Environmental conditions for the microbiological examination of non-sterile products

Document valid from:	**28.11.2025**	
Document number:	**I-SMI.TI.01e**	**Version 4.0**
Classification:	**Public**	
Replaced document:	**SMI.TI.01_03**	**dated:** 20.04.2015
Root SMI documents:	**--**	
Referenced QMI documents:	**--**	

Approval	Date:	Signature:
Author:	12.11.2025	Nicole Weyeneth
Technical verification:	12.11.2025	Federico Cimini
Formal verification (Release VS-QMI):	28.11.2025	Michelle Scheidegger

Index

1. Purpose and scope 2
2. Basics 2
3. Definitions and abbreviations 2
4. Interpretation 2
4.1 Need for laminar air flow cabinet (LAF) 2
4.2 Requirements for the testing area 3
4.3 Requirements for sample collection, storage and traceability 3
5. Changes to the previous version 4

1. Purpose and scope

In order to assure a harmonized conduct of inspections of microbiological laboratories, this document summarizes the conditions under which a laboratory has to perform microbiological examination of test samples claimed to be non-sterile. Non-sterile samples include samples from all stages of the manufacturing process, as well as monitoring samples from process water.

2. Basics

- Ph. Eur., 2.6.12 Microbiological examination of non-sterile products: microbial enumeration test
- Ph. Eur., 2.6.13 Microbiological examination of non-sterile products: test for specified micro-organisms
- Ph. Eur., 5.1.4 Microbiological quality of non-sterile pharmaceutical preparations and substances for pharmaceutical use
- Ph. Eur., 01 General notices
- PIC/S PE 009, Part I and II, Guide to good manufacturing practice for medicinal products

3. Definitions and abbreviations

Ph. Eur.	European Pharmacopoeia
PIC/S	Pharmaceutical inspection co-operation scheme

4. Interpretation

European guidelines do not set clear specifications concerning the conditions under which a laboratory has to perform microbiological examination of non-sterile samples. The following subsections outline the interpretation on the environmental condition of a microbiological laboratory and the microbiological examination of non-sterile samples. The interpretation further expands to sample collection and storage conditions.

4.1 Need for laminar air flow cabinet (LAF)

The general requirement is to minimize the risk of contamination during the microbiological examination of non-sterile samples. The environmental conditions should be designed to suit the operations carried out in them and should not add additional challenge to the sample results. Requirements for the environmental condition must therefore be designed in order to avoid extrinsic contamination of the samples to be tested.

An increase of the microbial load by a contamination of the test sample at the testing site can mask a low signal of bioburden stemming from the sample, as well as mask specific and slow growing microorganisms (e.g. by swarming microorganisms, overgrowth). Therefore, the sample to be tested must be adequately protected to prevent contamination from the testing process or the environment. On the other hand, measures must be in place to prevent the inhibition of growth of potentially present organisms.

A variety of tests, e.g. microbiological monitoring of samples of water for injection or from manufacturing steps that are close before sterile filtration, do require stringent measures due to low bioburden acceptance limits i.e. performance of the tests in a LAF with HEPA filter to reduce the risk of contamination is recommended.

In cases where the most-probable-number method, i.e. the least accurate method to analyze total aerobic microbial count, is used, the impact of contamination during the sample preparation and dilution shall be assessed.

For different non-sterile products, different microbial limits are accepted. In regards to these limits, microbiological laboratory areas must be designed, qualified and controlled to avoid a negative impact on the samples to be tested. A risk-based assessment of the necessary environmental requirements should be available to justify the laboratory layout and room classification, environmental controls and monitoring (e.g. temperature, viable monitoring), and measures to prevent contamination (e.g. cleaning, disinfection, gowning) during sample testing including sample preparation (e.g. weighing of non-liquid products, dilution, mixing). The analytical procedures must be carried out at room temperature (15 °C to 25 °C), which must be respected.

Therefore, in laboratories for the microbiological examination of non-sterile samples a LAF with HEPA filter may be used to protect the testing environment from outside contamination. Other measures to minimize the risk of contamination may be applied if a company can show documented evidence (e.g. qualification, validation, risk-assessment) that they can achieve the necessary level of quality and safety for the testing environment. Additionally, the air in laboratory areas, including within LAFs, where samples are prepared, diluted, mixed and processed, must be of appropriate quality, and acceptance criteria for the quality of air and environmental controls must be justified and be based on a risk assessment.

4.2 Requirements for the testing area

Quality control laboratories should normally be separated from production areas to avoid contamination.
All microbiological examinations must be performed under conditions designed to avoid accidental contamination of the product and of the sample to be examined. Such conditions include examination to take place in a dedicated room i.e. laboratory, physically separated from production areas by various means: by location and by restriction of access, by specified air supply and prevention of airflow between laboratories and adjacent areas. Further measures include dedicated gowns and other protective clothing, strict separation of production workflow from laboratory workflow.

Therefore, microbiological laboratories are to be physically separated from the production area.

Further, clean microbiological laboratory activities (e.g. handling of microbiological test samples, bioburden testing) should be performed in dedicated rooms separated from live culture activities (e.g. growth promotion testing, method suitability testing), handling of contaminated or potentially contaminated samples (e.g. subculturing for microbiological identification or further processing of the sample), or waste treatment areas. If separation by means of dedicated rooms cannot be achieved, a risk-based assessment must be available to address the necessary barriers (e.g. dedicated LAFs for clean and live culture activities) and measures to avoid the contamination of the clean activities.

4.3 Requirements for sample collection, storage and traceability

The sampling, the sample labelling, the sample handling, as well as the storage conditions for the sample should be in accordance with approved written procedures and documented. Full traceability including date, time and location of sample storage must be reflected in the samples'

chain of custody. The hold-time from sample collection to testing, as well as the storage condition, are critical for the microbiological examination of samples, therefore the following information should be documented (on paper or electronically):

- Date and time of sample collection
- Storage condition (temperature and container) and location (temperature controlled room or equipment) for each interim or long-term storage place
- Dates and times of sample testing steps

5. Changes to the previous version

- Chapter 1: Clarification on the scope of this technical interpretation addressing non-sterile products including all stages of the manufacturing process, as well as monitoring samples from process water
- Chapter 2: Adjustment of the list of guidelines on the basis of which the technical interpretation refers to
- Chapter 3: Chapter “Definitions and abbreviations” added
- Chapter 4: Subdivided into three chapters to outline the interpretation on the environmental condition of a microbiological laboratory and the microbiological examination of non-sterile samples
- Chapter 4.1: This chapter replaces the sub-chapter 3.1 of version 3.0 of this technical interpretation and clarifies under which circumstances a laminar air flow cabinet is recommended. Precising that a risk-based assessment of the necessary environmental requirements should be available to justify the environmental conditions for the microbiological examination of non-sterile samples
- Chapter 4.2: This chapter replaces the sub-chapter 3.2 of version 3.0 of this technical interpretation and precises the measures to be taken to avoid contamination between production and microbiological laboratory areas, as well as between clean and live culture activities in the microbiological laboratory
- Chapter 4.3: This chapter is new and outlines the requirements for sample collection and storage