ISO/DIS 11737-3 Sterilization of health care products — Microbiological methods — Part 3: Bacterial endotoxin testing

Foreword

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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.

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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). The predominant pyrogenic contaminants 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 will be covered in this document. Other endotoxin detection methodologies, such as monocyte activation and recombinant Factor C (rFc), are not included (see B.12).

Endotoxins are the high molecular weight lipopolysaccharide (LPS) components of the outer cell wall of Gram-negative bacteria, which can cause fever, meningitis, and a rapid fall in blood pressure if introduced into blood or 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 address microorganism viability on products, but often do not address endotoxin levels 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 (i.e. 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 intended use and/or non-pyrogenic label claim. 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 the application of the normative requirements (Annex A);

— the background on the bacterial endotoxins test (Annex B);

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

— guidance on in-process monitoring of manufacturing (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 ST72:2019. Several sections in both the normative and the informative parts have been restructured and extended or changed.

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 amoebocyte lysate reagents.

NOTE See Annex A for guidance on Clauses 1 to 10.

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.
NOTE Endotoxin limits are determined according to applicable regulatory requirements and should be consistent with the intended clinical application.
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 terminological 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 or Limulus amebocyte lysate (TAL/LAL) reagent and measuring the resulting proportional reaction via visual, turbidimetric, or chromogenic techniques
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]
3.3
chromogenic technique
BET methodology that quantifies endotoxins on the basis of a measured colour-producing reaction proportional to the interaction of LAL and endotoxin
3.4
control standard endotoxin (CSE)
endotoxin standard preparation whose potency has been standardized against the Reference Standard Endotoxin (RSE) for a specific batch of LAL
3.5
depyrogenation

process used to remove or deactivate pyrogenic substances to a specified level
Note 1 to entry: Pyrogenic substances include bacterial endotoxins.
[SOURCE:ISO 11139:2018, 3.77]
3.6
direct contact
medical device or medical device component that comes into physical contact with body tissue
[SOURCE:ISO 10993-1:2018, 3.6]
3.7
end product
product samples that have completed the entire manufacturing process
Note 1 to entry: For the purposes of this document, end product testing can be performed prior to sterilization (pre-sterilization samples) or after sterilization (post-sterilization samples). For limitations see 5.2.6
3.8
endotoxin or bacterial endotoxin
lipopolysaccharide component of the cell wall of Gram-negative bacteria that is heat stable and elicits a variety of inflammatory responses in animals and humans
[SOURCE:ISO 11139:2018, 3.101]
3.9
endotoxin limit
maximum allowable level of endotoxin specified for a product
3.10
endotoxin unit (EU)/ international unit (IU)
standard unit of measure for endotoxin activity initially established relative to the activity contained in 0.2 ng of the US. Reference Standard Endotoxin Lot EC-2 (USP standard reference material)
Note 1 to entry: Currently, the US. Reference Standard Endotoxin EC-6, USP Lot G, and the World Health Organization’s primary international endotoxin standard (IS) are sub-lots of the same endotoxin preparation, making the EU and IU equal [43].
3.11
end point
the most dilute concentration of a test or control solution for which a positive reaction for bacterial endotoxin is observed

Note 1 to entry: This definition is used for a concentration dependent aspect of bacterial endotoxin testing, in contrast to the end point methods described in A.6.1.1.

3.12

enhancement

BET anomaly in which a non-endotoxin related factor, usually attributable to a characteristic of the test sample, elicits a test reaction greater than the amount of endotoxin present

3.13

gel-clot technique

BET methodology that quantifies or detects endotoxin on the basis of a clot-producing reaction proportional to the interaction of LAL and endotoxin

3.14

geometric mean end point

the antilog of the average of the logarithmic values with respect to the end points from replicate dilution series converted back to a base 10 number used to establish the central tendency or typical value from a test solution

3.15

indirect contact

medical device or medical device component through which a fluid or gas passes, prior to the fluid or gas coming into physical contact with body tissue (in this case the medical device or medical device component itself does not physically contact body tissue)

[SOURCE:ISO 10993-1:2018, 3.11]

3.16

inhibition

BET anomaly in which a non-endotoxin related factor, usually attributable to a characteristic of the test sample, elicits a test reaction less than the amount of endotoxin present

3.17

inhibition/enhancement test (test for interfering factors)

method suitability

test used to determine whether a particular sample contains factors that diminish its accuracy by introducing enhancement or inhibition into the test system

3.18

interference

an interfering factor observed in the performance of the test that exceeds the acceptable threshold for a given BET technique (e.g. positive product control that indicates a detected endotoxin level less than 50 % or greater than 200 % or ± 2 lambda)

