

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



Microbiology chapters

Microbiology chapters: sterility, efficacy of antimicrobial preservation, microbiological quality of non-sterile products, rapid microbiological methods, viral safety, TSE

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Structure of the presentation

- Sterility
- Efficacy of antimicrobial preservation
- Microbiological quality of non-sterile products, rapid microbiological methods
- Viral safety
- TSE





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

Until 10.0

- ✓ "(...) The texts of the 3 pharmacopoeias are therefore considered *harmonised*."
- ✓ *NOTE: ICH has declared this method interchangeable within the ICH regions."*

From 10.0

No more specific information (information to be retrieved on the respective websites)

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 The culture media

- **Two fluid media:** **Fluid Thioglycollate medium** and **Soya-bean casein digest medium**
- **Sterility**
- **Growth promotion**

Table 2.6.1.-1. – Strains of the test micro-organisms suitable for use in the growth promotion test and the method suitability test

Aerobic bacteria	
<i>Staphylococcus aureus</i>	ATCC 6538, CIP 4.83, NCTC 10788, NCIMB 9518, NBRC 13276
<i>Bacillus subtilis</i>	ATCC 6633, CIP 52.62, NCIMB 8054, NBRC 3134
<i>Pseudomonas aeruginosa</i>	ATCC 9027, NCIMB 8626, CIP 82.118, NBRC 13275
Anaerobic bacterium	
<i>Clostridium sporogenes</i>	ATCC 19404, CIP 79.3, NCTC 532, ATCC 11437, NBRC 14293
Fungi	
<i>Candida albicans</i>	ATCC 10231, IP 48.72, NCPF 3179, NBRC 1594
<i>Aspergillus brasiliensis</i>	ATCC 16404, IP 1431.83, IMI 149007, NBRC 9455

2.6.1 The steps of the test

- Sample preparation
- Inoculation of sample to the two different liquid media
 - Membrane filtration (0.45 µm)
 - Or: direct inoculation
- Incubation (14 days)
- Observation and interpretation of results



"If no evidence of growth is found, the product to be examined complies with the test for sterility"

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 micro-organisms in the same conditions as for the growth promotion test. The organisms should **grow**.

This method suitability test is performed:

- a) when the test for sterility has to be carried out on a new product;*
- b) whenever there is a change in the experimental conditions of the test.*

The method suitability test may be performed simultaneously with the test for sterility of the product to be examined.

Please note:

- Method **suitability** is not the same as method **validation**!

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."

2.6.1 Minimum number of items to be tested

Table 2.6.1.-3. – *Minimum number of items to be tested*

Number of items in the batch*	Minimum number of items to be tested for each medium, unless otherwise justified and authorised**
<i>Parenteral preparations</i> <ul style="list-style-type: none"> - Not more than 100 containers - More than 100 but not more than 500 containers - More than 500 containers 	10 per cent or 4 containers, whichever is the greater 10 containers 2 per cent or 20 containers (10 containers for large-volume parenterals) whichever is less
<i>Ophthalmic and other non-injectable preparations</i> <ul style="list-style-type: none"> - Not more than 200 containers - More than 200 containers - If the product is presented in the form of single-dose containers, apply the scheme shown above for preparations for parenteral administration 	5 per cent or 2 containers, whichever is the greater 10 containers
<i>Catgut and other surgical sutures for veterinary use</i>	2 per cent or 5 packages whichever is the greater, up to a maximum total of 20 packages
<i>Bulk solid products</i> <ul style="list-style-type: none"> - Up to 4 containers - More than 4 containers but not more than 50 containers - More than 50 containers 	Each container 20 per cent or 4 containers, whichever is the greater 2 per cent or 10 containers, whichever is the greater

* If the batch size is not known, use the maximum number of items prescribed.

**If the contents of one container are enough to inoculate the 2 media, this column gives the number of containers needed for both the media

2.6.1 Minimum quantity to be used for each medium

Table 2.6.1.-2. – *Minimum quantity to be used for each medium*

Quantity per container	Minimum quantity to be used for each medium unless otherwise justified and authorised
<p><i>Liquids</i></p> <ul style="list-style-type: none"> - less than 1 mL - 1-40 mL - greater than 40 mL and not greater than 100 mL - greater than 100 mL <p><i>Antibiotic liquids</i></p>	<p>The whole contents of each container</p> <p>Half the contents of each container but not less than 1 mL</p> <p>20 mL</p> <p>10 per cent of the contents of the container but not less than 20 mL</p> <p>1 mL</p>
<i>Insoluble preparations, creams and ointments to be suspended or emulsified</i>	Use the contents of each container to provide not less than 200 mg
<p><i>Solids</i></p> <ul style="list-style-type: none"> - less than 50 mg - 50 mg or more but less than 300 mg - 300 mg to 5 g - greater than 5 g 	<p>The whole contents of each container</p> <p>Half the contents of each container but not less than 50 mg</p> <p>150 mg</p> <p>500 mg</p>
<i>Catgut and other surgical sutures for veterinary use</i>	3 sections of a strand (each 30 cm long)

