A guide through texts of relevance to biologicals

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Structure of the presentation

• General monographs
• General Chapters
  • Physico-chemical methods
  • Microbiological methods
  • Viral safety/TSE
• Animal welfare
Examples of general monographs of relevance to biologicals

- Substances for pharmaceutical use (2034)
- Pharmaceutical preparations (2619)
- Products of rDNA technology (0784)
- Products with risk of transmitting agents of animal spongiforms encephalopathies (1483)
Substances for pharmaceutical use (2034)

- **Definition:** Substances for pharmaceutical use are any organic or inorganic substances that are used as active substances or excipients for the production of medicinal products for human or veterinary use.

- Requirements laid down in this general monograph apply to all substances for pharmaceutical use whether or not the substance is covered by an individual monograph.

- Consists of the following sections: production, characters, identification, tests, assay, labelling.

Pharmaceutical Preparations (2619)

- Reference source of standards in the European Pharmacopoeia on active substances, excipients and dosage forms, which are to be applied in the manufacture/preparation of pharmaceuticals, but **not a guide on how to manufacture** as there is specific guidance available covering methods of manufacture and associated controls.

- **Does not cover investigational medicinal products,** but competent authorities may refer to pharmacopoeial standards when authorising clinical trials using investigational medicinal products.
Products of rDNA technology (0784)

Revision
- Not revised since 1990
- General update to take into account current practice and advances in rDNA technology (e.g. modified proteins, use of transgenic animals and plants, glycosylated proteins)
- take into account the relevant WHO, ICH and EMA guidelines

New General chapters
- 2.6.34: Host-cell protein assays
  See presentation from G. Cirélice
- Methods used for detection and assessment of residual substrate cell DNA (2.6.35) New chapter under elaboration
General chapters

2.2 Physical and physicochemical methods

- Electrophoresis (2.2.31)
- Chromatographic separation techniques (2.2.46)
- Peptide mapping (2.2.55)
- Amino acid analysis (2.2.56)
- Glycan analysis of glycoproteins (2.2.59)
- Isoelectric focusing (2.2.54)
- Size-exclusion chromatography (2.2.30)
- Capillary electrophoresis (2.2.47)
General chapters

• Chromatographic separation techniques (2.2.46)
  ➢ Provides definitions and calculations of common parameters (peak, retention time, resolution etc)
  ➢ Defines permitted deviations to adjust chromatographic conditions, e.g. composition of mobile phase, column length, particle size etc. without re-validation
  ➢ Provides general system suitability parameter, not given in the individual monograph, symmetry factor 0.8 to 1.5

2.6 Biological tests

• Sterility (2.6.1)
• Pyrogens (2.6.8)
• Microbiological examination of non-sterile products: microbial enumeration tests (2.6.12)
• Microbiological examination of non-sterile products: tests for specified micro-organisms (2.6.13)
• Bacterial endotoxins (2.6.14)
• Microbiological control of cellular products (2.6.27)
• Monocyte-activation tests (2.6.30)
2.6.1 Sterility

2.6.1 International Harmonisation
(see Q4B Annex 8)

"NOTE (1) This chapter has undergone pharmacopoeial harmonisation. See chapter 5.8. Pharmacopoeial harmonisation."

Chapter 5.8

"(...) The texts of the 3 pharmacopoeias are therefore considered harmonised.

NOTE: ICH has declared this method interchangeable within the ICH regions."
2.6.1: The steps of the test

- Sample preparation
- Inoculation of sample to two different liquid media (Fluid Thioglycollate medium and Soya-bean casein digest medium)
  - Membrane filtration
  - Direct inoculation
- Incubation (14 days)
- Observation and interpretation of results

2.6.1 Environment

Precautions against microbial contamination: test to be carried out under aseptic conditions.

Chapter 5.1.9 Guidelines for using the test for sterility. Aseptic conditions for performance of the test can be achieved using, for example, a class A laminar-air-flow cabinet located within a class B clean room, or an isolator.
2.6.1 Method suitability

Method suitability: the aim is to verify that the product will not interfere with the test: the product is tested in the presence of the test organisms in the same conditions as for the growth promotion test. The organisms should grow.

Please note:
- Method suitability is not the same as method validation!
- The Ph. Eur. does not specify when/how often to perform the verification of method suitability

2.6.1 Neutralisation

"If the product has antimicrobial properties, wash the membrane not less than three times by filtering through it each time the volume of the chosen sterile diluent used in the method suitability test. Do not exceed a washing cycle of 5 times 100 ml per filter, even if during method suitability it has been demonstrated that such a cycle does not fully eliminate the antimicrobial activity."

