis toxin activity in acellular pertussis vaccines, Pharmeur Bio Sci Notes 2016:97-114*)

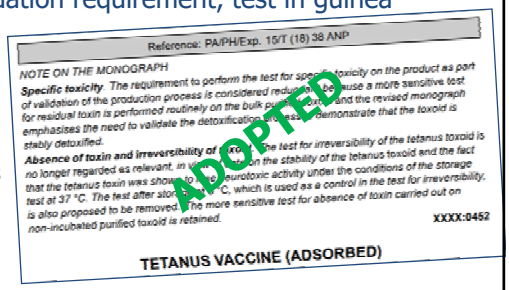
Rationalising toxicity testing requirements for aP vaccines

- Test for residual toxin on purified bulk: replacement of HIST by CHO clustering assay
  - Data from BSP collaborative study
  - *Standardised CHO assay method described as an example*
- Removal of the Test for irreversibility
  - Data confirming that the pertussis toxoid is stable and reversion is not an issue for marketed aP vaccines (→ *manufacturers & OMCLs survey*)
  - History of safe use of aP vaccines
- Removal of the requirement to test the final lot for residual toxin
  - Testing of the pre-adsorbed bulk is considered the most effective and robust approach

Coming next… Monograph revisions underway

- Review of toxicity testing requirements for Tetanus vaccines
  - Removal of the **Test for irreversibility** of toxoid (test in guinea pigs)
    - Tetanus toxin was shown to lose neurotoxic activity under the conditions of the storage test at 37°C
    - Data confirming the stability of Tetanus toxoid
  - Removal of the **Test for specific toxicity** (process validation requirement, test in guinea pigs)
    - Considered redundant because a more sensitive test for residual toxin is performed routinely on bulk purified toxoid
    - Emphasis on the need to validate the detoxification process to demonstrate that the toxoid is stably detoxified

    → To be published in Supplement 10.3


Reference: PA/PH/Exp. 15/T (18) 38 ANP

NOTE ON THE MONOGRAPH

Specific toxicity *The requirement to perform the test for specific toxicity on the product as part of validation of the production process is considered redundant because a more sensitive test for residual toxin is performed routinely on the bulk purified toxoid and the revised monograph emphasises the need to validate the detoxification process to demonstrate that the toxoid is stably detoxified.*

Absence of toxin and irreversibility of toxoid *The test for irreversibility of the tetanus toxoid is no longer regarded as relevant, in view of the data on the stability of the tetanus toxoid and the fact that the tetanus toxin was shown to lose its neurotoxic activity under the conditions of the storage test at 37 °C. The test after storage at 37 °C, which is used as a control in the test for irreversibility, is also proposed to be removed. The more sensitive test for absence of toxin carried out on non-incubated purified toxoid is retained.*

XXXX:0452

TETANUS VACCINE (ADSORBED)

ADOPTED

Current and future collaborative studies to validate 3Rs methods

Current/future BSP projects

- Rabies vaccine for human use: ELISA to replace *in vivo* potency assay
- Tetanus vaccines: BINACLE assay (*in vitro* test) to replace *in vivo* test for *Absence of toxin and irreversibility of toxoid*
- Tetanus- & diphtheria vaccines: ELISA to replace *in vivo* potency assay

→ Further information in *BSP work programme*

3Rs in pharmacopoeial testing

It is possible to use a 3R alternative method that is not described in the Ph. Eur.?

Yes.

The Use of 3R alternatives is encouraged by the Ph. Eur. Suitably validated alternate methods can be used in accordance with the General Notices and upon approval of the competent authority.

Thank you for your attention

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