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Standard Guide for Determination of Endotoxin on Sterile Medical Gloves¹

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INTRODUCTION

This guide is established and designed to determine the qualitative or quantitative presence of bacterial endotoxin on sterile medical gloves. Bacterial endotoxins are found in the outer membrane of gram negative bacteria and may contaminate gloves during the manufacturing process. Consequences of endotoxin introduced into a patient during invasive procedures are dose dependent and may include inflammation, fever, nausea, pain, clot formation, hypoglycemia and reduced perfusion of the heart, kidney, and liver as well as endotoxic shock. Endotoxins are not inactivated by routine methods utilized in the routine sterilization of medical gloves including irradiation (gamma or E-beam), ethylene oxide, or steam.

1. Scope

1.1 This guide covers a selection of methodologies for the determination of bacterial endotoxin on gloves when such a determination is appropriate.

1.2 As bacteria may continue to grow on non-sterile gloves, reportable endotoxin levels are only appropriate for sterile gloves.

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

1.4 The sample preparation method described must be used regardless of the test method selected. This method does not describe laboratory test method validation, analyst qualification, or reagent confirmation. Product-specific validation is addressed.

1.5 The safe and proper use of medical gloves is beyond the scope of this guide.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory requirements prior to use.*

2. Referenced Documents

2.1 EN Standard:²

EN 455-3:1999 Medical Gloves for Single Use—Part 3:

¹ This guide is under the jurisdiction of ASTM Committee D11 on Rubber and is the direct responsibility of Subcommittee D11.40 on Consumer Rubber Products. Current edition approved Dec. 1, 2004. Published January 2005. DOI: 10.1520/D7102-04.

² Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036.

Requirements and Testing for Biological Evaluation

2.2 ANSI Standard:²

ANSI/AAMI ST 72:2002 Bacterial Endotoxins—Test Methodologies, Routine Monitoring and Alternatives to Batch Testing

3. Terminology

3.1 Definitions:

3.1.1 *bacterial endotoxin test (BET)*—a method for determining the qualitative or quantitative presence of endotoxin in an aqueous test sample utilizing *Limulus* amoebocyte lysate (LAL) reagent and measuring the resulting proportional reaction.

3.1.2 *batch*—defined quantity of intermediate or finished product produced in a defined cycle of manufacture that is said to be of uniform quality.

3.1.3 *chromogenic (colorimetric) 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.

3.1.4 *control standard endotoxin (CSE)*—purified endotoxin product supplied at a known potency and utilized as a standard control in endotoxin testing.

3.1.5 *device*—with regard to medical gloves, a device is defined as a pair of gloves when they are packaged in pairs and a single glove when packaged singly.

3.1.6 *endotoxin*—high molecular weight, heat stable complex associated with the cell wall of gram-negative bacteria that is pyrogenic in humans and specifically interacts with LAL.

3.1.7 *endotoxin unit (EU)*—the standard unit of measure for endotoxin activity initially established relative to the activity in 0.2 ng of the U.S. Reference Standard Endotoxin (USP standard reference material).

3.1.7.1 *Discussion*—The FDA's endotoxin standard and that of the World Health Organization's International Endotoxin Standard (*IU*) are sub lots of the same endotoxin preparation, making *EU* and *IU* equal.

3.1.8 *endpoint (gel clot)*—last positive (coagulated or gel clot) tube in a series of dilutions.

3.1.9 *enhancement*—a type of interference that renders test results with higher values than the amount of endotoxin present.

3.1.10 *gel-clot technique*—*BET* methodology that can be used to detect or quantify the presence of endotoxin based on the proportional reaction of endotoxin with *LAL*.

3.1.11 *inhibition*—*BET* anomaly wherein a non-endotoxin substance, usually contributed by the sample, elicits a test reaction less than the amount of endotoxin actually present.

3.1.12 *inhibition/enhancement test*—test used to determine whether a particular *BET* sample contains factors that diminish its accuracy of the *BET* either by enhancement or inhibition of the results.

3.1.13 *interfering substances*—those substances that cause inhibition or enhancement.

