American National Standard

ANSI/AAMI ST72:2002

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

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AAMI Association for the Advancement of Medical Instrumentation

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It is most important that the objectives and potential uses of an AAMI product standard or recommended practice are clearly understood. The objectives of AAMI's technical development program derive from AAMI's overall mission: the advancement of medical instrumentation. Essential to such advancement are (1) a continued increase in the safe and effective application of current technologies to patient care, and (2) the encouragement of new technologies. It is AAMI's view that standards and recommended practices can contribute significantly to the advancement of medical instrumentation, provided that they are drafted with attention to these objectives and provided that arbitrary and restrictive uses are avoided.

A voluntary *standard* for a *medical device* recommends to the manufacturer the information that should be provided with or on the product, basic safety and performance criteria that should be considered in qualifying the device for clinical use, and the measurement techniques that can be used to determine whether the device conforms with the safety and performance criteria and/or to compare the performance characteristics of different products. Some standards emphasize the information that should be provided with the device, including performance characteristics, instructions for use, warnings and precautions, and other data considered important in ensuring the safe and effective use of the device in the clinical environment. Recommending the disclosure of performance characteristics often necessitates the development of specialized test methods to facilitate uniformity in reporting; reaching consensus on these tests can represent a considerable part of committee work. When a drafting committee determines that clinical concerns warrant the establishment of *minimum* safety and performance criteria, referee tests must be provided and the reasons for establishing the criteria must be documented in the rationale.

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Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific need of the user.

Particular care should be taken in applying a product standard to existing devices and equipment, and in applying a recommended practice to current procedures and practices. While observed or potential risks with existing equipment typically form the basis for the safety and performance criteria defined in a standard, professional judgment must be used in applying these criteria to existing equipment. No single source of information will serve to identify a particular product as "unsafe". A voluntary standard can be used as one resource, but the ultimate decision as to product safety and efficacy must take into account the specifics of its utilization and, of course, cost-benefit considerations. Similarly, a recommended practice should be analyzed in the context of the specific needs and resources of the individual institution or firm. Again, the rationale accompanying each AAMI standard and recommended practice is an excellent guide to the reasoning and data underlying its provision.

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

Developed by
Association for the Advancement of Medical Instrumentation

Approved 10 June 2002 by
American National Standards Institute, Inc.

Abstract Specifies general criteria to be applied in the determination of bacterial endotoxins (pyrogens) on medical devices, components, or raw materials. Bacterial endotoxin methodologies covered include both qualitative (limit) methods and quantitative (end-point) methods using *Limulus* amebocyte lysate methodology. Determination of pyrogens other than bacterial endotoxins is not covered and acceptable levels for bacterial endotoxins are not covered.

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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Association for the Advancement of Medical Instrumentation

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NOTE—Participation by federal agency representatives in the development of this Recommended Practice does not constitute endorsement by the federal government or any of its agencies.

Foreword

Pyrogens are any substance that can induce fever in a host. 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 most significant pyrogens have been found to be endotoxins from Gram-negative bacteria. Although Gram-positive bacteria, fungi, and viruses can induce fever in the host, they do so through a different mechanism (systemic effects) and to a lesser degree than Gram-negative bacteria. Only Gram-negative bacterial endotoxin testing will be covered in this document.

Endotoxin is the high molecular weight lipopolysaccharide (LPS) component of the outer cell wall of Gram-negative bacteria that causes 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 when Gram-negative bacteria divide or lyse. Endotoxin contamination is difficult to prevent because it is ubiquitous in nature, stable, and small enough to pass through conventional sterilizing filters.

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

1) use of manufacturing techniques that produce non-pyrogenic products,

2) minimization of the source of pyrogens in the manufacturing process, and

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

This document will focus primarily on product manufactured under conditions that do not require a depyrogenation step as part of the manufacturing process.

The purpose of this document is to consolidate the requirements for testing for bacterial endotoxins. This includes the selection of product units for testing, selection and validation of testing technique, use of technique for routine testing, and interpretation of test results. This document also addresses the requirements for manufacturing operation validation that would support alternatives to batch testing.

The information included in the annexes provides:

— the background/history of endotoxin testing,

— guidance on endotoxin test methodologies, and

— guidance on alternatives to batch testing and validating manufacturing operations.

