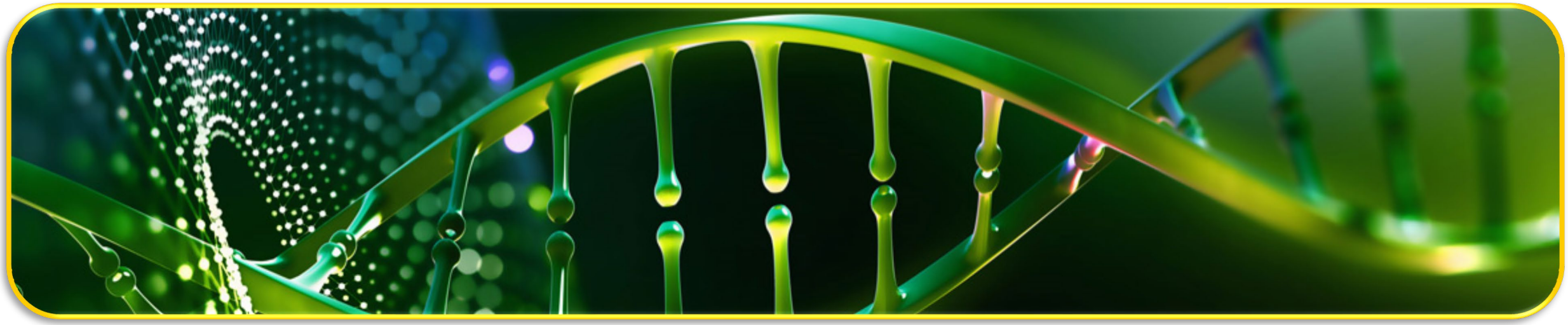
THE EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES & HEALTHCARE (EDQM)

Advancement in gene therapy: the European Pharmacopoeia's new approach

Olga Kolaj-Robin, PhD, EPD, EDQM

Marie-Thérèse Duffour, PhD, GTP WP Chair, ANSM



Tell us which group you are representing!

- ❑ Industry
- ❑ Regulatory assessors
- ❑ Inspectors
- ❑ CDMOs
- ❑ Independent laboratories
- ❑ Governmental organisations
- ❑ Academia
- ❑ Hospitals
- ❑ Other

Have you read/used general chapter *Gene transfer medicinal products for human use (5.14)*

- ❑ Yes
- ❑ No
- ❑ I don’t know

Before the webinar announcement, were you aware of the new approach of the Ph. Eur. to gene therapy?

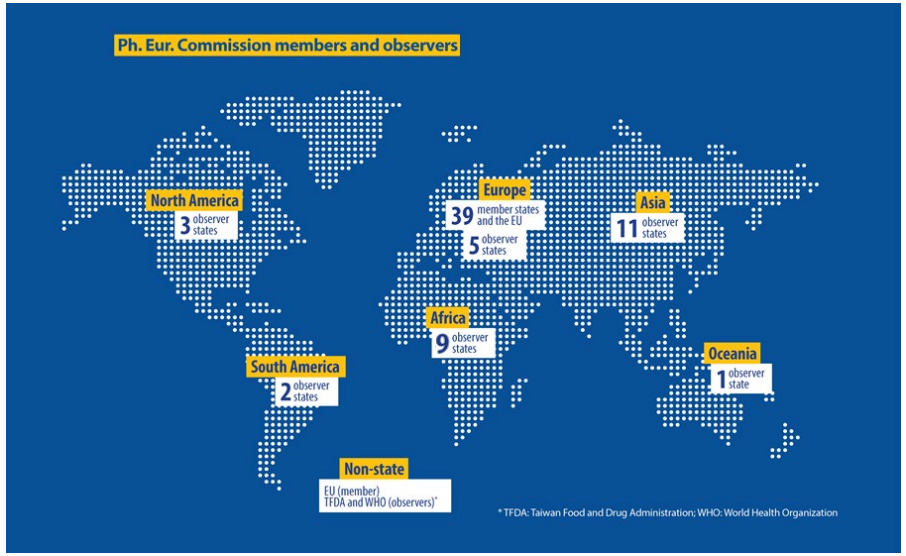
- ❑ Yes
- ❑ No, I became aware with the webinar announcement
- ❑ No, I don’t know the new approach
- ❑ I am not sure

European Pharmacopoeia (Ph. Eur.)

EDQM 1964 - 2024 — COUNCIL OF EUROPE 1949 - 2024 CONSEIL DE L'EUROPE

- Protecting public health – one common compulsory standard
- Official pharmacopoeia in Europe (complemented by national pharmacopoeias)
- Legally binding quality standards for ALL medicinal products i.e. raw materials, preparations, dosage forms, containers…
- Mandatory at the same date for all Members

- 40 Members (39 Member States & EU)
- 33 Observers (5 European, 26 non-European countries, TFDA, WHO)
- Supplement 11.7: 2520 monographs, 396 general texts



EDQM 1964 - 2024 — COUNCIL OF EUROPE 1949 - 2024 CONSEIL DE L'EUROPE

Ph. Eur. content and structure

Document en Français | PDF | Knowledge Database

General Notices apply to all monographs and other texts. See the information section on general monographs.

Ph. Eur. Reference standards / preparations & reagents

General chapters & general texts

- avoid repeating standard procedures or requirements in each monograph; aspects that cannot be treated in each monograph
- become mandatory when referred to in a monograph
- provide standard analytical procedures; guidance

Individual monographs

- Specific but not stand alone
- Analytical procedures and acceptance criteria represent required quality standards
- Based on approved specifications backed up by batch data
- Reliance on manufacturers' feedback (public consultation)

General notices

- Essential reading
- Apply to all texts
- Address general topics
- Provide basic information
- Include rules to understand texts, conventional expressions

General monographs

Dosage form monographs

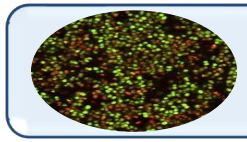
- Classes of substances/medicinal products
- Mandatory for all substances/preparations within the scope of the definition
- Not cross-referenced in individual monographs (exceptions)

Cell and gene therapy – Ph. Eur. PORTFOLIO Prior to Suppl. 11.7

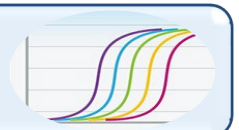
General overarching texts

- 5.14 Gene transfer medicinal products for human use

- 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
- 5.32 Cell-based preparations for human use



General methods: numeration & viability



- 2.7.23 Numeration of CD34+/CD45+ cells in haematopoietic products
- 2.7.24 Flow cytometry
- 2.7.28 Colony-forming cell assay for human haematopoietic progenitor cells
- 2.7.29 Nucleated cell count and viability
- 2.6.35 Quantification and characterisation of host-cell DNA

General chapters: Microbiology aspects & viral safety

- 2.6.1 Sterility
- 5.1.6 Alternative methods for control of microbiological quality
- 2.6.27 Microbiological examination of cell-based preparations
- 2.6.39 Microbiological examination of human tissues
- 5.1.13 Pyrogenicity - 2.6.14 BET - 2.6.30 MAT - 2.6.32 rFC
- 2.6.7 Mycoplasmas
- 5.1.7 Viral safety
- 5.2.8 TSE

Monographs

- Bovine serum (2262)
- Human haematopoietic stem cells (2323)

Under elaboration
Under revision
Recently adopted/published/revised
~~*Suppressed in Supplement 11.7*~~

Non exhaustive list

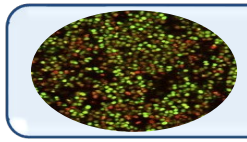
Cell and gene therapy – Ph. Eur.

