⟨161⟩ MEDICAL DEVICES—BACTERIAL ENDOTOXIN AND PYROGEN TESTS

INTRODUCTION

The methods and requirements in this chapter apply to assemblies or devices labeled sterile and nonpyrogenic that are in contact directly or indirectly with the cardiovascular system, lymphatic system, or cerebrospinal fluid. This includes, but may not be limited to, the following:

- Fluid pathways of catheters and administration sets such as solution administration sets, extension sets, transfer sets, blood administration sets, intravenous catheters, implants, extracorporeal oxygenator tubing, dialysis tubing, intramuscular drug delivery catheters, and transfusion and infusion assemblies
- Liquid medical devices such as dialysate
- Implantable medical devices such as heart valves and vascular grafts, and other medical devices with a nonpyrogenic claim that may come into contact with blood or cerebrospinal fluid
- Gels with a nonpyrogenic claim including demineralized bone matrices and drug delivery systems

BACTERIAL ENDOTOXINS

For the bacterial endotoxins test (BET) assay parameters, procedures, standards, and controls for the gel-clot and kinetic and endpoint assays, proceed as directed in *Bacterial Endotoxins Test* ⟨85⟩, substituting the medical device eluate for the product sample.

DEFINITIONS

Endotoxin limit

- The endotoxin limit for the finished device is NMT 20 USP Endotoxin Units per device and NMT 2.15 USP Endotoxin Units for devices in contact with cerebrospinal fluid.
- For devices that directly or indirectly contact the intraocular environment, a lower endotoxin limit may apply (*1*).
- The endotoxin limit for the device extract in Endotoxin Units/mL is calculated as:

$$\text{Result} = (K \times N)/V$$

K = limit for each device
N = number of devices tested
V = total volume of the extract

Kit: A kit is defined as a collection of individual devices in its primary package, or a variety of related devices. Each individual type of device may have its own product endotoxin limit and should be tested and evaluated on an individual basis; see *Table 1. Selection of Product Units for Testing* [refer to ANSI/AAMI ST72 (*2*)].

Table 1. Selection of Product Units for Testing

Product Unit	Item for Testing
Individual medical device in a primary package where each medical device is used individually in clinical practice	Individual medical device
Set of components in a primary package where components are assembled as a product and are used together in clinical practice	Combination of components
A number of identical medical devices in one primary package where each medical device is used independently in clinical practice	Single medical device taken from the primary package
Kit of procedure-related medical devices where each medical device is used independently in clinical practice and where each medical device may have a different endotoxin limit	Each type of medical device that has a nonpyrogenic claim, or all items together

Lambda (λ): λ is the test method sensitivity for gel-clot methods. For turbidometric or chromogenic technique, λ is equal to the lowest endotoxin concentration on the referenced standard curve.

Product families: Product families for the purposes of BET may be defined on the basis of common materials of construction and/or common components. The choice of family members should be justified and documented. If a product family contains devices of many sizes, the device with the largest surface area claimed to be nonpyrogenic is chosen as the representative member of the family to be used in suitability testing.

Extracting or rinsing solution: *Water for BET* [see *Bacterial Endotoxins Test* ⟨85⟩*, Reagents and Test Solutions, Water for Bacterial Endotoxins Test (BET)*] or other suitable solvent demonstrated to be noninterfering with the BET assay is used to extract or rinse the medical device.

Sampling

1. *Sample*: A sample for end-product testing must be in its final configuration and packaging, including all component parts that make up the final medical device.
2. *Representative sampling*: A representative sample is a sample plan that is created and justified based on the assumption that the manufacturing process is validated and in a state of control.
3. *Sample size*: The number of devices chosen for routine testing is dependent on the size of the lot, level of control, statistical considerations, and historical performance. In most cases, each lot of product must be tested using an appropriate number of samples, NMT 10, taken at random to represent the quality of the lot. Alternate sampling plans that utilize small sample sizes or do not test each lot of product must be clearly defined and must be supported/justified by a robust risk assessment.
4. *Pre-post sterilization samples*: When qualifying a BET assay for pre-sterilization samples, equivalency in test results must be shown with post-sterilization samples to ensure that sterilization and post-sterilization handling have no adverse impact on the accuracy of the test result. Pre-sterilization testing is inappropriate for products that support microbial growth.

Suitability testing (also known as test for interfering factors): Suitability studies of device extracts are designed to ensure that the device extract under test does not adversely impact the accuracy of the test result by either a) causing the assay to underestimate endotoxin (inhibition) or b) contributing to overestimation of endotoxin (enhancement).

REAGENTS AND TEST SOLUTIONS

See ⟨85⟩.

PREREQUISITES TO TESTING

Apparatus

All equipment used in the extraction or testing process must be properly qualified and/or calibrated as appropriate. This equipment includes but is not limited to water baths, heat blocks, mechanical pipetters (fixed, adjustable, and repeating pipetters), thermometers, and plate/tube/cartridge readers.

Depyrogenate all glassware and other heat-stable materials in a hot air oven using a validated process. If employing plastic apparatus such as microplates and pipet tips for automatic pipetters, use apparatus that is shown to be free of detectable endotoxin and does not interfere with the test.

Analysts must be properly trained to perform the assay.

The assay sensitivity must be confirmed as described in *Bacterial Endotoxins Test* ⟨85⟩*, Gel-Clot Technique, Preparatory Testing, Test for Confirmation of Labeled Lysate Sensitivity* for gel-clot reagents and *Bacterial Endotoxins Test* ⟨85⟩*, Photometric Quantitative Techniques, Preparatory Testing, Assurance of Criteria for the Standard Curve* for quantitative methods. Assay sensitivity confirmation must be carried out when a new batch of reagent (lysate or endotoxin) is used or when there is any change in the test conditions that could affect the outcome of the test.

