Recent Regulatory Issues Concerning Bacterial Endotoxin Testing

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Introduction

Within the last two years, there have been developments in two areas of regulatory significance to endotoxin testing. The first concerns changes to the Bacterial Endotoxins Test (BET) chapter in the United States Pharmacopeia (USP). The second is the release by the US Food and Drug Administration (FDA) of a guidance document on pyrogen and endotoxins testing in June of 2012.

Chapter <85> Bacterial Endotoxins, United States Pharmacopeia

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, and were incorporated into the BET chapter in USP 36 [1], which became effective on May 1, 2013. These changes are described and discussed in turn below.

The first three changes described were 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.)

Changes and Comments

1. Specification that the Default Endotoxin Test is the Gel-Clot Limit Test

In the introductory paragraphs to the BET chapter, the instruction that in the event of doubt or dispute, the final decision is made based upon "the gel-clot technique" was changed to specify "the gel-clot limit test", unless otherwise indicated.

This is a rather minor change and has little effect because the only other gel-clot technique in the BET chapter is 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 merit in 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, references to testing medical device extracts have been removed and specific mention of testing extracts has

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been eliminated. It should be noted that USP chapter <161> "Transfusion and Infusion Assemblies and Similar Medical Devices" [2] 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, the word "regression" has been deleted from the reference to 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, in the section on radiopharmaceutical products, the units EU have been added to read "For radiopharmaceutical products not administered intrathecally, the endotoxin limit is calculated as 175 EU/V…. 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". The change corrects the units, which otherwise work out as just "/mL".

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

Also in footnote number 2, in the section on formulations (usually anticancer products) administered on a per square meter of body surface, the definition of K in the formula for calculating the endotoxin limit has been changed from "K = 2.5 USP-EU/kg and M is the (maximum dose/m^2/hour x 1.80 m^2)/70 Kg" to "K = 100 EU/m^2 and M is the maximum dose/m^2".

If the values and formulae given in the BET chapter prior to the recent change are used to calculate K per square meter basis, a value of 97 EU/ m^2 is obtained. The change to the footnote rounds this value to 100 EU/m^2. This change results in slight increase in product specific endotoxin limits and MVDs, but is still almost half the value that obtained prior to the interim revision announcement that became effective in April of 2011 [3]).

This change has a number of advantages:

1. The value of K is a round number that incorporates the corrections for the surface area of a "typical" 70 kg person.
2. The calculation of the endotoxin limit is greatly simplified, which reduces the opportunity for error.
3. The structure of the formula is now 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 [2]).
4. The value of K is now expressed in the same units as the dose of the product, which is analogous to the value of K for drugs administered per kg body weight.

As the endotoxin limit for a product calculated using a value of K of 100 EU/ m^2 is slightly less stringent that that calculated using the previous value of K of 2.5 EU/Kg, it should not be necessary to change the limits in procedures and submissions to regulatory agencies unless desired. There is no risk to public health resulting from leaving in place a slightly more stringent limit than that which is required by the recent change.

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, a requirement has been added to repeat the test for interfering factors when any condition changes that is likely to influence the result of the test.

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 (1) of the criteria for the standard curve and (2) that the sample solution does not interfere with the test.)

FDA Guidance for Industry "Pyrogen and Endotoxins Testing: Questions and Answers" 2012

In June of 2012, the FDA released the long awaited question and answer (Q&A) guidance document on pyrogen and endotoxin testing [4], almost exactly a year after the withdrawal of the former guidance documents (the 1987 "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" and the 1991 "Interim Guidance for Human and Veterinary Drug Products and Biologicals: Kinetic LAL Techniques").

Comments

Introduction

The title and the Introduction section to the guidance make clear that the scope of the guidance includes the pyrogen test. One of the questions addresses when it is appropriate to use the pyrogen test. However, the Introduction states that the document does not cover the breadth of endotoxin and pyrogen testing. It focuses on specific issues that may be subject to misinterpretation and are not covered in compendial procedures or in currently available guidance documents.

The guidance refers to the USP Chapter <85> BET, the USP Chapter <161>, Transfusion and Infusion Assemblies and Similar Medical Devices [2], and the AAMI/ANSI standard ST72:2002/R2010 (the current version of which is ST72 2011 [5]). It states that these three documents describe the fundamental principles of the gel clot, photometric, and kinetic test methods, and that a thorough understanding of these documents is expected.

Background

The Background section does not mention the Interim Guidance document of 1991. The Interim Guidance was specific to testing of drugs and biological products by turbidimetric and chromogenic methods. It was withdrawn at the same time as the 1987 Guidance.