PORTFOLIO

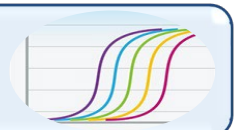
From Suppl. 11.7

General overarching texts

- ~~5.14 Gene transfer medicinal products for human use~~
- 3186 Gene therapy medicinal products for human use
- 5.34 Additional information on gene therapy medicinal products for human use
- 5.2.12 Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
- 5.32 Cell-based preparations for human use



General methods: numeration & viability



- 2.7.23 Numeration of CD34+/CD45+ cells in haematopoietic products
- 2.7.24 Flow cytometry
- 2.7.28 Colony-forming cell assay for human haematopoietic progenitor cells
- 2.7.29 Nucleated cell count and viability
- 2.6.35 Quantification and characterisation of host-cell DNA

General chapters: Microbiology aspects & viral safety

- 2.6.1 Sterility
- 5.1.6 Alternative methods for control of microbiological quality
- 2.6.27 Microbiological examination of cell-based preparations
- 2.6.39 Microbiological examination of human tissues
- 5.1.13 Pyrogenicity - 2.6.14 BET - 2.6.30 MAT - 2.6.32 rFC
- 2.6.7 Mycoplasmas
- 5.1.7 Viral safety
- 5.2.8 TSE

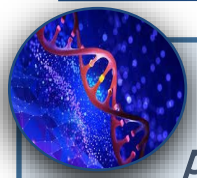
Monographs

- Bovine serum (2262)
- Human haematopoietic stem cells (2323)

Under elaboration
Under revision
Recently adopted/published/revised
~~*Suppressed in Supplement 11.7*~~

Non exhaustive list

General overarching texts – GTP texts: evolution



Gene transfer medicinal products for human use (5.14)

General chapter

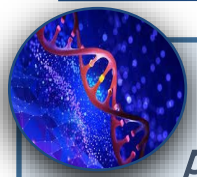
- Definition, Production
  - *Recombinant vectors*
  - *Genetically modified cells*
- Plasmid vectors for human use
- Bacterial cells used for the manufacture of plasmid vectors for human use
- Adenovirus vectors for human use
- Poxvirus for human use

- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

Adopted 2005
Ph. Eur. 5.6
(Jan 2007)

Adopted 2008
Ph. Eur. 6.6
(Jan 2010)

General overarching texts – GTP texts: evolution



Gene transfer medicinal products for human use (5.14)

General chapter

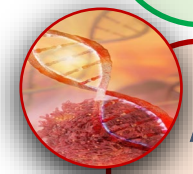
- Definition, Production
  - *Recombinant vectors*
  - *Genetically modified cells*
- Plasmid vectors for human use
- Bacterial cells used for the manufacture of plasmid vectors for human use
- Adenovirus vectors for human use
- Poxvirus for human use

- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

Gene therapy medicinal products for human use (3186)

General monograph

- Definition
- General requirements on:
  - the Production of GTMPs
  - Recombinant vectors
  - Genetically modified cells
- Genetically modified autologous human cells modified by integrating retroviral or lentiviral vectors
- Adeno-associated-virus vectors for human use
- Oncolytic herpes simplex virus for human use



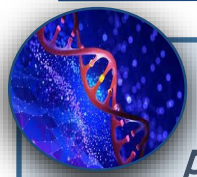
Additional information on gene therapy medicinal products for human use (5.34)

General chapter

- Plasmid vectors for human use including Bacterial cells used for the manufacture of plasmid vectors for human use
- Genetically modified bacterial cells for human use
- Adenovirus vectors for human use
- Poxvirus vectors for human use
- Retroviridae-derived vectors for human use

Revised from 5.14
New sections

General overarching texts – GTP texts: evolution



Gen… …cinal products … use (5.14)

General chapter

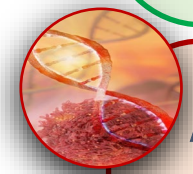
- Definitio…
  Re… …iant …
  Genetically modified cells
- Plasmid vectors for human use
- Bacterial cells used for the manufacture of plasmid vectors for human use
- Adenovirus vectors for human use
- Poxvirus for human use

- Adeno-associated-virus vectors for human use
- Retroviridae-derived vectors for human use

Gene therapy medicinal products for human use (3186)

General monograph

- Definition
- General requirements on:
  - the Production of GTMPs
  - Recombinant vectors
  - Genetically modified cells
- Genetically modified autologous human cells modified by integrating retroviral or lentiviral vectors
- Adeno-associated-virus vectors for human use
- Oncolytic herpes simplex virus for human use



Additional information on gene therapy medicinal products for human use (5.34)

General chapter

- Plasmid vectors for human use including Bacterial cells used for the manufacture of plasmid vectors for human use
- Genetically modified bacterial cells for human use
- Adenovirus vectors for human use
- Poxvirus vectors for human use
- Retroviridae-derived vectors for human use

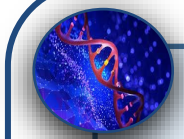
new general monograph (3186) and general chapter (5.34) replaced chapter 5.14 (Suppl. 11.7)

Revised from 5.14
New sections

Can *Gene therapy medicinal products for human use (3186)* be used for clinical trials?

- ❑ Yes
- ❑ No
- ❑ I don’t know

Can *Gene therapy medicinal products for human use (3186)* be used for clinical trials?



Gene transfer medicinal products for human use (5.14)

…The need for application of part or all of the texts to products used during the different phases of clinical trials is decided by the competent authority…

Gene therapy medicinal products for human use (3186)

- **Introductory text no longer present**
- **General monograph applicable for gene medicinal products for human use, i.e. in the context of marketing application**
- **Not mandatory for clinical trials**
- **Potential full or partial application of the monograph 3186 (and general chapter 5.34) for clinical trials up to the competent authority**

☑ Yes

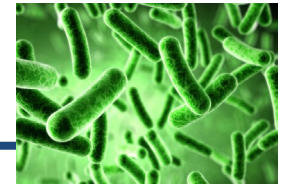
Definition

Gene therapy medicinal products for human use (3186)

- GTMP contains or consists of a **recombinant nucleic acid** used in/administered with a view to regulating, repairing, replacing, adding or deleting a genetic sequence
- activity **directly linked** to the recombinant nucleic acid sequence or the expressed product
- Vaccines against infectious diseases **excluded**
- Recombinant nucleic acid delivered using recombinant vectors or genetically modified cells

Are genetically modified bacteria covered in the scope of general monograph 3186?

- ❑ Yes, as genetically modified cells
- ❑ Yes, as recombinant vectors
- ❑ No
- ❑ I don’t know



Are genetically modified bacteria covered in the scope of general monograph 3186?

Gene transfer medicinal products for human use (5.14)

*Genetically modified cells. Genetically modified eukaryotic or **bacterial cells** are modified by vectors to express a product of interest*

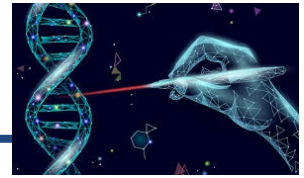
Gene therapy medicinal products for human use (3186)

Recombinant vectors

- viral vectors and recombinant oncolytic viruses (replication-competent, conditionally replication-competent and replication–incompetent) genetic material remaining cytoplasmic or episomal, integrated into the host genome or remaining extracellular
- DNA- and RNA-based nucleic acid vectors in simple formulation or complexed with various molecules
- **Genetically modified microorganism such as bacteria**

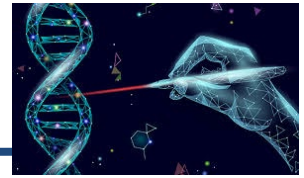
Dedicated section in *Additional information on gene therapy medicinal products for human use (5.34)*

☑ Yes, as recombinant vectors



Are gene-edited cells covered in the scope of general monograph 3186?

