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

edqm

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

2024 EDQM virtual training programme:

Independent modules on European Pharmacopoeia texts related to Biologicals and on Microbiology chapters

(Live Webinars)
Date: 30 January 2024 – 01 February 2024

Module 2
Blood products and vaccines for human use

31 January 2024

Agenda

Blood products

Solène Le Maux, EDQM, Council of Europe

Vaccines for human use

Gwenaël Ciréfice, EDQM, Council of Europe

A journey inside the blood product monographs

Blood products monographs in the Ph. Eur.

1 monograph for product from animal plasma

Anti-T lymphocyte immunoglobulin for human use, animal (1928)

5 monographs for rDNA blood products

- Alteplase for injection (1170)
- Human coagulation factor VIIa (rDNA) concentrated solution (2534)
- Human coagulation factor VIII (rDNA) (1643)
- Human coagulation factor IX (rDNA) concentrated solution (2522)
- Human coagulation factor IX (rDNA) powder for solution for injection (2994)

28 monographs for human plasma-derived products

It includes monographs for:

- Human plasma for fractionation
- Human normal immunoglobulins
- Human specific immunoglobulins
- Human coagulation factors
- Other fractionated products

Case study

Biological Reference Preparations (BRP)

Human plasma-derived products

Human plasma-derived products

Technical guide for the elaboration and use of monographs on human plasma-derived products (available on EDQM website)

Access to Technical guide:
https://www.edqm.eu/en/d/66882?redirect=%2Fen%2Fph-eur-technical-guides

- Authorities responsible for granting marketing authorisations for plasma-derived products
- Official Medicines Control Laboratories (OMCLs)
- Manufacturers of plasma-derived products
- Public and private analytical laboratories working for one of the above, for example
- …

Provide guidance to authors (and contributors) of Ph. Eur. monographs and general chapters on human plasma-derived products

Also provide help to users of Ph. Eur. monographs and general chapters on human plasma-derived products

Human plasma-derived product monographs

Anticoagulants and preservative solutions for human blood (0209)

Containers

- Sterile plastic containers for human blood and blood components (3.3.4)
- Glass container (3.2.1)
- Empty sterile containers of plasticised poly(vinyl chloride) for human blood and blood components (3.3.5)
- Sterile containers of plasticised poly(vinyl chloride) for human blood containing anticoagulant solution (3.3.6)
- Materials for containers for human blood and blood components (3.3.1)

Additional tests not restricted to plasma-derived products

- Sterility (2.6.1)
- Bacterial Endotoxins (2.6.14)
- Monocyte-Activation Test (2.6.30)
- Nucleic acid amplification techniques (2.6.21) :
  - ➢ *Validation of nucleic acid amplification techniques (NAT) for the detection of hepatitis C virus (HCV) RNA in plasma pools : guidelines*
  - ➢ *Validation of nucleic acid amplification techniques (NAT) for quantification of B19 Virus (B19V) DNA in plasma pools: guidelines*

non exhaustive list

Human plasma for fractionation (0853)

Human plasma (pooled and treated for virus inactivation) (1646)

Human normal immunoglobulin for intramuscular administration (0338)

- Human anti-D immunoglobulin (0557)
- Human Hepatitis A immunoglobulin (0769)
- Human varicella immunoglobulin (0724)
- Human rabies immunoglobulin (0723)
- Human rubella immunoglobulin (0617)
- Human tetanus immunoglobulin (0398)
- Human measles immunoglobulin (0397)
- Human Hepatitis B immunoglobulin (0722)

Human normal Immunoglobulin for subcutaneous administration (2788)

Human normal immunoglobulin for intravenous administration (0918)

- Human anti-D immunoglobulin for intravenous administration (1527)
- Human varicella immunoglobulin for intravenous administration (1528)
- Human Hepatitis B immunoglobulin for intravenous administration (1016)

Human Coagulation factors

- Human coagulation factor VII (1224)
- Human coagulation factor VIII (0275)
- Human coagulation factor IX (1223)
- Human coagulation factor XI (1644)

Other fractionated products

- Human albumin solution (0255)
- Human fibrinogen (0024)
- Fibrin sealant kit (0903)
- Human antithrombin III concentrate (0878)
- Human prothrombin complex (0554)
- Human von Willebrand factor (2298)
- Human α-1-proteinase inhibitor (2387)
- Human C1-esterase inhibitor (2818)

General Chapters/methods

- Prekallikrein activator (2.6.15.)
- Test for anticomplementary activity of immunoglobulin (2.6.17.)
- Anti-A and anti-B haemagglutinins (2.6.20.)
- Nucleic acid amplification techniques (2.6.21.)
- Activated coagulation factors (2.6.22.)
- Test for anti-D antibodies in human immunoglobulin (2.6.26.)
- Test for procoagulant activity of immunoglobulin (2.6.42.) *
- Assay of human coagulation factor VIII (2.7.4.)
- Test for Fc function of immunoglobulin (2.7.9.)
- Assay of human coagulation factor VII (2.7.10.)
- Assay of human coagulation factor IX (2.7.11.)
- Assay of heparin in coagulation factors (2.7.12.)
- Assay of human anti-D immunoglobulin (2.7.13.)
- Assay of human antithrombin III (2.7.17.)
- Assay of human coagulation factor II (2.7.18.)
- Assay of human coagulation factor X (2.7.19.)
- Assay of human von Willebrand factor (2.7.21.)
- Assay of human coagulation factor XI (2.7.22.)
- Assay of human plasmin inhibitor (2.7.25.)
- Assay of human protein C (2.7.30.)
- Assay of human protein S (2.7.31.)
- Assay of human alpha-1-proteinase inhibitor (2.7.32.)
- Assay of human C1-esterase inhibitor (2.7.34.)

*Under elaboration

Human plasma-derived product monographs

Source of the active substance: products obtained from plasma complies with 0853

Requirements given in 0853, 0338, 0918, 2788 not repeated – references included

Class-specific Ig monographs

Human plasma for fractionation (0853)

Human plasma (pooled and treated for virus inactivation) (1646)

Human normal immunoglobulin for intramuscular administration (0338)

- Human anti-D immunoglobulin (0557)
- Human Hepatitis A immunoglobulin (0769)
- Human varicella immunoglobulin (0724)
- Human rabies immunoglobulin (0723)
- Human rubella immunoglobulin (0617)
- Human tetanus immunoglobulin (0398)
- Human measles immunoglobulin (0397)
- Human Hepatitis B immunoglobulin (0722)

Human normal Immunoglobulin for subcutaneous administration (2788)

Human normal immunoglobulin for intravenous administration (0918)

- Human anti-D immunoglobulin for intravenous administration (1527)
- Human varicella immunoglobulin for intravenous administration (1528)
- Human Hepatitis B immunoglobulin for intravenous administration (1016)

Human Coagulation factors

- Human coagulation factor VII (1224)
- Human coagulation factor VIII (0275)
- Human coagulation factor IX (1223)
- Human coagulation factor XI (1644)

Other fractionated products

- Human albumin solution (0255)
- Human fibrinogen (0024)
- Fibrin sealant kit (0903)
- Human antithrombin III concentrate (0878)
- Human prothrombin complex (0554)
- Human von Willebrand factor (2298)
- Human α-1-proteinase inhibitor (2387)
- Human C1-esterase inhibitor (2818)

Monographs must be used and applied, taking account

- General Notices
- Applicable general monographs (e.g. Pharmaceutical preparations 2619)
- Applicable dosage form monographs (e.g. Parenteral preparations 0520)
- Relevant overarching human plasma derived monographs (0853, 0338, 0918, 2788)

non exhaustive list

General provisions applicable to human plasma-derived product

DEFINITION

The preparations are **obtained from plasma that complies with the monograph Human plasma for fractionation** (0853).

Allowed substances are included in the Definition section

- e.g. "*The preparation may contain excipients such as stabilisers, heparin and antithrombin*."
- By default, a substance or class of auxiliary substances (excipients, stabilisers, other ingredients, etc.) not mentioned in the Definition section, should not be used. Such substances are then included in the Production section.

General provisions applicable to human plasma-derived product – *cont'd*

PRODUCTION

Production section primarily addressed to manufacturers: describes essential features of the manufacturing process

No antimicrobial preservative or antibiotic is added

- unless otherwise mentioned in DEFINITION
- e.g. in the monograph Human normal immunoglobulin for intramuscular administration (0338)

  "*Multidose preparations contain an antimicrobial preservative*."