Question 1: How do I establish a sampling plan for in-process testing and finished product release?

The 1987 Guideline on the *Limulus* amebocyte Lysate Test, (which was withdrawn in 2011), stated: "Sampling technique selected and the number of units to be tested should be based on the manufacturing procedures

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and the batch size. A minimum of three units, representing the beginning, middle, and end, should be tested from a lot". The wording in the current guidance emphasizes the justification of an appropriate sampling technique and states that the sampling plan should be dynamic. This might result in the need for an increased level of testing compared to those based on the former document. The numbers of samples might be reduced once sufficient data has been collected to demonstrate that a process is under control. There is no mention in the response to Question 1 of sampling from the beginning, middle and end of a production run. However, this is included in the response to Question 4.

Question 2: When is retesting appropriate?

Prior to publication of the guidance, FDA speakers had stated at meetings that the outdated provisions for retesting in the former guidance documents was a principle reason for their withdrawal. The withdrawn documents were written before the Barr decision of 1993 and before the Out of Specification (OOS) Guidance was issued by FDA in 2006. They were inconsistent with the OOS guidance and current FDA thinking. The response to Question 2 agrees with and refers to the 2006 OOS Guidance document.

Question 3: Is sample storage and handling important?

Sample storage and handling is a point about which FDA has shown consistent concern for over 20 years (for example see Guilfoyle et al. [6]). This issue is important. Sample stability is also mentioned in the response to question 4 in the discussion of medical device extracts.

Question 4: Can finished product samples for analysis of bacterial endotoxins be pooled into a composite sample prior to analysis?

The response allows for pooling of products provided that the MVD for the sample pool (and, by logical extension, the endotoxin limit for the sample pool) is reduced proportionately. This is not a new point. The issue of pooling of drug products has been raised at meetings by FDA speakers for many years and it has appeared in the handouts of presentations. This is the first time it has appeared in a guidance document. FDA suggests pooling no more than three units and refers to testing representative finished product containers from the beginning, middle, and end of the production run. It is noted here that when vials/containers of drug product are tested individually there is no need to reduce the MVD. Also, testing individual units gives more information about variability between samples. That information is lost when units are pooled for testing.

For medical devices, the guidance refers to the standards ISO 10993-1 and ISO 10993-12 for rinsing/eluting and sampling techniques. The response to the question does not refer to USP Chapter <161>, Transfusion and Infusion Assemblies and Similar Medical Devices [2]. This USP chapter calls for pooling up to 10 medical device extracts and gives a formula for calculating the endotoxin limit for the extract pool. USP chapter <161> does not require adjusting the MVD (or the endotoxin limit) to account for pooling because that has been accounted for in the endotoxin limit of 20 EU/device. This is not stated in the USP chapter of the guidance document but it is explained clearly in the standard ANSI/AAMI ST72:2011 [5] in Annex A, item A.8.

Question 5: May a firm use alternative assays to those in the USP for a compendial article?

The response regarding alternate (i.e. non-compendial) methods states that such methods and/or procedures may be used if they provide advantages in accuracy, sensitivity, precision, selectivity, or adaptability to automation or computerized data reduction, and in other special circumstances. It makes clear that (1) such methods should be appropriately validated and (2) if a difference appears or in the event of a dispute, the final decision is made based upon the USP compendial gel-clot method unless otherwise indicated in the monograph for the product being tested.

As examples of alternative assays that require validation as alternate methods, the recombinant Factor C assay and the Monocyte Activation Test (MAT) are cited.

Question 6: What is the best process for transitioning from one alternate bacterial endotoxins test (BET) method to another?

The response to the question makes clear that firms should carefully consider the validation requirements for a method change. Consequently, it would be prudent to document the rationale for the approach taken to the validation, including whether or not to adopt the suggestion to test field samples. It suggests that comparing the two tests (the current test and the proposed new method) to verify the equivalence of the new method. In addition, it states that the sensitivity of the new method can be evaluated on spiked product samples. The response is not explicit whether "spiked product samples" refers to spiking of undiluted product or to spiking of product at the test dilution, as is typically done to prepare positive product controls.

It is a little surprising that the USP chapter <1225>, "Validation of Compendial Procedures" is referenced. Chapter <1225> describes the requirements for validation of procedures that are included in the USP (i.e. Compendial Procedures). The methods at issue in this question and answer are those that are included in the USP BET chapter and are therefore validated compendial procedures. A more appropriate reference would be chapter <1226> "Verification of Compendial Procedures" (which does refer to chapter <1225>).