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Sterility test Q&A session

2.6.8 Pyrogens
2.6.8
...replacement

2034 Substances for pharmaceutical use

**Pyrogens (2.6.8).** If the test for pyrogens is justified rather than the test for bacterial endotoxins and if a pyrogen-free grade is offered, the substance for pharmaceutical use complies with the test for pyrogens. The limit and test method are stated in the individual monograph or approved by the competent authority. Based on appropriate test validation for bacterial endotoxins and pyrogens, the test for bacterial endotoxins may replace the test for pyrogens.

0520 Parenteral preparations

**Bacterial endotoxins - pyrogens.** A test for bacterial endotoxins (2.6.14) is carried out or, where justified and authorised, the test for pyrogens (2.6.8).

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Replacement of/alternative to the rabbit pyrogen test

*(Suppl. 9.2)*

Recent revision of Chapter 5.1.10 Guidelines for using the test for bacterial endotoxins

- Recommendation is given to perform risk assessment when using the bacterial endotoxin test as a pyrogenicity test, due to the potential contamination by non-endotoxin pyrogens.
- A distinction is made between replacement methods already described in the Ph. Eur. and other alternative methods.
2.6.8
...replacement

“In accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm. Wherever possible and after product-specific validation, the pyrogen test is replaced by the monocyte-activation test (2.6.30).”

2.6.14 Bacterial endotoxins
2.6.14 Reagent used: LAL

LAL is a lyophilised product obtained from amoebocyte lysate from the horseshoe crab (*Limulus polyphemus* or *Tachypleus tridentatus*).

Lysate: defined sensitivity (IU/ml): \( \lambda \)

Use of alternative reagents to the Limulus amoebocyte

Use of recombinant factor C or other reagents  

This practice avoids the use of endangered animal species and can be considered in the context of the use of an alternative method as given in the General Notices.

This is referred to in the revised version of Chapter 5.1.10

“The use of alternative reagents such as recombinant factor C as a replacement to the amoebocyte lysate eliminates the use of a reagent extracted from live animals.

*Replacement of a rabbit pyrogen test or a bacterial endotoxin test prescribed in a monograph by a test using recombinant factor C reagent or any other reagent as a replacement of the amoebocyte lysate is to be regarded as the use of an alternative method in the replacement of a pharmacopoeial test, as described in the General Notices.*"
2.6.14 Testing procedure

- Confirmation of the labelled lysate sensitivity ($\lambda$)
- Calculation of the Maximum Valid Dilution (MVD)
- Testing for interfering factors
- Testing of the sample

5.1.10 GUIDELINES FOR USING THE TEST FOR BACTERIAL ENDO TOXINS

Calculation of Endotoxin limit

$\text{Endotoxin limit}$: the endotoxin limit for active substances administered parenterally, defined on the basis of dose, is equal to:

$$\frac{K}{M}$$

$K$ = threshold pyrogenic dose of endotoxin per kilogram of body mass;

$M$ = maximum recommended bolus dose of product per kilogram of body mass.
5.1.10 GUIDELINES FOR USING THE TEST FOR BACTERIAL ENDOTOXINS

“When the endotoxin concentration in the product exactly equals the threshold value, gelation will occur, as is the case when the endotoxin concentration is much higher, and the product will fail the test, because the all-or-none character of the test makes it impossible to differentiate between a concentration exactly equal to the threshold concentration and one that is higher. It is only when no gelation occurs that the analyst may conclude that the endotoxin concentration is below the threshold concentration.”

Therefore the limit in monograph is expressed as « less than X IU/mg »

New section: RISK ASSESSMENT

To assure that the product or substance does not contain non-endotoxin pyrogens

When the non-endotoxin pyrogens cannot be ruled out, MAT test is recommended.
2.6.14 Methods to be used

Method A. Gel-clot method: limit test
Method B. Gel-clot method: semi-quantitative test
Method C. Turbidimetric kinetic method
Method D. Chromogenic kinetic method
Method E. Chromogenic end-point method
Method F. Turbidimetric end-point method

“Proceed by any of the 6 methods for the test. In the event of doubt or dispute, the final decision is made based upon method A unless otherwise indicated in the monograph.”

