The new structure and content of the revised chapter 2.6.27 entitled “Microbiological examination of cell-based preparations”

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Scope of the revised 2.6.27 General Chapter

Cell based preparations

- Excluding the products covered by the Directive 2002/98/EC on Blood or Blood components.
- Excluding the medicinal products covered by the Regulation 1394/2007/EC (ATMP).

Preparations covered by the Tissue Directive 2004/23/EC

These products are industrially manufactured as routinely prepared according to a Standard Operating Procedure.

Due to the current legislation (human cell, tissue and organ cannot be sold) they are commonly prepared in public or non-for-profit entities.

Most of these entities are strictly linked to the national Health System as Hospitals or Blood Centres

Examples :

- Haematopoietic stem cells either from bone marrow, peripheral blood or cord blood
- Pancreatic Islets preparation
- Skin graft

Microbiological characteristics of cell based preparations

- Live cells cannot be subjected to a sterilization process.
- May carry infective agents either on the cell surface or absorbed in the cytoplasm.
- May harbour latent infective agents integrated in the genome.

Risk factors

Each batch is derived from a single individual with a peculiar medical history. (Variety of possible infective agents in the source material)

The media and physical conditions used to manipulate and store the cells are by definition able to allow survival, if not proliferation, of living organism. (possibility of rapid growth of microbial contamination)

2-2. HANDLING CONSTRAINTS

- **Shelf-life** - If not cryopreserved, the shelf-life range from hours or few days.
- **Sample composition** - In some cases, the cell-based preparation itself can inactivate contaminating micro-organisms resulting in a false negative.
- **Sample size** - The total volume of a batch could be reduce to less than 50mL, resulting in limitation to the sampling.

1-4. RATIONALE FOR METHOD SELECTION

The following approaches to microbiological examination may be applied:

- automated growth-based methods;
- a combination of preculturing and detection by alternative methods (5.1.6);
- direct detection by alternative methods (5.1.6);
- methods based on the sterility test prescribed in general chapter 2.6.1.

3-1-2. Method suitability

Due to the heterogeneity of the cell based preparation sourcing, content and manufacturing procedure the suitability of the method is to be confirmed in the presence of the specific sample composition.

Main Changes to the previous version

a) greater flexibility for the incubation temperature(s) and examples of temperature settings where the test volume allows 2 incubation conditions.

b) the list of micro-organisms used for method validation, Yersinia enterocolitica is replaced by Micrococcus sp., because it is more appropriate as an example of a common contaminant of cell-based preparations.

c) Information about the sensitivity to be achieved during validation has also been included.

Main Changes to the previous version

The revision has also been an opportunity to refer to general chapter 2.6.1. Sterility, which may be applied, and to introduce alternative rapid test methods, to be used with or without a pre-incubation step, by referring to general chapter 5.1.6. Alternative methods for control of microbiological quality.

Main Changes to the previous version

An introduction has been added with a rationale for method selection according to the characteristics and constraints inherent to the cell-based preparation to be tested.

The revised general chapter also includes considerations and recommendations concerning sampling, the sample composition, and ‘negative-to-date’ results.

Thans for your attention

Regulatory overview on Rapid Microbiological Methods for the control of cell therapy products

Violaine Closson, ANSM

11 October 2017, STRASBOURG, FRANCE

INTERNATIONAL MICROBIOLOGY SYMPOSIUM

1. **What is a cell therapeutic product, what is an Advanced Therapeutic Medicinal Product (ATMP)?**
2. How ATMPs are regulated in Europe
3. Microbiological testing
   1) Risk related to the cell therapy products
   2) French experience
   3) 2.6.27 versus 2.6.1

1- What is a cell therapeutic product, what is an Advanced Therapeutic Medicinal Product (ATMP)?

Definition: Products or medicinal products containing **cells** derived from human tissue and cells

Regulations:

- **Directive 2004/23/CE on human tissues and cells**

  Cell and tissue *preparations* (human tissues and cells)

  DIRECTIVE 2004/23/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
  of 31 March 2004
  on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells

- **Regulation 1394/2007**

  Advanced Therapeutic *Medicinal Products* (ATMPs)

  REGULATION (EC) No 1394/2007 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
  of 13 November 2007
  on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004

How to differentiate an ATMP from a cell preparation?

The differences between cell preparations and medicinal products are based on:

- the **level of manipulation** during manufacturing process (**non-substantial/substantial manipulation).**

*Have the cells or tissue(s) been manipulated during the manufacturing process so that their **biological characteristics, physiological functions or structural properties have been modified to be relevant for their intended function?***

- **the homologous/non-homologous use of the cells.**

*Are the cells used for the **same essential function in donor and recipient**?*

Classification depends not only on **the product** (substantial manipulation), but also on the **therapeutic application** (non homologous use)

The **Committee for Advanced Therapies (CAT)** is in charge to classify the ATMPs (*Regulation 1394/2007*)

What is a substantial manipulation during the manufacturing process?

Non substantial manipulations are listed in Annex I of Reg 1394/2007

- cutting
- grinding
- shaping
- centrifugation
- soaking in antibiotic or antimicrobial solutions
- sterilization
- irradiation
- cell separation, concentration or purification
- filtering
- lyophilization
- freezing
- cryopreservation
- vitrification

Substantial manipulations

- cell expansion (culture)
- genetic modification of cells
- differentiation/activation with growth factors,
- enzymatic digestion (to destroy cell to cell interactions)
- etc.