- ❑ Yes
- ❑ No
- ❑ I don’t know



Are gene-edited cells covered in the scope of general monograph 3186?

Gene therapy medicinal products for human use (3186)

- Genetically modified cells: autologous, allogeneic or xenogeneic genetically modified, including by **genome editing tools** (Definition)
- Requirement for description of genome editing tools and additional tests to those listed to be required by the CA depending on the tools used (General requirements)
- The individual section dedicated to *Genetically modified human autologous cells* ***modified by integrating retroviral or lentiviral vectors***

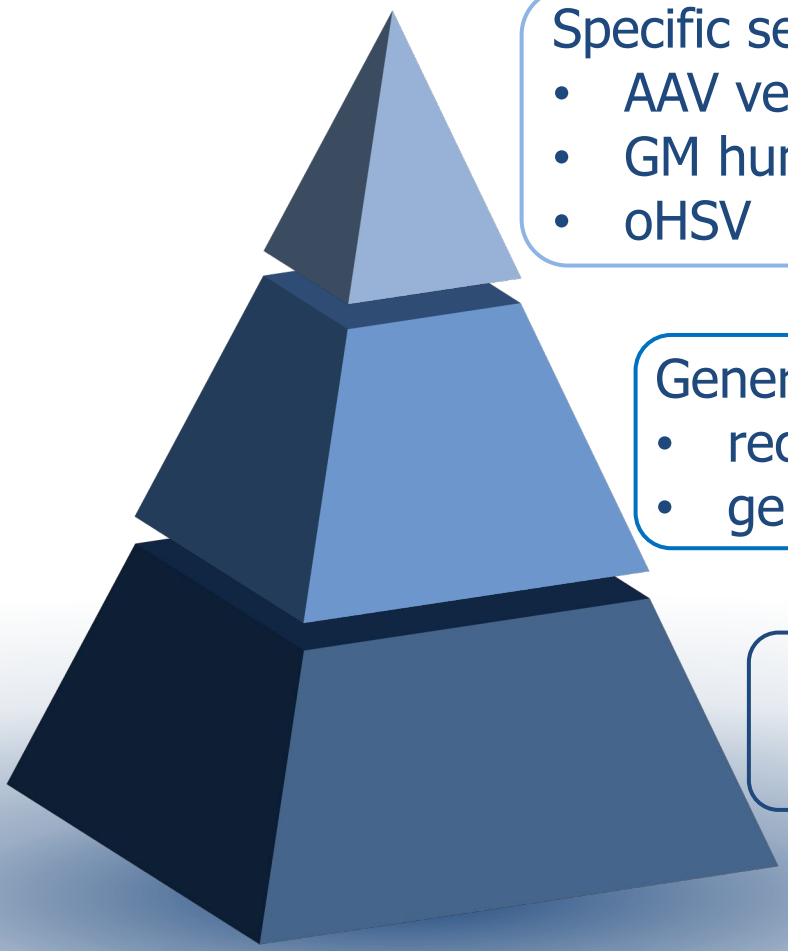
☑ Yes (general requirements)

Definition

Gene therapy medicinal products for human use (3186)

- GTMP contain or consist of a **recombinant nucleic acid** used in/administered with a view to regulating, repairing, replacing, adding or deleting a genetic sequence
- activity **directly linked** to the recombinant nucleic acid sequence or the expressed product
- Vaccines against infectious diseases excluded
- Recombinant nucleic acid delivered using **recombinant vectors** or **genetically modified cells**
- **Recombinant vectors:**
  - viral vectors and recombinant oncolytic viruses (replication-competent, conditionally replication-competent and replication–incompetent) genetic material remaining cytoplasmic or episomal, integrated into the host genome or remain extracellular
  - DNA- and RNA-base nucleic acid vectors in simple formulation or complexed with various molecules
  - **Genetically modified microorganism such as bacteria**
- **Genetically modified cells:** autologous, allogeneic or xenogeneic genetically modified, including by **genome editing tools**

New general monograph 3186 – requirement layers



Specific sections
- AAV vectors
- GM human autologous cells
- oHSV

General requirements
- recombinant vectors
- genetically modified cells

General provisions for GTMP production

Revised from 5.14
New sections

General monograph

Gene therapy medicinal products for human use (3186)

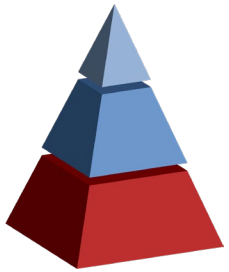
- Definition
- General requirements on:
  - the Production of GTMPs
  - Recombinant vectors
  - Genetically modified cells
- Genetically modified autologous human cells modified by integrating retroviral or lentiviral vectors
- Adeno-associated-virus vectors for human use
- Oncolytic herpes simplex virus for human use

General chapter

Additional information on gene therapy medicinal products for human use (5.34)

- Plasmid vectors for human use
  including
  Bacterial cells used for the manufacture of plasmid vectors for human use
- Genetically modified bacterial cells for human use
- Adenovirus vectors for human use
- Poxvirus vectors for human use
- Retroviridae-derived vectors for human use

General requirements



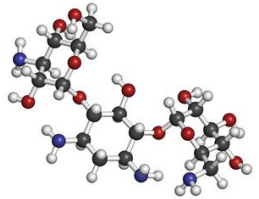
Gene therapy medicinal products for human use (3186)

2. General requirements

- **Applicability of the section to all GTMPs; requirements not necessarily repeated in the individual sections of the general monograph 3186 or general chapter 5.34**
- **Applicability of a risk-based approach according to Directive 2001/83/EC to meet the quality requirements as defined in the general monograph**

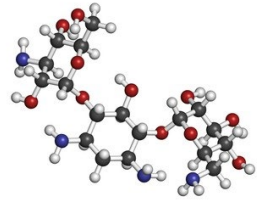
2-1. General provisions for GTMP production

- **Requirement for the production process to yield a GTMP of consistent quality and stability**
- **Substances used in production**:
  - raw and starting materials shown **suitable for their intended use**; **specifications** established to control their identity, purity and where applicable potency or strength;
  - **absence** of extraneous agents to be demonstrated
  - Use of **antibiotics avoided** during production; penicillin, other **β-lactams and streptomycin not to be used**
  - raw materials of biological origin comply with *Raw materials of biological origin for the production of cell-based and gene therapy medicinal products (5.2.12)* unless otherwise justified and authorised



Can kanamycin or other antibiotics be used in production of a plasmid used for the production of a recombinant vector ?

- ❑ Yes
- ❑ No
- ❑ I don’t know



Can kanamycin or other antibiotics be used in production of a plasmid used for the production of a recombinant vector ?

Gene therapy medicinal products for human use (3186)

- Antibiotics to be **avoided** during production → can be used if justified and authorised
- Penicillin, other β-lactams and streptomycin **not to be used** in production → in line with EMA Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products
- If antibiotic is authorised for use during production, residues are determined → covered by residual reagents in individual sections and by process-related impurities in general requirements

☑ Yes

General requirements

Gene therapy medicinal products for human use (3186)

2-1. General provisions for GTMP production (cont.)

