General Chapter 5.2.12. Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

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General Chapter 5.2.12 Regulatory framework
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

- Directive 2001/83/EC

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on the Community code relating to medicinal products for human use
A biological medicinal product is a product, the active substance of which is a biological substance. A biological substance is a substance that is produced by or extracted from a biological source and that needs for its characterisation and the determination of its quality a combination of physicochemical-biological testing, together with the production process and its control.

The following shall be considered as biological medicinal products: (…); advanced therapy medicinal products as defined in Part IV of this Annex.

Any other substances used for manufacturing or extracting the active substance(s) but from which this active substance is not directly derived, such as reagents, culture media, foetal calf serum, additives, and buffers involved in chromatography, etc. are known as raw materials.
General Chapter 5.2.12  Regulatory framework: Ph. Eur.
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

- 5.14 Gene transfer medicinal products for human use
  - Chapter for information

**PRODUCTION**
Substances used in production. The raw materials used during the manufacturing process, including viral seed lot and cell bank establishment, where applicable, are qualified. Unless otherwise justified, all substances used are produced within a recognised quality management system using suitable production facilities. Suitable specifications are established to control notably their identity, potency (where applicable), purity and safety in terms of microbiological quality and bacterial endotoxin contamination. The quality of water used complies with the relevant corresponding monographs (Purified water (0088), Highly purified water (1927), Water for injections (0169)). Where bovine serum is used, it complies with the monograph Bovine serum (2262). The use of antibiotics is avoided wherever possible during production.

SUBSTANCES USED IN PRODUCTION
The quality of substances used in production may be critical with respect to the quality, safety and efficacy of the final product, particularly for substances of biological origin. This is of particular importance for:
- proteins, including enzymes and antibodies;
- cryopreservation reagents;
- purification reagents.
Quality assurance. All substances must be produced within a recognised quality management system using suitable production facilities.

**Quality specifications.** A suitable quality specification must be presented for each substance, including notably:
- identity;
- potency (where applicable);
- purity;
- determination of bacterial endotoxins (2.6.14) (where applicable);
- microbiological quality (total viable count, tests for specified micro-organisms);
- sterility (2.6.1) (where applicable).

**Viral safety.** The requirements of chapter 5.1.7 apply.

Transmissible spongiform encephalopathies (5.2.8). A risk assessment of the product with respect to transmissible spongiform encephalopathies is carried out, and suitable measures are taken to minimise any such risk.

Water. Water used in the preparation of cellular products complies with the relevant monograph (Water for injections (0169), Water, highly purified (1927), Purified water (0088)). Water incorporated into the final product complies with the section on Water for injections in bulk in the monograph Water for injections (0169), and in addition is sterile.
General Chapter 5.2.12 Regulatory framework: Ph. Eur.

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

- 5.14 Gene transfer medicinal products for human use
- Human haematopoietic stem cells (2323)

Monographs on
- Insulins (0838, 1637, 1638)
- G-CSF (2206)
- GM-CSF (1641)
- Erythropoietin (1316)
- Interferon gamma (1440)
- Bovine serum (2262)
- Trypsin (0694)

Caution: these requirements for substances used as APIs might not be adapted to raw materials.

=> Need for requirements specific to raw material

General Chapter 5.2.12 Initial phases

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

Cooperation between EDQM and EMA

- User’s needs identified
- Classes of raw materials defined
- Current variable practices to be harmonised

RCG WP

2012 June
Ph. Eur. Commission established
Raw Materials for the Production of Cellular and Gene Transfer Products Working Party

14 experts from Member States
+ 1 EMA representative
+ 1 observer

Survey to National Pharmacopoeia Authorities

2012 December

EDQM / EMA Symposium

2013 April
International Symposium
With manufacturers, regulators and users
**General Chapter 5.2.12  Aim**

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

- Overarching general chapter covers the quality requirements of raw materials of biological origin used for the production of cell-based and gene therapy products:
  - For information / Purpose is not to increase regulation
  - To harmonize variable practices
  - To identify the critical quality attributes of raw materials
  - To encourage raw materials manufacturers
    - to provide consistent, predefined quality
    - to record and share information on the origin and quality of the raw material
  - To help users to manage batch-to-batch variations and changes in raw materials