Examples

- **Haematopoietic stem cells**
  - Collected from bone marrow, blood cord, apheresis
  - No substantial manipulation
  - The essential function of the HSC is the « hematopoietic or immune reconstitution" = homologous use
    - For HSC transplantation/graft, it is a homologous use **= not ATMP**
    - For orthopedic use, regeneration of cartilage and bone, it is not homologous use **= ATMP**

- **Skin / keratinocytes**
  - Homologous use: skin substitute
  - Substantial modification
    - Skin graft used such as = **not ATMP**
    - keratinocytes isolated from skin biopsy by enzymatic digestion with the destruction of the tissues architecture and functional interactions of the cells = **ATMP**

Definition of ATMPs

Regulation 1394/2007/EC modifying Directive 2001/83/EC

A new class of medicinal products

- Gene therapy medicinal product (GTMP)
- Somatic cell therapy medicinal product (SCMP)
- Tissue engineered product (TEP)
- Combined advanced therapy medicinal product

1. Gene therapy medicinal product (GTMP)

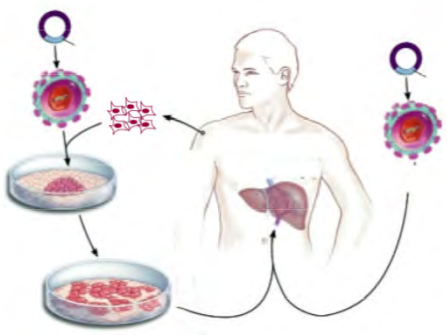
Gene therapy medicinal product means a **biological** medicinal product which has the following characteristics:

(a) *it contains an active substance which **contains or consists of a recombinant nucleic acid** used in or administered to human beings with a view to **regulating, repairing, replacing, adding or deleting a genetic sequence;***

(b) *its therapeutic, prophylactic or diagnostic **effect relates directly to the recombinant nucleic acid sequence** it contains, or to the product of genetic expression of this sequence.*

Gene therapy medicinal products **shall not include vaccines against infectious diseases.**

EX VIVO GENE THERAPY



IN VIVO GENE THERAPY

STRIMVELIS TG
ZALMOXIS TC
CAR-T cells

GLYBERA
IMLYGIC

Genetically modified cells are usually considered as GTMP

2. Somatic cell therapy medicinal product

Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics:

*(a) contains or consists of cells or tissues that have been subject to **substantial manipulation** so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that **are not intended to be used for the same essential function(s) in the recipient and the donor;***

*(b) is presented as having properties for, or is used in or administered to human beings with a view to **treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.***

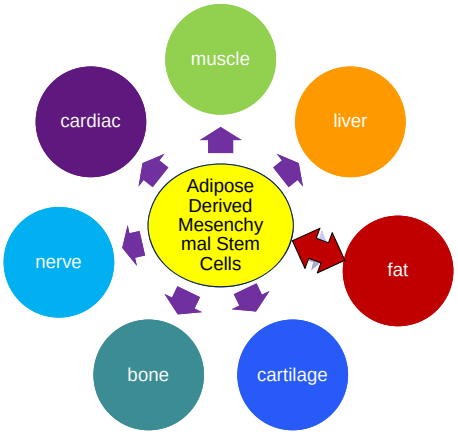
3. Tissue engineered product

Tissue engineered products means a product that:

*(a) contains or consists of **engineered** cells or tissues, and*

*(b) is presented as having properties for, or is used in or administered to human beings with a view to **regenerating, repairing or replacing a human tissue.***

Case of the adipose derived mesechymal stem cells



Essential function of fat tissue is to restore fat tissue

- **As a natural lipofiller = not ATMP**
- **For autologous treatment or auto-immune diseases = SCTP**
- **For cheloid scars = TEP**

4. Combined Advanced Therapy Medicinal Products

A Combined advanced therapy medicinal product means an advanced therapy medicinal product that fulfils the following conditions:

a) *it must incorporate, as an integral part of the product,* ***one or more medical devices*** *within the meaning of Article 1(2)(a) of Directive 93/42/EEC or one or more active implantable medical devices within the meaning of Article 1(2)(c) of Directive 90/385/EEC, and*

b) *its cellular or tissue part must contain* ***viable cells or tissues****, or*

c) *its cellular or tissue part containing* ***non-viable cells or tissues must be liable to act upon the human body with action that can be considered as primary*** *to that of the devices referred to.*

To summarize

ATMPs	Characteristics/effects	
Gene therapy medicinal product	recombinant nucleic acid	To regulate, repair, replace, add or delete a genetic sequence (direct effect)
Somatic cell therapy medicinal products	Substantial manipulation or/and Non homologous use	To treat, prevent or diagnose a disease through pharmacological, immunological or metabolic action
Tissue engineered products	Substantial manipulation or/and Non homologous use	To regenerate, repair or replace a human tissue
Combined ATMP	Medical device + cell/tissue part	

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2 - How ATMPs are regulated ?

- **Regulation 1394/2007/EC** modifying Directive 2001/83/EC

Specific rules concerning the authorisation supervision and pharmacovigilance of ATMPs

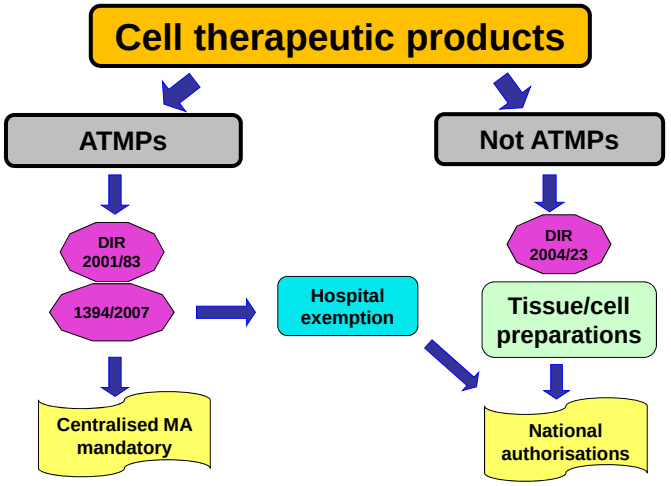
- Creation of the **Committe for Advanced Therapies (CAT) =** the committee at the European Medicines Agency that is responsible for assessing the quality, the safety and efficacy of ATMPs and following scientific developments in the field
- Centralised procedure mandatory
- Risk management Plan and follow-up of safety and efficacy
- Cell and tissue donation procurement and testing in compliance with Directive 2004/23

