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# Sterilization of health care products — Microbiological methods —

## Part 3: Bacterial endotoxin testing

*Stérilisation des produits de santé — Méthodes microbiologiques — Partie 3: Essai des endotoxines bactériennes*

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

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# Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

ISO draws attention to the possibility that the implementation of this document may involve the use of (a) patent(s). ISO takes no position concerning the evidence, validity or applicability of any claimed patent rights in respect thereof. As of the date of publication of this document, ISO had not received notice of (a) patent(s) which may be required to implement this document. However, implementers are cautioned that this may not represent the latest information, which may be obtained from the patent database available at www.iso.org/patents. ISO shall not be held responsible for identifying any or all such patent rights.

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 198, *Sterilization of health care products*.

A list of all parts in the ISO 11737 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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# Introduction

A pyrogen is any substance that can induce fever. Testing for pyrogens is required for release of many health care products. Pyrogens can be classified into two groups: microbial (e.g. bacteria, fungi, viruses) and non-microbial (e.g. drugs, device materials, steroids, plasma fractions; see the ISO 10993 series). The predominant pyrogenic contaminants encountered in the manufacturing of health care products are bacterial endotoxins, which are components of the cell walls of Gram-negative bacteria. Although Gram-positive bacteria, fungi, and viruses can be pyrogenic, they do so through different mechanisms (systemic effects) and to a lesser degree than Gram-negative bacteria. Only the Gram-negative bacterial endotoxins test (BET) using amebocyte lysate reagents from *Limulus polyphemus* or *Tachypleus tridentatus* is covered in this document. Other endotoxin detection methodologies, such as monocyte activation and recombinant Factor C (rFc), are not included (see B.12) in this document.

Endotoxins are the molecular weight lipopolysaccharide (LPS) components of the outer cell wall of Gram-negative bacteria, that can cause fever, meningitis, and a rapid fall in blood pressure if introduced into the blood stream or certain other tissues of the body. The outer cell wall components, which are composed primarily of proteins, phospholipids and LPS, are constantly released by the cell into the surrounding environment. Endotoxins are ubiquitous in nature, stable, and small enough to pass through conventional sterilizing filters. Sterilization processes will inactivate microorganisms on or in products, but usually do not inactivate endotoxin on products. With controlled processes, endotoxin contamination can be prevented.

The non-pyrogenicity of a health care product can be achieved through the following:

a) manufacturing techniques that prevent or control endotoxin contamination (e.g. contamination with Gram-negative bacteria);

b) depyrogenation by endotoxin inactivation (e.g. dry heat) or physical removal (e.g. rinsing, distillation, ultrafiltration).

The purpose of this document is to describe the requirements and guidance for testing for bacterial endotoxins. This includes product required to be non-pyrogenic based on either intended use or non-pyrogenic label claim, or both. Guidance is also provided on selection of product units, method suitability, use of techniques for routine testing, interpretation of test results, and alternatives to batch testing and risk assessment. Information on the following is provided in the annexes:

— guidance on bacterial endotoxin testing (Annex A);

— the history and background on the BET (Annex B);

— guidance on out of specified limits (OSL) and failure investigation (Annex C);

— guidance on in-process monitoring of manufacturing or component testing (Annex D);

— guidance on conducting a risk assessment to support alternatives to batch testing (Annex E);

— typical assignment of responsibilities (Annex F).

This document is based on ANSI/AAMI ST72. Several sections in this document have been restructured and extended or changed from ANSI/AAMI ST72.


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# Sterilization of health care products — Microbiological methods —

# Part 3: Bacterial endotoxin testing

## 1 Scope

### 1.1 Inclusions

This document specifies general criteria to be applied in the determination of bacterial endotoxins on or in health care products, components or raw materials using bacterial endotoxins test (BET) methods, using amebocyte lysate reagents.

### 1.2 Exclusions

**1.2.1** This document is not applicable to the evaluation of pyrogens other than bacterial endotoxins. Other endotoxin detection methodologies are not included (see B.12).

**1.2.2** This document does not address setting specific endotoxin limit specifications.

## 2 Normative references

There are no normative references in this document.

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

**3.1**
**bacterial endotoxins test**
**BET**
assay for measuring bacterial endotoxins by combining an aqueous test sample or test sample extract with *Tachypleus* amebocyte lysate *(TAL)* (3.41) or *Limulus* amebocyte lysate *(LAL)* (3.28) reagent and measuring the resulting proportional reaction via visual, *turbidimetric* (3.42) or *chromogenic techniques* (3.3)

**3.2**
**batch**
defined quantity of a product intended or purported to be uniform in character and quality produced during a specified cycle of manufacture

[SOURCE: ISO 11139:2018, 3.21]

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