- **Viral safety (5.1.7)**
- **Transmissible spongiform encephalopathies (5.2.8)**
- **Containers *Materials used for the manufacture of containers* (3.1 and subsections) and *Containers* (3.2 and subsections)**
- **Labelling according to relevant EU or other applicable regulations**

General chapters, when referred to in a monograph, become part of the standard

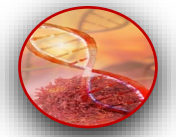
General monograph 3186 – other cross-references

Substrates used in the manufacture of recombinant vectors

(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

(5.34) Additional information on GTMPs for human use (for bacterial cells for plasmid production)



(5.34) Additional information on GTMPs for human use

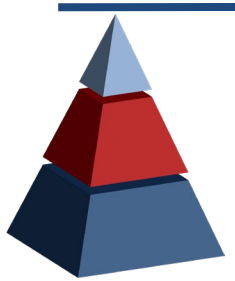
- Informative cross-reference to other individual sections
- *Plasmid vectors for human use* for bacterial cell banks
- RV & LV section for production of vectors for genetic modification of cells

Gene therapy medicinal products for human use (3186)

Methodology chapters

e.g. NAT (2.6.21), SEC (2.2.30), Flow cytometry (2.7.24), Nucleated cell count & viability (2.7.29), Mycoplasmas (2.6.7), Sterility (2.6.1), Microbiological examination (**2.6.27**), BET (2.6.14, **2.6.32**)…

General requirements for rec. vectors and GM cells



Gene therapy medicinal products for human use (3186)

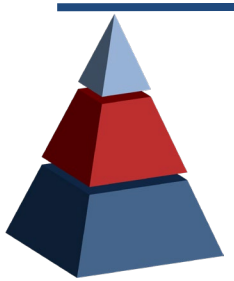
2-2. Recombinant vectors for human use

2-2-1. General provisions on recombinant vector production
2-2-2. Characterisation of the vector
2-2-3. Vector production
- 2-2-3-1. Vector harvest
- 2-2-3-2. Purified harvest
- 2-2-3-3. Final lot

- Definition of the **production system** (consisting of the components used to obtain the recombinant vector);
- Requirement for **genetic and phenotypic stability** (if applicable, e.g. for the cells of a stable production system) at or beyond the maximum number of passage levels used for production is demonstrated for the production system and for the recombinant vector
- requirements for **substrates** used in the manufacture of recombinant vectors (5.2.2, 5.2.3 and *Plasmid vectors for human use* of 5.34 for bacterial cells)

- In justified cases, the tests listed under respective heading can be performed at an **alternative stage** of the process under a suitable testing scheme as authorised by the competent authority
- In cases where it is not possible to distinguish between the vector harvest, purified harvest and/or final lot, the **requirements** listed under the respective heading **remain applicable** and are performed under a **suitable testing scheme** approved by the competent authority

General requirements for rec. vectors and GM cells



Gene therapy medicinal products for human use (3186)

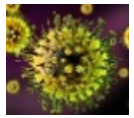
2-3. Genetically modified cells for human use

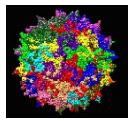
2-3-1. Vectors and tools used for GM of cells
2-3-2. Source cells used for production of GM cells
2-3-3. Production of GM cells
2-3-4. Final lot

- Additional tests **required** as approved by the competent authority, depending on the recombinant vector and tools used for genetic modification
- Sterility requirement for the recombinant vector or genome editing tools

- Tests to be performed at **appropriate stages** depending on the manufacturing process
- Additional tests **may be required** as approved by the competent authority depending on the genetically modified cells and the tools used for their modification
- **RCV** when a replication-incompetent vector used for modification; test **may be omitted** as authorised by the competent authority if appropriate testing is performed at the level of the viral vector used for genetic modification of cells

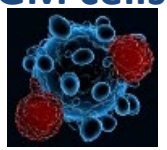
General monograph 3186: built-in flexibility

oHSV 

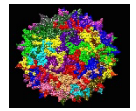
AAV 

Residual reagents. **Based on risk analysis,** tests for residues of reagents used during production and posing safety concerns are carried out on the purified harvest. **Where relevant,** the residual bovine serum albumin is determined by a **suitable** immunochemical **method** (*2.7.1*). If authorised for use in production, residual antibiotic concentration **is determined by liquid chromatography** or by **other suitable methods**.

Assay. The number of transduced cells **is determined by a suitable method, such as** flow cytometry (*2.7.24*). The number of transduced cells **is within the limits approved for the particular product**. (...)

GM cells 

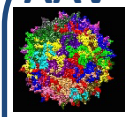
Biological activity. Biological activity **is determined by a suitable test** [...]. The biological activity **is within the limits approved for the particular product**.

AAV 

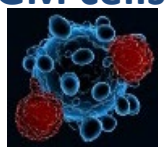
✓ **Flexible wording**
✓ **Reference to suitable methods**

Gene therapy medicinal products for human use (3186)

✓ **No numerical acceptance criteria**

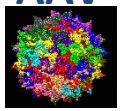
AAV 

Capsid titre (empty and full particles). The capsid titre **is determined by a suitable method such as** ELISA (*2.7.1*), size-exclusion chromatography (*2.2.30*) or analytical ultracentrifugation.

GM cells 

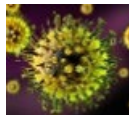
Genetically modified autologous human cells comply with the requirements given below under Identification, Tests and Assay. The following **tests are performed at appropriate stages depending on the manufacturing process.**

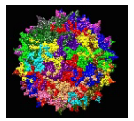
oHSV 

AAV 

Virus/Vector aggregates. Virus/vector aggregates **are determined by a suitable method such as** light scattering, size-exclusion chromatography (*2.2.30*), analytical ultracentrifugation or electron microscopy.

General monograph 3186: built-in flexibility

oHSV 

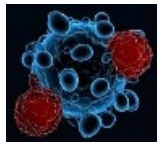
AAV 

Residual reagents. Based on risk analysis, tests for residues of reagents used during production and posing safety concerns are carried out on the purified harvest. **Where relevant,** the residual bovine serum albumin is determined by a **suitable** immunochemical **method** (*2.7.1*). If auth… antibiotic concent… **chromatograp…**

oHSV 

Virus concentration is determined by a **suitable method such as** NAT (*2.6.21*) or immunochemical methods (*2.7.1*).

Assay … The … of … cells … is

GM cells 

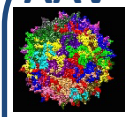
Expression of the genetic insert is determined by suitable immunochemical (*2.7.1*) or biochemical assays or by flow cytometry (*2.7.24*) if it encodes a protein. If the genetic insert codes for an RNA as an end product, methods such as quantitative reverse transcription polymerase chain reaction (RT-qPCR) (*2.6.21*) can be used. Expression of the genetic insert **complies with the pre-defined acceptance criteria for the particular product.**

Biologic… suitable … limits ap…

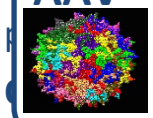
✓ **Flexible wording**
✓ **Reference to suitable methods**

Gene therapy medicinal products for human use (3186)

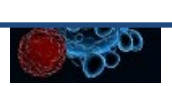
✓ **No numerical acceptance criteria**

AAV 

… suitable method such as ELISA (*2.7.1*), size-exclusion chromatography (*2.2.30*) or analytical ultracentrifugation.

AAV 

The ratio of viral genomes to infectious particle titre is within the limits approved for the particular product.