- **Regulation 668/2009/EC**

  Evaluation and **Certification** of Non-clinical & Quality data of ATMPs for micro-small and medium-sized enterprises (SME)

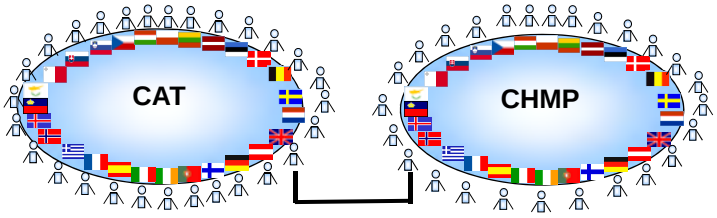
- **Directive 2009/120** amending Directive 2001/83/EC

  replaces part IV of Annex 1 (Module 3, 4 & 5**, Risk Based Approach** Guideline)



Hospital exemption *= ATMPs which are prepared on a* ***non-routine basis*** *according to specific quality standards, and* ***used within the same Member State*** *in a* ***hospital*** *under the* ***exclusive professional responsibility of a medical practitione****r, in order to comply with an* ***individual medical prescription*** *for a* ***custom-made product*** *for an* ***individual patient (Reg. 1394/2007 art 28).***

CAT: Committee for Advanced Therapies



Composition:
Multidisciplinary scientific experts representing all European members states as well as patients and medical association:

- 28 national experts and alternates including 5 joint members from the CHMP and 2 joint members from the scientific advice working party + Norway and Iceland
- 2 members and alternates represent patients organisations and
- 2 members and alternates represent clinicians

CAT covers the scientific areas relevant to advanced therapies, including medical devices, tissue engineering, gene therapy, cell therapy , biotechnology, surgery, pharmacovigilance, risk management and ethics.

First meeting in January 2009

Tasks of the CAT

- Classification: to provide advice on whether a product falls within the definition of an ATMP
- Marketing **Authorisation** Evaluation: to formulate a **draft opinion** on the quality, safety and efficacy of an ATMP for final approval by the CHMP
- Scientific Advice: to advise on any medicinal product which may require, for the evaluation of its quality, safety or efficacy, expertise in one of the scientific areas
- Certification (quality and non-clinical) incentative for Small and Medium Enterprises
- Guidelines to assist scientifically in the elaboration of any documents related to the fulfilment of the objectives of this Regulation
- Support to other Committees

ATMP overview in Europe (2009-2016)

- > 500 clinical trials using ATMPs in EU
- ~ 230 ATMP classifications
- 215 scientific advice requests
- 15 MAAs reviewed
- **9 ATMPs approved**, but 4 withdrawn
- **5 licensed ATMPs**

Approved ATMPs under centralised procedure

Name	ATMP	MA	Product/indication
ChondroCelect	**TEP**	2009 Withdrawn 2016	**Autologous cartilage cells** **Repair of cartilage defects of the knee**
Glybera	**GTMP**	2012 Withdrawn 2017	**alipogène tiparvovec (AAV-1 – Lipoproteine lipase gene)** **Familial lipoprotein lipase deficiency (LPLD)**
MACI	**TEP**	2013 Suspended 2014	**Autologous chondrocytes** **Repair of cartilage defect sof the knee**
Provenge	**SCTMP**	2013 Withdrawn 2015	**sipuleucel-T (autologous PBMC activated with PAP-GMSF colony-stimulating factor)** **Prostate Cancer**
Holoclar	**TEP**	2015	**Autologous human corneal epithelial cells** **Moderate to severe limbal stem-cell deficiency caused by burns**
Imlygic	**GTMP**	2015	**talimogene laherparepvec (HSV-1-derived virus GM-CSF)** **Melanoma**
Strimvelis	**GTMP**	2016	**Autologous CD34+ cells transduced with retroviral vector encoding the human adenosine deaminase (ADA) cDNA sequence** **ADA-SCID deficiency**
Zalmoxis	**SCTMP**	2016	**Allogenic T cells modified with a suicide gene** **Adjunctive treatment in haplo-identical haematopoeitic stem cells transplantation of adult patients with high-risk haematological malignancies**
Spherox	**TEP**	2017	**Autologous matrix associated chondrocytes** **Repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee**

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3- Microbiological testing

3.1 Risk related to the cell therapy products

- **Specificity of cell therapy products:**
  - Fragile
  - Precious
  - Limited shelf life
  - Many cell therapy products cannot be cryopreserved without affecting viability and potency
  - Small size of the batch / limited sample volume
  - Cannot be terminally sterilized by filtration or other physical /chemical means
  - Microbial contaminants may be found out or inside the cells (microbiological /sterility testing cannot be limited to cell supernatant)
- **Safety issues: Infection**
- **Microbiological testing is a critical quality parameter for cell therapy products**

