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# Revisions to the **Pharmacopeial Bacterial Endotoxins Test Chapters**

by *Michael Dawson, Ph.D., RAC*  
Director of Regulatory Affairs

December, 2010

# LAL Update

volume 26, number 1

## Letter From The Editor

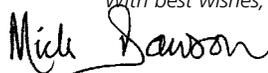
Dear LAL User:

*This Update describes the changes to the Bacterial Endotoxins Test (BET) chapter were published in USP 33 and became effective on October 1, 2010. Despite the fact that when the proposed changes were first published in Pharmacopeial Forum in 2007 the entire text of the chapter was struck out and complete new text was given, the changes are actually rather minor. The principles and the major requirements are unchanged. In addition to the changes in the USP BET, changes were also made quite recently to the Bacterial Endotoxins chapter in the EP. These are also discussed.*

*Other proposed changes to the USP BET were announced in the November/December 2010 issue of USP's publication Pharmacopeial Forum (volume 36, number 6). The most significant revision concerns a tightening of the endotoxin limit for products dosed per unit of body surface area. The change is a recommendation from FDA and is proposed for an interim revision announcement (i.e. a fast track change) with an official date of April 1, 2011. It is proposed that the value of K be reduced from to 2.5 EU/kg for drugs administered on a per square meter basis. It will remain 5 EU/kg for drugs administered on a per kg basis. Thus the endotoxin limits for such product will be halved. This is a significant change for affected manufacturers.*

*Also included in the proposed revision announcement is the reinstatement of the requirement that "the test for interfering factors must be repeated when any condition changes that is likely to influence the result of the test." This requirement was in the BET of USP 32 but it was omitted from the USP 33 chapter, as is noted in the article.*

*With very best wishes for 2011 from all of us at Associates of Cape Cod,*

With best wishes,  
  
*Michael Dawson, Ph.D., RAC*

**IMPORTANT**

The information in this article is given for informational purposes and does not include all the minor changes made to the chapters. It is not intended to be a substitute for the pharmacopeia, regulations or regulatory guidance documents. Procedures, decisions and actions should be based on the relevant regulations, guidance documents and pharmacopeial chapters, not on this article.

**Introduction**

This article reviews the changes made to the bacterial endotoxins test (BET) chapters in the United States Pharmacopeia (USP) and in the European Pharmacopoeia (EP). It also makes comparisons between these chapters and the BET chapter in the Japanese Pharmacopoeia.

The recent changes do not alter the fundamental principles of the chapter. These principles were established when the BET chapters in the USP, EP and JP were harmonized. The harmonized BET Text was published in the second supplement to USP 24 and became official in 2001, at the same time as the revised EP chapter. The revised JP chapter became official on April 1 of the same year. The current changes clarify the chapters somewhat and improve the degree of harmonization. However, there are still minor differences between the chapters.

The principle elements of the chapter are: first, a general section that addresses LAL reagents, the various test methods, apparatus (glass and plastic labware), the standard endotoxin and dilutions of standard, sample solutions, maximum valid dilution (MVD) and endotoxin limits. This is followed by a section specific to the gel-clot technique, followed by one specific to the photometric quantitative techniques (turbidimetric and chromogenic methods). The two technique specific sections each include a subsection on preparatory testing, which is subdivided into (i) confirmation of test method performance and (ii) the sample type specific test for interfering factors. Finally, each technique section includes a subsection on the actual test. In the case of the gel-clot technique, there are options for either limit test or a quantitative test.

The USP first published proposed changes to the BET in 2007 (Pharmacopeial Forum 33, No. 3). In 2009 the approved stage 6 draft was published in Pharmacopeial Forum 35(3) and scheduled for publication in USP 33. The recall and republication of USP 33 resulted in a delay of the date on which the revised BET chapter became official until October 1, 2010.<sup>1</sup>

The changes to the EP chapter were initially published in PharmEuropa 19(2), also in 2007. The final version was published in 2009 in Supplement 6.6 to EP 6 and became effective on January 1, 2010. The chapter is essentially unchanged in EP 7.0<sup>2</sup>, which becomes effective on Jan. 1st, 2011.

**Summary of Changes to the BET Chapters**

1. The USP chapter now describes three techniques (the gel-clot, turbidimetric technique, and chromogenic) in the initial, general section, not two (gel-clot and photometric). The EP continues to describe 6 methods: A. Gel-clot limit test; B. Gel-clot: quantitative test; C. Turbidimetric kinetic method; D. Chromogenic kinetic method; E. Chromogenic end-point method; F. Turbidimetric end-point method.
2. The revised USP chapter specifies use of an amoebocyte reagent "manufactured in accordance with the regulations of the competent authority," which is the same wording as the EP chapter. In USP 32, the BET stated LAL "... which has been prepared and characterized for use as an LAL Reagent." The wording of the revised USP chapter is interesting because the term "competent authority" has specific meaning in Europe but not in the United States (US) context, though the Food and Drug Administration is the US equivalent of a European Competent Authority. This requirement reflects the statement in the 1987 FDA Guideline on Validation of the Limulus Amoebocyte Lysate Test ... that reagent licensed by the Center for Biologics Evaluation and Research (CBER) should be used for validation, release and in-process testing.
3. In the USP, the note on  $\beta$ -glucans has been moved from a footnote into the main text and refers to lysates with factor G removed or added glucan blockers, which is like the EP.
4. USP now refers to reconstituted lysate as lysate TS, like the JP (but not the EP, which refers to lysate solution).
5. The USP footnote on "LAL Reagent Water" has been replaced with "Water for BET" in the text, like the EP.
6. Details of reconstitution, use and storage of USP Endotoxin Reference Standard (Endotoxin RS) are replaced with reference to instructions. There are no changes to this section of the EP chapter.
7. In the USP, the caution "Do not store dilutions ... in the absence of supporting data ..." has been removed and replaced by: "Use dilutions as soon as possible to avoid loss of activity by adsorption," which is in accordance with the EP.



