Microbial Safety of Advanced Therapy Medicinal Products (ATMPs) – Screening strategies of cell and tissue based products

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Advanced Therapy Medicinal Products – what does it mean?

**Legislation**
- Medical Devices 93/42/EEC
- Medicinal Products 2001/83/EC

**Science**
- Medical Devices
- Tissue Engineering
- Cell Therapy
- Gene Therapy
- Biotech (e.g. insulin)
- Chemicals (e.g. aspirin)

**Advanced Therapies**
Tissue Engineered Products

- **Cartilage repair**
  Autologous chondrocyte transplantation (ACT)
  1st & 2nd & 3rd generation products

- **Skin regeneration**
  Different skin cells (keratinocytes, fibroblasts)
  in combination with a sheet-like matrices/scaffolds
  Acute wounds, diabetic foot skin ulcers

- **Bone regeneration**
  Osteoblasts or bone-marrow-derived stem cells combined
  with ceramic-based scaffolds or biomaterials

- **Cardiovascular regeneration**
  Hematopoetic stem cells for heart regeneration
  Engineered autologous/allogeneic blood vessels or heart valves

- **Complete organ engineering**
  Artificial lymph node
  Artificial liver
  Artificial engineered trachea

autologous coronary vessel
Cell Therapy Medicinal Products

- **Liver repair**
  Allogenic liver cell suspension for treatment of acute sepsis or inherited metabolic liver failure

- **Type I Diabetes**
  Allogenic pancreatic islets to restore insulin production

- **Skin repair**
  Different skin cell suspension for treatment of acute wounds

- **Immunotherapeutics**
  CTLs or NK cell transfer for adoptive immunotherapy

- **Cell-based therapeutic Vaccines**
  Peptide-loaded DC used as tumor vaccines to induce immunity towards tumor-associated antigens
  Fused Tumor/DC hybrid cells

- **Adult stem cells**
  MSC of different origin, e.g. treatment of anal fistula
1) Isolation of target viable human cells (autologous or allogeneic)

2) Human cells undergoing a manufacturing process or used not same essential function

3) Re-Infusion of viable human cells, may be associated with non-cellular components biomolecules, chemical substances or combined with structural materials
Mandatory virus tests for all tissue/cell donors

Directive 2006/17/EC ...as regard certain technical requirements for the donation, procurement and testing of human tissues and cells

The following tests must be performed at a minimum requirement:
- anti HIV 1/2
- HBsAg
- anti Hbc
- Anti HCV*
- Anti HTLV-1 for donors living in, originating from, high incidence areas or sexual partners originating from those areas or where the partners originate from those areas

* When anti-HBc is positive and HBsAg is negative, further investigations are necessary with a risk assessment to determine eligibility for clinical use.

No exclusion of viraemic window phase donations
No common European regulation for virus NAT
## Concept for Virus NAT in Germany

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Serology¹</th>
<th>HCV NAT²</th>
<th>HIV NAT²</th>
<th>HBV NAT²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Musculoskeletal tissue</td>
<td>Yes³</td>
<td>Yes⁴</td>
<td>Yes⁴</td>
<td>Yes⁴</td>
</tr>
<tr>
<td>Cardiovascular tissue</td>
<td>Yes³</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cornea</td>
<td>Yes⁵</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

¹Anti-HIV-1 and -2, anti-HCV, HBsAg, anti-HBc, TP HA.
²Single-sample or minipool NAT assays can be used. The required detection limit of the assay should, however, be based on the individual donation in analogy to blood donation.
³If anti-HBc is reactive, but anti-HBs $\geq 100$ IU/L and HBV DNA NAT is negative, the tissue can be used.
⁴NAT can be omitted if validated virus inactivation procedures are used that have been approved by the national regulatory authority.
⁵If anti-HBc is reactive and HBV DNA NAT is negative, the tissue can be used, additionally when the recipient has immunity against HBV.