Risk identification

- **Origins of the contamination:**
  - Starting material (donor, collection practices)
  - Raw materials
  - Manufacturing process
  - Cleaning process
- **How should the risks and risk factors be addressed?**
  - Testing of the starting and raw materials
  - Validation of the aseptic manufacturing process,
  - Control of the manufacturing (Good Manufacturing Practices)
  - Microbiological testing in process
  - Microbiological testing on the final product (results after administration)
- **Limits**
  - Limited amount of material for testing
  - Limited time for batch release due to the short shelf life

3.2 French experience from tissue and cell preparations (2001-2013)

- 1998: French « Good Manufacturing Practices for tissue and cell preparations »
  ➡ Microbiological control mandatory.
- 1998: a questionnaire was sent to the cell producers to collect the practices
- 1999: establishment of a working groups with experts to establish standard recommendations for microbiological controls
- 1999-2013: market survey
  - 31 centers (cell banks)
  - Each operator sent cell products of clinical grade to be controlled in parallel by the French Agency >1600 products were controlled
- 2001-2013: collaborative studies and external controls to standardize and validate the microbiological methods
- 2006-2012: collection of contaminants found in cell preparations
- In 2013: 90% of the French sites use an automated growth-based system (BACTEC® or BacT/ALERT®) with an incubation ranging from 7 to 10 days

Automated growth-based system – results of the French studies

- Compared to direct innoculation, the results from the automated growth-based systems show:
  - More adapted to the low available sample volume
  - Better detection for slow growth germs
  - More sensitive 1 to 10 ufc/ml
  - Less false positive results
- Results confirmed by literature
- French standardized recommendations published in 2002
- Publication of the first version of 2.6.27 chapter in 2007

Contamination rate of the haematopoietic stem cells after collection (before manufacturing)

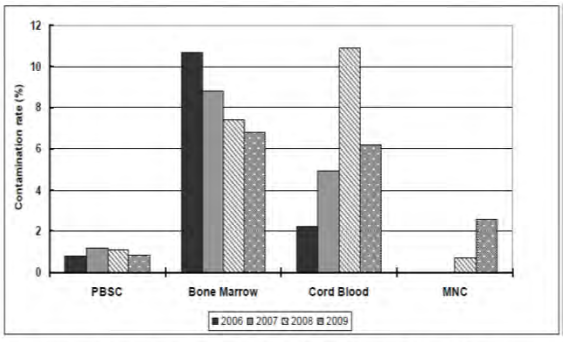

Fig. 9. Contamination rates are represented according to the year and to the haematopoietic product in 2006, 2007, 2008 and 2009.

Follow-up from 2006 to 2009
French sites

Contamination rate in 2009:
Peripheral Blood Stem Cells (PBMC) 1%
Bone Marrow 7%
Cord blood 6%
Mononuclar cells (MNC) 2%

Importance of disinfection procedure and aseptic practices for cell procurement

Panterne B., Richard M.-J., Sabatini C., Ardiot S., Huyghe G., Lemarié C., Pouthier F. and Mouillot L. (2011). Ten Years of External Quality Control for Cellular Therapy Products in France, Progress in Molecular and Environmental Bioengineering - From Analysis and Modeling to Technology Applications, Angelo Carpi (Ed.), ISBN: 978-953-307-268-5, InTech

3.3 How to choose between 2.6.1 and 2.6.27?

EUROPEAN PHARMACOPOEIA

- **2.6.1 Sterility**
- **2.6.27 Microbiological Examination of Cell-based Preparations**

2.6.1

- Sample size:
  - 1 ml for 1-40ml batch size
  - **20 ml for 40-100ml** batch size
  - 10% for >100ml but not les than 20ml
- Cannot be filtered on membrane (incompatibility)
- Direct innoculation of the cell suspension
- Detection: measure of the turbidity (false positive)
- Long incubation period (14 days)

2.6.27

- Small sample size – adapted for a batch size between 1ml and 1000 ml
  - 100 µl for a total cell volume <10ml
  - 1% of total cell volume between 10 and 1000 ml (**1ml for 100ml**)
- Detection: measure of the CO_2: less false results
- More sensitive
- Fastest

2.6.27 more adapted to cell therapeutic products

- **ATMP**
  - Not a « sterile product » as sterility is defined
  - No legal requirements for ATMP in Ph. Eur.

  (monography of *Human Haematopoetic Stem Cells* is the only one that refers to 2.6.27).

- **ATMP microbiological testing**
  - 2.6.1 or 2.6.27
  - 2.6.27 more adapted
  - No cross validation of 2.6.27 with 2.6.1 is required.
  - Case by case validation is required for 2.6.27 regarding the method suitability

Conclusion

- ATMPs are medicinal products
- Centralised procedure except for the « hospital exemption »
- Dedicated Committee: CAT
- Microbiological contamination is a **critical safety risk** for the patient
- 2.6.27 more adapted
- Need to have robust and fastest methods

Thank you for your attention

ansm
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Paul-Ehrlich-Institut

Microbiological Safety of Cell and Gene Therapy Medicinal Products – From *Status Quo* to Paradigm Change

EDQM
International Microbiology Symposium
11th October 2017, Strasbourg, France

Jan-Oliver Karo
Paul-Ehrlich-Institut

Disclaimer

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Selection of presented methods does not reflect in any way endorsement of a particular technology.