2.6.30 Monocyte-activation test (MAT)
2.6.30 History


Supplement 6.7, April 2010

3 Methods

Method A. Quantitative test
Method B. Semi-quantitative test
Method C. Reference lot comparison test

2.6.30 Monocyte-activation test

Cell sources

- whole blood
- peripheral blood mononuclear cells (PBMC)
- cells pooled from a number of donors
- cryo-preserved cells
- monocytic continuous cell lines
2.6.30 Current activities

- EDQM Survey (February/March 2013)
- Revision Supplement 9.2
- Revision of chapter 5.1.10 to include references to 2.6.30
- Revision of chapter 2.6.8 to include that after product specific validation the pyrogen test replaced by the MAT 2.6.30
- Use of the MAT for inherently pyrogenic preparations

Deferoxamine mesilate

Reference of the MAT chapter in an individual monograph

**Bacterial endotoxins** (2.6.14): less than 0.025 IU/mg, if intended for use in the manufacture of parenteral preparations without a further appropriate procedure for the removal of bacterial endotoxins. Since deferoxamine mesilate has an inhibitory effect on BET, a suitable procedure is in place to remove this inhibitory effect. The MAT (2.6.30) has been found suitable to overcome this issue.
Pyrogen/BET/MAT Q&A session

2.6.12, 2.6.13 and 5.1.4
Microbiological quality of non sterile preparations
Microbiological quality

- Microbiological examination of non-sterile products: total viable aerobic count (2.6.12)
  (Harmonised with JP and USP, see Q4B Annex 4A)
- Microbiological examination of non-sterile products: test for specified micro-organisms (2.6.13)
  (Harmonised with JP and USP, see Q4B Annex 4B)
- Microbiological quality of pharmaceutical preparations and substances for pharmaceutical use (5.1.4)
  (Harmonised with JP and USP, see Q4B Annex 4C)

5.1.4 Acceptance criteria

When an acceptance criterion for microbiological quality is prescribed it is interpreted as follows:

- $10^1$ CFU: maximum acceptable count = 20;
- $10^2$ CFU: maximum acceptable count = 200;
- $10^3$ CFU: maximum acceptable count = 2000, and so forth.
5.1.4 Acceptance criteria

Table 5.1.4.-1. – Acceptance criteria for microbiological quality of non-sterile dosage forms: The table gives acceptance criteria for TMC, TYMC and specified micro-organisms for all Ph. Eur. routes of administrations

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>TAMC (CFU/g or CFU/mL)</th>
<th>TYMC (CFU/g or CFU/mL)</th>
<th>Specified micro-organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sterile preparations for oral use</td>
<td>$10^6$</td>
<td>$10^6$</td>
<td>Absence of Esherichia coli (1 g or 1 mL)</td>
</tr>
<tr>
<td>Aqueous preparations for oral use</td>
<td>$10^5$</td>
<td>$10^4$</td>
<td>Absence of Esherichia coli (1 g or 1 mL)</td>
</tr>
</tbody>
</table>

5.1.4 FAQ

Question What if I find a specific microorganism which is not indicated in this table? Strictly speaking, can I conclude that my product complies with the European Pharmacopoeia?
5.1.4 Other micro-organisms

In addition to the micro-organisms listed in Table 5.1.4.-1, the significance of other micro-organisms recovered is evaluated in terms of:

— use of the product
— nature of the product
— method of application
— intended recipient
— use of immunosuppressive agents, corticosteroids
— presence of disease, wounds, organ damage.

5.1.4 Risk assessment

"Where warranted, a risk-based assessment of the relevant factors is conducted by personnel with specialised training in microbiology and the interpretation of microbiological data. For raw materials, the assessment takes account of processing to which the product is subjected, the current technology of testing and the availability of materials of the desired quality."
5.1.4 Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use

Table 5.1.4.2 – Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use

<table>
<thead>
<tr>
<th>Substances for pharmaceutical use</th>
<th>TAMC (CFU/g or CFU/mL)</th>
<th>TYMC (CFU/g or CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^3</td>
<td>10^2</td>
<td></td>
</tr>
</tbody>
</table>

5.1.4 FAQ

Question According to 5.1.4, should all substances for pharmaceutical use be tested according to Table 5.1.4-2?

