ACILA AG

Service Catalog 2025

Microbiological Test Methods
Physico-chemical Test Methods

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1. Tests using Microbiological Methods

1.1 Testing of sterile products

This test is carried out on aqueous, sterile solutions (parenteral drugs, extraction or rinsing solutions and other sterile solutions), water-soluble solids, oily liquids as well on ointments and creams. A sterile test is performed to show that the products examined are free of microbiological contamination.

Service	Test Code
Sterility Test with membrane filtration (MCE Membrane)	7130015
Sterility Test with membrane filtration (PDF Membrane)	7130016
Sterility Test with direct inoculation	7130005
Sterility Test - Validation per test germ (6 germs are necessary)	7130020

1.2 Testing of non-sterile products

This test is carried out to determine the microbial load (counting the population of viable microorganisms) on or in products (especially medical devices), components, raw materials or packaging.

Testing of non-sterile products is carried out according to the following specifications:

- DIN EN ISO 11737-1 10-2021: Sterilization of health care products - Microbiological methods - Part 1: Determination of a population of microorganisms on products
- Ph. Eur. 2.6.12: Microbiological examination of non-sterile products: microbial enumeration tests
- Ph. Eur. 5.1.4: Microbiological quality of non-sterile pharmaceutical preparations
- USP 61: Microbial limit tests

Further tests are stability tests regarding antimicrobial preservation according to following procedures:

- Ph. Eur. 5.1.3: Efficacy of antimicrobial preservation
- USP 51: Antimicrobial Effectiveness Testing

Service	Test Code
Determination of microbial load (Bioburden)	7140005
Surface colony count	7150025
Efficacy of antimicrobial preservation	7130018
Spore strips test	7100011

1.3 Testing for Bacterial Endotoxins

1.3.1 Ph. Eur.: 2.6.14 LAL test - Detection or determination of endotoxins of Gram-negative bacteria using horseshoe crab amebocyte lysate

1.3.1.1 Gel-clot methods

Gel-clot methods are divided into limit testing (Method A) and quantitative determination (Method B). These methods are based on a reaction of the horseshoe crab amebocyte lysate with bacterial endotoxins at approximately 37 °C, which results in gel formation.

Service	Test Code
Validation of Endotoxin Determination, method A, Ph. Eur. & USP (Gel-Clot Test)	7111017
Endotoxin Determination (quantitative), method B, Ph. Eur. & USP (Gel-Clot Test)	7112005
Endotoxin Limit Test, method A, Ph. Eur. & USP (Gel-Clot Test)	7113004
Endotoxin Product Characterization, method B, Ph. Eur. & USP (Gel-Clot Test)	7112024

(Test Codes 7112005 & 7113004 are intended for testing products whose test methods have previously been successfully validated on three production batches)

1.3.1.2 Kinetic Turbidimetric method

The test is based on a reaction of horseshoe crab amebocyte lysate with bacterial endotoxins at approximately 37°C, resulting in turbidity of the solution. The turbidity is measured photometrically. The onset time is the time until a desired threshold turbidity value is reached; it corresponds to the endotoxin content of a sample. This reaction time and the logarithms to the base 10 of the different concentrations of the standard solution used in the test can be represented with a Cartesian plot of the turbidity evolution as a function of time. The lower limit of detection of endotoxins in a test solution is 0.001 I.U./mL.

Service	Test Code
Validation of Endotoxin Determination, method C, Ph. Eur. & USP (kinetic turbidimetric)	7111018
Endotoxin Determination (quantitative), method C, Ph. Eur. & USP (kinetic turbidimetric)	7112010
Endotoxin Limit Test, method C, Ph. Eur. & USP (kinetic turbidimetric)	7113005
Endotoxin Product Characterization, method C, Ph. Eur. & USP (kinetic turbidimetric)	7112025

(Test Codes 7112010 & 7113005 are intended for testing products whose test methods have previously been successfully validated on three production batches)

1.3.1.3 Kinetic Chromogenic method

Endotoxins catalyze the activation of a proenzyme in the horseshoe crab amebocyte lysate. Activation is determined by the concentration of endotoxins. The activated enzyme catalyzes the cleavage of p-nitroaniline (pNA) from the colorless substrate (Ac-Ile-Glu-Ala-Arg-pNA). The endotoxin content of a sample is calculated by comparing its reaction time with the reaction time of standard solutions containing known amounts of endotoxin (standard curve). The range of the standard curve generally includes endotoxin concentrations from 0.001 to 1.0 I.U./mL.