General provisions applicable to human plasma-derived product – *cont'd*

CHARACTERS

Not legally binding

Define the physical status & appearance of the product

e.g. in Human normal immunoglobulin for intramuscular administration (0338)

"– **liquid preparation**: clear or slightly opalescent, colourless or pale-yellow or light-brown liquid; during storage it may show formation of slight turbidity or a small amount of visible particulate matter;

– **freeze-dried preparation**: white or slightly yellow powder or friable mass, hygroscopic."

IDENTIFICATION

Immunoelectrophoresis or compliance with the limit of the assay

General provisions applicable to human plasma-derived product – *cont'd*

TESTS

General tests to be performed

- Solubility "*dissolves completely with gentle swirling within 10 min*"
- pH "*6.8 to 7.4*"
- Osmolality: lower limit defined "*minimum 240 mosmol/kg*"
- Total protein: specification limit depending on product (not always provided in monograph)
- Water: specification limit as approved by competent authority
- Sterility: complies with the test for sterility (2.6.1)
- Pyrogenicity: *currently under revision, see Webinar module 4
- Other specific tests, if applicable, are prescribed

Product must comply with these requirements throughout its shelf-life

General provisions applicable to human plasma-derived product – *cont'd*

ASSAY/POTENCY

Included in each specific monograph

- Activity of a plasma-derived product is expressed either in
  - ➢ International Units per container or per volume
  - ➢ Units of mass per volume (g/mL, or g per container)
- Potency assay may be described in a separate chapter which is then referred to in the specific monograph

  e.g. immunoassays for immunoglobulin preparations (described in 2.7.1 and 2.7.13) or chromogenic assays developed for coagulations factor determinations (e.g. 2.7.4)
- Statistical methods for calculation of results and terms "*estimated potency*" and "*stated potency*" in general chapter *5.3. Statistical analysis of results of biological assays and tests*

General provisions applicable to human plasma-derived product – *cont'd*

STORAGE

Not legally binding

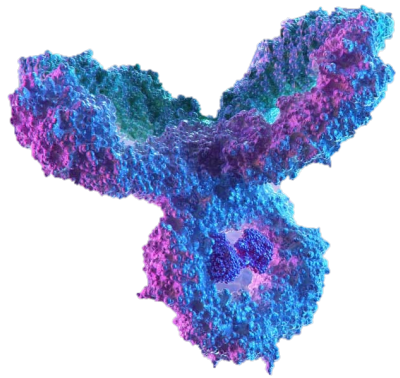
Products comply throughout the period of validity

- storage conditions
- e.g. light protection, type of glass container, vacuum or inert gas

LABELLING

- Status of the labelling defined in the General Notices: "The statements in the Labelling section are not therefore comprehensive. In addition, for the purposes of the Ph. Eur., only those statements that are necessary to demonstrate compliance or non-compliance with the monograph are mandatory. Any other labelling statements are included as recommendations."
- e.g. in Human antithrombin III concentrate (0878): "*The label states:*
  - *the number of International Units of antithrombin III in the container;*
  - *the name and volume of the liquid to be used for reconstitution;*
  - *where applicable, the amount of albumin added as a stabiliser*."

Human immunoglobulins



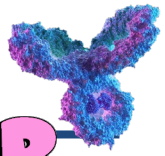
SLIDO

- **A Human Rubella immunoglobulin :**

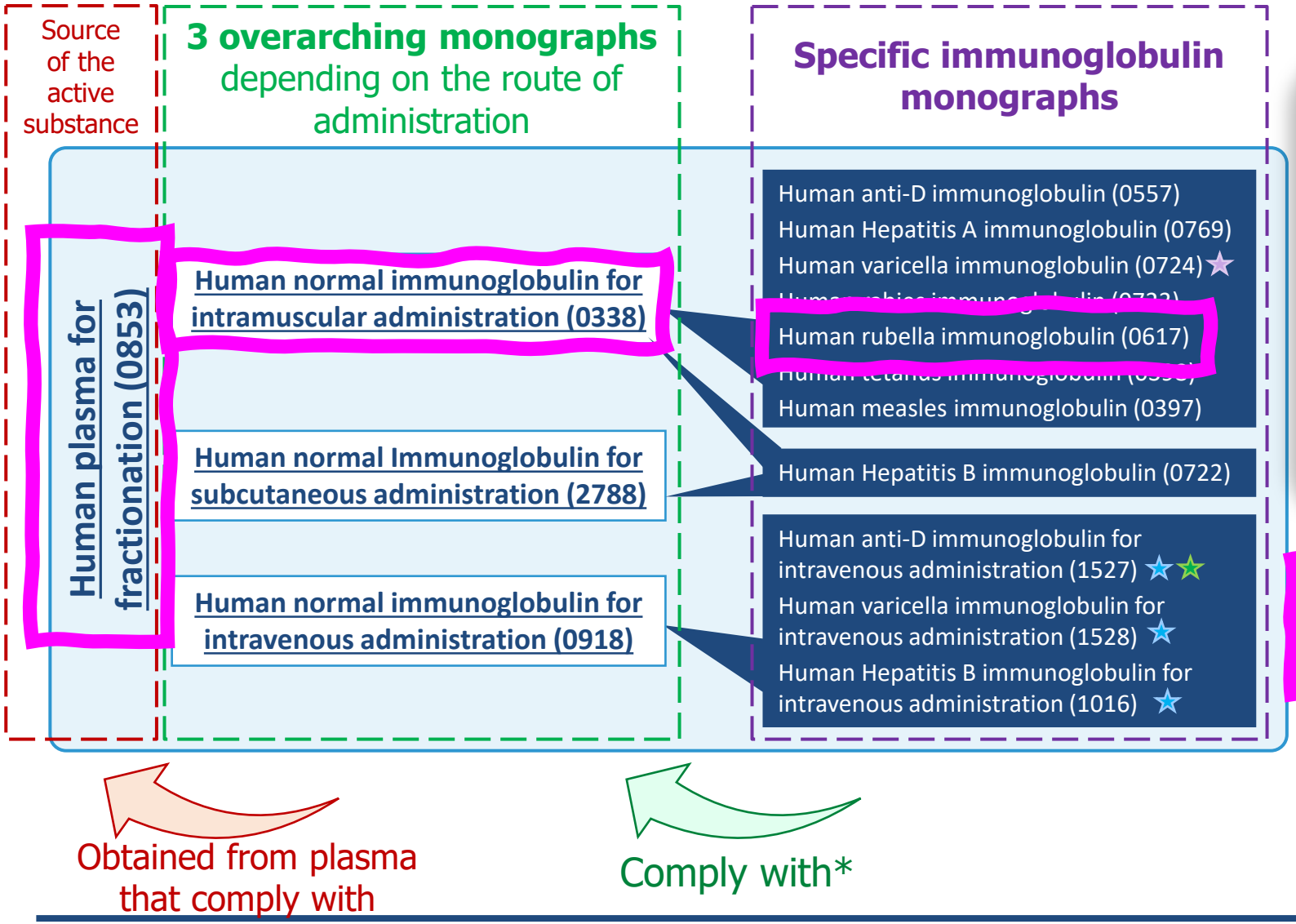
✓Complies with Human measles immunoglobulin (0397)
✓Complies with Human normal immunoglobulin for intramuscular administration (0338)
✓Complies with Human rubella immunoglobulin (0617)
✓Complies with Human albumin solution (0255)
✓is obtained from plasma that complies with Human plasma for fractionation (0853)
✓I don’t know

Correct answers in green!

Human immunoglobulins



QUIZ ANSWER



A Human Rubella immunoglobulin:

- complies with *Human rubella immunoglobulin* (*0617*)
- complies with *Human normal immunoglobulin for intramuscular administration* (*0338*), except for the minimum number of donors and the minimum total protein content
- is obtained from plasma that complies with *Human plasma for fractionation* (*0853*)

Also complies with applicable general monograph and dosage form monograph

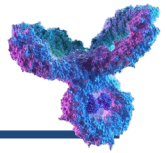
*Except for:

- the minimum number of donors
- the minimum total protein content

And in some cases:

- the limit for osmolality (1527, 1528, 1016)☆
- the limit for prekallikrein activator (1527)★
- where authorised, the test for antibody to hepatitis B surface antigen (0724)☆

Immunoglobulins: PRODUCTION section



Specific provisions for manufacturing process – Patient Safety

"The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses, it shall have been shown that any residues present in the final product have no adverse effects on the patients treated with the immunoglobulin."