3.19

intraocular

located or occurring within or administered through the eye

3.20

interfering factors

non-endotoxin related factor, usually attributable to a characteristic of the test sample, that causes inhibition or enhancement

3.21

intravascular

located or occurring within or administered through the heart or blood vessels

3.22

intralymphatic

located or occurring within or administered through a lymph vessel

3.23

intrathecal

located, or occurring within or administered through the space under the arachnoid membrane of the brain or spinal cord

3.24

kinetic method

photometric quantitative techniques (turbidimetric or chromogenic) for BET

3.25

LAL reactive material (LAL-RM)

any non-endotoxin compound that will activate the LAL clotting cascade and cause enhancement

3.26

lambda (λ)

labeled sensitivity of an LAL gel-clot reagent expressed in EU/mL or, for chromogenic or turbidimetric tests, the lowest point (endotoxin concentration) on the referenced standard curve

3.27

Limulus amebocyte lysate (LAL)

reagent extracted from amebocytes taken from hemolymph of the horseshoe crab, Limulus polyphemus, which reacts with endotoxin, to form a gelatinous clot and is used to estimate endotoxin levels in BET methods
Note 1 to entry: The term LAL is sometimes used to describe TAL, as both are similar lysates that are used in the BET.
3.28
lipopolysaccharide
Gram-negative bacterial cell wall component composed of lipid A, a core polysaccharide, and an O-side chain
3.29
maximum valid dilution (MVD)
maximum amount a sample can be diluted or the total extraction volume used relative to the sensitivity of a BET in which the specified endotoxin limit can be detected
3.30
non-pyrogenic
does not induce a fever
Note 1 to entry: Describes an item or product that contains endotoxin levels that comply to specified limits.
3.31
out of specification limits (OSL)
a sample with a valid BET result that exceeds a product endotoxin limit specification
Note 1 to entry: The term OSL applies only within the context of this document and does not imply compliance with any other regulatory guidance dealing with out of specification (OOS) results.
3.32
product positive control (PPC)
a sample spiked with a known amount of endotoxin used for confirmation that the product being tested is not subject to interfering factors
3.33
product realization
all processes consistent with the quality system requirements that are needed to develop, manufacture, and deliver the end product
3.34
pyrogen
any substance that induces a fever

3.35
pyrogenic
induces a fever
Note 1 to entry: Describes an item or product that contains endotoxin levels above specified limits.
3.36
Reference Standard Endotoxin (RSE)
USP Endotoxin Reference Standard that has a defined potency of 10,000 USP EUs per vial
3.37
repeat test
an analysis of additional product samples from a previously tested batch or another batch
3.38
retest
a reanalysis of previously tested product samples or product sample preparation
3.39
standard control series
serial dilution series of RSE or CSE used to verify LAL sensitivity
3.40
Tachypleus amebocyte lysate (TAL)
reagent extracted from amebocytes taken from hemolymph of the horseshoe crab, Tachypleus tridentatus, which reacts with endotoxin, to form a gelatinous clot and is used to estimate endotoxin levels in BET methods
Note 1 to entry: The term TAL is sometimes used to describe LAL, as both are similar lysates that are used in the BET.
3.41
turbidimetric technique
BET methodology that quantifies or detects endotoxin on the basis of a measured turbidity reaction proportional to the interaction of LAL and endotoxin
3.42
validation
confirmation process, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

Note 1 to entry: The objective evidence needed for a validation is the result of a test or other form of determination such as performing alternative calculations or reviewing documents.
Note 2 to entry: The word “validated” is used to designate the corresponding status.
Note 3 to entry: The use conditions for validation can be real or simulated.
[SOURCE:ISO 11139:2018, 3.313]
3.43
verification
confirmation, through the provision of objective evidence, that specified requirements have been fulfilled
Note 1 to entry: The objective evidence needed for a verification can be the result of an inspection or of other forms of determination such as performing alternative calculations or reviewing documents.
Note 2 to entry: The word “verified” is used to designate the corresponding status.
[SOURCE:ISO 9000:2015, 3.8.12, modified — The original Note 2 to entry has been deleted and Note 3 has been renumbered as Note 2 accordingly.]
3.44
water for bacterial endotoxins test (WBET)
purified water employable as a solvent, diluent, and/or extractant that is non-reactive with the lysate employed at the detection limit of the reagent, and does not elicit interference with methodology in use (typically LAL reagent water, water for injection, or other appropriate solution meeting these requirements)

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