There is no mention of the testing required to support changing to reagent from a different manufacturer (without a change of test method). Thus, it is left to the firm to appropriately validate and document the change. A reagent transfer protocol is available from Associates of Cape Cod, Inc., that can assist with this process.

Finally, the response gives useful information on the expectations for reporting such changes to FDA.

Question 7: What happened to the endotoxins limit table in Appendix E of the 1987 Guidance?

The response explains the rational for withdrawal of the table of endotoxins limit that was Appendix E of the 1987 Guidance. Withdrawal of the Appendix E is a positive step as it forces users to refer to primary sources of information, including USP monographs. The response includes important recognition of the fact that the endotoxin limit in a USP monograph may not be appropriate for a particular product because the product strength or dosage regime differs from that used to calculate the limit in the USP monograph. Consequently, it is always prudent to verify endotoxin limits by calculating them using the maximum dosage stated in the package insert for the product.

Question 8: How can Quality by Design concepts support endotoxins limits?

The response emphasizes the importance of process control and the role of endotoxin testing of raw materials, product components and of in-process samples in assuring the quality of finished product. Also, preference for quantitative testing as opposed to over limit testing is indicated.

Question 9: When is the USP Chapter <151> Pyrogenicity Test (the rabbit pyrogen test) appropriate?

The response notes that for some products, a pyrogen test is specified in the USP monograph or may be necessary in cases in which a valid BET cannot be performed. The response also raises the possibility of contamination of products by non-endotoxin pyrogens. While this cannot be ruled out by endotoxin testing, actual cases of contamination by non-endotoxin pyrogens are rare. But for this fact, the BET would not have been accepted as an alternative to (or have largely replaced) the pyrogen test. (It is worth noting that some therapeutic agents are known pyrogens, such as interleukin-2.)

Question 10: How would an appropriate endotoxins limit be determined for a veterinary product that targets multiple species?

The response states: "For a veterinary product labeled for use in multiple species, the limit should be based on the maximum product dose used on the smallest species. If … the product may be used on juvenile and adult animals, the juvenile is considered the worst case". The statement that the limit should be based on the maximum product dose used on the smallest species is surprising since it leaves open the potential for confusion if the maximum dose per unit mass is specified in the package insert (PI) for a larger species. In the event of such confusion, a conservative approach is to use the maximum dosage specified in the PI to determine endotoxin limit. This will result in the most stringent endotoxin limit, even if the limit is not based on the product dose for the smallest species – juvenile or otherwise.

Question 11: What are the endotoxins limits for medical devices?

In addition to the limits given in USP chapter <161> (which are 20 EU/device and 2.15 EU/device respectively for devices that contact the cardiovascular or lymphatic system and for those that contact cerebrospinal fluid), the response also gives limits of 0.5 EU/mL and 0.06 EU/mL. These limits are linked to an extract volume of 40 mL, which is recommended in the next paragraph. It is not clear why an extract volume of 40 mL is given, even though provision for reduced or increased volumes is made to accommodate smaller or larger medical devices. The response states that the endotoxin limit can be adjusted if the extract volume is changed, but it does not mention that USP chapter <161> provides a formula for determining the endotoxin limit from any extract volume.

The response states, "For inhibition/enhancement testing, both the rinse/extract solution and the device eluate/extract should be tested". Thus, the guidance recommends that the solution to be used for extracting the device as well as the solution after extracting the device should be tested. The initial test of the solution will serve as a control in the event that the device extract gives a positive test result.

The response to Q11 states that more stringent limits should be applied to devices for which multiple units of the same device from one manufacturer are intended for use in a single procedure. The multiple units should meet the same endotoxins limit as a single device. This implies that the USP limit for a single device (e.g. 20 EU) should be divided by the maximum number of devices likely to be used in the single procedure. The resulting reduced limit would then be applied to each of the devices that are expected to be used together.

Question 12: What is the FDA's expectation for regular screening of therapeutic drug products?

The response to this question indicates that FDA is encouraging endotoxin tests to be a sensitive as possible. This means testing at the highest product concentration as reasonably possible (i.e. as far from the MVD as possible). It seems clear that intent is to get as much information from the test as possible.

The response suggests testing at a dilution of 1:30 for a product for which the first dilution that does not interfere with the test is 1:20. This could result in interference problems if subsequent batches show slightly greater levels of interference. A more common recommendation in the industry is to test at a dilution of at least a twofold greater than that at which interference was overcome (unless that dilution exceeds the MVD). In the case of the example given, that would be 1:40.