**General Chapter 5.2.12  Final phases**

Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

- **Public consultation**
  - Pharmeuropa (Issue 26.4: Q4 2014 – Q1 2015)
- **Final version preparation**
  - Comments received during public consultation were evaluated
- **Publication**
  - Ph. Eur. 9th Edition
  - Implementation date: 1 January 2017
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

1. Scope

- Applies to raw materials of biological origin
- Raw materials are used for manufacturing or extracting the active substance(s) but are not intended to form part of the active substance

- Applies to:
  - sera and serum replacement;
  - proteins produced by recombinant DNA technology;
  - proteins extracted from biological materials;
  - vectors.

- Not in the scope:
  - chemically synthesised raw materials: e.g. basal media (purely composed of chemicals);
  - Synthetic peptides or polynucleotides;
  - medical devices and plastics.

2. Risk Assessment

- Evaluation of the impact must be performed by the user

- Origin

- Risk factor evaluated in relation to the clinical benefit/risk

2. RISK ASSESSMENT

Evaluation of the impact of the raw material on the quality, safety and efficacy of cell-based/gene therapy medicinal products must be performed by the user of the raw material. No single measure or combination of measures can guarantee the quality, functionality and safety of a raw material for its intended use. Therefore, a risk assessment must consider the biological origin and traceability of the raw material, the production steps applied to it and the ability of the drug product manufacturing process to control or remove the raw material from the final medicinal product.

Any risk factor must be evaluated in relation to the clinical benefit/risk of the cell-based or gene therapy medicinal product. When evaluating the risk posed by the raw material to the final medicinal product, the exposure of a patient to residual amounts of raw material with potential harmful effects (e.g. adverse immune reactions) should be considered in relation to the clinical benefit/risk of the cell-based or gene therapy medicinal product.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

3. General requirements
Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Mat-batch), Storage, Labelling

➤ Three categories

The origin of the raw material and if relevant any biological substances used for the production of the raw material must be known. Special attention must be paid to risks related to the sourcing (including pooling) of the substances used for the production of the raw material. Depending on the source of the raw material and the substances used in its production, raw materials can be divided into 3 categories:

1) raw materials of human or animal origin;
2) raw materials produced using substances of human or animal origin;
3) raw materials free from substances of human or animal origin.

➤ Risks to be minimized
Due to the inherent risk of transmitting adventitious agents, it is recommended to minimise, wherever possible, the use of raw materials of human or animal origin. If such raw materials are required for the production of cell-based/gene therapy medicinal products, appropriate measures are taken to minimise the risks of transmitting adventitious agents such as viruses, prions, bacteria and protozoa.

➤ Traceability
For human blood and tissue-derived materials, only carefully evaluated donors who have been adequately tested for infectious transmissible agents may be used. These materials comply with appropriate EU and/or national legislation applicable to transplantation and transfusion. Tracability measures ensure each donation to be followed from the donation to the raw material and to the final product, and vice versa.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

3. General requirements
Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Mat-batch), Storage, Labelling

- Suitable quality management system

The production process is optimised to consistently minimise and/or remove adventitious agents and harmful impurities, whilst retaining the quality of the raw material. This can be achieved using one or a combination of the following measures:
- using validated inactivation/removal procedures such as gamma sterilisation or low pH during chromatography, where possible;
- demonstrating the ability of a production process to minimise, remove or inactivate adventitious agents or harmful impurities;
- testing for adventitious agents or harmful impurities.

A raw material is sterile and produced under aseptic conditions and/or subject to terminal sterilisation, unless otherwise justified. If the raw material is not sterile, the level of microbial contamination must be known.

Additives, such as stabilisers, may be added to the raw material. In cases where antibiotics and stabilisers of biological origin are used in the production of the raw material, their presence is justified and careful consideration is given to their selection, use, quality and concentration in the raw material, as well as their impact on the actual raw material itself.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

3. General requirements
Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Mat-batch), Storage, Labelling

3-3. GENERAL QUALITY REQUIREMENTS
Raw materials must meet pre-defined quality requirements for identity, purity and biological activity. In order to ensure the function of the raw material, it is subject to testing using appropriately qualified methods. The identity test must reflect the uniqueness of the raw material and distinguish it from other related or similar substances. Impurities include both process-related substances (e.g. in the case of recombinant proteins: host-cell-derived proteins (HCP), host-cell-derived DNA and vector-derived DNA (residual DNA), other biological or chemical substances) and product-related substances (e.g. aggregates and degradation products). The content of a raw material may be expressed either in absolute or relative terms. The assay for determination of biological activity may be used to establish the content.

