American National Standard

ANSI/AAMI ST72:2019

Bacterial endotoxins—
Test methods, routine monitoring, and alternatives to batch testing

AAMI
Advancing Safety in Health Technology

Erratum

American National Standard
Bacterial endotoxin -Test methods, routine monitoring and alternatives to batch testing

TECHNICAL CORRIGENDUM 1

(ANSI/AAMI/ISO ST72:2019)

Erratum issued: 16 April 2020

Page 7, *Equation 2 and Equation 3*

On each equation replace the denominator, “1”, with a lambda, “λ”.

Bacterial endotoxins – Test methods, routine monitoring, and alternatives to batch testing

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Abstract: Specifies general criteria to be applied in the determination of bacterial endotoxins on or in medical devices, components, or raw materials employing bacterial endotoxins test (BET) methods using amebocyte lysate reagents *from Limulus polyphemus* or *Tachypleus tridentatus*. The document is not applicable to the evaluation of pyrogens other than bacterial endotoxins.

Keywords: Limulus amebocyte lysate, LAL, pyrogenic labeling, maximum valid dilution, MVD, RSE:CSE standardization, analyst qualification, product qualification, gel-clot technique, chromogenic technique, turbidimetric technique, medical device, batch testing, laboratory quality system, product family, set, sample frequency, kinetic assay

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Contents

Page

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Association for the Advancement of Medical Instrumentation

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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 A.12).

Endotoxin is the high molecular weight lipopolysaccharide (LPS) component 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 into the environment. Because it is ubiquitous in nature, stable, and small enough to pass through conventional sterilizing filters, endotoxin contamination is difficult to prevent.

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

a) manufacturing techniques that prevent or control endotoxin contamination,

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

The purpose of this document is to consolidate the requirements and guidance for testing for bacterial endotoxins. This includes product required to be non-pyrogenic due to intended use and non-pyrogenic labelling. Details are 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:

— the background/history of endotoxin testing (Annex A),

— guidance on endotoxin test methods, routine monitoring, and alternatives to batch testing (Annex B),

— guidance on out of specification test results and investigation (Annex C),

— guidance on in-process monitoring of manufacturing processes and component testing (Annex D), and

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

The annexes to this document are for information only.

Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing

1 Scope

1.1 This document specifies general criteria to be applied in the determination of bacterial endotoxins on or in medical devices, components, or raw materials employing bacterial endotoxins test (BET) methods using amebocyte lysate reagents *from Limulus polyphemus* or *Tachypleus tridentatus*.

NOTE Although the scope of this standard is limited to medical devices, it also includes requirements and provides testing guidance that might be applicable to other health care products, such as, biologics, tissue-based products and combination products.

1.2 This document is not applicable to the evaluation of pyrogens other than bacterial endotoxins.

2 Normative references

The following documents contain provisions that, through reference in this text, constitute provisions of this guideline. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this guideline are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below.

The United States Pharmacopoeia (USP) <85>, current edition, United States Pharmacopeial Convention (USP), Rockville MD.

The United States Pharmacopoeia (USP) <161>, current edition, United States Pharmacopeial Convention (USP), Rockville MD.

U.S. Food and Drug Administration:1998*, Quality System Regulation*, 21 CFR, Part 820.

3 Definitions

For the purpose of this document, the following definitions apply.

3.1 bacterial endotoxins test (BET): Assay for measuring bacterial endotoxins by combining a liquid 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 product intended or purported to be uniform in character and quality produced during a specified cycle of manufacture.

[Source: ISO 11139:2018]

3.3 chromogenic technique: BET methodology that quantifies or detects endotoxins on the basis of a measured color-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: Validated process designed to remove or inactivate endotoxin.

3.6 direct contact: Term used for a device or device component that comes into physical contact with body tissue

3.7 end product testing: Testing carried out on product samples that have completed the entire manufacturing process.

3.8 endotoxin or bacterial endotoxin: High molecular weight complex associated with the cell wall of Gram-negative bacteria that is pyrogenic in humans and specifically interacts with an endotoxin detection system.