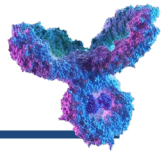
Removal/inactivation of **infectious agents**

No adverse effects of **residual inactivation agents** (when used)

No exhaustive list of methods or specifications

Suitability of production process established **by competent authorities** i.e. by examination of data and/or inspection

Immunoglobulins: PRODUCTION section – *cont'd*



Specific provisions for manufacturing process – Patient Safety

Removal of **procoagulant agents**

"The method of preparation also includes a step or steps that have been shown to remove thrombosis-generating agents. Emphasis is given to the identification of activated coagulation factors and their zymogens and process steps that may cause their activation. Consideration is also to be given to other procoagulant agents that could be introduced by the manufacturing process."

The product "does not exhibit thrombogenic (procoagulant) activity."

Intravenous and subcutaneous immunoglobulin products (0918, 2788)

"does not exhibit"= acceptable safety level defined by the competent authority

Elaboration of a new general chapter *Test for procoagulant activity of immunoglobulin (2.6.42)*
→ description of test procedures used to determine procoagulant activity

Immunoglobulins: protein composition

Protein composition

- test procedure updated in supplement 11.4 (implementation date: 1 April 2024)
- take into account current practices:
  - **removal of the cellulose acetate gel electrophoresis** procedure, which is phasing out
  - description of **suitable agarose gel** unchanged
  - addition of a reference to **capillary zone electrophoresis procedure as an alternative procedure**, in order to address the increasing use of this state-of-the art technology in the field

Definition of 'alternative analytical procedures' in General Notices:

"The tests and assays described are the official analytical procedures upon which the standards of the Ph. Eur. are based. With the agreement of the competent authority, alternative analytical procedures may be used for control purposes, provided that they enable an unequivocal decision to be made as to whether compliance with the standards of the monographs would be achieved if the official procedures were used. In the event of doubt or dispute, the analytical procedures of the Ph. Eur. are alone authoritative."

NEW CHAPTER 5.27 "COMPARABILITY OF ALTERNATIVE ANALYTICAL PROCEDURES" Supplement 11.5 (publication in January 2024)

rDNA blood products

rDNA Blood products monographs

Overarching general monograph for rDNA products:
Recombinant DNA technology, products of (0784)

5 monographs for rDNA blood products

- Alteplase for injection (1170)
- Human coagulation factor VIIa (rDNA) concentrated solution (2534)
- Human coagulation factor VIII (rDNA) (1643)
- Human coagulation factor IX (rDNA) concentrated solution (2522)
- Human coagulation factor IX (rDNA) powder for solution for injection (2994)

SLIDO

- **As there is no monograph for recombinant human albumin, could we use the monograph Human albumin solution (0255) as standard of reference for a recombinant human albumin?**

✓Yes
✓No
✓I don’t know

Correct answer in green!

QUIZ ANSWER

There is a monograph for human albumin solution (0255).
There is no monograph for recombinant human albumin.

Could we use the monograph human albumin solution (0255) as standard of reference for a recombinant human albumin?

No, recombinant human albumin is not in the scope of human albumin solution (0255):

"Sterile liquid preparation of a **plasma protein fraction** containing human albumin. It is obtained from plasma that complies with the monograph Human plasma for fractionation (0853)."

Overarching general monograph for rDNA products:
Recombinant DNA technology, products of (0784)

Alteplase

Alteplase (1170)

Covers both
active substance and
medicinal product

Alteplase concentrated solution (3197)

active substance

Alteplase for injection (1170)

medicinal product

Human coagulation factor VIII (rDNA)

Human coagulation factor VIII (rDNA) (1643)

Covers both **active substance** and **medicinal product**

Applies to **full-length** and **B-domain-modified** rFVIII

Human coagulation factor VIII (rDNA), **concentrated solution** (3105)

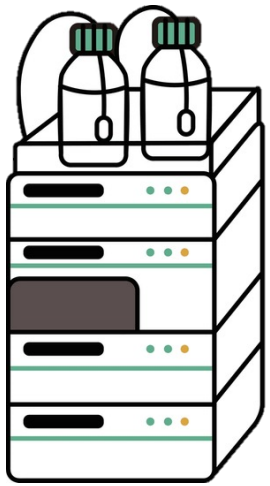
Human coagulation factor VIII (rDNA), *B-domain modified*, **concentrated solution** (3107)

Human coagulation factor VIII (rDNA), **powder for injection (3106)**

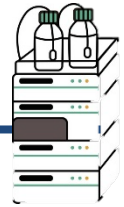
Human coagulation factor VIII (rDNA), *B-domain modified*, **powder for injection** (3108)

Human albumin solution 0255: molecular-size distribution

Case study



Molecular-size distribution revision



Human albumin monograph 0255 was revised to reflect analytical improvement by:

- adjusting the sample preparation, chromatographic parameters and test conditions
- deleting the use of sodium azide (classified as a CMR) as preservative in the mobile phase described in the monograph
- including tools for peak identification and assessment of system suitability
- amending the acceptance criterion

Supplement 10.6 – implementation date 1 January 2022

Human albumin solution: molecular-size distribution

"**Molecular-size distribution**. Size-exclusion chromatography (*2.2.30*): use the normalisation procedure."

- Reference to 2.2.30, which itself reference to general chapter Chromatographic separation techniques (2.2.46) → **both chapters become mandatory** (see General notices)
- **Criteria for assessing the suitability** of the system described in 2.2.46
- Extent to which **adjustments of chromatographic conditions** can be made described in 2.2.46.

Normalisation procedure definition in 2.2.46
"the percentage content of a component of the substance to be examined is calculated by determining the area of the corresponding peak as a percentage of the total area of all the peaks, excluding those due to solvents or reagents, those arising from the mobile phase or the sample matrix, and those at or below the reporting threshold."

Human albumin solution: molecular-size distribution

"Reference solution (a). Dilute *human albumin (molecular size) BRP* with the mobile phase […]
Column:
– *size*: *l* = 0.30 m, Ø = 7.8 mm;
– *stationary phase*: *hydrophilic silica gel for chromatography R* (5 µm) with a pore size of 25-30 nm and of a grade suitable for fractionation of globular proteins in the relative molecular mass range of 10 000 to 500 000."

- Intended for identification of the peaks due to the monomer and the dimer as described in the monograph
- System sensitivity
- Reporting threshold

EUROPEAN PHARMACOPOEIA 11.4

FR Document en Français | PDF | Knowledge Database

General Notices apply to all monog
See the information section on ger

HUMAN ALBU

Albumini hu

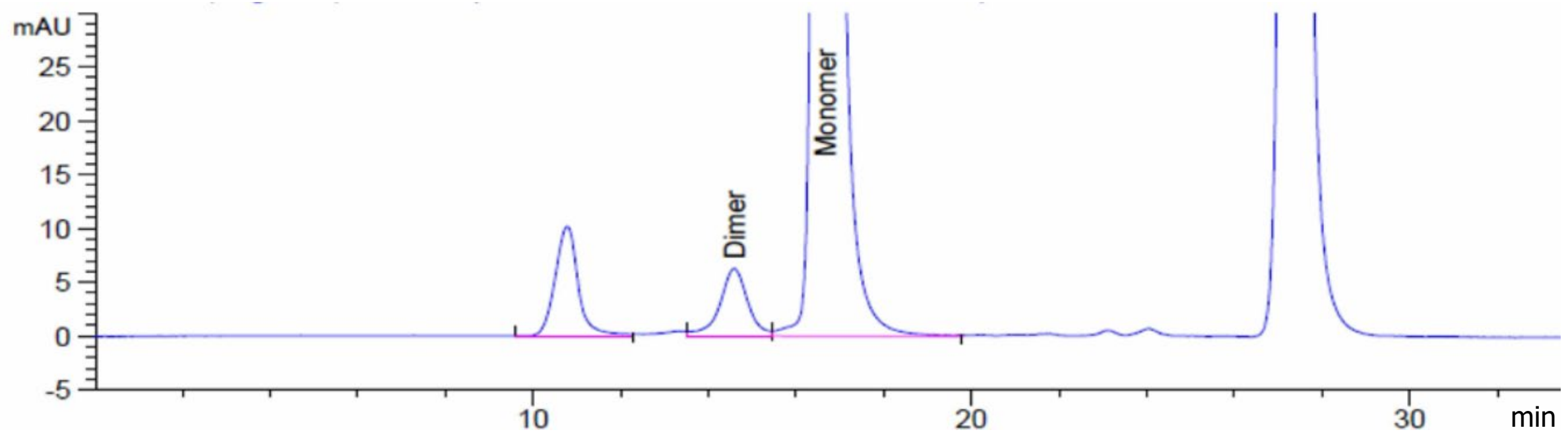
	Available since	Cat. No.	Name	Batch No.	Unit Quantity	Price	SDS Product Code
Reference standards		H0900000	Human albumin for electrophoresis BRP	1	50 mg	79 EUR	201700506
		Y0001913	Human albumin (molecular size) BRP	1	50 mg	90 EUR	201700506

Practical Information	**Test(s)**	**Brand Name/Information**
	Molecular size distribution	TSK G2000 SW, TSK G3000 SW (l = 0.6 m, Ø = 7.5 mm) and TSK G3000 SWXL (l = 0.3 m, Ø = 7.8 mm) are suitable. From 10.6: TSKgel G3000 SWXL or BioSep-SEC-s3000 are suitable.