HCV, hepatitis C virus; NAT, nucleic acid amplification testing; HIV, human immunodeficiency virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HBc, hepatitis core antigen; TP HA, Treponema pallidum hemagglutination assay.

[Pruss, Caspari, Krüger, Blümel, Nübling, Gürtler, and Gerlich. Transplant Infectious Disease12: 275-386, 2010]
Additional Viruses to be considered in specific cases

Directive 2006/17/EC:
“In certain circumstances, additional testing may be required depending on the donor’s history and the characteristics of the tissue or cells donated (e.g. RhD, HLA, malaria, CMV, toxoplasmosis, EBV, *Trypanosoma cruzi)*.“

Transmission of HSV by cornea has been reported

Transmission of rabies by cornea has been reported

Risk for HCMV, EBV, HHV6 etc. from leukocyte donations?
**Human/ animal-derived reagents in cell culture**

- Avoid human/animal serum whenever possible
- **Often, human donor serum (e.g. pooled AB serum) is used instead of fetal bovine serum. However, human serum is not considered more safe with respect to virus infections**
- Use only virus-inactivated serum whenever possible
- Use only virus inactivated porcine trypsin (e.g. 45 kGy or UV treated)
- Replace porcine trypsin by recombinant trypsin
- Evaluate other (research grade) reagents on their virus/TSE risk
Adventitious agents tests
Problems with cell therapy medicinal products

- Contamination from reagents (FBS, Trypsin etc.)
- Contamination from starting tissue/blood
- MCB or individually donated cells for direct culture
- Contamination from handling

14d Ph. Eur test on microbial sterility
14d to 28d *in vitro* adventitious virus assay?
*In vivo* virus assay?
Microbial Safety of (cell based) ATMPs – How to square the circle?

1. Revision of / amendments to existing regulations and establishment of new regulations
   International discussions (EDQM, EMA, FDA/CBER ...)

2. Advice of manufacturers

3. Development of novel approaches for Reliable Rapid Sterility Testing

4. Pyrogen testing of ATMPs by novel alternative tests (Monocyte Activation Test / MAT)

Sterility of source material cannot be guaranteed comparably to transfusion medicine where 0.2 to 0.5% of cellular blood components are bacterially contaminated.

Source material and final product cannot be sterilised e.g. by heat, gas, X-rays, filtration through 0.2 µm membranes.

Sterility testing is subject to “sampling error” i.e. the only potential result is: “The test sample is sterile” but gives no significant information on the whole volume of the product or intermediates.

Shelf life is frequently extremely short in comparison to usual drugs i.e. the established methods for sterility testing are not applicable.

Conventional pyrogen tests are not applicable for ATMPs rabbit test false positive endotoxin test false negative.

CONCLUSION:
Sterility of (cell based) ATMPs cannot be guaranteed!
3. Development of novel approaches for Significant Rapid Sterility Testing
February 2008: FDA Guidance

Many cell-based products cannot be cryopreserved or otherwise stored without affecting viability and potency. Most cell-based products are manufactured using aseptic manipulations because they cannot undergo sterile filtration or terminal sterilization (Ref. 2). Rapid and effective testing is needed because many cell-based products have a potentially short dating period, which often necessitates administration of the final product to a patient before sterility test results are available. Because of the challenges associated with cell-based products, there is a significant need to develop, validate, and implement sterility test methods that are more rapid than the sterility test methods described in 21 CFR 610.12.