3-3-1. IDENTIFICATION
The identity tests are specific for the particular raw material and address the molecular structure/composition or other relevant physico-chemical, biological or immunochemical properties. Methods used in the determination of biological activity and purity may also serve to identify the raw material. Identification may be carried out by comparison with a defined reference material or a representative batch of the raw material.

3-3-4. REFERENCE MATERIAL OR REFERENCE BATCH
An appropriate reference material or a representative batch of the raw material is used to perform the above-mentioned identification, tests and assay. Where available, the use of established reference standards, such as European Pharmacopoeia reference standards or WHO International Standards, is recommended.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

3. General requirements

*Origin, Production, General quality requirements (ID/Tests/Assay/Ref. Mat-batch), Storage, Labelling*

<table>
<thead>
<tr>
<th>3.3.2. TESTS</th>
<th>3.3.3. ASSAY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td><strong>Content.</strong> The content (e.g. protein content) composition of the raw material is determined by an appropriate qualified method.</td>
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<tr>
<td>Liquid or reconstituted freeze-dried raw materials comply with the limits defined for the particular raw material with regard to degree of opalescence (2.2.1) and degree of coloration (2.2.2).</td>
<td>Biological activity. Where relevant, the biological activity is determined by a suitable assay. Where relevant (e.g. for enzymes), the biological activity is expressed per milligram of total protein (specific activity).</td>
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<tr>
<td><strong>Solubility.</strong> Freeze-dried raw materials dissolve completely in the prescribed volume of reconstituting liquid within a specified time, at a specified temperature, as defined for the particular raw material.</td>
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4. Sera and serum replacements

4.1 Definition / 4.2 Production / 4.3 Identification / 4.4 Tests / 4.5 Assay

Typically complex biological mixtures

Sera from human or animal sources and serum replacements (including platelet lysates and other undefined growth additives, conditioned media, blood and other cellular components) are used as growth additives for cell culture. Sera and serum replacements used to promote cellular growth are typically complex biological mixtures, whose exact composition is not always possible to define. Due to this complex nature, special attention is given to verifying the consistency and performance of every batch.

4.2. PRODUCTION

Due to potential differences in quality between batches of serum, cell or tissue lysate, suitable measures are implemented to verify the consistency of each batch before using them as raw materials for the production of cell-based/gene therapy medicinal products. Because of the inherent risk of transmitting infectious agents from pooled plasma, pooled sera, or other derivatives from pooled allogeneic human blood or plasma, consideration is given to limit the number of donations which are pooled, unless sufficient methods for inactivation/removal of viruses are applied during production, where applicable.

For conditioned media, a cell bank system is preferred. The removal of the cells from the media must be ensured and potential impurities originating from these cells determined if possible.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

4. Sera and serum replacements

4.1 Definition / 4.2 Production / 4.3 Identification / 4.4 Tests / 4.5 Assay

4.3. IDENTIFICATION
It is recognised that the exact qualitative composition of sera and serum replacements may be difficult to determine. However, the approximate protein composition in both cases may be determined by, for example, protein electrophoresis. Where relevant, tests for total protein content or any chemical additives are performed. For human serum, the electrophoretic pattern corresponds to that of an appropriate serum reference batch. Alternatively, identity may be determined by comparison of albumin content with an appropriate serum reference batch. For serum replacements, the electrophoretic pattern or the use of markers secreted by cells/platelets may be used. Human origin is determined by a suitable immunochemical method (2.7.1), unless otherwise justified.

4.4. TESTS
See section 3.3-2.

Haemoglobin: where relevant, within the limits defined for the particular raw material.

Cell-derived impurities: where relevant, within the limits defined for the particular raw material.