Human albumin solution: molecular-size distribution

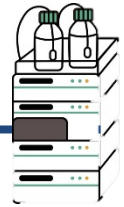
"*Identification of peaks*: use the chromatogram supplied with *human albumin (molecular size) BRP* and the chromatogram obtained with reference solution (a) to identify the peaks due to the monomer and the dimer. *Relative retention* with reference to the monomer (retention time = about 17 min): dimer = about 0.88."

2.2.46: "When retention times and relative retentions are provided in monographs, they are for information purposes only, unless otherwise stated in the monograph"



Chromatogram extracted from the Information leaflet of Human albumin (molecular size) BRP batch 1

Human albumin solution: molecular-size distribution



"System suitability:
– the chromatogram obtained with reference solution (a) is qualitatively similar to the chromatogram supplied with human albumin (molecular size) BRP;
– signal-to-noise ratio: minimum 10 for the peak due to the monomer in the chromatogram obtained with reference solution (b);
– resolution: minimum 1.5 between the peaks due to the dimer and the monomer in the chromatogram obtained with reference solution (a)."

System suitability criteria of the monograph and of general chapter 2.2.46 must be met.

2.2.46: "Compliance with the system suitability criteria is required throughout the chromatographic procedure. No sample analysis is acceptable unless the suitability of the system has been demonstrated."

Human albumin solution: molecular-size distribution

"*Limit*:
– *peak due to the polymers and aggregates eluted with the void volume*: maximum 10 per cent;
– *reporting threshold*: 0.5 per cent (peak due to the monomer in the chromatogram obtained with reference solution (b))."

Up to the user to determine the void volume in order to identify the polymers and aggregates peak
e.g. The void volume may be experimentally determined using blue dextran.

Void volume defined in 2.2.30
"Molecules apparently larger than the maximum pore size of the packing material migrate along the column only through the spaces between the particles of the packing material without being retained and elute at the retention volume of an unretained compound V0 (also known as exclusion volume or void volume)".

Blood products monographs in the Ph. Eur.

After vaccine presentation

Agenda

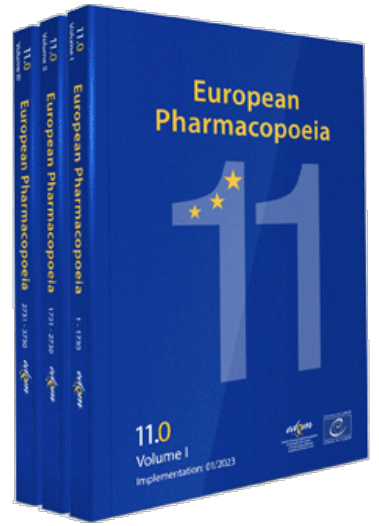
Blood products

Solène Le Maux, EDQM, Council of Europe

Vaccines for human use

Gwenaël Ciréfice, EDQM, Council of Europe

Vaccines for human use in the Ph. Eur.

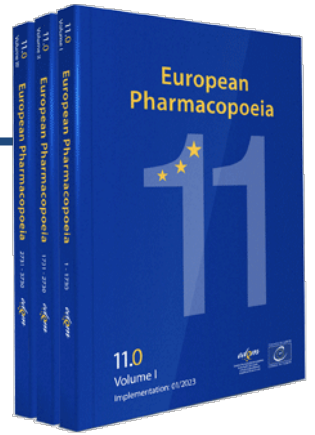


Outline

- Ph. Eur. standards for vaccines for human use
- General chapters supporting vaccine monographs
- General monograph *Vaccines for human use* (0153)
- Individual vaccine monographs
- Adjuvants
- Reduction of animal testing (3Rs)

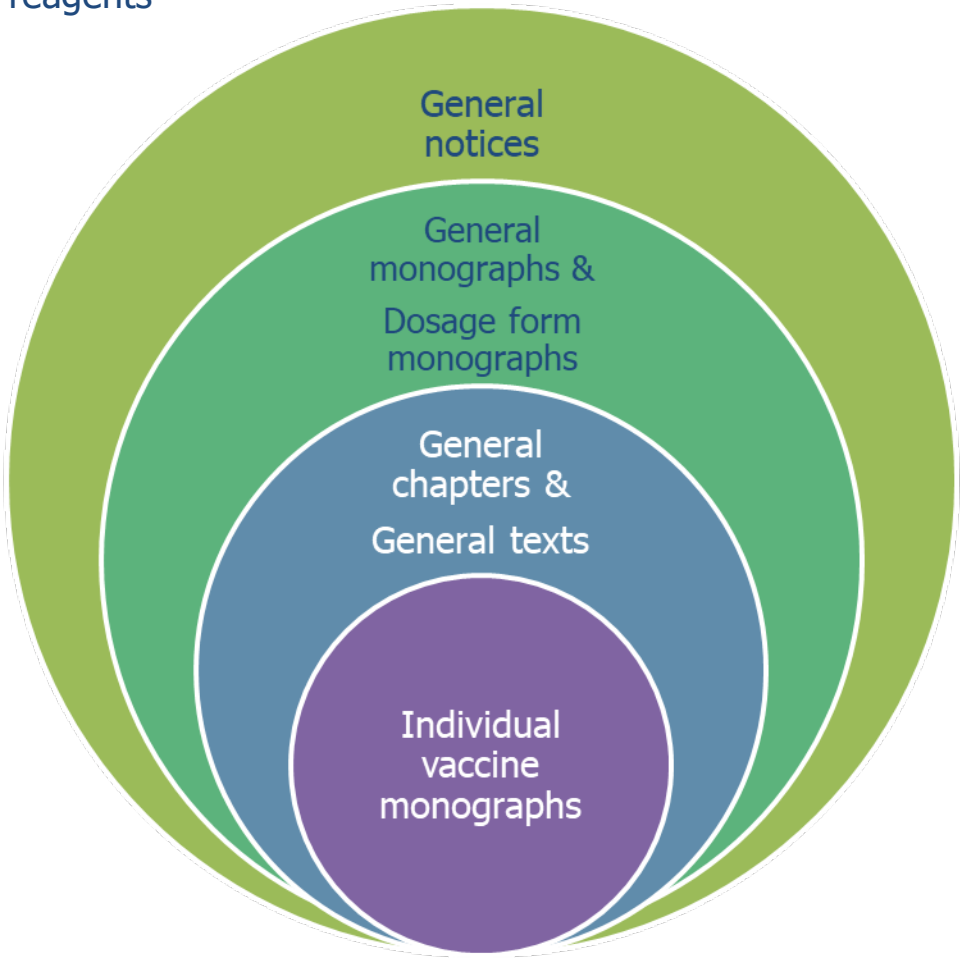
- Coming next

Ph. Eur. standards for vaccines



- European Pharmacopoeia 11.4
  - European Pharmacopoeia 11.4
    - 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 starti...
    - 11 Sutures
    - 12 Herbal drugs and herbal drug preparations
    - 13 Homoeopathic preparations
    - 14 Monographs A-C
    - 15 Monographs D-K
    - 16 Monographs L-P
    - 17 Monographs Q-Z
    - 18 Index

