// **Requirements** for sterile production - **depyrogenation** of **primary packaging materials.** ///

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Elimination and detection of pyrogens and endotoxins

High standards of purity must be maintained in the production of pharmaceuticals. Biopharmaceuticals in particular, which derive from fermentation and cell culture processes and, contrary to regulatory guidelines, cannot be sterilized in the final vessel, must be prepared from absolutely pure ingredients under aseptic conditions (European Commission 25.11.2008; U.S. Department of Health and Human Services Food and Drug Administration 2004).

Pyrogens are a highly complex group of impurities that form during fermentation and cell culture processes and can sometimes cause severe adverse reactions when administered to the bloodstream.

They are chemically highly heterogeneous substances that, after entering the bloodstream, trigger an immune reaction in humans and produce, among other things, fever. Pyrogens can be either endogenous (IL-1, IL-6 and TNF-alpha) or exogenous (LPS (endotoxin) / particles deriving from material abrasion). Endotoxins can be considered a subclass of pyrogens and are actually a part of the cell membrane in gram-negative bacteria. They are the most common source of pyrogenicity and are among the most potent pyrogens.

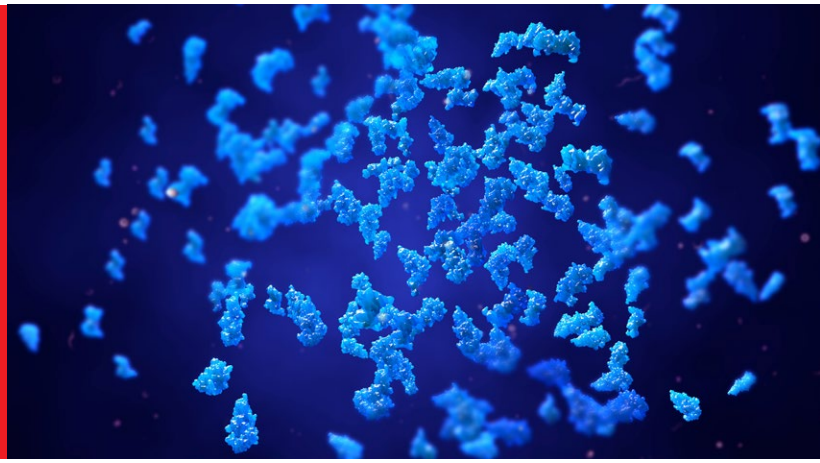
During the production of biopharmaceuticals, it is therefore important to determine and minimize the presence of pyrogens and especially endotoxins. There are various ways to do this:

Classical detection of pyrogens

The procedure for the detection of pyrogens as well as the process of depyrogenation are described in USP General Chapters <151> Pyrogenicity Test and <1228> Depyrogenation (The United States Pharmacopeial Convention; The United States Pharmacopeial Convention). USP <151>, the rabbit pyrogen test (RPT), involves intravenously injecting the test substance into rabbits and then measuring the increase in their body temperature. The Limulus amebocyte lysate (LAL) test (according to USP <85> / USP <1085> or the European equivalent Ph. Eur. 8, 2.6.14 Testing for Bacterial Endotoxins) is considered the gold standard in the industry for the detection of specific endotoxins (The United States Pharmacopeial Convention 01 Dec., 2012, 2018_Entwurf).