Agenda

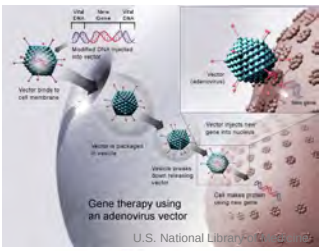
- **Introduction**
- Microbiological Safety of ATMPs
- Case Studies & Experiences

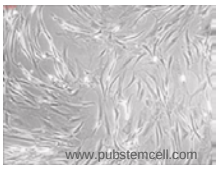
Advanced Therapy Medicinal Products (ATMPs)

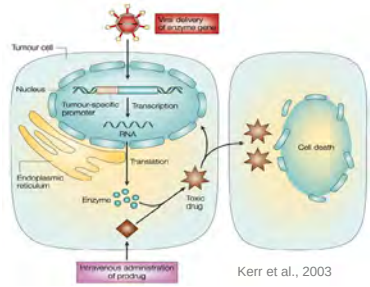
Medicinal products (for human use): "*Any substance or combination of substances presented for treating or preventing disease in human beings* […]" Dir. 65/65/EEC

Gene therapy medicinal products	Somatic cell therapy medicinal products	Tissue engineered products
Genetically modified cells		
		
Regulation, repair, replace-ment, addition or deletion of a genetic sequence	Pharmacological, immunological, metabolic action	Regeneration, repair or replacement of cells / tissues

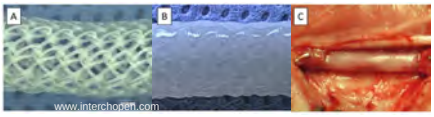
ATMPs - Examples







Kerr et al., 2003



Agenda

- Introduction
- **Microbiological Safety of ATMPs**
- Case Studies & Experiences

Agenda

- Introduction
- **Microbiological Safety of ATMPs**
  - Source / Starting Materials
  - Manufacturing Process & Controls
  - Mycoplasmas
- Case Studies & Experiences

Source / Starting Materials

- Heterogeneous material
  - e.g. solid organs, bone marrow, adipose tissue, mucosal cells, skin cells,…
  - Banking system (cell banks) ⟺ primary origin
  - Primary “sterile” ⟺ primary “unsterile”
  - Autologous ⟺ allogeneic
  - Living ⟺ deceased donors

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  - Autologous ⟺ allogeneic
  - Living ⟺ deceased donors
- Variable conditions at procurement (type of biopsy, procurement procedures,..)

Source / Starting Materials

Donor Exclusion

- *Systemic infection which is not controlled at the time of donation, including bacterial diseases, systemic viral, fungal or parasitic infections, or significant local infection in the tissues and cells to be donated.*
- Chronic bacterial infections e.g. brucellosis, typhus, leprosy, relapsing fever, melioidosis and tularemia

Tissues & cells: Dir. 2004/23/EC, Dir. 2006/17/EC, “German Hemotherapy GL”

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Certain bacterial agents may not be detectable in classical sterility test

Source / Starting Materials

- Limitations of donor exclusion criteria -> subclinical infections
- Establishment of (well-characterized) cell banks usually not possible
- Viable human starting material (cells / tissues)
  - Sterilization not possible (> 90 %)

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- Viable human starting material (cells / tissues)
  - Sterilization not possible (> 90 %)

➡ **Sterility of source material cannot be "guaranteed"**

Microbiological contamination rates of over 90% have been reported – after "aseptic" procurement of "primary sterile" material

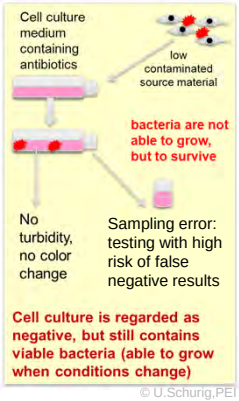
Antibacterial / -fungal Agents

Antibacterial / -fungal Agents

Pro:
Reduction / elimination of initial bioburden

Cons:

- Masking of contamination
  -> micro-organisms not detectable in control due to growth depression, false negative results ("improved sample error")
- Concentration of antibiotics in vitro usually higher than in vivo
  -> depression in culture but growth in vivo causing infection of patient ("tissue level of antibiotics")
- Different antibiotic susceptibility profiles
  -> resistances, no cidal activity, intracellular location



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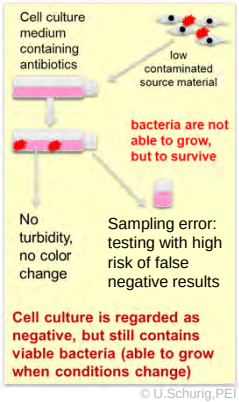

Exclude antimicrobial agents as early as possible from the manufacturing process (e.g. in 2nd half)

Use adequate microbial testing strategies (low-binding membranes, neutralization agents, etc.)

Agenda

- Introduction
- **Microbiological Safety of ATMPs**
  - Source / Starting Materials
  - Manufacturing Process & Controls
  - Mycoplasmas
- Case Studies & Experiences

Manufacturing Process – Challenges for the Microbial Safety

- Large sample amounts are often not available or of great “value”
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➡ Culture supernatants are tested, which may not represent the microbial situation of the product (cell-associated / intracellular micro-organisms)

Always include the cellular matrix, if not otherwise justified.

Raw Materials

- Challenge: research / non-GMP grade
- Raw materials of human / animal origin (e.g. FCS, peptones)
- Trend: switch to plant-derived materials

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Provide CoAs with submission docs

Raw Materials – Case Studies

Case #1:

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➡ **Routine sterility test for incoming materials**

Raw Materials – Case Studies

Case #2:

- Three contaminated GT-Product batches (CAR-T Cells)
- MO: *Leifsonia* sp.
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-> False CoAs

Sterilisation

Sterilisation

- For CTMPs and most GTMPs not possible (> 90 %)
- Terminal sterilization is not applicable

Sterile Filtration:

- Ph. Eur. 5.1.1, EudraLex Vol.4 Annex 1
- Nominal pore size of 0.22 µm (or less) or equivalent retention capacity
- Monitor the bioburden prior filtration
- Filter integrity: verify before and after use (e.g. bubble-point test)

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0.2 µm-rated filter do not remove all viruses or mycoplasmas

ATMPs: Sterile Products?