2.6.1 Quiz

Question *What is the total quantity to be tested for a batch of a parenteral preparation consisting of 3000 vials filled with 1.5 ml?*

Answer: 40 ml

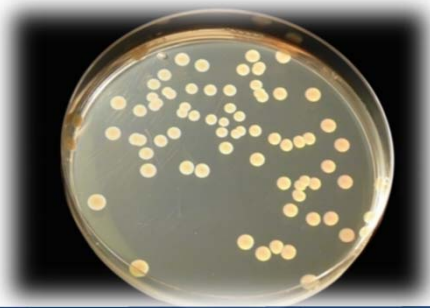
Quiz

Sterility test Q&A session



Q&A

2.6.12, 2.6.13 and 5.1.4 Microbiological quality of non sterile preparations



Microbiological quality

- Microbiological examination of non-sterile products: microbial enumeration tests ([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)

2.6.12 Microbiological examination of non-sterile products: enumeration

- Negative control
- Growth promotion of media
- Suitability of the method in the presence of the product
 - Neutralisation/removal of antimicrobial activity
- Testing and examination of the product
- Interpretation of results
 - Total Aerobic Microbial Count (**TAMC**): number of Colony Forming Units (CFU) found using casein soya bean digest agar
 - Total combined yeasts/mould count (**TYMC**): number of CFU found on Sabouraud-dextrose agar

2.6.13 Microbiological examination of non-sterile products: specified

micro-organisms

- Negative control
- Growth promotion and inhibitory properties of media
- Suitability of the test method
- Testing of products
 - Bile-tolerant gram-negative bacteria
 - Escherichia coli
 - Salmonella
 - Staphylococcus aureus
 - Clostridia
 - Candida albicans

5.1.4 Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use

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

Table 5.1.4.-1. – Acceptance criteria for microbiological quality of non-sterile dosage forms

Route of administration	TAMC (CFU/g or CFU/mL)	TYMC (CFU/g or CFU/mL)	Specified micro-organisms
Non-aqueous preparations for oral use	10 ³	10 ²	Absence of <i>Escherichia coli</i> (1 g or 1 mL)
Aqueous preparations for oral use	10 ²	10 ¹	Absence of <i>Escherichia coli</i> (1 g or 1 mL)

Etc...

2.6.12/5.1.4 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 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

	TAMC (CFU/g or CFU/mL)	TYMC (CFU/g or CFU/mL)
Substances for pharmaceutical use	10^3	10^2

5.1.4 Quiz

Question *I am a manufacturer of a API produced by chemical synthesis. According to table 5.1.4-1, do I have to comply with the limits for TAMC and TYMC?*

Quiz

5.1.4 Quiz

General Monograph 2034 Substances for pharmaceutical use

- **Microbiological quality.** Individual monographs give acceptance criteria for microbiological quality wherever such control is necessary. Table 5.1.4.-2. – Acceptance criteria for microbiological quality of non-sterile substances for pharmaceutical use in chapter 5.1.4. *Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use* gives recommendations on microbiological quality that are of general relevance for substances subject to microbial contamination. Depending on the nature of the substance and its intended use, different acceptance criteria may be justified.

5.1.4 Quiz

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?*

Quiz

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."*

Microbial contamination of non-sterile products Q&A session



Efficacy of antimicrobial preservation (5.1.3)

5.1.3 Scope

- Aimed at verifying the efficacy of preservatives in pharmaceutical preparations
- 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

- Challenge of the sample by inoculation of micro-organisms

Test micro-organisms

<i>Pseudomonas aeruginosa</i>	ATCC 9027; NCIMB 8626; CIP 82.118.
<i>Staphylococcus aureus</i>	ATCC 6538, NCTC 10788, NCIMB 9518; CIP 4.83.
<i>Candida albicans</i>	ATCC 10231; NCPF 3179; IP 48.72.
<i>Aspergillus brasiliensis</i>	ATCC 16404; IMI 149007; IP 1431.83.

- Incubation
- Sampling at different time intervals
- Acceptance criteria at each time of testing: fall of the count 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 5.1.3-1 Acceptance criteria for parenteral preparations

		Log reduction				
		6 h	24 h	7 d	14 d	28 d
Bacteria	A	2	3	-	-	NR
	B	-	1	3	-	NI
Fungi	A	-	-	2	-	NI
	B	-	-	-	1	NI

NR: no recovery.

NI: no increase in number of viable micro-organisms 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.