Reference standards & reagents

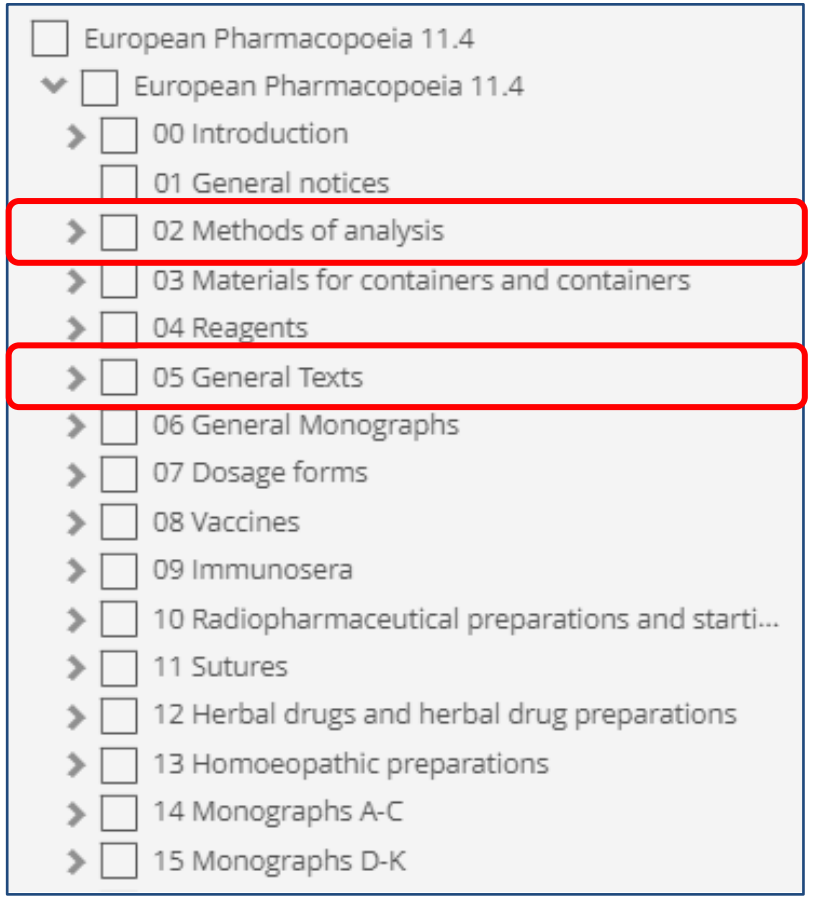


Technical guide for the elaboration of monographs on vaccines
(not part of Ph. Eur.)

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*

New! MAT for vaccines containing inherently pyrogenic components *2.6.40*
(published in Suppl. 11.5 of Jan 2024)

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

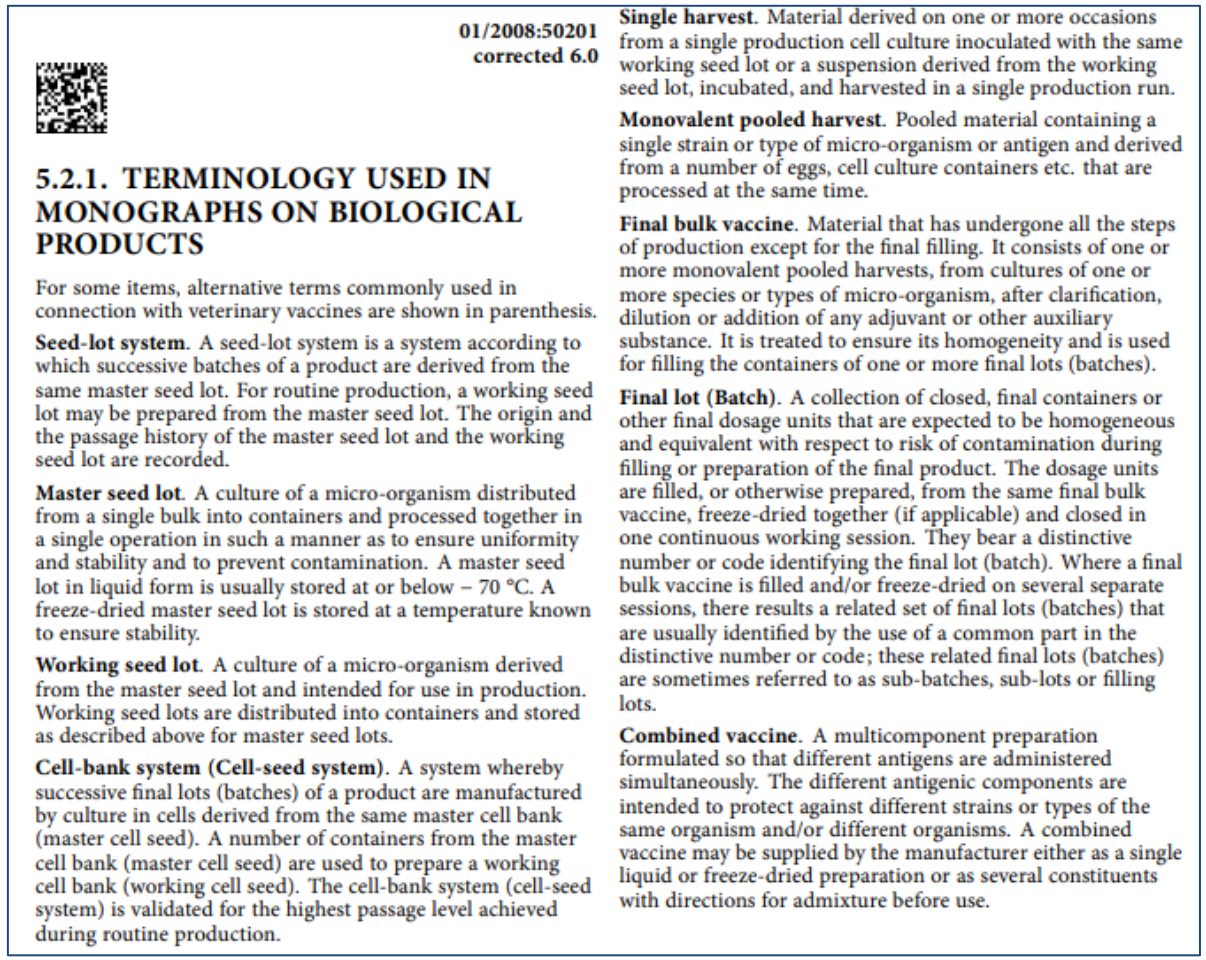
Efficacy of antimicrobial preservation *5.1.3*

(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



01/2008:50201
corrected 6.0

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.

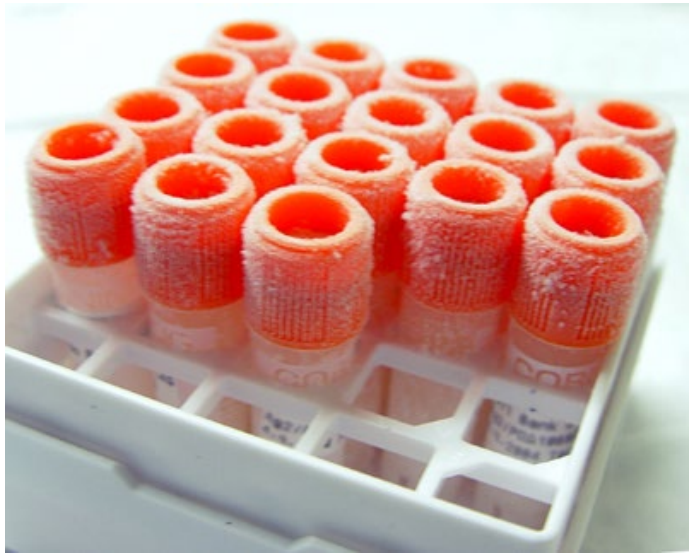
Combined vaccine. A multicomponent preparation formulated so that different antigens are administered simultaneously. The different antigenic components are intended to protect against different strains or types of the same organism and/or different organisms. A combined vaccine may be supplied by the 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)

(1) The diploid character is established for each WCB but using cells at or beyond the maximum population doubling level used for production.

(2) If the cells are susceptible to infection with *Mycobacterium tuberculosis* or other species.

(3) If insect cells or raw materials of plant origin are used.

(4) Testing is carried out for the MCB, but using cells at or beyond the maximum population doubling level used for production.

(5) Testing is carried out for each WCB, but using cells at or beyond the maximum population doubling level used for production.

(6) Specific tests for possible contaminants (e.g. viruses) defined according to a risk assessment based on the origin of the cells and on the potential extraneous agents inadvertently introduced during production processes or through the use of animal or plant derived raw materials. The appropriate testing stages should be selected based on the risk assessment.

