ASSOCIATES OF CAPE COD INCORPORATED
Specialists in Endotoxin and Glucan Detection

Revision of the United States Pharmacopoeia Bacterial Endotoxin Test Chapter

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Letter From The Editor

On December 1, 2012 the latest version of the USP Bacterial Endotoxins Test chapter became effective. The changes are not major but some of them are interesting. One simplifies the determination of endotoxin limits for certain products. While rather minor itself, this change provides a reminder that limits for these products were substantially tightened in 2011. In this article each of the changes are considered and their impact on endotoxin testing is assessed.

With best wishes for 2013,

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Revision of the United States Pharmacopoeia Bacterial Endotoxin Test Chapter

The Second Supplement to United States Pharmacopoeia (USP) 35 included a few changes to chapter <85>, Bacterial Endotoxins Test (BET). The changes became effective on December 1, 2012.

This article describes and discusses the changes in turn. Changes are shown in redline in each case.

1. **Specification that the default endotoxin test is the gel-clot limit test.**

   In the introductory paragraphs to the BET chapter: "Proceed by any of the three techniques for the test. In the event of doubt or dispute, the final decision is made based upon the gel-clot ~~technique~~ limit test unless otherwise indicated in the monograph for the product being tested."

   This is a rather minor change and has little effect because the only other alternative gel-clot technique in the BET chapter was the Quantitative Test, which includes all the elements of the limit test plus additional standard endotoxin concentrations and dilutions of the specimen. The limit test is simpler to perform. If the specification is to be met, the specimen must test negative, so there is no value obtained from testing dilutions of the specimen.

2. **Elimination of Reference to Testing of Extracts**

   In the REAGENTS AND TEST SOLUTIONS section, under the sub-heading Sample Solutions: "Prepare the Sample Solutions by dissolving or diluting drugs~~, or taking washes from medical devices~~ using Water for BET. Some substances or preparations may be more appropriately dissolved, or diluted~~, or extracted~~ in other aqueous solutions."

   References to testing extracts have been removed and specific mention of testing extracts of medical devices has been eliminated. It should be noted that USP chapter <161> "Transfusion and Infusion Assemblies and Similar Medical Devices" refers to the BET chapter for testing of medical device extracts. The removal of references to extracts from the BET chapter does not change this or mean that it is not appropriate to test medical device extracts using the method described in the BET.

3. **Change from "Standard Regression Curve" to "Standard Curve"**

   In the DETERMINATION OF MAXIMUM VALID DILUTION (MVD) section, under the sub-heading Concentration of Sample Solution: "λ: the labeled sensitivity in the Gel-Clot Technique (EU/mL) or the lowest concentration used in the standard ~~regression~~ curve for the Turbidimetric Technique or Chromogenic Technique."

   The deletion of "regression" has no impact on the meaning or intent of the sentence.

4. **Correction of Units for the Endotoxin Limits for Radiopharmaceuticals**

   In footnote number 2, which explains endotoxin limits for different categories of product:

   "For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as 175 EU/V, where V is the maximum recommended dose in mL. For intrathecally administered radiopharmaceuticals, the endotoxin limit is obtained by the formula 14 EU/V."

   The insertion of "EU" in the formula for calculating endotoxin limits for radiopharmaceutical products means that the resulting limit will have units of EU/mL, not just /mL without the "EU."

5. **Simplification of the Endotoxin Limit for Products Administered per Square Meter of Body Surface Area**

   Also in footnote number 2: "For formulations (usually anticancer products) administered on a per square meter of body surface, the formula is K/M, where K =~~2.5 USP-EU/kg~~ 100 EU/m^2 and M is the ~~(~~maximum dose/m^2~~/hour x 1.80 m2)/70 Kg~~."

   If the values and formulae given in the BET chapter prior to the recent change are used to calculate K on a per square meter basis, a value for K of 97 EU/ m^2 is obtained. The recent change to the footnote rounds this value to 100 EU/ m^2. This change has the advantage of a round number and simplifies the calculation required to determine a product specific limit, reducing the potential for errors. Also, it gives a formula structure that is similar to that for radiopharmaceuticals (and to that for medical device extracts given in USP chapter 161, "Transfusion and Infusion Assemblies and Similar Medical Devices"). This change will result in slightly increased product specific endotoxin limits and MVDs.

6. **Requirement to Repeat the test for Interfering Factors for the Gel-Clot Technique**

   In section on the GEL-CLOT TECHNIQUE, under the sub-heading Test for Interfering Factors: "The test for interfering factors must be repeated when any condition changes that is likely to influence the result of the test."

This portion of the BET is not actually a change as it first appeared in the interim revision announcement of 2011 and was included in the BET in USP 35. The requirement brings the section on the GEL-CLOT TECHNIQUE into agreement with the section on PHOTOMETRIC QUANTITATIVE TECHNIQUES, which states under the sub-heading Preparatory Testing "Validation for the test method is required when conditions that are likely to influence the test result change." (Validation includes verification of the criteria for the standard curve and that the sample solution does not interfere with the test.)

The changes regarding the Gel Clot Limit Test being the referee method in the event of a dispute, the deletions regarding medical devices and extracts and the deletion of "regression" from the reference to the standard curve (numbers 1, 2 and 3 above) were all made in the interests of harmonization with the European and Japanese Pharmacopeia endotoxin test chapters. These changes were announced on the USP website in late 2011 (see http://www.usp.org/usp-nf/harmonization/stage-6/bacterial-endotoxins-test.)

Conclusion

Most of the changes to the BET chapter made in Second Supplement to USP 35 are quite minor and will not impact the majority of laboratories performing the bacterial endotoxins test. An exception concerns drugs that are administered per square meter of body surface. In this case the change from an endotoxin limit calculated using a value of K of 2.5 EU/kg to one based on K = 100 EU/m^2 slightly raises that limit and consequently increases the maximum valid dilution (MVD). As the new limit for a product (calculated using a value of K of 100 EU/m^2) is slightly less stringent that that calculated using a value of K of 2.5 EU/Kg, it is not necessary to change the limits in procedures and submissions to regulatory agencies unless desired. There is no risk to public health that results from leaving in place a slightly more stringent limit than that which is required.

More important than this small difference is the change from a value of K of 5 EU/kg, which was made in the interim revision announcement in 2011. That change halved the endotoxin limits for this category of product, making it substantially more stringent. If limits (and MVDs) were never reduced from those determined using a value of K of 5 EU/Kg, they should be promptly recalculated using the new value of K and the changes applied to procedures and submissions.