Question What shall we do if a specific monograph exists but does not include a test for microbial contamination?
Microbial contamination of non-sterile products Q&A session
Efficacy of antimicrobial preservation (5.1.3)

5.1.3

• Referred to in production section of monographs

**PRODUCTION**

During the development of an eye preparation whose formulation contains an antimicrobial preservative, the necessity for and the efficacy of the chosen preservative shall be demonstrated to the satisfaction of the competent authority. A suitable test method together with criteria for judging the preservative properties of the formulation are provided in chapter 5.1.3. **Efficacy of antimicrobial preservation.**

• The test is not intended to be used for routine control purposes.
5.1.3: The steps of the test

- Aimed at verifying the efficacy of preservatives in pharmaceutical preparations
- Challenge of the sample by inoculation of micro-organisms
- Incubation
- Sampling at different time intervals
- Acceptance criteria at each time of testing: fall or no increase of the count

5.1.3 Acceptance criteria

In chapter 5.1.3, the criteria for evaluation of antimicrobial activity are given in terms of the log reduction of viable microorganisms.

<table>
<thead>
<tr>
<th></th>
<th>Log reduction</th>
<th>6 h</th>
<th>24 h</th>
<th>7 d</th>
<th>14 d</th>
<th>28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>2</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>NR</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>NI</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>NI</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>NI</td>
</tr>
</tbody>
</table>

NR: no recovery
NI: no increase in number of viable microorganisms compared to the previous reading.

The A criteria express the recommended efficacy to be achieved. In justified cases where the A criteria cannot be attained, for example for reasons of an increased risk of adverse reactions, the B criteria must be satisfied.
Vaccines for human use  
(general monograph 0153)

If neither the A criteria nor the B criteria (in chapter 5.1.3) 
can be met, then in justified cases the following criteria 
are applied to vaccines for human use

• bacteria, no increase at 24 h and 7 days, 3 log reduction 
at 14 days, no increase at 28 days;
• fungi, no increase at 14 days and 28 days.

The criteria in monograph 0153 were included when the current 
antimicrobial preservatives section was introduced in 2001 (Ph. Eur. 
edition 4.0) because the preservatives in many vaccines on the 
market did not comply with criteria A or B of chapter 5.1.3 although 
they had been satisfactorily used for many years.

FAQ’s on chapter 5.1.3

Question: In order to fulfil the A criteria, 2 log reductions for Fungi after 14 days should be 
achieved. Can a reduction from of 1.8 log 1.8 be rounded up to 2 and therefore be considered 
acceptable?
FAQ’s on chapter 5.1.3

Response: Strictly speaking, logarithmic values should not be rounded. We recommend you to approach this problem on a case by case basis, a specific borderline result might be considered acceptable when taking into account the preservative efficacy test as a whole and the precision of the method. As part of a laboratory investigation, you may repeat testing and avoid reacting on a single potentially faulty figure.

FAQ’s on chapter 5.1.3

Question: If the environment does not allow microorganisms to grow or if the product possesses some inherent antimicrobial activity and when there is no specific inactivator known to neutralise this residual activity, how should I proceed with the test?
FAQ’s on chapter 5.1.3

Response: In those instances alternative strategies for method validation including dilution and membrane filtration with filter rinsing should be studied. If no suitable neutralising method can be found, it can be assumed that the failure to isolate the inoculated organism is attributable to the microbicidal activity of the product. This information serves to indicate that the product is not likely to be contaminated with the given species of the micro-organism. The test can be performed with only those organisms for which adequate recovery could be obtained.
5.1.6. Alternative methods for control of microbiological quality

5.1.6. Introduction

The following chapter is published for information.

The objective of this chapter is to facilitate the implementation and use of alternative microbiological methods where this can lead to cost-effective microbiological control and improved assurance for the quality of pharmaceutical products. These alternative methods may also find a place in environmental monitoring.
5.1.6. Outline

The chapter gives:

- the principle of detection, enumeration, isolation and identification of the methods which have successfully been used in the QC of pharmaceuticals
- Guidance on how to validate alternative methods against Ph. Eur. compendia

5.1.6. Revision

(Suppl. 9.2)

- Update and addition of new methods
- Guidance on how to validate an alternative microbiological methods
- Requirements on databases used for identification tests & their validation
- Example validation of alternative methods added in knowledge database
### 2.6.27 Microbiological control of cellular products

*Suppl. 9.2*

<table>
<thead>
<tr>
<th>Topic or modification</th>
<th>Improvements</th>
</tr>
</thead>
</table>
| New title (Microbiological examination of cell-based preparations) and Extensive revision to take account of technological developments in rapid microbiological methods and their benefits for the cell therapeutic products | - Guidance on how to select the microbiological examination method depending on the characteristics and constraints inherent to the cell-based preparation characteristics:  
  - 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. |

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Rapid Methods Q&A session
5.1.7 Viral safety

5.1.7

- Published in final supplement to 5th edition
- Emphasises the importance of carrying out a risk assessment on viral safety of materials of human or animal origin
- Cross reference to 5.1.7 in general monographs on preparations, i.e. allergens, extracts, immunosera, monoclonal antibodies, products of recombinant DNA technology, vaccines and substances for pharmaceutical use

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5.2.8 Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products

- Identical with the EMA Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products
- 5.2.8 is referred to in General monograph 1483 Products with risk of transmitting agents of animal spongiform encephalopathies
FAQ

**Question** I have looked at the monograph on Trypsin: it does not contain any warning about possible viral contamination, nor does it refer to any BSE related issue: how can this be, knowing that the substance is of bovine origin?