Testing methods for cell cultures:

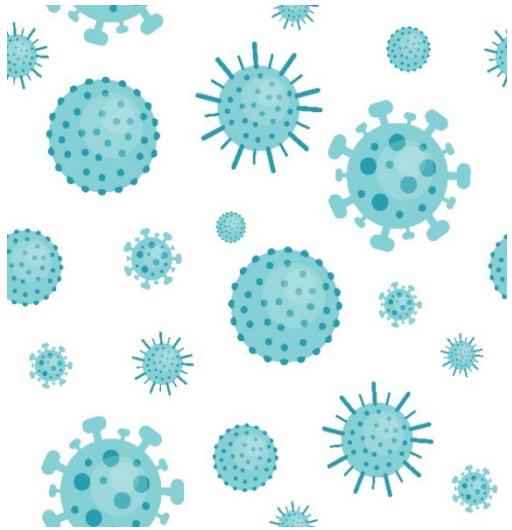
- Identity and purity
- Extraneous agents
- Tumorigenicity

- 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*")
- A strategy is given in Table 5.2.3-1. Alternative strategies could focus on more extensive testing of the MCB or WCB

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): to be justified if maintained
  - Molecular methods (for specific extraneous agent or broad virus detection)

- Testing strategy (package of suitable tests) is to be built based on a risk assessment
  - Includes a package of tests able to detect different families of extraneous agents that may infect the source of virus strains
  - Takes into account the capacity of the process to remove / inactivate viruses

2.6.16 Tests for extraneous agents in viral vaccines for human use

GC

- The list of tests (in Table 2.6.16-1) is to be adapted depending on the extraneous agents that have the potential to contaminate the product

Table 2.6.16.-1. – *Relevant tests for extraneous agents at various production stages*

Tests	Virus seed lots	Virus harvests	Production substrates	
			Control cells	Control eggs
Bacterial and fungal contamination	+	+	-	-
Mycoplasmas	+	+	-	-
Spiroplasmas(1)	+	-	-	-
Mycobacteria	+	+(9)	-	-
Test in suckling mice(2)	+	-	-	-
Avian viruses(3)	+	+	-	-
Test for extraneous agents in cell cultures(4)	+	+(9)	+	+
Insect viruses(5)	+	+	-	-
Examination of control cells	-	-	+	-
Haemadsorbing viruses	+	+	+	-
Test for haemagglutinating agents on control eggs	-	-	-	+
Avian leucosis viruses(6)	+	-	+	+
Test for specific viruses by NAT(7)	+	+	-	-
Test for viruses using broad molecular methods(8)	+	+	-	-

(1) If insect cells or raw materials of plant origin are used.

(2) If the risk assessment indicates that this test provides a risk mitigation taking into account the overall testing package.

(3) If the virus is propagated in avian or primary avian tissues. If the risk assessment indicates that this test provides a risk mitigation taking into account the overall testing package.

(4) Test performed in suitable permissive cell cultures based on a risk assessment.

(5) If the virus is propagated in insect cells.

(6) If the virus is propagated in primary avian tissues (including eggs).

(7) Based on a risk assessment.

(8) These methods may be used either as an alternative to *in vivo* tests and specific NAT or in addition/as an alternative to *in vitro* culture tests based on the risk assessment and in agreement with the competent authority.

(9) Not applicable for inactivated viral vaccines.

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.

SLIDO

- **Can Next Generation Sequencing (NGS) be used for broad detection of viruses in virus seed lots / virus harvests, as per Ph. Eur. chapter 2.6.16?**

✓Yes
✓No
✓I don’t know

Correct answer in green!

General chapters supporting vaccine monographs

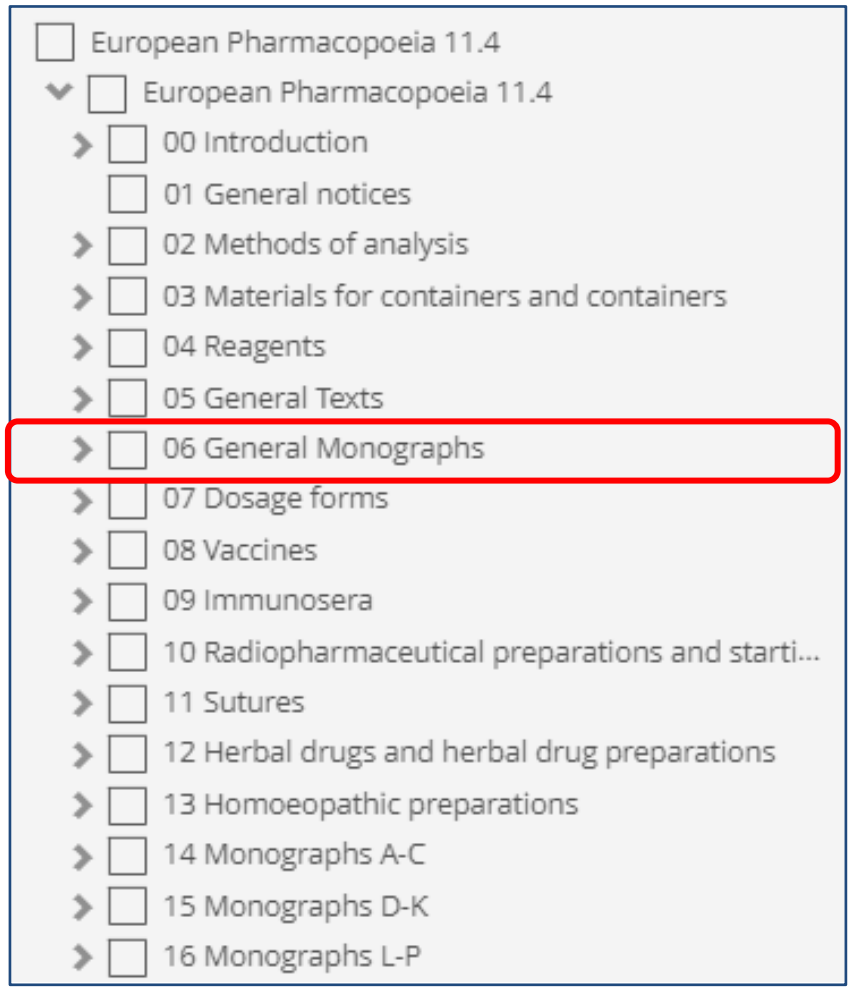
GC

Can Next Generation Sequencing (NGS) be used for broad detection of viruses in virus seed lots / virus harvests, as per Ph. Eur. chapter 2.6.16?

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

General monograph *Vaccines for human use* (0153)

GM



07/2018:0153
corrected 10.0

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

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

- *Note: other general monograph(s) may apply (e.g. Pharmaceutical preparations (2619), Products with risk of transmitting agents of animal spongiform encephalopathies (1483), products of recombinant DNA technology (0784)) – see Module 1*

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

SLIDO

- **According to the General Monograph *Vaccines for human use* (0153):**

✓Carrier proteins used in conjugated vaccines comply with 5.2.11

✓Inclusion of a preservative in single-dose preparations is acceptable

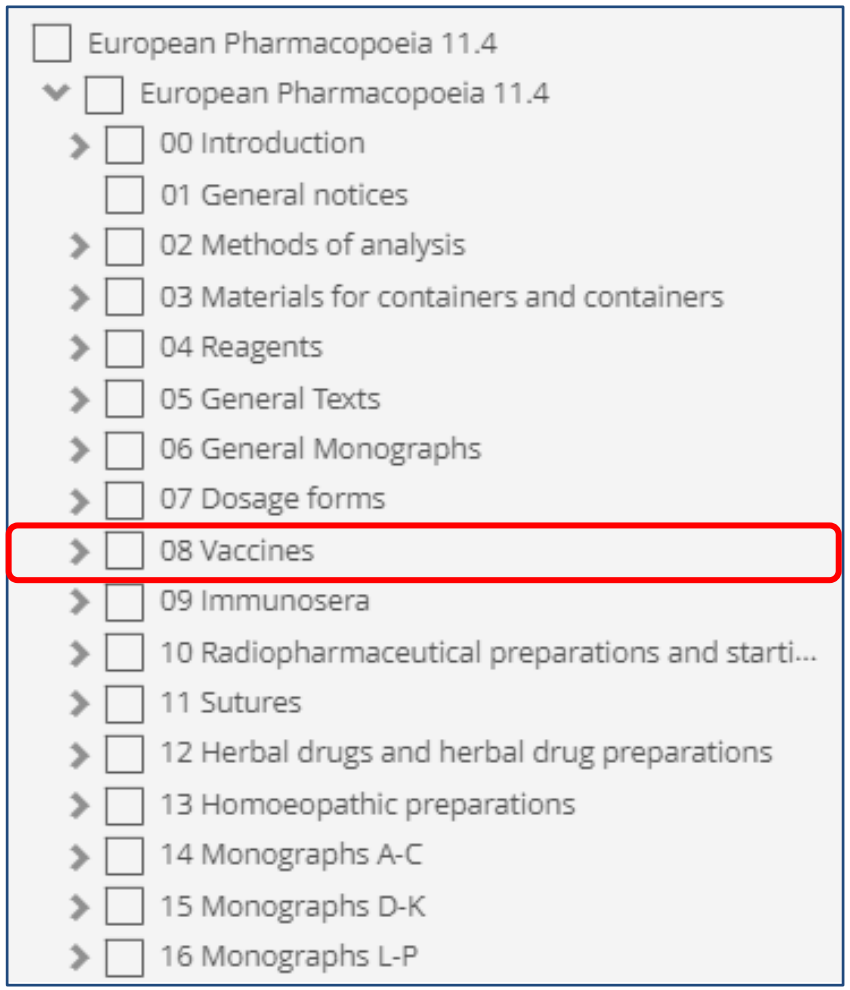
✓Tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain / suffering

✓I don’t know

Correct answers in green!