PREPARATION OF DEVICES: STANDARD METHODS

Endotoxin Limit

The endotoxin limit for the extracting or rinsing solution is:

$$\text{Result} = (K \times N)/V$$

K = amount of endotoxin allowed per device (20 Endotoxin Units per device; 2.15 for intrathecal devices)
N = number of devices tested
V = total volume of the extract or rinse

[NOTE—The volume of the rinse or extract may be adjusted for the size and configuration of the device.]

The standard extracting fluid for extracting, rinsing, or soaking medical devices is *Water for BET*. If necessary, other solvents may be used after it has been demonstrated that they do not interfere with the performance of the assay. Extraction is not required for liquid medical devices. Either the entire device if claimed to be nonpyrogenic or all components of the device that are claimed to be nonpyrogenic, including fluid pathways, must come in contact with the extraction fluid for the entire course of the extraction.

The standard extraction method is to soak or immerse the device or flush the fluid pathway with extracting fluid that has been heated to 37 ± 1.0°, keeping the extracting fluid in contact with the relevant surface(s) for NLT 1 h at controlled room temperature. Alternate extraction or rinsing methods may be used, but must be demonstrated to be equivalent to or better than the standard method. Extracts from individual medical devices are typically pooled for testing.

Suitability

Suitability of the BET assay must be demonstrated for each product or product family. The number of batches of product chosen for the suitability study is reflective of the level of control of the process and must be justified.

For the suitability test, medical device extracts or liquid medical devices are considered to be the sample solutions under test and are tested as outlined for the chosen technique in *Bacterial Endotoxins Test* ⟨85⟩*, Gel-Clot Technique, Preparatory Testing, Test for Interfering Factors*.

If necessary, adjust the pH of the solution to be examined (or dilution thereof) so that the pH of the mixture of the lysate and sample solution falls within the pH range specified by the lysate manufacturer, usually pH 6.0–8.0. The pH may be adjusted by use of an acid, base, or suitable buffer as recommended by the lysate manufacturer. Acids and bases may be prepared from concentrates or solids with *Water for BET* in containers free of detectable endotoxin. Buffers must be shown to be free of detectable endotoxin and interfering factors.

If the undiluted rinsing or extracting solution or liquid medical device is determined to be unsuitable for ⟨85⟩, the test for interfering factors may be repeated utilizing dilution or a method that will neutralize or eliminate the interfering substance.

If glucan interference is suspected, the use of a glucan-blocking agent or endotoxin-specific lysate is permissible.

Dilution in *Water for BET* or other validated solvent by a factor not to exceed the maximum valid dilution (MVD) is permissible. The MVD for medical devices is the following:

$$\text{MVD} = \text{Endotoxin limit (of the rinsing, extracting, or diluting solution in Endotoxin Units/mL)}/\lambda$$

λ = sensitivity of the test method in Endotoxin Units/mL

Interference may be overcome by suitable treatment of the device extract. Methods for overcoming interference and the qualification of such treatments are described in ⟨85⟩. To establish that the chosen treatment effectively eliminates interference without loss of endotoxins, perform the assay described in ⟨85⟩ using the preparation to be examined to which standard endotoxin has been added and which has then been submitted to the chosen treatment.

DEMONSTRATION OF CONTINUED SUITABILITY

The continued suitability of the assay should be re-assessed when significant changes are made to the product, process, raw material supply, manufacturing and/or testing site, BET reagent source, or any other change that could adversely affect the accuracy of the BET result. The results of this re-assessment should be documented, and suitability studies re-executed as necessary.

Changes to extraction techniques require a new suitability study. Because of possible changes in interference patterns between standard BET methods, a change in BET methodology (e.g., from gel-clot limits test to kinetic chromogenic) requires a re-execution of the suitability study.

Routine Testing

Routine testing of the finished medical devices must utilize the same extraction techniques that were documented in the successful suitability study.

Routine testing is to be performed as outlined in ⟨85⟩, following instructions for incubation and controls listed under the chosen assay technique.

Interpretation of Test Results

See ⟨85⟩. The preparation under test complies with the test if the mean endotoxin concentration of the replicates of *Solution A*, after correction for dilution and concentration, is less than 20 Endotoxin Units per medical device (2.15 Endotoxin Units per medical device for those devices that come in contact with the cerebrospinal fluid.) For liquid medical devices, the limit meets the requirements set forth in *Bacterial Endotoxins Test* ⟨85⟩*, Determination of Maximum Valid Dilution (MVD), Endotoxin Limit, footnote 2*.

If a device fails to meet this requirement, an investigation may be initiated per documented procedures. A failed, valid BET assay may not be repeated using *Pyrogen Test* ⟨151⟩ in its place.

PYROGENS

For samples that cannot be tested by BET because of nonremovable inhibition or enhancement of the test, ⟨151⟩ is applied. Select an appropriate number of devices, NMT 10, and obtain a pooled extract utilizing preparation methods appropriate to the device as directed for bacterial endotoxins, but with volumes of rinse or extraction fluid not to exceed 40 mL of sterile saline test solution per device. The requirements of ⟨151⟩ must be met.

REFERENCES

1. U.S. Food and Drug Administration. Guidance for industry. Pyrogen and endotoxins testing: Questions and answers. Rockville, MD: Food and Drug Administration; June 2012. www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm314718.htm. Accessed 12 July 2016.

2. ANSI/AAMI ST72. Bacterial endotoxins—Test methods, routine monitoring, and alternatives to batch testing. Arlington, VA: Association for the Advancement of Medical Instrumentation; 2011.