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CAPE COD  
INCORPORATED**

*Specialists in Endotoxin and Glucan Detection*

**Corporate Headquarters**  
Associates of Cape Cod, Inc.  
124 Bernard E. Saint Jean Drive,  
East Falmouth, MA 02536  
T (508) 540-3444  
F (508) 540-8680  
www.acciusa.com

**UK Office**  
Associates of Cape Cod Int'l Inc.  
Deacon Park, Moorgate Road,  
Knowsley, Liverpool L33 7RX  
United Kingdom  
T (44) 151-547-7444  
F (44) 151-547-7400

**European Office**  
PYROQUANT DIAGNOSTIK GmbH  
Opelstrasse 14,  
D-64546 Morfelden-Walldorf,  
Germany  
T (49) 61 05-96 10 0  
F (49) 61 05-96 10 15

8. The section on pH has been reworded. The statement that the pH is usually within range if the sample pH is between 6.0–8.0 has been removed. The USP BET and the EP chapter both now state that the pH of the sample/lysate reaction mixture should be within the range specified by the lysate manufacturer, usually 6.0 to 8.0.
9. In the section on the MVD, under the explanation of M (the maximum dose), the USP and EP refer to the maximum recommended bolus dose of product per kilogram of body mass. The chapters continue “When the product is to be injected at frequent intervals or infused continuously, M is the maximum total dose administered in a single hour period.”
10. The explicit requirement to repeat the test for interfering factors if experimental conditions change is omitted from the USP section of the revised chapter on the gel-clot technique. It was in USP 32 and is in both the former and the revised EP chapters. The requirement is explicit for photometric methods. There is also such a requirement for the confirmation of lysate sensitivity in the revised USP BET.  
  
However, the requirement that the test for interfering factors should be repeated if conditions change is implicit in the statement that “If the sensitivity of the lysate ... is not less than  $0.5 \lambda$  and not greater than  $2 \lambda$ , the Sample Solution does not contain factors that interfere under the experimental conditions used.” (Emphasis added.) The test is only valid if the specific conditions of the test are unchanged and consequently it should be revalidated if conditions do change.
11. The requirement to use the gel-clot limit test (as opposed to the quantitative test) when a monograph contains a requirement for endotoxin limits has been removed from the USP. This is required by the EP in the event of a dispute when another method is not specified in the product monograph.
12. A provision for retesting has been added to both the USP and EP chapters for a gel-clot limits test in which one of the two replicates of the sample [solution A] tested positive in an initial test. In a retest, if one or both replicates test positive the preparation does not comply with the test. This is consistent with the requirements for a gel-clot quantitative test (ie. an assay), in which both replicates must test negative at the endotoxin limit.
13. Under the Photometric Quantitative Techniques section, the requirement to perform the test for the Assurance of Criteria for the Standard Curve for each lysate lot has been added to the USP and EP chapters. This is (and was previously) specified for the gel-clot technique (and in the JP chapter).
14. In the EP, the section titled “Test for bacterial endotoxins: guidelines” has been removed from the BET chapter, number 2.6.14, and is now a separate new chapter under General texts, number 5.1.10.<sup>3</sup>

### Conclusion

The revisions to the BET chapters do not change the fundamental principles of the test. This is despite the fact that, in the case of the USP, the revised versions published in Pharmacopeial Forum show deletion of all of the current text and complete replacement. The changes are relatively minor but still leave some small differences between the BET chapters in the three pharmacopeia.

### References for LAL Update USP changes

1. United States Pharmacopeia (2010) Bacterial Endotoxins Test. Chapter <85>. USP 33: pp. R65-R69. United States Pharmacopeial Convention, Rockville, MD.
2. 2.6.14. Bacterial Endotoxins (2010) European Pharmacopoeia 7.0, p. 171 -175. European Pharmacopoeia Commission, Strasbourg, France.
3. 5.1.10. Guidelines for using the Test for Bacterial Endotoxins (2010), European Pharmacopoeia 7.0, p. 520 -523. European Pharmacopoeia Commission, Strasbourg, France.

*This article is based upon a presentation given at the Parenteral Drug Association Europe Conference on Endotoxin in Barcelona, Spain in April, 2010.*