Individual vaccine monographs

IM



01/2012:2417

ROTAVIRUS VACCINE (LIVE, ORAL)

Vaccinum rotaviri vivum perorale

DEFINITION

Rotavirus vacc
suitable virus
and presented
The vaccine is
preparation to
stated on the l
ready for adm
presence of a

PRODUCTIO

GENERAL PR

The vaccine st
been shown to
the vaccine of
vaccine is form
fluids. Where
of the solvent
The productio
and a cell-ban
authorised, th
no more passa
prepare the va
with respect to
If purification
process-relate
host-cell prote
serum, trypsin
consistency of

REFERENCE

A suitable refe
batches of vac
established for
The difference
rotavirus vacc

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.

VIRUS PROPAGATION, SINGLE HARVEST, MONOVALENT

07/2019:0158
corrected 10.0

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.

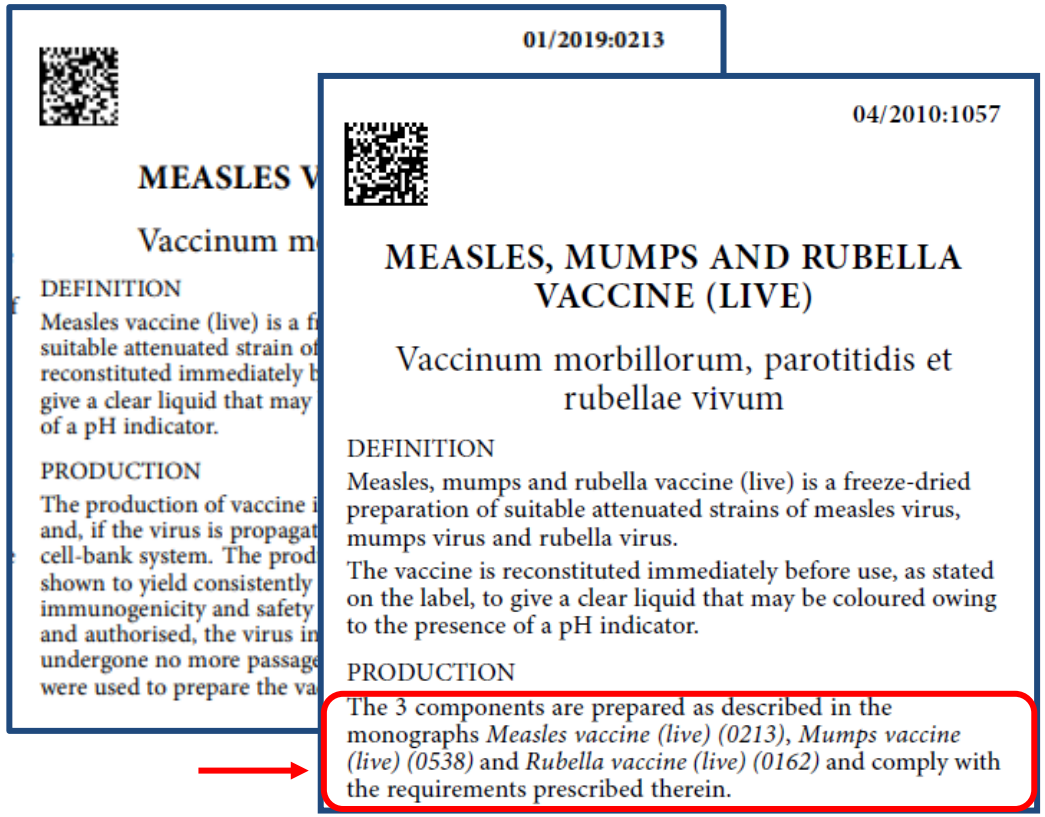
Individual vaccine monographs

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.*


01/2019:0213

MEASLES V

Vaccinum m

DEFINITION

Measles vaccine (live) is a f
suitable attenuated strain of
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 passage
were used to prepare the va

04/2010:1057

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.

Individual Monograph
Rotavirus vaccine (live, oral)

01/2012:2417

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

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…

Individual Monograph
Rotavirus vaccine

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 must comply throughout its shelf life.

Individual Monograph
Rotavirus vaccine (live, oral)

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.

Individual Monograph Influenza vaccine (surface antigen, inactivated, prepared in cell cultures)

TESTS

Residual infectious virus. Carry out an amplification test for residual infectious influenza virus by inoculating not less than 0.2 mL of the vaccine into cell cultures of the same type as used for production of the vaccine; incubate for not less than 4 days at 37 °C. Inoculate not less than 0.2 mL of the cell culture harvested medium into a new semiconfluent cell culture and incubate as before. At the end of the incubation period, examine for live virus by a haemagglutination test. If haemagglutination is found for any of the fluids, carry out for that fluid a further passage on cell cultures and test for haemagglutination; no haemagglutination occurs.

Antimicrobial preservative. Where applicable, determine the amount of antimicrobial preservative by a suitable chemical method. The content is not less than the minimum amount shown to be effective and is not greater than 115 per cent of the quantity stated on the label.

Free formaldehyde (*2.4.18*): maximum 0.2 g/L, where applicable.

Bovine serum albumin: maximum 50 ng per human dose, determined by a suitable immunochemical method (*2.7.1*).

Total protein: maximum 40 µg of protein other than haemagglutinin per virus strain per human dose, unless otherwise justified and authorised.

Sterility (*2.6.1*). It complies with the test.

Bacterial endotoxins (*2.6.14*): less than 25 IU per human dose.

Individual vaccine monographs

IM

ASSAY or LIVE VIRUS/BACTERIA CONCENTRATION or PS CONTENT

- Potency test (in specific 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 Individual Monograph Rotavirus vaccine (live, oral)

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

- *As per the General Notices (see Module 1).*
- 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 (live, oral)

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.

SLIDO

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

✓Specific vaccine monograph(s)
✓General Notices
✓General monograph vaccines for human use
✓2.7.15 Assay of hepatitis B vaccine (rDNA)
✓I don’t know

Correct answers in green!

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)
    - 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

Access to Technical guide: here

Alternatives to Animal Testing at EDQM/Ph. Eur.

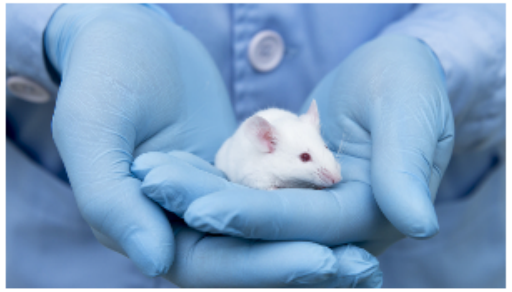
COUNCIL OF EUROPE

Home | About us | European Pharmacopoeia | Reference Standards | Certification of Suitability | OMCL Network | Transfusion & Transplantation | Patient & Consumer Health Protection

Home > European Pharmacopoeia > Focus > Alternatives to Animal Testing

Alternatives to Animal Testing

- The Council of Europe on the protection of animal rights
- Categories of medicines concerned by animal testing for Quality Control purposes
- The contributors to the introduction of the 3Rs in the European Pharmacopoeia
- Achievements of the Ph.Eur. Commission for 3Rs
- Achievements of the Biological Standardisation Programme for 3Rs



The Council of Europe on the protection of animal rights

The protection of animal rights and in particular those used for experimentation has long been a subject of interest for the Council of Europe. The first milestone was achieved in 1986, when the European Convention (ETS 123) for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes was open for signature. This Convention is designed to reduce both the number of experiments and the number of animals used for such purposes and it encourages not to experiment on animals except where there is no alternative and it promtoes research into alternative methods.

This Council of Europe Convention paved the way for the EU's Directive 86/609/EEC, adopted in 1986, as the provisions in it are based on the Convention. In September 2010, the EU adopted a new Directive 2010/63/EU on the same subject replacing Directive 86/609/EEC, which came into effect in 2013.

The EDQM in particular is actively involved in the application of the 3Rs principles in its areas of activity:

- the elaboration of the European Pharmacopoeia itself
- the Biological Standardisation Programme (BSP)
- the Official Medicines Control Laboratory (OMCL) network – in particular, the networks for Official Control Authority Batch Release (OCABR) for human and veterinary biologicals.