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"Sterility is the absence of viable micro-organisms, as defined by a sterility assurance level equal to or less than 10^{-6}."

(Ph. Eur. 5.1.1 Methods of Preparation of Sterile Products)

"*Sterility of the medicinal product cannot be assured by testing […]*"

(EMA Draft "GL on the sterilisation of the medicinal product, active substance, excipient and primary container " EMA/CHMP/CVMP/QWP/BWP/850374/2015)

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*"…**nonsterile** does not mean the product is contaminated with microorganisms, but rather that its contents **have not been sterilized**, or treated with a process during manufacturing to eliminate potential microorganisms*." (FDA Q&A doc https://www.fda.gov/Drugs/DrugSafety/ucm374838.htm)

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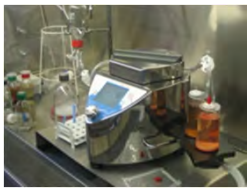
Testing via 2.6.1. does not render an ATMP "sterile"

Sterility Testing (Ph. Eur. 2.6.1; USP <71>)

Membrane Filtration



Direct Inoculation

Incubation: aerobic 20 to 25 °C
anaerobic 30 to 35 °C

14 d incubation: visual readout of turbidity ⇒ detection at ~10e7 - 10e8 CFU/mL

Turbidity of sample matrix
may impede readout

⇓

Sub-culture for at least 4 d -> ≥ 18 days incubation

CBMPs: 2.6.1. Sterility Test – Gold Standard or Old Standard?

Known deficiencies:

- Not all MOs are detected -> not a test for "sterility"
- Parameter turbidity: not adequate for all preparations & MOs
  - ➢ Subjective
  - ➢ Turbidity of sample may impede readout
    - -> No method performance -> sub-cultivation (risk for secondary contamination, extended cultivation)
  - ➢ Not all MOs reveal turbidity despite high titres!
    - -> Mycoplasma
    - -> Spiroplasma (detectable in Bactec, Aquilino et al. 2014)
- MF: Plugging of filter membranes (e.g. cells)
- Incubation period not suitable for many innovative products

Microbiological Control of Cellular Products (Ph. Eur. 2.6.27)

Direct Inoculation (DI)

Incubation: aerobic + anaerobic at 35 – 37 °C

REVISED

Manual

- 14 d incubation
- visual readout of turbidity

-> detection at 10e7 - 10e8 CFU/mL

Turbidity of sample matrix may impede readout

Comparable to 2.6.1 DI, but limited to one temperature -> benefit?

Automated detection systems

- Only 7 d incubation
- Continuous automated readout: e.g. CO_2-level via fluorescence or colorimetric sensor

Turbidity of sample matrix has no impact on readout

- Limited to one temperature
- 7 d sometimes not sufficient

Manufacturing Process & Release Control – The Impact of Time

Image from www.aufstellung-loesungsweg.de

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Duration of manufacturing process & shelf life of final ATMP are often extremely short compared to classical drugs (24 - 48 h, sometimes only a few hours)

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Manufacturing Process & Release Control – The Impact of Time

Duration of manufacturing process & shelf life of final ATMP are often extremely short compared to classical drugs (24 - 48 h, sometimes only a few hours)

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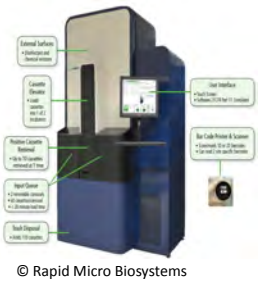
Innovative 21st century products / therapies pose novel demands towards microbiological methods.
How to address?

Image from www.aufstellung-loesungsweg.de

Rapid Microbiological Methods (RMMs)

Def.: Detection system yielding equivalent or better results than conventional (microbiological) methods, in less time.



© Rapid Micro Biosystems

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© bioMérieux

RMMs – Why?

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- Reduced time-to-result
  - Shorter production cycle -> ↑product availability
    -> Emergency cases (e.g. pandemic outbreaks, bioterrorism)
  - Faster investigations -> CAPAs
  - Results available before administration
    -> Improved product / patient safety

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  - ➢ Faster investigations -> CAPAs
  - ➢ Results available before administration
    -> Improved product / patient safety
- Potentially broader microbial detection range (non-growth-based methods)
  -> Viable but non-culturable (VBNC)

RMMs – Present Situation

- Broad range of technologies available, but often limited with respect to certain aspects (limit of detection, matrix-interferences, time-to-result, etc.)

  ⇒ Development / establishment of AMMs providing rapid results within 24 to 48 h is crucial for short shelf-lived products

  Proposed USP <71.1>

  ⇒ Broadening the range of similar methods -> backup option!

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    Proposed USP <71.1>

  - ➡ Broadening the range of similar methods -> backup option!
- There will be no “Jack of All Trades”- Approach, each method may have limitations, similar to traditional compendia methods (e.g. sterility test)

RMMs – Present Situation

- Need for paradigm change: “we don´t have to detect everything, but what is (clinically) relevant, within the given time frame”
- Introduction of RMMs is welcome by regulatory authorities
- Detailed guidance documents available (Ph. Eur. 5.1.6, PDA TR 33, USP <1223>, etc.), but different validation approaches may be acceptable
  - ➡ Early advice strongly recommended

Establishing RMMs – Case Study #1

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- Marketing Authorization Application (MAA)
- Cell therapy product
- Shelf life: < 20 h
- Release tests: culture automate & Gram stain

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Major objection:
Implementation of RMM providing results before product administration

Establishing RMMs – Case Study #1

- Seven methods, representing four distinct technologies were evaluated
- Result: Sample matrix showed high interference (background signals)

➡ None of the methods met the requirements

One promising method was not included. Vendor was "*unresponsive to attempts to collaborate on feasibility*"

Establishing RMMs – Case Study #1

- Attempts to “mediate” were without success
- Vendor obviously not “interested” -> other priorities
- No RMM currently “suitable” for the product / manufacturing-characteristics of the product (?)