As used within the context of this document, "shall" indicates requirements strictly to be followed to conform to the recommended practice. "Should" indicates that among several possibilities, one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action should be avoided but is not prohibited. "May" is used to indicate that a course of action is permissible within the limits of the recommended practice. "Can" is used as a statement of possibility and capability. Finally, "must" is used only to describe "unavoidable" situations, including those mandated by government regulation.

The annexes to this Recommended Practice/American National Standard are for information only.

Suggestions for improving this Recommended Practice are invited. Comments and suggested revisions should be sent to Technical Programs, AAMI, 1110 N. Glebe Road, Suite 220, Arlington, VA 22201-5762.

NOTE—This foreword does not contain provisions of ANSI/AAMI ST72:2002, *Bacterial endotoxins—Test methodologies, routine monitoring, and alternatives to batch testing*, but it does provide important information about the development and intended use of the document.

Bacterial endotoxins—Test methodologies, 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 using bacterial endotoxin test methodology.

NOTE—Although the scope of this standard is limited to medical devices, it specifies requirements and provides guidance that may be applicable to other health care products.

1.2 The bacterial endotoxin test methodologies covered in this document include both qualitative methods and quantitative methods.

1.3 This document is not applicable to the determination of pyrogens other than bacterial endotoxins.

1.4 This document does not specify acceptable levels for bacterial endotoxins.

NOTE—For acceptable levels for bacterial endotoxins, reference the appropriate regulatory standards.

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.

ISO 9001:2000, *Quality Management Systems—Requirements.*

The United States Pharmacopoeia (USP). Easton: Mack, 2000, 24th ed., ⟨85⟩ & ⟨161⟩.

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

U.S. Food and Drug Administration: *Guideline on validation of the* Limulus *amebocyte lysate test as an end-product endotoxin test for human and animal parenteral drugs, biological products, and medical devices*. DHHS, December (1987).

FDA Interim Guidance for Human and Veterinary Drug Products and Biologicals, *Kinetic LAL Techniques*, July 15, 1991.

3 Definitions

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

3.1 bacterial endotoxins test (BET): Assay for measuring active endotoxin by combining a liquid test sample with *Limulus* amebocyte lysate (LAL) reagent and measuring the resulting proportional reaction via visual, turbidimetric, chromogenic, or other validated means of detection.

3.2 batch: Defined quantity of bulk, intermediate, or finished product, intended or purported to be uniform in character and quality, which has been produced during a defined cycle of manufacture.

3.3 chromogenic technique: BET methodology that quantifies or detects endotoxin on the basis of a measured color-producing reaction proportional to the interaction of LAL and endotoxin.

3.4 control standard endotoxin (CSE): Endotoxin preparation other than the reference standard endotoxin (RSE) that has been standardized against the RSE.

3.5 depyrogenation: Validated process designed to remove or inactivate endotoxin.

3.6 endotoxin: High molecular weight complex associated with the cell wall of Gram-negative bacteria that is pyrogenic in humans and specifically interacts with LAL.

3.7 endotoxin unit (EU): Standard unit of measure for endotoxin activity initially established relative to the activity contained in 0.2 ng of the U.S. Reference Standard Endotoxin Lot EC-2 (USP standard reference material).

NOTE—FDA's reference 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 (Poole et al., 1997).

3.8 endpoint (gel clot): Last positive tube in a series of dilutions.

3.9 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.

3.10 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.

3.11 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.

3.12 inhibition/enhancement test: Test used to determine whether a particular BET sample contains factors that diminish its accuracy by introducing enhancement or inhibition into the test system.

3.13 interfering factors test: See inhibition/enhancement test.

3.14 LAL reactive material (LAL-RM): Any non-endotoxin compound that will activate the LAL clotting cascade and therefore has the potential to cause enhancement.

3.15 LAL reagent water (LRW): Purified water or other qualified solutions employable as a solvent, diluent, and/or extractant in a BET, and as such must be non-reactive in the methodology in use.

3.16 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.

3.17 *Limulus* amebocyte lysate (LAL): Reagent extracted from circulating amebocytes of the horseshoe crab, *Limulus polyphemus* or *Tachypleus tridentatus* (TAL), that interacts with endotoxin to form a gelatinous clot and is used to estimate endotoxin levels via the bacterial endotoxins test.