Chapter 5.1.3 Quiz

Question: *In order to fulfil the A criteria, 3 log reductions for bacteria at 24 hours should be achieved. Can a reduction of 2.8 log be rounded up to 3 log and therefore be considered acceptable?*

Quiz

Chapter 5.1.3 Quiz

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.*

Preservative efficacy Q&A session

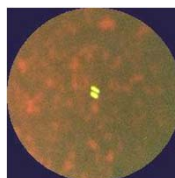


Q&A

Rapid microbiological methods

5.1.6. Alternative methods for control of microbiological quality

- *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:

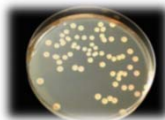
- the principle of detection, enumeration, isolation and identification of the methods which have successfully been used in the QC of pharmaceuticals
- Guidance on how to validate alternative methods against Ph. Eur. compendia
- First published in 2006
- Then revised following a survey among users in 2010
- Major revision in Supplement 9.2 (2017)

*Note: Not in the programme of International Harmonisation.
Not in contradiction with USP Chapter*

3.1 Validation: introduction

"Any given method will usually provide an indirect and conditional measure of microbiological quality. For example, the total number and viability of micro-organisms can be indicated by the number of colonies appearing under a certain set of conditions of sample preparation, cultivation and incubation; reproduction in classical microbiology is hence taken as the general indicator for viability. There are other parameters, however, that can be used as a viability measure, such as the level of ATP or the accumulation or metabolism of substrates in living cells. The results from different viability-indicating methods may not always be identical"

CFU



3. Validation of Alternative Microbiological Methods

3-1. INTRODUCTION

3-2. VALIDATION PROCESS

3-2-1. Description of the technique

3-2-2. Risk-benefit analysis

3-2-3. **Primary validation**

3-2-4. **Validation for the intended use**

3-3. TYPES OF MICROBIOLOGICAL TESTS

3-3-1. Validation of alternative qualitative test for the presence and absence of micro-organisms

3-3-2. Validation of alternative quantitative tests for enumeration of micro-organisms

3-3-3. Validation of alternative identification tests

3.2.3 Primary validation

The supplier, using a panel of **test micro-organisms appropriate for the intended use**, must characterise the principle of detection. Depending on the type of alternative method, relevant validation criteria shall be selected from those listed below:

- prerequisite treatment of sample or micro-organisms;
- type of response;
- specificity;
- detection limit;
- quantitation limit;
- range;
- linearity;
- accuracy and precision;
- robustness of the method in a model system.

3-2-4. Validation for the intended use

Validation for the intended use should encompass the entire process, from the decision to change any aspects of a microbiological testing programme to on-going routine use. It should consist of the following phases:

- user requirement specification (URS);
- design qualification (DQ);
- installation qualification (IQ);
- operational qualification (OQ);
- performance qualification (PQ).

3.2 Validation Process: tasks and responsibilities

Table 5.1.6.-1 – Tasks to be undertaken during the validation process

Activity	normally carried out by	
	Supplier	User
Primary validation	+	-1
URS (instrument, application)	-	+
Description of the technique	+	-2
Risk benefit analysis	-3	+
Design Qualification	-	+
Installation Qualification	-4	+
Operational Qualification	-4	+
Performance Qualification:		
- Verification of primary validation data given by the supplier	-	+
- Verification for the intended use (e.g. sterility testing, TAMC/TYMC, ...)	-	+
- Method Suitability Test	-	+

- (1) The user performs primary validation if they employ the alternative method for a use other than that defined by the supplier.
- (2) The user shall critically review information provided by the supplier.
- (3) As part of commercialisation, the supplier may list advantages of the alternative method over conventional techniques.
- (4) IQ / OQ for complex equipment, IQ/OQ is often outsourced to supplier.

**Use of pharmacopoeial test strains,
Use of the product to be analysed (« product-specific validation »)**

3.3. Types of microbiological tests

Criterion	Qualitative test	Quantitative test	Identification test
Accuracy	+ ¹	+	+
Precision	-	+	-
Specificity	+	+	+
Detection limit	+	- ²	-
Quantitation limit	-	+	-
Linearity	-	+	-
Range	-	+	-
Robustness	+	+	+
Suitability testing	+	+	-
Equivalence testing	+	+	-

Table 5.1.6.-2 – Validation criteria for qualitative, quantitative and identification tests

(1) Performing an accuracy test of the alternate method with respect to the compendial method can be used instead of the validation of the limit of detection test.
(2) may be needed in some cases

Suitability testing

➤ Aim: to show the suitability of the alternative method in the presence of the product

It must be shown that the test sample does not interfere with the system's detection capacity or microbial recovery. Specific points to be addressed are:

- the ability of the test to detect micro-organisms in the presence of the sample matrix;
- verifying if the sample matrix interferes with the alternative system (e.g. background signal or inhibiting chemical reactions).