Guidance for Industry
Validation of Growth-Based Rapid Microbiological Methods for Sterility Testing of Cellular and Gene Therapy Products

We, the Food and Drug Administration (FDA), are providing you, manufacturers of cellular therapy and gene therapy products, with recommendations on the validation of growth-based Rapid Microbiological Methods (RMMs) for sterility testing of your products. This guidance addresses considerations for method validation and determining equivalence of an RMM to sterility assays described in Title 21 Code of Federal Regulations, 610.12 (21 CFR 610.12). This guidance, when finalized, will address relevant issues and facilitate the implementation of an RMM for sterility testing. This guidance applies to somatic cellular therapy and gene therapy products. …
Computer based, automated culturing
sterility testing within 7 days

BACTEC
BD Diagnostic Systems

BacT/Alert
BioMerieux
Can we speed up Sterility Testing?
### Sterility

**Regulation:**
- Incubation for (at least) **14 days**

**Readout:**
- Turbidity

**Limit of detection:**
- \(10^7 - 10^8\) CFU/ml

**Culture Media:**
- **Anaerobic culture medium:** Fluid Thioglycollate Medium
- **Aerobic culture medium:** Soya-bean Casein Digest Medium (Tryptone Soy Broth, TSB)

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e.g. European Pharmacopoeia

2.6.1. Sterility
Question: Would it work to use **Flow Cytometry** instead of **turbidity** in order to detect bacteria much earlier in sterility testing?

**Klebsiella pneumoniae**

bacterial growth curve (determined by computer based impedance monitoring)

sample from early growth curve which was analysed in flow cytometer see yellow arrow in left graph
Solution:

Combination of
short pre-incubation of test sample
applying pharmacopoeial conditions
(aerobic and anaerobic liquid media)
and
rapid bacteria detection methods
(developed for PCs)
Rapid detection of different bacteria using flow cytometry after short pre-incubation in pharmacopoeial aerobic and anaerobic liquid media.
**Time points of first detection in flow cytometer after pre-incubation in pharmacopeial media**

*used in pharmacopoeias for growth promotion test*

<table>
<thead>
<tr>
<th>bacteria species</th>
<th>strain</th>
<th>initial number</th>
<th>time of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spore forming bacteria (aerobe and anaerobe)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. <em>Bacillus cereus</em></td>
<td>PEI-B-07-23</td>
<td>2,4 CFU/ml</td>
<td>7 h</td>
</tr>
<tr>
<td>2. <em>Bacillus subtilis</em></td>
<td>ATCC 6633</td>
<td>2,9 CFU/ml</td>
<td>9 h</td>
</tr>
<tr>
<td>3. <em>Clostridium perfringens</em></td>
<td>ATCC 13124</td>
<td>7,0 CFU/ml</td>
<td>8 h</td>
</tr>
<tr>
<td>4. <em>Clostridium sporogenes</em></td>
<td>ATCC 19404</td>
<td>0,8 CFU/ml</td>
<td>15 h</td>
</tr>
<tr>
<td>GRAM-negative Bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. <em>Escherichia coli</em></td>
<td>ATCC 25922</td>
<td>3,6 CFU/ml</td>
<td>11 h</td>
</tr>
<tr>
<td>6. <em>Klebsiella pneumoniae</em></td>
<td>PEI-B-08-07</td>
<td>4,5 CFU/ml</td>
<td>6 h</td>
</tr>
<tr>
<td>7. <em>Pseudomonas aeruginosa</em></td>
<td>ATCC 9027</td>
<td>0,6 CFU/ml</td>
<td>14 h</td>
</tr>
<tr>
<td>8. <em>Serratia marcescens</em></td>
<td>ATCC 43862</td>
<td>2,5 CFU/ml</td>
<td>5 h</td>
</tr>
<tr>
<td>GRAM-positive Bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9. <em>Enterococcus faecalis</em></td>
<td>ATCC 20478</td>
<td>1,1 CFU/ml</td>
<td>12 h</td>
</tr>
<tr>
<td>10. <em>Staphylococcus aureus</em></td>
<td>ATCC 6538</td>
<td>1,8 CFU/ml</td>
<td>6 h</td>
</tr>
<tr>
<td>11. <em>Staphylococcus epidermidis</em></td>
<td>PEI-B-06-07</td>
<td>0,3 CFU/ml</td>
<td>16 h</td>
</tr>
<tr>
<td>12. <em>Streptococcus pyogenes</em></td>
<td>PEI-B-20-05</td>
<td>5,7 CFU/ml</td>
<td>20 h</td>
</tr>
</tbody>
</table>