EMA decision: “conditional” approval
-> Continue seek for RMM; provide updates with PSURs

Agenda

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- **Microbiological Safety of ATMPs**
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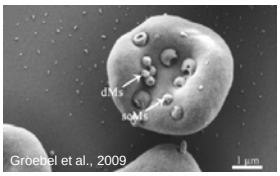
- Case Studies & Experiences

Mycoplasmas



- Ubiquitous bacteria (humans, animals, insects, plants, etc.)
- Smallest and simplest self-replicating organism (0.15 – 0.3 µm)
- Auxotrophic; fastidious and slow growth
- Lack of rigid cell wall
- Cell-associated / intracellular pathogen

Mycoplasmas – A Challenge

- Limitations of donor exclusion criteria
  -> Up to 20% of *M. pneumoniae* infections are subclinical (Spuesens EBM et al., 2013)
- Antimicrobial treatment:
  - Unsusceptible to “commonly” used antimicrobial agents (cell culture) that target the cell wall
  - Escape from antimicrobial treatment -> cell invasion (Hegde et al. 2014)

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- Usually no “visible” signs of contamination (media / culture changes), despite high titres (e.g. 10e7 CFU/mL)

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Probably the most prevalent & serious microbial contaminants in cell culture

Mycoplasma Control

- **Traditional**

  Ph. Eur. 2.6.7 culture methods (culture + indicator cell culture method)
  - Laborious (several sub-cultivations, evaluation of growth)
  - Long incubation period (up to 28 days)
  - Recommended sample amounts not available
  - Known limitations of growth-based methods (mycoplasmas are fastidious)

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⇨ **Usually not suitable for short shelf-life ATMPs**

Mycoplasma Control

- **Alternative Methods (Ph. Eur. 2.6.7)**

  (a) NAT-based concept

  Pros: - Significantly faster (several hours)
  - Potentially broader detection range (non-growth based)
    -> Detection of fastidious “uncultivable” strains (cultivar alpha)

  Cons: - Direct NAT not distinguishes between DNA from viable or dead mycoplasma -> risk of false-positives (quality of ingredients)
  - Careful primer selection

Mycoplasma Control

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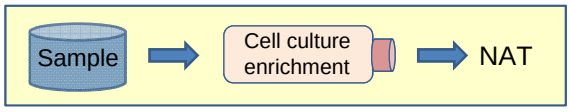
  (b) Hybrid approach



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  - Mycoplasmas
- **Case Studies & Experiences**

Lessons learned from Microbiological Assessment / Inspections

Case #1

Initial info: mycoplasma testing, validated & compliant with Ph. Eur. 2.6.7

After request: For the culture method, the sample is filtered through a 0.2 µm-rated membrane followed by two membrane washing steps (via filtration). Then the membrane is transferred to liquid medium for detection of potential mycoplasmas.

OK ?

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OK ?

- Not a compendial approach!
- Mycoplasmas can penetrate 0.2 µm-rated filters
- Filter not certified for that purpose

Lessons learned from Microbiological Assessment / Inspections

- Mycoplasma NAT: LOD > 10.000 CFU/ mL
- Sterile filtration with 0.45 µm-rated filters
- Method suitability test w/o intended sample
- Wrong incubation conditions
- False data evaluation -> false-negative (!)
- …

Image: www.goodleadership.com

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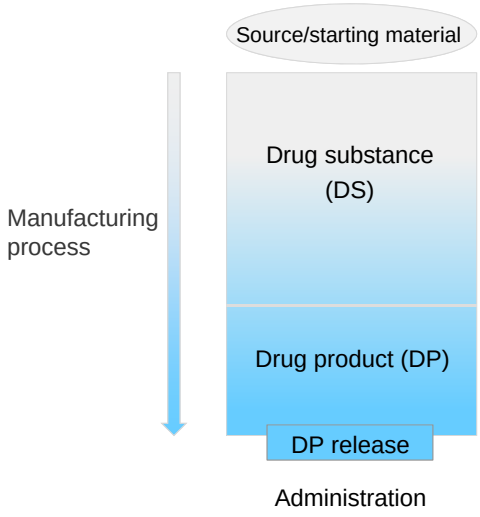
How far can you stretch safety?

Image: www.goodleadership.com

ATMPs - Manufacturing Flow Chart



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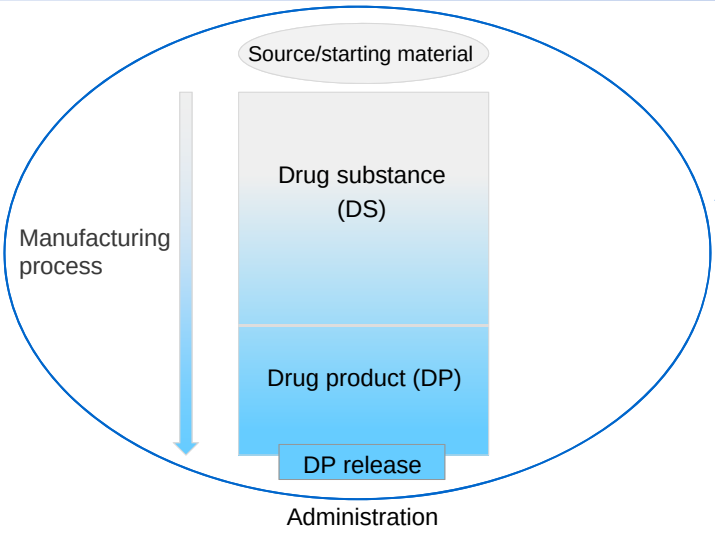