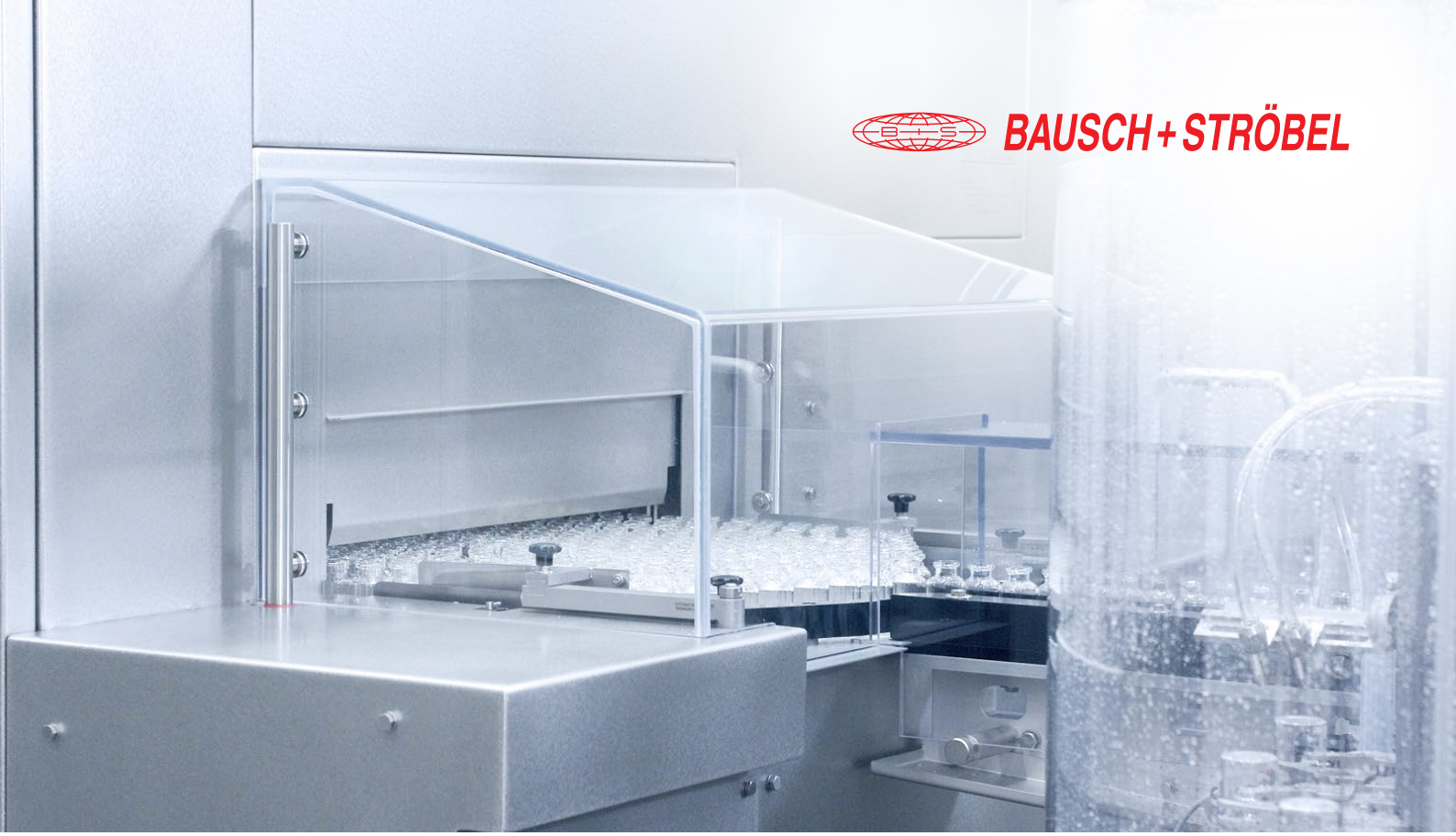
The use of laboratory animals (USP <151>) is subject to increasing ethical scrutiny, but the use of a reagent derived from the blood of the endangered species of horseshoe crab (USP <85> / USP <1085>) has also come under criticism. In Europe, however, there is a possible alternative to the LAL test: the recombinant factor C test (as per Ph. Eur.- 2.6.32 Test for bacterial endotoxins using recombinant factor C). In addition, there are issues with the robustness and reproducibility of LAL assay. One of the biggest challenges in this context are the so-called masking effects of ingredients in drugs, which alter the structure of the endotoxin in such a way that it cannot be detected by LAL assay (Low Endotoxin Recovery-LER, Reich 2016). It is crucial to be able to contain these effects, which are anything but trivial.

One of the biggest **challenges** in this context are the so-called **masking effects** of ingredients in drugs.



However, the most obvious and significant limitation of the tests (LAL and recombinant factor C) may be that there are many classes of pyrogens other than endotoxins and that the LAL test, unlike the RPT, does not provide information on the presence of these other non-endotoxin pyrogens (NEPs).

A real alternative to the RPT is therefore the monocyte activation test (MAT). The key advantage of the MAT is that it additionally detects NEPs. These include peptidoglycanes, flagellin or lipoteichoic acid. This in-vitro method is based on the immunological response of monocytes, which release pro-inflammatory cytokines (e.g. IL-6) upon contact with pyrogenic substances, such as endotoxin or peptidoglycan. These endogenous substances can then be detected using various analytical systems, such as the „Enzyme-linked Immuno Sorbent Assay“ (ELISA).



Pyrogens and pharmaceutical machine manufacturing

As a valued partner to our customers, we understand that reliable depyrogenation of the primary packaging material is critical to the biopharmaceutical manufacturing process. For this reason, we like to collaborate with world leaders in the field of pyrogen and endotoxin detection. In cooperation with Microcoat Biotechnologie GmbH, for instance, we were able to demonstrate the extent to which packaging materials can be depyrogenised during various process steps, such as ultrasonic treatment, washing and heat treatment. The results show that for successful depyrogenation, the primary packaging material must be sterilized with dry heat. Washing on its own is not, therefore, sufficient to achieve a 3-log reduction in endotoxin. Ultrasound can also be used to assist in the cleaning of primary packaging materials.

References **March 2022**

European Commission (25.11.2008): Volume 4 EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use- Annex 1 Manufacture of Sterile Medicinal Products (corrected version). Online available: https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-4/2008_11_25_gmp-an1_en.pdf, last checked on 10. November 2021.

The United States Pharmacopeial Convention (2018_Entwurf): <1085> Guidelines on Endotoxins Testing.

The United States Pharmacopeial Convention: <1228> Depyrogenation.

The United States Pharmacopeial Convention: <151> Pyrogen Test. Online available: http://www.pharmacopeia.cn/v29240/usp29nf24s0_c151.html, last checked on 10. November 2021.

The United States Pharmacopeial Convention (01 Dec., 2012): <85> Bacterial Endotoxins Test.

U.S. Department of Health and Human Services Food and Drug Administration (2004): Guidance for Industry- Sterile Drug Products Produced by Aseptic Processing- Current Good Manufacturing Practice. Online available: https://www.fda.gov/media/71026/download, last checked on 10. November 2021.

Questions? Let's talk!

If you would like more information about this topic please feel free to contact us.

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