**Response**: Trypsin has to not only comply with the monograph on Trypsin (0694) but also with the general monograph 1483 Products with risk of transmitting agents of animal spongiform encephalopathies, which refers to Chapter 5.2.8
Viral safety/BSE/TSE Q&A session

ANIMAL WELFARE
Legal situation (1)

“European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes”

*Council of Europe, 1986*

Starting point for
Change of national/European legislation
Ph. Eur. for replacement of animal tests

Legal situation (2)

  - Current legislation implemented in all EU Member States
  - Requires use of alternative methods where available
Direct towards 3Rs (1)

**General statement on animal usage**

*(in II. Introduction)*

- Ph. Eur. refers to European Convention (Council of Europe, 1986)
- “Commission is committed to the reduction of animal usage wherever possible .., and encourages those associated with its work to seek alternative procedures.”

Direct towards 3Rs (2)

“In accordance with European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986) and the European Directive on the same principles, tests must be carried out in such a way as to use the minimum number of animals and to cause the least pain, suffering, distress or lasting harm”

*Statement in 3 general monographs 0153, 0030 and 0062*
Direct towards 3Rs (3)

- Specific monographs encourage alternative “3R” methods, humane endpoints
- When a validated method is available (e.g. via BSP collaborative study), a detailed protocol is given as an example

General Notices (07/2014)

Demonstration of compliance with the Pharmacopoeia

(1) An article is not of Pharmacopoeia quality unless it complies with all the requirements stated in the monograph. This does not imply that performance of all the tests in a monograph is necessarily a prerequisite for a manufacturer in assessing compliance with the Pharmacopoeia before release of a product. The manufacturer may obtain assurance that a product is of Pharmacopoeia quality on the basis of its design, together with its control strategy and data derived, for example, from validation studies of the manufacturing process.
The “consistency approach”  
De Fabrizio et al., 2011

The consistency approach is a concept which includes the strict application of GMP rules and Guidelines, process validation and in process and final product tests and is aimed at verifying if a manufacturing process produces final batches which are consistent with one that fulfils all the criteria of Quality, Safety and Efficacy as defined in the Marketing Authorisation, ultimately resulting in the replacement of routinely used in vivo tests.

General Notices Consistency of Production (07/2014)

(3) Reduction of animal testing: the European Pharmacopoeia is dedicated to phasing out the use of animals for test purposes, in accordance with the 3Rs (Replacement, Reduction, Refinement) set out in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. In demonstrating compliance with the Pharmacopoeia as indicated above (1), manufacturers may consider establishing additional systems to monitor consistency of production. With the agreement of the competent authority, the choice of tests performed to assess compliance with the Pharmacopoeia when animal tests are prescribed is established in such a way that animal usage is minimised as much as possible.
Follow PhEur 3R’s activities on the EDQM website

Alternatives to Animal Testing

Categories of medicines concerned by animal testing for Quality Control purposes
- Vaccines for human use and for veterinary use
- Blood products
- Biological and biotechnological products
- Antibiotics
- Radiopharmaceuticals

Why the Council of Europe can be considered a pioneer in this field

The protection of animal rights and in particular those used for experimentation has long been a subject of interest for the Council of Europe. The first initiative was achieved in 1986, when the European ConventionETS 123 for the Prevention of Cruelty to Animals used for Experimental and Other Scientific Purposes was open for signature.

It is interesting to note that the European ConventionETS 123 was adopted before the EU’s Directive 86/609/EEC (adopted on 24 November 1986) and that the provisions of this Directive are based on the Convention. In September 2010, the EU adopted a new Directive 2010/63/EU on the same subject that replaces the 1986 Directive 86/609/EEC and it comes into effect on 1 January 2013.

The main area of activity of the EDQM that are actively involved in the application of 3Rs principles includes:

- Alternatives to Animal Testing
- Reduction of Animal Use
- Replacement of Animal Use

Animal welfare Q&A session
Thank you for your attention!