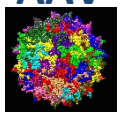
… are pe… depend… proces…

oHSV 

Virus identity is verified by a **suitable method such as** immunochemical methods (*2.7.1*) targeting viral proteins. **The genetic insert and the modified viral sequences** are identified by a **suitable method such as** NAT (*2.6.21*) or restriction-enzyme analysis.

oHSV 

Virus/Vector aggregates. Virus/vector aggregates **are**

AAV 

Residual DNA from plasmids, bacmids and viruses used during production. **Where applicable**, the contents of residual DNA from plasmids, bacmids and viruses used in production, are **determined by a suitable method, such as** qPCR (*2.6.21*).

General monograph 3186: built-in flexibility

oHSV **Residual reagents. Based on risk analysis,** tests for residues of reagents used during production and posing

GM cells **Identification.** Cell identity and transgene identity are verified **by suitable meth** as flow cytometry (2.7.24) and nu amplification techniques (NAT) (2.6.21).

antibiotic concent **chromatograp**

oHSV determined **method such** immunochemi

EXAMPLES

Assay The number of transduced cells is

Expression of the genetic insert is determined cells

AAV **The vector genome titre** (full particles) is determined **with a suitable method such as** NAT (*2.6.21*) or size-exclusion chromatography (*2.2.30*). The vector genome titre is **within the limits approved for the particular product.**

AAV **The genomic integrity** of the plas or bacmids is **verified by suit methods such as** NAT (*2.6.21*) restriction-enzyme analysis of the region corresponding to *rep*, *cap*, the genetic insert and the helper functions, **where applicable**.

(*2.6.21*) can be used. Expression of the genetic insert **complies with the pre-defined acceptance criteria for the particular product.**

✓ **Flexible wording**
✓ **Reference to suitable methods**

Gene therapy medicinal products for human use (3186)

✓ **No numerical acceptance criteria**

AAV

AAV **The ratio of viral genomes to infectious particle titre is within the limits approved**

suitab

as ELIS chromat analytica

AAV **Percentage of full and empty particles** is calculated using th capsid titre and ve **determined by a** percentage of full **within the limit particular product**

oHSV **Virus identity** is verified by a **suitable method such as** immunochemical methods (*2.7.1*) targeting viral proteins. **The genetic insert and the**

oHSV **The physical particle titre** is determined by a **suitable technique such as** liquid chromatography (*2.2.29*), NAT (*2.6.21*) or immunochemical methods (*2.7.1*). The physical particle titre **is within the limits approved for the particular product.**

oH

AAV

GM cells **The differentiation potential** (for products containing stem or progenitor cells) is determined by a suitable method. The differentiation potential **complies with the pre-defined acceptance criteria for the particular product.**

such as qPCR (*2.6.21*).

For both, recombinant vectors and genetically modified cells, the following requirement is stated:

The process demonstrates adequate clearance of impurities.

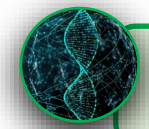
Is complete clearance of impurities expected?

- ❑ Yes
- ❑ No
- ❑ It depends
- ❑ I don’t know

For both, recombinant vectors and genetically modified cells, the following requirement is stated:

The process demonstrates adequate clearance of impurities.

Is complete clearance of impurities expected?



Gene therapy medicinal products for human use (3186)

- **'Adequate clearance' does not mean complete removal of impurities**
- **reduction to the levels acceptable by the competent authority**
- **DL of the analytical procedure has to be taken into account**

☑ **No**

Do I have to perform all the tests included in the monograph?

- ❑ Yes
- ❑ No, if indicated so in the monograph
- ❑ No
- ❑ I don’t know

Do I have to perform all the tests included in the monograph?

Gene therapy medicinal products for human use (3186)

- Examples of when **test** may be **omitted** outlined (above example RCV on genetically modified cells if appropriate testing performed at the level of the viral vector used);
- The article has to **comply** with all the requirements stated in the monograph;
- **Not all the tests** have to be **performed** when assessing compliance with the Ph. Eur. before release
- **Assurance** that an article is of Ph. Eur. quality may be **obtained** on the basis of its **design**, together with its **control strategy** and data derived e.g. from **validation studies**

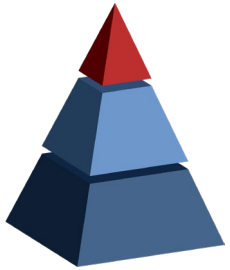
1. GENERAL NOTICES

1.1.2.2 Demonstration of compliance with the Ph. Eur.

☑ No*

***Justification needed**

Individual sections – section outline

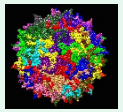


Recombinant vectors

DEFINITION
PRODUCTION SYSTEM/MATERIALS USED
VECTOR PRODUCTION AND HARVEST
PURIFIED HARVEST
FINAL LOT
- Identification
- Tests
- Assay

Gene therapy medicinal products for human use (3186)

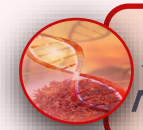
AAV



Vector construction
Vector production
Plasmids used for production
Viruses used in production
Cell lines used for production

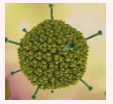
oHSV

Vector construction
Virus seed lot



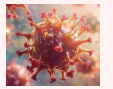
Additional information on gene therapy medicinal products for human use (5.34)

AdV



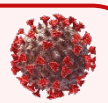
Vector construction
Vector production
Cell lines used in production
Vector seed lot
Helper virus

Pox



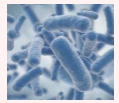
Vector construction
Cell substrate
Vector seed lot

LV/RV



Vector construction
Vector production
Plasmids used for production
Cells used for production

GMBC

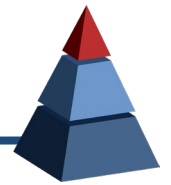


Bacterial cells used for production
Plasmids
Other genetic materials
MCB
WCB

pDNA

Vector construction
Bacterial cells used for production

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-1. Definition

Adeno-associated virus (AAV) vectors for human use are freeze-dried or liquid preparations of recombinant AAV (rAAV), genetically modified to transfer genetic material to human somatic cells *in vivo*.

4-2. Production system

4-2-1. Vector construction

- Replacing *rep* and *cap* with a genetic insert (gene of interest + relevant regulatory elements); provision of *rep* and *cap in trans*;
- rAAV vector = vector genome + capsid that may originate from various serotypes or combinations thereof
- Capsid consisting of VP1, VP2 and VP3 AAV proteins
- Sequence homology in the starting materials should be limited to reduce the risk of rcAAV in final lot

4-2-2. Vector production

- Examples of production strategies (in mammalian or insect cell lines by transient co-infection or using a stable cell line)
- Manufacturing strategy designed to minimise the risk of rcAAV generation; process designed to effectively eliminate plasmids and viruses used in production

Individual sections – focus on AAV section

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-2. Production system

4-2-3. Plasmids used for production of AAV vectors

- Complete description including identification of the plasmid DNA, its source, means of isolation and nucleotide sequence established for each plasmid; documentation of source and function of plasmid component parts (e.g. ori, promoters, genes coding for selection markers)
- Production based on bacterial cell-bank system; MCB requirements according to 5.34

EXCEPT

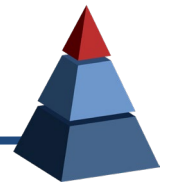
- copy number
- percentage of cells retaining the plasmid

- If bacmids used → requirement for plasmids apply unless otherwise justified and authorised

Additional information on gene therapy medicinal products for human use (5.34)