**Reliable Rapid Sterility Testing within 20 hours**
Novel Approaches in Rapid Sterility Testing
Pilot Studies at PEI

Combination of short pre-incubation with:

1. Flow Cytometry
2. Universal Bacteria PCR/NAT

BD Biosciences
BactiFlow
AES Chemunex
Rapid Sterility Testing using BactiFlow

inoculum 250 CFU/ml in jurkat cells (human lymphocytic cell line), no pre-incubation

jurkat control

S. epidermidis (PEI-B-06)
Novel Approaches in Rapid Sterility Testing
Pilot Studies at PEI

Combination of short pre-incubation with:

1. Flow Cytometry

- BD Biosciences
- BactiFlow
- AES Chemunex

2. Universal Bacteria PCR/NAT
**Klebsiella pneumoniae PEI-B-08-06,**
inoculum 0.4 CFU/ml, detection after 6 and 8 h pre-incubation at 37°C in thioglycolate medium
Real Time PCR in LightCycler 480 (Roche)
Novel Approaches in Rapid Sterility Testing
Pilot Studies at PEI

Detection of Micro-colonies

3. Milliflex Rapid
(coming from food microbiology)

4. Milliflex Quantum
Procedure Milliflex

1. Lysis
2. Filtration
3. Incubation

4. Application of Reagents
   - Rapid System
     - AutoSpray Station
   - Quantum System
     - manual
     - Incubation 30 min

5. Detection
   - Detection Tower: automatic enumeration of colonies
   - Detection System: manual enumeration of colonies

[pictures provided by Millipore]
Results microcolonies: Examples

**Enterobacter cloacae**
Incubation at 32.5°C

- 4 h
- 5 h
- 6 h

**Bacillus cereus**
Incubation at 32.5°C

- 7 h
- 9 h
- 11 h
Results: Detection Time in CHO cells

- 8 different bacterial species and 1 yeast were analysed in both Milliflex Rapid and Milliflex Quantum
- Artificial contamination of CHO cell suspensions mimicking a cell therapeutic
- Detection time in Milliflex Rapid: 4 to 7 hours
- Detection time in Milliflex Quantum: 7 to 12.5 hours
Currently:

Revision of

European Pharmacopeia Chapter 2.6.27. “Microbiological control of cellular products”

initiated by PEI

Rapid Methods will be implemented
Conclusions

1. It’s impossible to guarantee sterility of (cell based) ATMPs!
2. One has to consider bacterial species as contaminants unknown in microbial safety of “classical (biological) parenterals”.
3. We need a complex strategy in order to improve microbial safety of cell based ATMPs which includes (examples)
   - donor qualification/donor selection (taking over of experiences from transfusion medicine !)
   - selection, preparation, disinfection of harvest side
   - evaluation of every step (up to the last small detail) in manufacturing process
   - novel approaches for Rapid Sterility Testing
4. We need a paradigm shift in thinking regarding sterility testing of ATMPs!
   Established thinking (requirements): We have to find everything.
   New thinking: We have to find as much as possible as fast as possible within the existing time frame.
   The latter requires novel approaches.
5. The medical ethics forbids to withhold the patients from the novel products considering the fascinating new opportunities in therapy. In consequence, we have to be creative and diligent in order to solve the problems still existing.
Many thanks to:

Utta Schurig  
Melanie Störmer  
Julia Brachert  
Monika Bubenzer  
Rekia Beshir  
Ute Sicker  
Sven-Boris Nicol  
Oliver Karo

Bettina Löschner  
Gabriele von Eschwege  
Valerija Gubic  
Ingo Spreitzer  
Björn Becker  
Waldemar Hägel

and to you for attention!