3.1.14 *Limulus amoebocyte lysate (LAL)*—the reagent extracted from amoebocytes in the circulatory system of the horseshoe crab *Limulus polyphemus* or *Tachyplesus tridentatus* (*TAL*), which forms a clot when brought into contact with substances containing endotoxin.

3.1.15 *lot*—see *batch*.

3.1.16 *lipopolysaccharide (LPS)*—the gram-negative cell wall component typically composed of lipid A, a core polysaccharide, and an O-side chain sugar moiety.

3.1.17 *LAL reagent water (LRW)*—*LAL* reagent water that has been validated to contain no detectable endotoxin.

3.1.18 *maximum valid dilution (MVD)*—the highest dilution a sample is permitted to attain in diluting out interfering substances while still being capable of detecting endotoxin in the sample extract.

3.1.19 *non-pyrogenic*—describes a product that does not induce a fever. Also used to label medical devices that contain endotoxin below a specified level.

3.1.20 *pyrogen*—any substance that can induce a fever. Endotoxins are one type of pyrogen.

3.1.21 *pyrogenic*—a term used to describe healthcare products with endotoxin levels above specified limits.

3.1.22 *reference standard endotoxin (RSE)*—the USP endotoxin reference standard defined to have a potency of 10 000 USP *EUs* per vial.

3.1.23 *turbidimetric technique*—*BET* methodology that detects or quantifies endotoxin based on the level of turbidity created proportional to the interaction of *LAL* and endotoxin.

4. Summary of Guide

4.1 A standard method of sample preparation is specified in this guide.

4.2 Four variations of endotoxin determination test methods are identified and briefly described to facilitate selection of the

appropriate method. The reader is referred to the referenced standards for complete instructions.

5. Significance and Use

5.1 This guide establishes a standard sample preparation method and provides a description of four established and recognized test methods for the determination of endotoxin on medical gloves. If interferences in a sample yield suspect results, a second method should be used.

5.2 This guide is appropriate for testing final product that has been subjected to all processes that could influence the final endotoxin level (either microbial contamination or processing agents/raw materials contaminated with endotoxin). As raw materials and processing conditions vary from lot to lot with regard to these parameters, it is appropriate to test for endotoxin on a routine basis if a product endotoxin claim is to be made (for example, non-pyrogenic). The user may find it beneficial to incorporate endotoxin testing for vulnerable areas of their manufacturing process as an alert mechanism.

6. Sampling, Sample Preparation, and Extraction

NOTE 1—All gloves must follow this sampling plan, sample preparation, and extraction method regardless of assay method chosen.

6.1 *Sampling*—The bacterial endotoxin test shall be carried out for each batch of gloves where a limit has been set. The sampling plan should be based on the batch size. Three percent of the batch should be tested with a minimum of 3 pairs of gloves and a maximum of 10 pairs of gloves per batch. For batch sizes under 30 units, two pairs of gloves may be analyzed.

6.1.1 Samples selected for testing should be produced and selected in the finished form. This includes all factors that might affect or contribute to the levels of endotoxin (for example, post-packaging and sterilization).

6.2 Sample Extraction:

6.2.1 Handle everything with pyrogen-free instruments. Perform all extractions in non-pyrogenic containers.

6.2.2 A sample extraction is prepared by immersing the outside surface of the gloves in *LRW*. The extractions should be performed with 40 mL of *LRW* per pair of gloves, for 40 to 60 min at a temperature between 37 to 40°C.

6.2.3 The extraction should be performed in a way to ensure that all surfaces of the gloves that would have patient contact, come in contact with the extraction medium. For example, the glove may be lowered into a flask containing 40 mL of *LRW* with the exterior of the glove in contact with the water and the cuff (≤ 2 cm) folded over the opening at the neck of the flask.

6.2.4 After the sample has been extracted, remove the glove. If appropriate, the second glove of the pair can then be extracted in the same flask with the extraction fluid of the first glove or both gloves may be extracted in the 40 mL *LRW* at the same time by both being suspended simultaneously in the flask, held in place by a non-pyrogenic stopper by the cuff at the opening of the flask.

6.2.5 Powder and other particulate matter can interfere with endotoxin determination assays. Interference should be overcome with sample dilution. Neither extract filtration nor centrifugation for clarification or the removal of particulates