Specific tests for viral contaminants. For bovine serum, the tests for viral contaminants specified in the monograph Bovine serum (2262) apply. For human serum, the tests for viral safety specified in the monograph Human plasma for fractionation (0853) apply.

4.5. ASSAY
The serum or serum replacement must show cell growth promoting properties that are within the limits defined for the particular raw material. More than one type of assay may be necessary to show suitability for the intended use.
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

5. Proteins produced by recombinant DNA technology

5.1 Definition / 5.2 Production / 5.3 Identification / 5.4 Tests / 5.5 Assay

- e.g. growth factors, cytokines, hormones, other proteins, mAbs

5.1. DEFINITION
Proteins and peptides produced by recombinant DNA technology, which are used as raw materials, include growth factors, cytokines, hormones, enzymes and monoclonal antibodies.

Growth factors, cytokines and hormones are substances typically used for stimulation or inactivation, growth promotion or differentiation of cells in cell culture systems.

Other proteins: Enzymes (e.g. collagenases), as raw materials, may be used for extraction of active substances from tissues and/or fluids. Other proteins (e.g. fibronectin) may be used as culture supports or media components.

Monoclonal antibodies: Used as raw materials, they include immunoglobulins and fragments of an immunoglobulin with defined specificity. Antibodies can either be conjugated (chemically modified) or non-conjugated. Typical chemical modifications include fluorescent labelling and conjugation to magnetic beads. Antibodies, as raw materials, may be used for selection, activation/stimulation, isolation or purification of cells in cell culture.

5.4. TESTS
See section 3-3.2.

Host-cell-derived proteins and residual host-cell or vector DNA: Where relevant for the particular raw material, the content of residual host-cell or vector DNA and/or protein is determined using a suitable method unless the production process has been qualified to demonstrate suitable clearance. The content is within the limits defined for the particular raw material.

Related proteins: Related proteins (e.g. polyclonal antibodies with undefined specificities, glycoforms, degradation and oxidation products, oligomers and aggregates) are determined using liquid chromatography, electrophoretic or immunological methods and are within the limits defined for the particular raw material.

5.5. ASSAY
Content. The protein content is determined by an appropriate qualified method, for example by liquid chromatography (2.2.29) or UV spectrophotometry (2.2.25).

Biological activity: The biological activity of a recombinant protein is determined using, for example, cell proliferation, cell differentiation or an enzyme assay. Several acceptable bioassays may exist for a particular protein. For antibodies, cell-based immunoassays and assays based on ligand-binding and affinity may be used.

Where relevant, the biological activity is expressed per milligram of total protein (specific activity).
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

6. Proteins extracted from biological material

6.1 Definition / 6.2 Production / 6.3 Identification / 6.4 Tests / 6.5 Assay

- e.g. enzymes, polyclonal Abs, other proteins (e.g. albumin)
General Chapter 5.2.12 Overview
Raw materials of biological origin for the production of cell-based and gene therapy medicinal products

6. Proteins extracted from biological material
   6.1 Definition / 6.2 Production / 6.3 Identification / 6.4 Tests / 6.5 Assay

6-5. ASSAY
Content: The protein content is determined using an appropriate qualified method, for example by liquid chromatography (2.2.29) or UV spectrophotometry (2.2.25).
Biological activity: Where relevant, the biological activity of a protein is determined using, for example, enzyme assays, immunoassays or assays based on cell proliferation/differentiation. For trypsin, the assay may be performed as described in the monograph Trypsin (0694).
Where relevant, the biological activity is expressed per milligram of total protein (specific activity).

7. Vectors

- Usually considered as starting materials

- Reference to general chapter 5.14

Vectors that may be used as raw materials in the production of cell-based and gene therapy medicinal products include DNA vectors (e.g. plasmids, transposon vectors) as well as viral vectors and bacteria (e.g. modified Lactococcus species).
Vectors are usually considered as starting materials, thus not under the scope of this general chapter. In cases where vectors are not considered as starting materials, such as vectors used as helper plasmids or helper viruses, the principles of this general chapter and the principles of production and quality control as outlined in General Chapter 5.14 Gene transfer medicinal products for human use are to be followed.
Acknowledgement

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- RCG Working Party Members

Thank you for your attention