Service	Test Code
Validation of Endotoxin Determination, method D, Ph. Eur. & USP (kinetic chromogenic)	7111016
Endotoxin Determination (quantitative), method D, Ph. Eur. & USP (kinetic chromogenic)	7112015
Endotoxin Limit Test, method D, Ph. Eur. & USP (kinetic chromogenic)	7113006
Endotoxin Product Characterization, method D, Ph. Eur. & USP (kinetic chromogenic)	7112026

1.3.2 Ph. Eur.: 2.6.32 Testing for bacterial endotoxins using recombinant factor C (rFC)

This test is based on the gene sequence of the horseshoe crab. A fluorometric method is used for this purpose.

In the field of the determination of bacterial endotoxins, the rFC method has been implemented in the European Pharmacopoeia as a separate chapter (Ph. Eur. 2.6.32).
It offers the following advantages over the LAL test:

- No false-positive results due to the presence of β-glucans
- Higher sensitivity
- Resource-saving and sustainable alternative method

The measurement range generally includes endotoxin concentrations from 0.001 to 1.0 I.U./mL.

Service	Test Code
Validation of Endotoxin Determination, rFC method, Ph. Eur.	7213001
Endotoxin Determination (quantitative), rFC method, Ph. Eur.	7112015
Endotoxin Limit Test, rFC method, Ph. Eur.	7213005
Endotoxin Product Characterization, rFC method, Ph. Eur.	7213015

1.4 Water testing

ACILA's lab performs purity tests on water for injections (WFI).

The following test methods are accredited:

1. Tests according to European Pharmacopeia (Ph. Eur.)
    - Sterility
    - Examination of non-sterile products
    - Bacterial Endotoxins
    - Particle Test

2. Tests according to standard procedures:
    - DIN ISO 11737-1 10-2021: Sterilization of health care products - Microbiological methods – Part 1: Determination of a population of microorganisms on products
    - DIN EN ISO 9308-1 2017-09: Water quality - Enumeration of *Escherichia coli* and coliform bacteria – Part 1: Membrane filtration method for waters with low bacterial background flora
    - DIN EN ISO 7899-2 2000-11: Water quality - Detection and enumeration of intestinal enterococci – Part 2: Membrane filtration method
    - DIN EN ISO 16266 2008-05: Water quality - Detection and enumeration of *Pseudomonas aeruginosa* - Method by membrane filtration
    - DIN EN ISO 6222 1999-07: Water quality - Enumeration of culturable micro-organisms — Colony count by inoculation in a nutrient agar culture medium
    - DIN EN ISO 11731 2019-03: Water quality - Enumeration of Legionella

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2. Tests using Physico-chemical Methods

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2.1 Particulate contamination – Sub-visible particles

Determination of sub-visible particles (measured sizes between 10µm and 25µm), which have been unintentionally introduced into solutions and containers because of contamination with foreign, undissolved particles (exceptions: gas bubbles).

Service	Test Code
Particle count - light obscuration	7100025
Particle count - using a microscope	7100026

Limit values according to Ph. Eur.:

Vial Volume	Particle size	Method	
		Light obscuration	Microscope
≤ 100 ml	≥ 10 µm	< 6,000 Particles / Vial	< 3,000 Particles / Vial
	≥ 25 µm	< 600 Particles / Vial	< 300 Particles / Vial
> 100 ml	≥ 10 µm	< 25 / mL	< 12 / mL

2.2 Determination of chlorides

2.2.1 Limit test

Determination of the content of chloride ions in water by photometric measurement of the turbidity occurring during precipitation with silver nitrate in the presence of diluted nitric acid. Due to the high sensitivity, this test is preferably carried out as a limit test for purity testing.

Service	Test Code
Determination of chlorides (limit test)	7210012

2.2.2 Total content

The chloride content is determined by means of potentiometric titration. A metal combination electrode for halogenide titrations AgCl 65 Glass shaft, platinum junction is used as the electrode, which is immersed in the analytical solution. The content is determined by adding silver nitrate solution to nitric acid.
At the equivalent point, the potential increases significantly and can therefore be evaluated using the usual methods.

Service	Test Code
Determination of chloride (total content) in water or aqueous solutions	7210010