INTERLABOR
BELP AG

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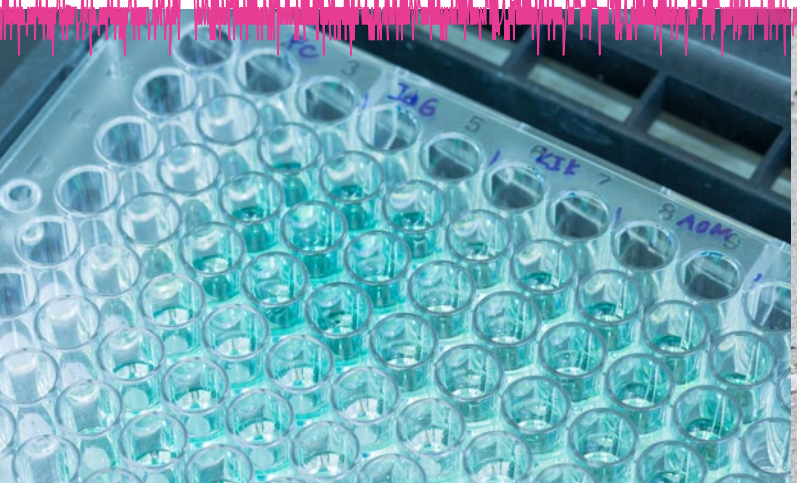
FACTSHEET



Quantitative Determination of Endotoxins

Introduction

Endotoxins belong to the heterogeneous family of pyrogens (see Box 1) and are components of the outer cell membrane of gram-negative bacteria. In contrast to exotoxins (e.g. botulinum toxin), endotoxins can trigger an immune response only when the bacterium lyses (disintegration of the cell). Lysis releases membrane components that can be recognized by the human immune system and cause an inflammatory reaction. The most common symptom after contact with endotoxins is fever, as the endotoxins stimulate the release of cytokines. Other reactions such as an increased heart rate or a reduction in the number of lymphocytes may result. Large doses of endotoxins can even lead to liver failure or death from hemorrhagic shock. If the exposure occurs via the gastrointestinal tract, the immune reaction triggered by endotoxins is usually harmless for healthy people. However, if endotoxins enter the bloodstream directly, e.g. through contaminated intravenous drugs, the consequences can be severe.

Test principle

To ensure that medicinal products such as intravenously administered antibiotics and other injection solutions are free of endotoxins, a highly sensitive and specific assay based on the lysate of amoebocytes obtained from the blood of the horseshoe crab (Limulus polyphemus) is most commonly used. Amoebocytes are the invertebrate counterpart to white blood cells in vertebrates. Amoebocytes trigger a coagulation reaction when they encounter a foreign substance in the bloodstream. This reaction is nowadays used for the detection of endotoxins. Table 1 shows an overview of the methods based on this reaction.

Box 1:

Difference between pyrogens and endotoxins

Pyrogen is a collective term for inflammatory substances that cause the immune system to react. They can originate from microbial or non-microbial contaminants. In general, pyrogens are divided into two groups: endotoxins and non-endotoxin pyrogens (NEP). While endotoxins originate exclusively from gram-negative bacteria, NEPs may orginate from gram-positive bacteria, viruses, yeasts or moulds. Even substances of non-biological origin, such as plastic particles or metal compounds, can act as a pyrogen. If you are interested in the general detection of pyrogens, we will be glad to advise you.

Table 1: Test methods for the determination of endotoxins

	Gel-clot	Chromogenic	Turbidimetric
Analysis	Qualitative or semi-quantitative	Quantitative	Quantitative
Measuring principle	Visual	Photometric (change of colour)	Photometric (turbidity)
Reference	Ph. Eur. 2.6.14, Method A or B	Ph. Eur. 2.6.14, Method D or E	Ph. Eur. 2.6.14, Method C or F
Advantages	- Technically simple - Most frequently used method	- Very sensitive (detection limit at 0.005 EU/ mL) - Automatic data acquisition - Higher dilution the enables reduction of interfering properties	
Disadvantages	- Limited sensitivity - Susceptible to vibrations	- Technically more demanding - Interference with turbid or coloured samples	

Determination of endotoxins at Interlabor Belp AG

In addition to the gel clot test, Interlabor now also offers the chromogenic kinetic method, which allows for a quantitative determination of the endotoxin content. The test principle is based on the enzymatic cleavage of a chromophore (p-nitroaniline) in the presence of endotoxins, causing a yellowish discoloration of the solution which can be measured photometrically. Due to its high sensitivity, the method is suitable for the analysis of challenging sample matrices, as interfering properties can be neutralized by a higher dilution. On request, we also perform the turbidimetric method.

Analysis within the scope of GMP

For a quantitative determination of endotoxins within the scope of GMP, a product-specific verification is required. In preliminary tests, we first check the solubility of the product and whether reaction-influencing properties occur. Based on the findings from the preliminary tests, the verification is then carried out according to the criteria of the pharmacopoeia (Ph. Eur. 2.6.14[1] and USP <85>[2]).

Key facts at Interlabor Belp AG

Depending on the project, different analysis qualities and processing times can be offered:

- Analysis quality: ISO 17025 or GMP (after verification)
- Delivery time: Standard approx. 8 - 10 working days or Express approx. 5 working days; Verification approx. 8 - 12 weeks
- Costs of analysis: method setup CHF 120.- (once per sample series) and each routine sample CHF 90.-; costs for verification upon request
- Sample quantity: routine approx. 1 g and verification approx. 5 g; depends on desired specification

We would be pleased to advice you in a personal meeting.

References

1. *https://gmpua.com/Validation/Method/LAL/EUPHARMACOPOEIA.pdf*
2. *https://www.usp.org/harmonization-standards/pdg/general-methods/bacterialendotoxins*

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