ADDITIONAL INFORMATION

- Article: Replacement, Reduction, Refinement - Animal welfare progress in European Pharmacopoeia monographs: activities of the European Pharmacopoeia Commission from 2007 to 2017 (May 2018)
- Vaccines for veterinary use (Supplement 7.7): what has changed and why (Pharmeuropa, February 2013)
- The Directive 2010/63/EU of the European Parliament and of the Council on the Protection of animals used for scientific purposes will take full effect from 1 January 2013
- Report published by the Veterinary Medicines Directorate (UK): Animal usage in quality control tests for the batch release of Immunological Veterinary Medicinal Products (IVMPs) via the UK from 2007 to 2012.
- Article: C. Milne & KH Buchheit. EDQM's 3R activities in the field of quality control of vaccines. ALTEX proceedings, 1/12:65-69 (2012)
- Article: E. Charton: Alternatives to Animal Testing: New Approaches in the Development and Control of Biologicals.
- Article: P. Castle. Replacement, Reduction, Refinement (3Rs), Animal welfare progress in European Pharmacopoeia monographs. Pharmeuropa 2007;19.3;430-441.
- Article: E. Charton and P. Castle. Reduction, Replacement and Refinement of animal tests in the European Pharmacopoeia: recent developments for monographs on biological substances and preparations. Pharmeuropa 2001;13.1;94-96.
- Article: P. Castle: The European

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 fosters and enables 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 5.2.14 on concept of "substitution" of animal tests for QC of vaccines

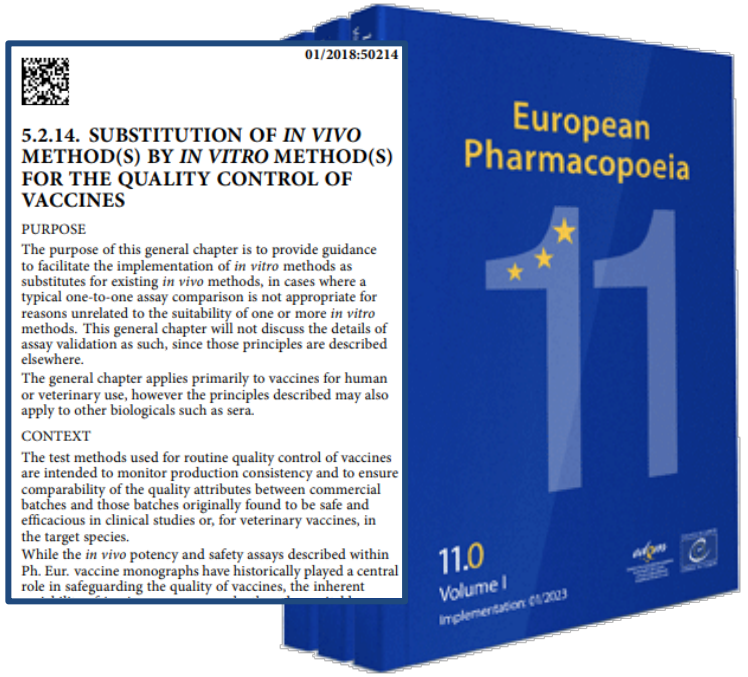
5.2.14 Substitution of in vivo methods for the QC of vaccines

- The introduction of *in vitro* methods to replace *in vivo* methods often prevented due to the characteristics of *in vivo* methods (e.g. variability, validation status of *in vivo* methods, product attributes assessed differently)

- Demonstration of equivalence may not only be problematic, but also of limited relevance

→ General chapter 5.2.14

→ Chapter elaborated to facilitate the transition to *in vitro* methods



5.2.14 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 relevance and performance of the *in vitro* method be demonstrated without such head-to-head comparison: concept of "substitution" as an alternative approach for replacement

- Focus on the scientific rationale behind the *in vitro* methods and the validation package

Some major milestones for 3Rs included in Ph. Eur.

Updates to toxicity testing requirements

Based on review and rationalisation of strategies, accumulated info and some BSP studies

	Development	Bulk	Final lot	Notes
Diphtheria vaccines	Test for specific activity in GP **Removed**	Absence of toxin Irreversibility of toxoid (*in vitro*)	-	**Applicable as of 01/07/22** *No more in vivo test for toxicity*
Tetanus vaccines (human and vet)	Test for specific activity in GP **Removed**	Absence of toxin in GP Irreversibility of toxoid **Removed**	-	**Applicable as of 01/01/21** Test for human and vet aligned. *In vivo still present but reduced*
Acellular Pertussis Vaccines	-	Residual toxin *in vivo* – **replaced** with *in vitro* Irreversibility of toxoid **Removed**	Residual toxin **Removed**	**Applicable as of 01/01/20** *No more in vivo test for toxicity* BSP114 study Isbruker et al. *Pharmeur Bio Sci Notes 2016:97-114*
Abnormal Toxicity Test (ATT)	Deletion for regular release tests in > 80 monographs in 1998 **Complete deletion** based on lack of scientific relevance			**Applicable as of 01/01/19** *No more ATT in Ph. Eur.*

GP: Guinea Pig

SLIDO

- **Is it possible to use a 3R alternative method that is not described in the Ph. Eur.?**

✓Yes
✓No
✓I don’t know

Correct answer in green!

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.

NEW! MAT for inherently-pyrogenic vaccines

- New Chapter **2.6.40**, published in January 2024 (Supplement 11.5), implementation date: 1 July 2024
- To cover the use of MAT to monitor the consistent pyrogenicity of a vaccine where pyrogens are an integral part of the product (→ *use as consistency test rather than safety test*)
- Aim: Facilitate the implementation of MAT method 2 (*Reference lot comparison test*) for inherently pyrogenic vaccines
- Intended to complement the information given in MAT chapter 2.6.30
- Until now, using chapter 2.6.30, users had to read between the lines to understand how to apply the test in this context

07/2024:20640

2.6.40. MONOCYTE-ACTIVATION TEST FOR VACCINES CONTAINING INHERENTLY PYROGENIC COMPONENTS

1. INTRODUCTION

This general chapter describes the use of the monocyte-activation test (MAT) to test vaccines containing inherently pyrogenic components (e.g. outer membrane vesicles, lipidated proteins).

The method described below can be used to monitor the consistency of pyrogen levels when the pyrogens in question are an integral part of the vaccine. The relevance of the MAT for this purpose is based on a risk assessment that takes into consideration the nature of the pyrogenic components, the magnitude of the pyrogenic activity and the control strategy applicable for these pyrogens (where appropriate, see the additional risk assessment factors outlined in general chapter *5.1.10*, section 3).

This general chapter is to be used in conjunction with general chapter *2.6.30. Monocyte-activation test*.

2. IN-HOUSE REFERENCE LOT

The in-house reference lot is a vaccine lot that has been found to be safe and efficacious through clinical studies, or is representative thereof.

Coming next… New and revised Ph. Eur. texts underway

1) New texts under elaboration

Title	General chapter / monograph	Scope	Current proposed timelines
Recombinant viral vectored vaccines for human use (5.37)	New general chapter	Viral-vectored vaccines	Public consultation in Pharmeuropa 36.2 (April 2024)
mRNA vaccines for human use (5.36)	New general chapter	mRNA-LNP vaccines	
mRNA substances for the production of mRNA vaccines for human use (5.39)	New general chapter	mRNA substances	
DNA template for the preparation of mRNA substances (5.40)	New general chapter	DNA template	
Aluminium phosphate, hydrated, for adsorption (3065)	New monograph	Aluminium phosphate as vaccine adjuvant	
High throughput sequencing for the detection of viral extraneous agents (2.6.41)	New general chapter	General chapter describing the technology and providing guidelines for method validation	

2) Revised texts in preparation
(selected examples)

Title	General chapter / monograph	Scope	Current proposed timelines
Vaccines for human use (0153)	Revised general monograph	- to take into account new vaccine classes (such as viral-vectored vaccines and mRNA vaccines) - to extend to the final bulk the possibility to replace the sterility test with a test for bioburden with a stringent limit	Public consultation in Pharmeuropa 36.2 (April 2024)
Pyrogenicity: revision of vaccine monographs	Revised general monograph & Revised vaccine monographs (17 texts)	- revision of vaccine monographs as part of the Ph. Eur. exercise to phase out the rabbit pyrogen test	Public comments under review. Target date for adoption: June 2024

Thank you for your attention

Stay connected with the EDQM

EDQM Newsletter: **https://go.edqm.eu/Newsletter**
LinkedIn: **https://www.linkedin.com/company/edqm/**
X: **@edqm_news**
Facebook: **@EDQMCouncilofEurope**