Acceptance criteria for the method in routine use will need to be defined as a function of the application and the validation data.

Equivalence testing

➤ Aim: to show that the alternative method is equivalent to the official method

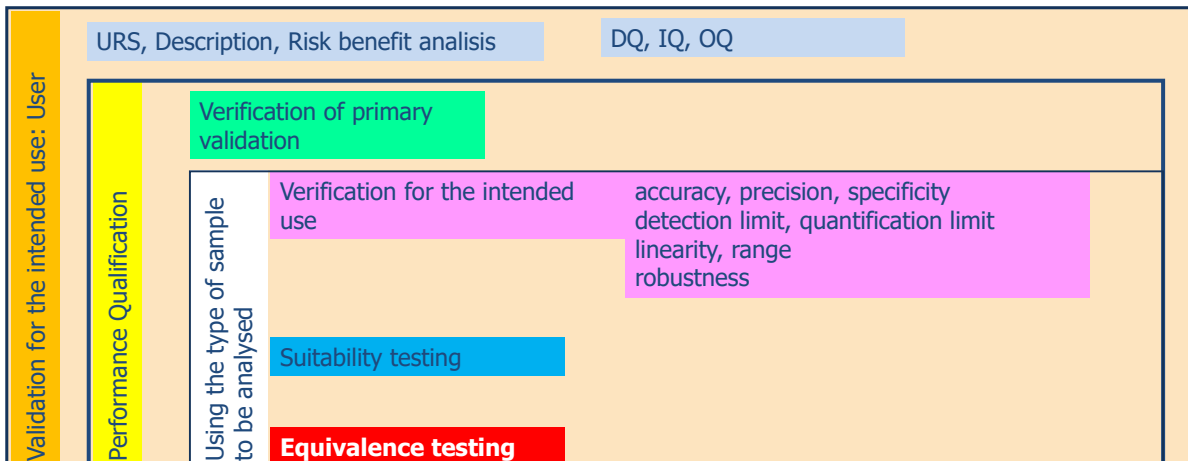
Can be conducted:

- directly on the validation parameters (sufficient numbers of replicates for relevant strains of test micro-organisms are required)
- or: parallel testing of samples for a predefined period of time or a predefined number of samples

The results of the alternative method must be equivalent to those of the pharmacopoeial method

Validation of an alternative method: summary

Primary validation: Supplier



Examples of validation protocols



- The detailed laboratory protocol for the implementation of a bioluminescence method has been removed from Chapter 5.1.6
- 3 **examples** are outlined on EDQM website
 - **rapid sterility test** based on membrane filtration;
 - quantitative test for the **enumeration** of micro-organisms using solid phase cytometry;
 - a molecular-based microbial **identification** method.

Examples of validation protocols



- This document might be updated with validation protocols of relevant, breakthrough AMM technologies (based on new principles) ensuring a better microbiological quality control by pharmaceutical companies
- This document is a support to the users on what may be performed during the validation of an alternative microbiological methods as described in chapter 5.1.6
- This document is not intended to be a compilation of all available equipment used for alternative microbiological methods on the market

Rapid Methods Q&A session



5.1.7 Viral safety

Chapter 5.1.7

- Published in final supplement to 5th edition (2007)
- Scope: **medicinal products** whose manufacture has involved the use of materials of human or animal origin
- Emphasises the importance of carrying out a **risk assessment** on viral safety of materials of human or animal origin
- Makes reference to the *Note for guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses (CPMP/BWP/268/95)* of the CPMP, and the *ICH guideline Q5A: Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin* (including any subsequent revisions of these documents).
- 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

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

5.2.8. Minimising the risk of TSE

- First published in 2001
- Identical with the EMA Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products
- Both texts revised in 2011 with same implementation dates
=> full alignment between Ph. Eur. and EU legislation



PRODUCTS WITH RISK OF TRANSMITTING AGENTS OF ANIMAL SPONGIFORM ENCEPHALOPATHIES

Producta cum possibili transmissione vectorium encephalopathiarum spongiformium animalium

DEFINITION

Products with risk of transmitting agents of animal spongiform encephalopathies are those derived from tissues or secretions of animals susceptible to transmissible spongiform encephalopathies other than by experimental challenge. This monograph applies to all substances or preparations obtained from such animals and to all substances or preparations where products obtained from such animals are included as active substances or excipients or have been used during production, for example as raw or source materials, starting materials or reagents.

PRODUCTION

Production complies with chapter 5.2.8. *Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products*

Chapter 5.2.8 is referred to in General monograph 1483 and renders **chapter 5.2.8 mandatory for all substances and preparations**

Quiz

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?*

Quiz

Quiz

- **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



Thank you for your attention



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