3.18 lipopolysaccharide: Gram-negative bacterial cell wall component typically composed of lipid A, a core polysaccharide, and an O-side chain.

3.19 lot: See **batch**.

3.20 maximum valid dilution (MVD): Maximum amount a sample can be diluted relative to the sensitivity of an LAL assay in which the specified test endotoxin limit can be detected.

3.21 non-pyrogenic: Term used to describe a health care product that does not induce a fever.

NOTE—May also be used to describe and label health care products that contain endotoxin levels less than specified limits.

3.22 product endotoxin limit: Maximum allowable level of endotoxin specified for a product, established below the threshold pyrogenic dose of endotoxin in humans.

3.23 pyrogen: Any substance that induces a fever.

3.24 pyrogenic: Term used to describe a health care product that induces a fever.

NOTE—May also be used to describe a health care product with an endotoxin level above specified limits.

3.25 reference standard endotoxin (RSE): USP Endotoxin Reference Standard that has a defined potency of 10,000 USP EUs per vial.

3.26 standard control series: Serial dilution series of RSE or CSE used to verify LAL sensitivity.

3.27 test endotoxin limit: Maximum endotoxin concentration allowable in a BET sample extract.

NOTE—This limit is determined by dividing the product endotoxin limit by the volume of the LRW used per unit for sample extraction.

3.28 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.

3.29 validation: Documented procedure for obtaining, recording, and interpreting the results needed to demonstrate that a process will consistently yield a product complying with predetermined specifications.

4 General

4.1 Documentation

4.1.1 Approved procedures and instructions on the testing techniques to be employed and the use and operation of all relevant equipment shall be available and shall be controlled as specified in quality system requirements.

4.1.2 Calculations and data transfers shall be subject to appropriate checks.

NOTE—If calculations are performed by electronic data processing techniques, the application of the software should be validated for the intended uses, and records of this validation should be retained.

4.1.3 Records of original observations, calculations, derived data, and final reports shall be retained as specified in quality system requirements. The records shall include the identity of personnel involved in the preparation and testing of samples.

4.2 Personnel

4.2.1 Responsibility for performing a BET shall be assigned to personnel as specified in quality system requirements.

4.2.2 Training shall be performed in accordance with documented procedures. Records of the relevant qualifications, training, and experience of technical personnel shall be maintained.

4.2.3 Analyst qualification shall be conducted prior to performance of a BET (see 8.3.3).

4.3 Equipment

4.3.1 All equipment required for performance of the specified tests and measurements shall be available, and planned maintenance and calibration shall be performed in accordance with documented procedures. Records of maintenance and calibration shall be retained.

4.3.2 All equipment shall be documented to perform according to specified criteria.

4.4 Reagents and materials

4.4.1 Methods shall be established and documented for the preparation of reagents, controls, and reference materials used in a BET, including appropriate quality tests.

NOTE—Appropriate quality tests should include confirmation of lambda.

4.4.2 Methods shall be established, validated, and documented for the depyrogenation of glassware and other heat-stable equipment used in a BET.

NOTE—Appropriate guidelines for depyrogenation of materials are referenced in USP, Weary, and PDA Technical Report No. 7.

4.4.3 Materials used in a BET shall be demonstrated to be free of detectable endotoxin if depyrogenation is not performed. This may be performed, for example, by testing a sample of purchased materials to demonstrate that they are non-pyrogenic or by accepting an appropriate vendor certificate.

NOTE—Containers used for sampling, storing, or diluting should be free of interference. For example, polypropylene has been shown to inhibit endotoxin detection (Rolansky, 1991).

5 Non-pyrogenic labeling

5.1 Products that directly or indirectly contact the cardiovascular system, lymphatic system, or cerebrospinal fluid, or present the potential for similar systemic exposures (e.g., solution administration sets, transfer sets, catheters, implants, and infusion assemblies), or ophthalmic products for intraocular use (e.g., silicone oil, viscoelastic products, intraocular lenses) shall be evaluated for endotoxin.

5.2 Application of any non-pyrogenic product label statement or claim shall require explicit substantiation. Such substantiation may include:

— direct testing of the product employing a validated BET by qualified personnel,