1. Plasmid vectors for human use
1-2-2. Bacterial cells used for production of plasmid vectors for human use

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

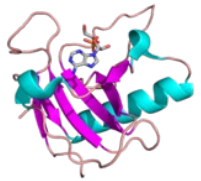
4. Adeno-associated virus vectors for human use

4-2. Production system

4-2-3. Plasmids used for production of AAV vectors

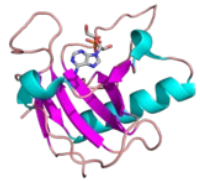
Only plasmids that comply with the following requirements may be used in the production of AAV vectors:

- **Identification** using a suitable method such as restriction-enzyme analysis, sequencing of NAT (2.6.21)
- **Genetic integrity** verified by a suitable method such as sequencing, NAT (2.6.21) or restriction-enzyme analysis of the region corresponding to *rep, cap,* the genetic insert and the helper functions, where applicable
- **DNA concentration** determined by a suitable method
- **DNA purity** determined using a ratio A_{260} and A_{280}; ratio between 1.8-2.0
- **Residual host-cell DNA** (2.6.35) determined by a suitable method such as qPCR (2.6.21)
- **Residual host-cell RNA** determined by a suitable method such as RT-qPCR (2.6.21)
- **Residual host-cell proteins** (2.6.34) determined by a suitable immunochemical method (2.7.1)
- **Mycoplasmas (2.6.7)** for material of biological origin unless otherwise justifies and authorised
- **BET (2.6.14 or 2.6.32)** less than the limit approved for the particular preparation
- **Microbiological examination** – compliance with the sterility (2.6.1) test or determination of bioburden (2.6.12)



If RNAse from *E. coli* is used, is the test for mycoplasmas required for plasmids or bacmids used in production of AAV vectors?

- ❑ Yes
- ❑ No
- ❑ I don't know



If RNAse from *E. coli* is used, is the test for mycoplasmas required for plasmids or bacmids used in production of AAV vectors?

Gene therapy medicinal products for human use (3186)

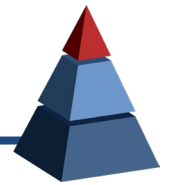
- **Mycoplasmas** (2.6.7). If materials of **biological origin** are used during the production of plasmids or bacmids, the plasmids or bacmids comply with the test, **unless otherwise justified and authorised.**

- *E. coli* not infected by mycoplasma

☑ No*

***Justification needed**

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-2. Production system

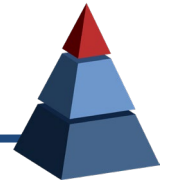
4-2-4. Viruses used for production of AAV vectors (baculoviruses or helper viruses)

- Production based on a seed lot and a cell-bank system compliant with general chapter 5.2.3.
- Strain of virus used is identified by historical records including information on its origin and its subsequent manipulation, notably deleted or modified regions; nucleotide sequence of each virus is documented.

Only viruses that comply with the following requirements may be used in the production:

- **Identification** using a suitable method such as an immunochemical method, NAT (2.6.21) or RE analysis
- **Genomic integrity** verified by a suitable method such RE analysis; for modified baculoviruses expressing *rep, cap* or genetic insert, assessed by sequencing or qPCR (2.6.21) of these regions
- **Infectious titre** determined by a suitable assay (each virus)
- Absence of **wtAAV** in helper virus seed lots verified using NAT (2.6.21)
- No **rc helper viruses** detected by a suitable method when replication-incompetent vector is used as a helper virus
- Compliance with **Extraneous agents (2.6.16)** (each virus)
- **BET (2.6.14 or 2.6.32)**: less than the limit approved for the particular preparation

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

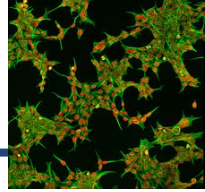
4-2. Production system

4-2-5. Cell lines used for the production of AAV vectors

- Absence of **wt AAV** for cells of mammalian origin verified by NAT (2.6.21)

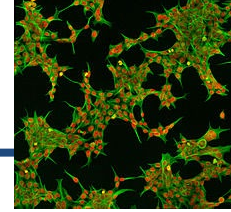
For stably genetically modified cell lines:

- **Copy number** of the inserted sequences per cell determined on genomic DNA isolated and purified from a known number of cells by a suitable method such as qPCR (2.6.21)
- **Sequence integrity of the viral genes and genetic insert** verified by nucleotide sequencing of the inserted sequences



Do requirements for copy number and sequence integrity of the viral genes apply to HEK293 cell line?

- ❑ Yes
- ❑ No
- ❑ I don’t know



Do requirements for copy number and sequence integrity of the viral genes apply to HEK293 cell line?

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

- Requirement for copy number and sequence integrity for stably genetically modified cell lines used for production of AAV vectors
- HEK293 cell line well characterised
- **Compliance ≠ Testing;** Assurance that an article is of Ph. Eur. quality may be obtained on the basis of its **design**, together with its control strategy and data derived e.g. from validation studies

1. GENERAL NOTICES

☑ **Yes**

Do the tests for copy number and sequence integrity of the viral genes have to be performed for HEK293 cell line?

☑ **No (justification needed)**

Do requirements of general chapter
Cell substrates for the production of vaccines for human use (5.2.3)
apply to cell lines used for the production of AAV vectors?

- ❑ Yes
- ❑ No
- ❑ I don’t know

Do requirements of general chapter 5.2.3 apply to cell lines used for the production of AAV vectors?

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

- Reference to general chapter *Cell substrates for the production of vaccines for human use (5.2.3)* for cells used in production of viruses used for production of AAV vectors (section 4-2-4)
- No reference to *5.2.3* for cell lines used for the production of AAV vectors (section 4-2-5)

Gene therapy medicinal products for human use (3186)

2-2. Recombinant vectors for human use

- requirements for **substrates** used in the manufacture of recombinant vectors (5.2.2, 5.2.3 and *Plasmid vectors for human use* of 5.34 for bacterial cells)

☑ **Yes**

Individual sections – focus on AAV section

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-3. Vector production and harvest

Portion of the production cell cultures set aside before transfection/infection and cultured in parallel (control cells)

Only single harvests that comply with the following requirements may be used in preparation of the purified harvest:

Identification by a suitable method such as immunochemical method (2.7.1), NAT (2.6.21) or RE analysis

Vector concentration (vector genome titre) determined

The single harvest and the control cells comply with the tests for **Extraneous agents (2.6.16)**

Is control cells testing required for every production run?

- ❑ Yes
- ❑ No
- ❑ I don’t know

Is control cells testing required for every production run?