ATMPs – The Applicant as Challenge

- > 80% small & medium enterprises (SME), start-ups, research facilities, universities
- Focused on research, clinic / non-clinic aspects
- High percentage with < 50 employees

-> Limited human & financial resources
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Less than ~25% seek national advice before initial submission (PEI experience)

Conclusion

- The heterogeneous group of ATMPs provides promising new treatment concepts and opportunities
- Unsterile source material, sampling limitations and short shelf-lives represent major challenges for the microbiological safety concept
- Development / establishment of RMMs providing results within 24 to 48 h is crucial for short shelf-lived ATMPs
- There will be no “Jack of All Trades”- Approach, each method may have limitations, similar to traditional compendia methods (e.g. sterility test)

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“Never keep hurdles too high…nor too low!”

Thanks for Your Attention!

Acknowledgements

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Federal Institute for Vaccines and Biomedicines

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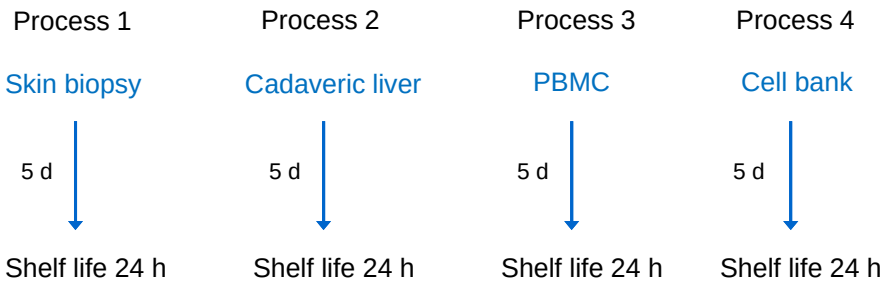
Agenda

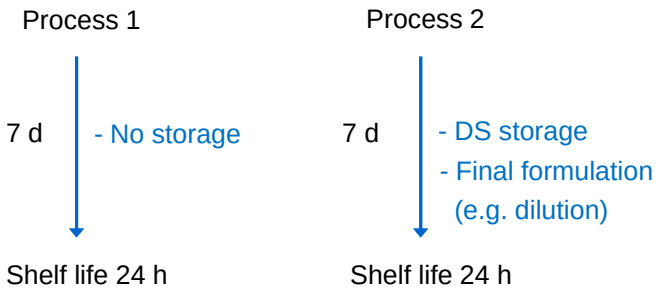
- Introduction
- **The Product is the Process**
- Microbiological Safety of ATMPs
- Case Studies & Experiences

Applications are different – Starting Material



Initial bioburden?

Applications are different – Shelf Life



Final microbiological results not available

Difference?

Applications are different

The Applicant´s (& Authorities) wishes:

- One clear microbiological safety concept
  - ➡ Processes & products are different “The product is the process”
- Clear requirements & statements
  - ➡ Guidance documents need some flexibility: Revision takes long time; no blocking of present **and** future development

Future: Development of Microbiological Safety Concepts specific for certain product groups (?)

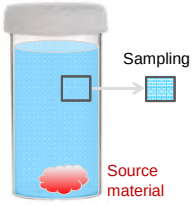
Source / Starting Materials – Challenges for the Microbiological Control

- Sample amount / matrix usually limited & of great “value”
  - ➡ Testing restricted to transport / storage solutions

Impact of transport / storage conditions on sampling error:

- Media composition -> growth support?
- Duration (e.g. 3 or 24 h)
- Temperature (e.g. 8 or 22 °C)
- Relative volume

➢ Detection of matrix-associated / intracellular micro-organisms?



Is this representative for the microbial status of the starting material?

Raw Materials

[…] substances such as reagents, culture media, foetal calf serum, additives, and buffers involved in chromatography, etc. used in the manufacturing or extraction of the active substance, but from which this active substance is not directly derived […].

(EMA/CHMP/BWP/429241/2013)

New Ph. Eur. 5.2.12.

"Raw Materials for the Production of Cell-based and Gene Therapy Medicinal Products"

Lessons learned from Microbiological Assessment / Inspections

Case #2

Initial info: microbiological control, validated & compliant with Ph. Eur. 2.6.27

After request: Submitted method suitability data (selection)

Microorganism	Titre (CFU)	Test start date	Test end date	Bactec vial	Result
Bacillus subtilis	100	11.10.2015	12.10.2015	Bactec Peds Plus/F	POSITIVE
Bacillus subtilis	100	11.10.2015	12.10.2015	Bactec + Anaerobic/F	POSITIVE
Clostridium sporogenes	100	11.10.2015	12.10.2015	Bactec Peds Plus/F	POSITIVE
Clostridium sporogenes	100	11.10.2015	12.10.2015	Bactec + Anaerobic/F	POSITIVE
Aspergillus brasiliensis	100	11.10.2015	12.10.2015	Bactec Peds Plus/F	POSITIVE
Aspergillus brasiliensis	100	11.10.2015	12.10.2015	Bactec + Anaerobic/F	POSITIVE

OK ?

- *Cl. sporogenes* grows aerobic
- *A. brasiliensis* grows anaerobic
- *A. brasiliensis* is detected in one day (aerobic & anaerobic)!

All shown in 3 repetitions!