Question 13: Are control standard endotoxins still acceptable for use in running bacterial endotoxins tests?

In the response to this question, the FDA provides a clear statement that use of appropriately calibrated CSE is encouraged.

Omitted Topics

In addition to the comments made on the Q&A document, it is notable that some topics that were addressed in the withdrawn 1987 and 1991 guidance documents are not included in the Q&A.

In the discussion on Question 6, it was noted the Q&A document does not address changing reagent manufacturer (while retaining the test method). This was included in the withdrawn guidelines.

The withdrawn 1987 FDA Guideline included a section on Initial Qualification of the Laboratory in the section on Drugs and Biological Products. It called for an assessment of the variability of the testing laboratory and for qualification of analysts. These are general GMP requirements and are not addressed specifically in the Q&A document. The USP BET chapter specifies verification of the performance of each lot of LAL reagent but it does not address qualification of laboratory and analysts.

Perhaps the most notable omission is the lack of any guidance on archived standard curves or the controls that should be used to verify their validity. There is now no mention of archived standard curves in any regulatory document, guidance or standard. The pharmacopeial BET chapters (USP,

European Pharmacopoeia and Japanese Pharmacopoeia) specify inclusion of a standard series with every photometric endotoxin test.

Conclusion

The issues addressed in the recent BET version and in the FDA's Q&A guidance document do not fundamentally change the way endotoxin testing should be performed.

Most of the changes to the BET chapter made in the Second Supplement to USP 35 and incorporated into USP 36 are quite minor and are not likely to impact the majority of laboratories. An exception concerns drugs that are administered per square meter of body surface. In this case, the change slightly raises the endotoxin limit and consequently increases the maximum valid dilution (MVD). More important than this small difference was the previous halving of the endotoxin limits that resulted from the change in the value of K (for these products only) from 5 EU/kg to 2.5 EU/kg. This change had been made by USP in an interim revision announcement in 2011. If limits (and MVDs) have not been reduced from those determined using a value of K of 5 EU/Kg, they should be promptly recalculated using the current value of K (100 EU/m^2) and the changes applied to procedures and submissions.

The Q&A document refers to the USP chapter <85>, Bacterial Endotoxins Test and to the standard, ANSI/AAMI, ST72 and makes it clear that the document is not intended to be all inclusive and that it only addresses a number of specific issues. As well as providing useful information on a number of subjects, the document contributes to a climate in which firms are expected to have justification for their testing activities (including sampling plans and validation of method change), as opposed to simply referring to a guidance document. It emphasizes scientifically defensible decisions and process control. While a number of topics that were previously addressed in the withdrawn guidance documents are not included (most notably the use of archived standard curves), it is anticipated that scientifically defensible and appropriately controlled procedures will be expected in these areas, too.

References

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6. *Guilfoyle, D. E., J. F. Yager and S. L. Carito. 1989. The effect of refrigeration and mixing on detection of endotoxin in parenteral drugs using the limulus amebocyte lysate (LAL) test. J. Parenter. Sci. Technol. 43(4):183-187)*

BEST QC Microbiology Training: A Review

Elizabeth Thomas, Editor

Where: Chicago, IL
When: September 11, 2013
What: Day 2 of BEST QC Microbiology Training – The Bacterial Endotoxins Test

EMD Millipore and Associates of Cape Cod offer microbiologists the opportunity for personalized training in their educational program: BEST QC Microbiology Training. The program is geared towards improving lab techniques and applications for in-process and product release quality control tests. Last month's Chicago event was the fourth in a series of six sessions scheduled in 2013; the remaining two will be held in San Francisco and San Juan. The collaborative training spans three days: the first focuses on Bioburden, the second on Endotoxins, and the third on Sterility.

The endotoxin-focused segment provided familiarization with endotoxin detection, including step-by-step instructions for utilizing Associates of Cape Cod's testing equipment. Troubleshooting techniques, method comparisons, and summaries of relative regulatory guidelines with a special focus on USP Chapter <85> Bacterial Endotoxins Test were also highlighted. The session was interactive, featuring videos, live demonstrations and hands-on trials by attendees, all of whom had at least rudimentary laboratory experience. Through the open forum environment, participants shared best practices and asked company representatives specific questions regarding the systems presented. The meeting was straight-forward and comprehensive, allowing attendees to grasp the basics of endotoxin testing.