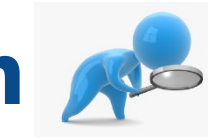
- **Not all the tests** have to be **performed** when assessing compliance with the Ph. Eur. before release
- **Assurance** that an article is of Ph. Eur. quality may be **obtained** on the basis of its **design**, together with its **control strategy** and data derived e.g. from **validation studies**
- **Harvest** maybe tested instead, provided that the **absence of interference** with the viral vector for the detection of extraneous agents at the level of the single harvest has been demonstrated

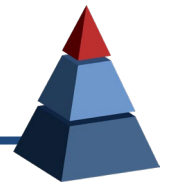
1. GENERAL NOTICES

☑ **No***

***Clarifications incorporated in the monograph (see next slide)**

Individual sections – focus on AAV section





Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-3. Vector production and harvest

Portion of the production cell cultures set aside before transfection/infection and cultured in parallel (control cells) **unless the absence of interference with the viral vector for the detection of extraneous agents at the level of the single harvest has been demonstrated.**

Only single harvests that comply with the following requirements may be used in preparation of the purified harvest:

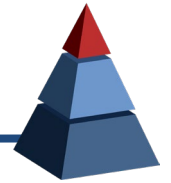
Identification by a suitable method such as immunochemical method (2.7.1), NAT (2.6.21) or RE analysis

Vector concentration (vector genome titre) determined

The single harvest and the control cells comply with the tests for **Extraneous agents (2.6.16). The control cells might not be used when the absence of interference with the viral vector for the detection of extraneous agents at the level of the single harvest has been demonstrated. In that case, the requirement is applicable only to the single harvest.**

- **Approach also applied in sections on adenovirus, poxvirus, lenti/retrovirus vectors**
- **Control cells to be tested for each run for oHSV**

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

- Several single harvests may be pooled before the purification process that is to demonstrate adequate clearance of impurities
- The entire genome of the vector is sequenced for **a suitable number of purified harvests** and the analytically determined sequence compared to the theoretical sequence

4-5. Final lot

- Several purified harvests may be pooled, stabiliser and other excipients may be added during preparation of final lot; formulated final lot is sterile-filtered
- A suitable batch of the formulated AAV vector, preferably one that has been clinically evaluated is fully characterised and retained for use a reference standard

Individual sections – focus on AAV section

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

Tests:

- Identification
- Genomic integrity
- Capsid titre
- Vg titre
- Infectious particle titre
- Capsid proteins
- rcAAV
- Residuals (viruses, DNA, HCP, reagents)
- Microbiological contamination
- BET

4-5. Final lot

4-5-1. Identification

4-5-2. Tests

- Appearance
- Particulate contamination
- Vector aggregates
- Osmolality
- Extractable volume
- pH
- Water
- Sterility
- BET

4-5-3. Assay

- Capsid titre
- Vg titre
- Full/empty particles
- Infectious particle titre
- Vg titre/infectious particle titre
- Expression from the genetic insert
- Biological activity

❖ **Vector identity to be verified**

❖ **Vector capsid identity and gene of interest identity to be verified**

by a suitable method such as:

an immunochemical method (2.7.1), NAT (2.6.21) or RE analysis

an immunochemical method (2.7.1) (capsid) and NAT (2.6.21) or RE analysis (gene of interest)

Individual sections – focus on AAV section

Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

Tests:

- Identification
- Genomic integrity
- Capsid titre
- Vg titre
- Infectious particle titre
- Capsid proteins
- rcAAV
- Residuals (viruses, DNA, HCP, reagents)
- Microbiological contamination
- BET

❖ **Sterility (2.6.1) or the bioburden (2.6.12) depending on the preparation**

4-5. Final lot

4-5-1. Identification

4-5-2. Tests

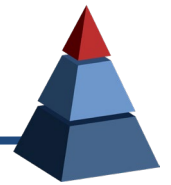
- Appearance
- Particulate contamination
- Vector aggregates
- Osmolality
- Extractable volume
- pH
- Water
- Sterility
- BET

4-5-3. Assay

- Capsid titre
- Vg titre
- Full/empty particles
- Infectious particle titre
- Vg titre/infectious particle titre
- Expression from the genetic insert
- Biological activity

❖ **Sterility (2.6.1)**

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

Tests:

- Identification
- Genomic integrity
- Capsid titre
- Vg titre
- Infectious particle titre
- Capsid proteins
- rcAAV
- Residuals (viruses, DNA, HCP, reagents)
- Microbiological contamination
- BET

4-5. Final lot

4-5-1. Identification

4-5-2. Tests

- Appearance
- Particulate contamination
- Vector aggregates
- Osmolality
- Extractable volume
- pH
- Water
- Sterility
- BET

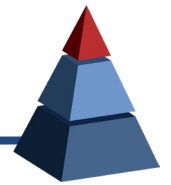
4-5-3. Assay

- Capsid titre
- Vg titre
- Full/empty particles
- Infectious particle titre
- Vg titre/infectious particle titre
- Expression from the genetic insert
- Biological activity

❖ **BET (2.6.14 or 2.6.32) - Possibility of testing for BET using rFC introduced alongside the classic (LAL)-based methods**

❖ **Introduced throughout 3186 and 5.34**

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

Tests:

- Identification
- Genomic integrity
- Capsid titre
- Vg titre
- Infectious particle titre
- Capsid proteins
- rcAAV
- Residuals (viruses, DNA, HCP, reagents)
- Microbiological contamination
- BET

4-5. Final lot

4-5-1. Identification

4-5-2. Tests

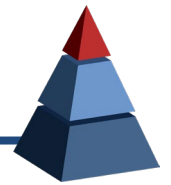
- Appearance
- Particulate contamination
- Vector aggregates
- Osmolality
- Extractable volume
- pH
- Water
- Sterility
- BET

4-5-3. Assay

- Capsid titre
- Vg titre
- Full/empty particles
- Infectious particle titre
- Vg titre/infectious particle titre
- Expression from the genetic insert
- Biological activity

❖ **Detection performed by a replication assay on a permissive cell line previously infected with a helper virus; analysis by Southern blot, or by detection of the *rep* by qPCR. The limit approved by the competent authority**

Individual sections – focus on AAV section



Gene therapy medicinal products for human use (3186)

4. Adeno-associated virus vectors for human use

4-4. Purified harvest

Tests:

- Identification
- Genomic integrity
- Capsid titre
- Vg titre
- Infectious particle titre
- Capsid proteins
- rcAAV
- Residuals (viruses, DNA, HCP, reagents)
- Microbiological contamination
- BET

4-5. Final lot

4-5-1. Identification

4-5-2. Tests

- Appearance
- Particulate contamination
- Vector aggregates
- Osmolality
- Extractable volume
- pH
- Water
- Sterility
- BET

4-5-3. Assay

- Capsid titre
- Vg titre
- Full/empty particles
- Infectious particle titre
- Vg titre/infectious particle titre
- Expression from the genetic insert
- Biological activity

❖ **Determined by suitable *in vitro* test using a clinically relevant cell line. Determination by *in vivo* when justified and only if an *in vitro* test not possible.**

❖ **An appropriate surrogate assay demonstrated to correlate to biological activity may be used**

❖ **Possibility of surrogate assays applied throughout 3186 and 5.34**

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-1. DEFINITION

3-2. VECTORS USED IN PRODUCTION OF GENETICALLY MODIFIED HUMAN AUTOLOGOUS CELLS

3-3. HUMAN AUTOLOGOUS CELLS – COLLECTION AND INITIAL PREPARATION

3-4. PRODUCTION

3-5. FINAL LOT

- Identification
- Tests
- Assay

Is the section *Plasmid vectors for human use* applicable for plasmids used for preparation of vectors for genetic modification of cells?

- ❑ Yes
- ❑ No
- ❑ I don’t know

Is the section *Plasmid vectors for human use* applicable for plasmids used for preparation of vectors for genetic modification of cells?

Gene transfer medicinal products for human use (5.14)

Plasmid vectors for human use

DEFINITION

(…) They are used to transfer genetic material into human somatic cells *in vivo* **or to genetically modify autologous, allogeneic, xenogeneic or bacterial cells before administration to humans.** (…)

Additional information on gene therapy medicinal products for human use (5.34)

1. Plasmid vectors for human use

- Applicable to plasmids as **medicinal products**
- Now contains **bacterial cells** used for production of plasmid vectors for human use referred to in sections on recombinant vectors with the exception of copy number and percentage of cells retaining the plasmid

Gene therapy medicinal products for human use (3186)

- Requirements for plasmids used for production of recombinant vectors outlined in **individual sections**.

☑ No

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-2. Vectors used in production of genetically modified autologous cells

- reference to 5.2 of the *Retroviridae-derived vectors for human use*
- List of requirements for **vectors** used for the production of genetically modified human autologous cells **– to be performed at appropriate stages of vector production**

Additional information on gene therapy medicinal products for human use (5.34)

5. Retroviridae-derived vectors for human use
5-2. Production system
5-2-1 Vector construction
5-2-2 Vector production
5-2-3 Plasmids used for production of vectors

List of requirements for plasmids

…MCB complies with the requirements for **bacterial cells** used for production of plasmid vector for human use of *Plasmid vectors for human use* with the exception of copy number and % of cells retaining the plasmid.

5-2-4 Cells used for the production of vectors

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-2. Vectors used in production of genetically modified autologous cells

- **Identification**
- **Genomic integrity**
- **Physical particle titre**
- **Functional titre**
- **Functional/physical particle titre**

- **RCV**
- **Residual hc DNA, pDNA, HCP, reagents**
- **Sterility (2.6.1)**
- **BET (2.6.14 or 2.6.32)**
- **Biological activity or expression from the genetic insert**

❖ **The vector used for autologous cell modifications is sterile**

❖ **rFC or LAL-based method can be used in testing for endotoxins**

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-2. Vectors used in production of genetically modified autologous cells

- Identification
- Genomic integrity
- Physical particle titre
- Functional titre
- Functional/physical particle titre

- RCV
- Residual hc DNA, pDNA, HCP, reagents
- Sterility (2.6.16)
- BET (2.6.14 or 2.6.32)
- Biological activity or expression from the genetic insert

❖ **RCV detection by a suitable method, typically by amplification in permissive cells followed by NAT (2.6.21), detection of a viral antigen (e.g. by p24 protein ELISA) or marker-rescue assay. No RCV are detected.**

❖ **Reference to 5.34 implies RCV testing on producer cells used for retro-/lentiviral vector production. No RCV are detected**

Additional information on gene therapy medicinal products for human use (5.34)

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-2. Vectors used in production of genetically modified autologous cells

- **Identification**
- **Genomic integrity**
- **Physical particle titre**
- **Functional titre**
- **Functional/physical particle titre**

- **RCV**
- **Residual hc DNA, pDNA, HCP, reagents**
- **Sterility (2.6.16)**
- **BET (2.6.14 or 2.6.32)**
- **Biological activity or expression from the genetic insert**

❖ **Biological activity determined by a suitable *in vitro* test using a suitable cells or cell line.**
***In vivo* test – when justified and only if *in vitro* not possible.**
Expression from the genetic insert(s) determined on a permissive cell line following inoculation of the vector ensuing consistent transduction (e.g. at a predetermined MoI)

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-3. Human autologous cells – collection and initial preparation

Collecting centres to comply with all regulations and directives applicable to the donation, procurement and testing of human cells

General provisions for production of GTMPs (2.1) also **applicable to the initial cell** isolation and separation steps

Cell **storage** and **shipment** from the site of procurement under **suitable and controlled conditions**

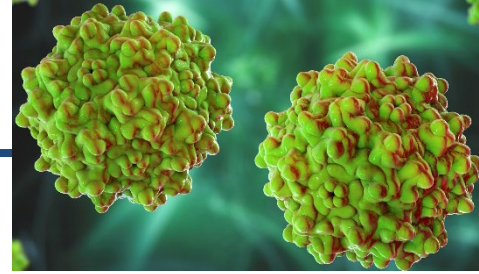
3-4. Production

Chain of identity ensured; **Surplus** of cell **can** be stored at the production site under suitable conditions; Relevant tests for cell identity, viability and other tests at critical production steps

3-4-1 Cell culture
Culture of cells under defined conditions and time to induce activation or proliferation → populations of interest may be enriched or actively selected

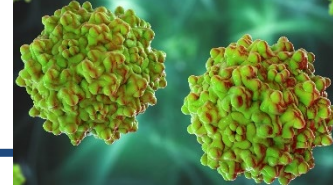
3-4-2 genetic modification
Consistent transduction e.g. using predetermined MoI using a validated and controlled process; limits for % transduced cells and VCN/transduced cell defined

3-4-3 expansion and harvest
performed under validated and controlled culture conditions for a pre-defined duration; process demonstrates adequate clearance of process-related impurities; process ensures the vector particles in the final product below a suitable level



Can RCV be tested on the vector used for genetic modification instead of on genetically modified autologous cells?

- ❑ Yes
- ❑ No
- ❑ I don’t know



Can RCV be tested on the vector used for genetic modification instead of on genetically modified autologous cells?

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-5. Final lot

3-5-1 Identification

3-5-2 Tests

Appearance e.g. (2.2.1 & 2.2.2)	Process-related imp.
Particulate contamination	BET (2.6.14 or 2.6.32)
Nucleated cell count (2.7.29)	Mycoplasmas (2.6.7)
Viability (2.7.29)	VCN/transduced cell
Cell population purity (e.g. 2.7.24)	% transduced cells
Microbiological examination (2.6.27)	**RCV**

3-5-3 Assay

- RCV on the list of tests to be performed for autologous GM cells
- Single list of tests to be performed at appropriate stages
- RCV to be tested for vectors used for modifications
- Clarification included in the monograph

*If appropriate testing is performed at the level of the viral vector used for genetic modification of cells, the **test** for replication-competent viruses **may be omitted** in the final lot as authorised by the competent authority.*

☑ Yes*

***as authorised by the CA**

Individual sections – focus on GM autologous cells section

Gene therapy medicinal products for human use (3186)

3. Genetically modified human autologous cells modified by integrating retroviral or lentiviral vectors

3-5. Final lot

3-5-3 Assay

- **Number of transduced cells** determined by a suitable method (e.g. flow cytometry) within the limits approved
- **Expression from a genetic insert** determined by a suitable method and complies with the pre-defined acceptance criteria
- **Biological activity** determined by a suitable method within the limits approved. Appropriate surrogate assays correlating to biological activity may be used
- **Differentiation potential** (for products containing stem or progenitor cells) is determined unless otherwise justified and authorised and it complies with the pre-defined acceptance criteria

RCV – an overview

Genetically modified cells

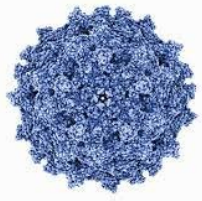
Vectors used for modification: RCV absent

Final lot: RCV absent*

* omission possible if appropriate testing performed on the viral vector

Recombinant vectors

Product-related impurities determined by suitable methods



AAV/AdV

Vector construction: reduction of rcAAV risk by limiting sequence homology between starting materials

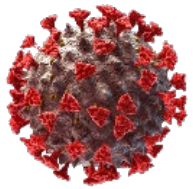
Viruses used for production: No rc helper viruses detected*

* if replication-incompetent vector used

Purified harvest: The limit as approved by the CA

Vector seed lot*: no RCV detected

* AdV only



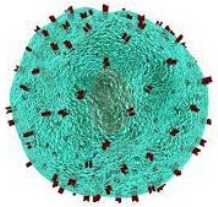
RV/LV

Vector construction: design to prevent RCV

Producer cells: no RCV detected

Single/purified harvest/final lot*: no RCV detected

*if no dilution and no pooling



GM autologous cells modified by LV/RV

Vectors used for modification: RCV absent

Producer cells for vector production: RCV absent*

* By reference to 5.34

Final lot: RCV absent*

* omission possible if appropriate testing performed on the viral vector

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**